The potentiation and broadening the effect of low molecular hypoglycemic drugs through interaction with some CNS stimulant molecules

Mohammad Mohiuddin
Registration Number: 73
Session: 2008 – 2009

Analytical Research Laboratory
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Dhaka
Dhaka – 1000

Dissertation submitted for the partial fulfilment of the requirements for the degree of Doctor of Phylosophy in Pharmaceutical Chemistry from University of Dhaka

19th September, 2013

CERTIFICATE

This is to certify that the thesis entitled 'The potentiation and broadening the effect of low molecular hypoglycemic drugs through interaction with some CNS stimulant molecules' has been carried out under our joint supervision by Mohammad Mohiuddin, Registration Number: 73, Session: 2008-2009 for the fulfilment of the requirements for the award of the degree of Doctor of Phylosophy from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh.

The results and findings incorporated here have not been submitted to anyother institution for the award of any degree. The style and format are in accordance with the practices of this university. The contents and results of the thesis are satisfactory. We recommend the work for the award of the degree of Doctor of Phylosophy in Pharmaceutical Chemistry under the University of Dhaka.

Prof. Dr. Md. Shah Amran
Co-supervisor

Department of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Dhaka
Dhaka-1000

Prof. Dr. Muhammad Amjad Hossain
Supervisor

Department of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Dhaka
Dhaka-1000

ABSTRACT

The in-vitro study on the interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin has been carried out by IR spectroscopy, UV spectrophotometric, continuous variation, mole ratio, conductometric and Ardon's spectrophotometric methods at room temperature and at different pHs to investigate the invitro complex formation and also to study the nature & strength of complexes which could be formed due to interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin. The results of the present study indicated that caffeine and theophylline form 1:1 complexes with gliclazide, glipizide, glyburide and metformin.

Observations of infrared and ultraviolat spectral data have revealed the possibility of interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin. The spectra of target molecules alone and the 1:1 mixture of caffeine and theophylline each with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities including some shifts in the absorption maxima. This may be due to interaction of caffeine and theophylline with the drugs that alter the absorption intensities. Study of continuous-variation, mole-ratio and conductometric methods has indicated initial complexation. Continuous-variation plots have conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin, 1:2 complexes of caffeine with glyburide, the formation of 2:1 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide and 1:1 complexes of theophylline with metformin. Mole-ratio plots conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin, 1:2 complexes of caffeine with glyburide, the formation of 1:2 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide & metformin.The conductometric method was used to further ascertain about the nature of interaction and stoichiometries. Conductometric titrations in demineralized water system at differe0nt pHs were carried out to find out the molar ratios at which complexation occurred. Conductrometric titrations have showed that 1:1 complexes are formed between caffeine and each of the interacting species. It has also showed that 1:1 complexes are formed between theophylline and each of the interacting species. The Ardon's spectrophotometric method confirmed the formation of 1:1 molecular complexes and led to calculate the stability constants. It has been observed that the stability constants for caffeine-gliclazide system were higher than that of caffeine-glipizide, caffeine-glyburide, caffeine-metformin, theophylline-gliclazide, theophylline-glipizide, theophylline-glyburide and theophylline-metformin system in all pHs conditions.

The in-vitro study of protein binding of gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures with caffeine and theophylline have been conducted by equilibrium dialysis method performing measurement by direct spectrophotometric method at temperature $37^0 \pm 0.5^0$ C and at pH 7.4. The number of binding sites and affinity constants of gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures with caffeine and theophylline have been calculated by scatchard method.Scatchard plots were prepared to reveal the number of binding sites and the affinity for protein binding. It has been found that both caffeine and theophylline cause lowering the affinity and percentage of binding of the drugs in the mixture to bovine serum albumin. Thus, the interaction of gliclazide, glipizide, glyburide and metformin with caffeine and theophylline can increase the free drug concentration of gliclazide, glipizide, glyburide and metformin in blood plasma. This may change the pharmacokinetic and pharmacodynamic properties of the drugs.

An in-vivo study has been conducted in rats to observe the influence of caffeine and theophylline on plasma concentration of gliclazide, glipizide, glyburide and metformin. The plasma concentration of gliclazide, glipizide, glyburide and metformin were determined by UV spectrophotometric method after oral single administration of gliclazide, glipizide, glyburide, metformin alone and with caffeine & theophylline in rats.

The in-vivo study for determination of plasma cocentration showed that concurrent administration of caffeine and theophylline with gliclazide and glyburide have not made noticeable changes in plasma concentration of gliclazide and glyburide. But administration of caffeine and theophylline with glipizide and metformin in rats has showed a significant change in plasma concentration of glipizide and metformin. So, a competitive inhibition of the binding to plasma protein by caffeine and theophylline increases the plasma concentration of glipizide and metformin.Thus any change in plasma concentration may affect the pharmacological effects of the drug.

An in-vivo study has been conducted in rats to observe the effect of caffeine and theophylline on hypoglycemic activity of gliclazide, glipizide, glyburide and metformin. The blood sugar levels of animals were measured after administration of a drug alone and in combination. The blood sugar levels were estimated in two stages; firstly, after 2 weeks and secondly, after 4 weeks of the administration of drug. The results in this study have shown that caffeine and theophylline can enhance hypoglycemic effect of gliclazide, glipizide, glyburide and metformin in rats. It has also been found that the influence of caffeine on metformin is stronger than on gliclazide, glipizide and glyburide in respect of hypoglycemic activity.Thus, the hypoglycemic activities of gliclazide, glipizide, glyburide and metformin are potentiated and broadened by caffeine and theophylline concurrent application. The potentiation of the antidiabetic properties of gliclazide, glipizide, glyburide and metformin may be due to relaxation effect of caffeine and theophylline on smooth muscles of the rat as well as some sort of modification of the molecular conformations of the antidiabetic agents.

Thus, the interaction of caffeine and theophylline with the potent antidiabetic drugs gliclazide, glipizide, glyburide and metformin hydrochloride may greatly influence the activity of these molecules. The data obtained from the present study would help to recommend that low molecular hypoglycemic drugs gliclazide, glipizide, glyburide as well as metformin may result into compatible combination therapies with CNS stimulant molecules caffeine and theophylline.

ACKNOLEDGEMENT

I would like to gratefully acknowledge my supervisor at the University of Dhaka, Professor Dr. Mohammad Amjad Hossain for his meticulous guidance, generous assistance and encouragement for meaningful completion of the study. I would also like to gratefully acknowledge my co-supervisor at the University of Dhaka, Professor Dr. Md. Shah Amran for his constant support, suggestions and co-operations for succesfully completion of the study.I am ever grateful and highly indebted to Professor Dr. Md. Abdur Rashid, Ex-Dean, Faculty of Pharmacy, University of Dhaka, Bangladesh for completion of the part-1 examination of the study.I am also grateful to all of my beloved teachers Prof. Dr. A.T.M. Zafrul Azam, Prof. Dr.Khalid Hosain, Prof. Dr. Saila Kabir, Prof. Dr. Md. Abdul Majid, Assistant Prof. Dr Abu Asad Chowdhury, Assistant Prof. Dr Mohammad Rashedul Haque, Assistant Prof. Dr Md. Gias Uddin and Assistant Prof. Dr Mohammad Sharifur Rahman for instruction and suggestion in completion of my course works. I am grateful to Professor Dr. Monira Ahasan, Chairperson, Department of Pharmaceutical chemistry, University of Dhaka for arrangement of seminars for presentation of my research works. I am also grateful to Professor Dr. Md. Saiful Islam, Dean, Faculty of Pharmacy, University of Dhaka for his help in submission procedure of the thesis paper.

I would like to thank the 'International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) for supplying the test animals. I am also grateful to the authority of the 'Orion Laboratories Ltd.', Dhaka, Bangladesh and 'Beximco Pharmaceuticals Ltd.', Gazipur, Bangladesh for providing the test drugs.

I would also like to express my highest grateful and thanks to all my collegues and collaborators at laboratory specially Nur Nabi, Ferdous Islam, Nurul Huda, Golam Murad, Golam Kibria, Sharif Mohammad Rasel, Rakib Ali, Arifur Rashid, Abdus Salam, Saiful Islam, Abdur Rahim, Zakia Sultana, Khalid Hossain, Abdulla-al Mamun, Abu Sayed, Liton Chandhra Paul, Faisal Hossain, Maksuda Haque and Anjuman Ara for all of their help in my research works and preparation of my thesis paper.I would especially like to thank Abdul Malek, Anwarul Islam and Sajjad Hossain for their tireless work to make the laboratory function. I would like to thank Mikhir, Shahid and Ruhul for their co-operation of departmental works. My sincere appreciation and thanks to Asma Rahman, PhD Research fellow, CARS, University of Dhaka for her information and suggestion.

I am delighted to pay tribute to my entire family especially my beloved parents for their support, encouragement and love all throughout. I would like to express my highest grateful and thanks to my Aunt Mrs. Parveen Akhter, uncle Mr. Shah Alam and cousin Farzana Alam Priyanka for their tireless support and love. I would also like to express my highest indebted thanks to my youngest uncle Mr.Jashim Uddin for his encouragement and love to complete of the study.

.

.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CH,APTER 1:

GENERAL INTRODUCTION

INTRODUCTION

1.1 INTERACTION OF DRUGS

A **drug interaction** is a situation in which a substance (usually another drug) affects the activity of a drug when both are administered together. This action can be synergistic (when the drug's effect is increased) or antagonistic (when the drug's effect is decreased) or a new effect can be produced that neither produces on its own.

Typically, interactions between drugs come to mind (drug-drug interaction). However, interactions may also exist between drugs and foods (drug-food interactions), as well as drugs and medicinal plants or herbs (drug-plant interactions). People taking antidepressant drugs such as monoamine oxidase inhibitors should not take food containing tyramine as hypertensive crisis may occur (an example of a drug-food interaction). These interactions may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances.

A very broad definition of a **drug interaction** may be defined as the altering of the effects of one of the drug by the prior or simultaneous administration of another drug. Usually this results in an adverse reaction. But in a good number of cases drug interaction may prove beneficial. The drug, which precipitates an interaction, is called precipitant drug and the drug whose action is affected is called the object drug.[1] [2]

Drug interactions may be the result of various processes. These processes may include alterations in the pharmacokinetics of the drug, such as alterations in the absorption, distribution, metabolism, and excretion (ADME) of a drug. Alternatively, drug interactions may be the result of the pharmacodynamic properties of the drug, e.g. the co-administration of a receptor antagonist and an agonist for the same receptor.

Drug interaction occurs not only by the effects of a different drug but also by some other factors. On this basis, drug may be classified as follows.[1] [2]

1.1.1 Drug-Drug interaction
1.1.2 Drug-Food interaction
1.1.3 Drug-Plant interaction
1.1.4 Drug-Metal interaction
1.1.5 Drug-Environment interaction
1.1.6 Drug-Diseases pathogen interaction

1.1.1 Drug – Drug interaction

Drug-drug interaction result when one drug alters the known therapeutic response of another that has been administered concurrently or before or after the drug. The next result may be enhanced or diminished effects of one or both the drugs.

Drug interactions are generally encountered in the following stages:

1.1.1.1 Pharmaceutical interaction
1.1.1.2 Pharmacokinetic interaction
1.1.1.3 Pharmacodynamic interaction

1.1.1.1 Pharmaceutical interaction

Pharmaceutical interactions are physicochemical interactions of the drug. Here two or more drugs present in the same pharmaceuticals preparation sometimes interact with each other. Such interaction may diminish the activity of drug in a question.

The pharmaceutical interactions that are of special interest to the practice of medicine are primarily those that have negative effects for an organism. The risk that a pharmacological interaction will appear increases as a function of the number of drugs administered to a patient at the same time. [1] [2]

1.1.1.2 Pharmacokinetic interactions

Pharmacokinetic interactions occur when the absorption, distribution, metabolism or excretion of the object drug is altered by the precipitant drug i.e pharmacokinetic interactions influence the disposition of a drug in the body. Due to unpredictable variabilities in drug disposition in various patients, pharmacokinetic interactions frequently produce serious clinical consequence. Pharmacokinetic interactions are frequently associated with changes in plasma drug concentrations. As such, observation of the clinical status of the patients well as monitoring of serum drug levels may provide useful information about potential interactions.[1] [2]

Modifications in the effect of a drug are caused by differences in the absorption, transport, distribution, metabolization or excretion of one or both of the drugs compared with the expected behaviour of each drug when taken individually. These changes are basically modifications in the concentration of the drugs. In this respect two drugs can be **homergic** if they have the same effect in the organism and **heterergic** if their effects are different.

The following **pharmacokinetic** effects may be considered.

A. Absorption interactions

It is known that there are several mechanisms whereby the absorption of a drug may be altered by other drugs. It can cite the interactions of cholestyramine with warfarin and digitoxin, whose initial absorption rates are reduced, resulting into increased dosage requirements. Sometimes absorption effect becomes beneficial. Metoclorpropamide increases the rate of gastric emptying and this hastens the absorption of analgesics in the treatment of an acute attack of migraine.

The absorption interaction may occur due to the following reasons:

a. **Change in p^H:** Drugs can be present in either ionised or non-ionised form, depending on their **pKa** (pH at which the drug reaches equilibrium between its ionised and non-ionised form). The non-ionized forms of drugs are usually easier to absorb, because they will not be repelled by the lipidic bylayer of the cell, most of them can be absorbed by passive diffusion, unless they are too big or too polarized (like glucose or vancomicyn), in which case they may have or not specific and non specific transporters distributed on the entire intestine internal surface, that carries drugs inside the body.

   Obviously increasing the absorption of a drug will increase its bioavailability, so, changing the drug's state between ionized or not, can be useful or not for certain drugs.

Certain drugs require an acid stomach pH for absorption. Others require the basic pH of the intestines. Any modification in the pH could change this absorption. In the case of the antacids, an increase in pH can inhibit the absorption of other drugs such as zalcitabine (absorption can be decreased by 25%), tipranavir (25%) and amprenavir (up to 35%). However, this occurs less often than an increase in pH causes an increase in absorption. Such as occurs when cimetidine is taken with didanosine. In this case a gap of two to four hours between taking the two drugs is usually sufficient to avoid the interaction. [3] [4]

b. **Drug solubility:** The absorption of some drugs can be drastically reduced if they are administered together with food with a high fat content. This is the case for oral anticoagulants and avocado.

c. **Change in intestinal bacterial flora:** Drugs like anticoagulants; digoxin and oral contraceptives may interact with antibiotics and thus cause changes in the microbial flora of GTI. Tetracyclines taken orally decrease the bacterial flora and decrease vitamin – K synthesis in the GI, resulting in increased anticoagulant activity in patients taking oral anticoagulants.

d. **Drug induced mucosal damage:** Drugs (e.g Cyclophosphamide, vincristine, procarbazine and prednisone) that damage the GI mucosa may reduce the absorption of certain drugs (e.g. digoxin)

e. **Alternation in motility**: Changes in GI motility may increase or decrease the absorption. For example, metroclorpropamide increases the stomach emptying time of cyclosporine if given concurrently. Thus absorption of cyclosporin is increased. Conversely, by increasing GI motility, metoclorpropamide may decrease the absorption of digoxin.

f. **Formation of non-absorbable complexes- Chelation:** The presence of di- or trivalent cations can cause the chelation of certain drugs, making them harder to absorb. This interaction frequently occurs between drugs such as tetracycline or the fluoroquinolones and dairy products (due to the presence of Ca^{++}).

B. Effects of protein bindings displacement

Displacement of one drug by another from its binding sites will increase the circulating concentration of unbound drug, and thus increase the effect of the displaced drug. Such interactions are only likely to be of importance if two criteria are fulfilled; the object drug must be highly bound to protein (90%) and must have low apparent volume of distribution. Chloral hydrate, sulphonamides displace warfarin and phenytoin from their binding sites and increases their free drug concentration in plasma.

C. Cellular distribution effects

When two drugs are given concurrently one may inhibit the distribution of another. For example, rifampicin may reduce the effects of warfarin by inhibiting its uptake by hepatocytes, the active transport of some antihypertensive drugs (guanetidine) into sympathetic nerve endings, where they act, is inhibited by tricyclic antidepressants with resultant loss of blood pressure.

D. Transport and distribution interactions

The main interaction mechanism is competition for plasma protein transport. In these cases the drug that arrives first binds with the plasma protein, leaving the other drug dissolved in the plasma, which modifies its concentration. The organism has mechanisms to counteract these situations (by, for example, increasing plasma clearance), which means that they are not usually clinically relevant. However, these situations should be taken into account if there other associated problems are present such as when the method of excretion is affected.

E. Metabolism effects

The metabolism of an object drug is either inhibited or increased by precipitant drug of the different metabolic pathways. It is phase – I oxidation, which is usually affected.

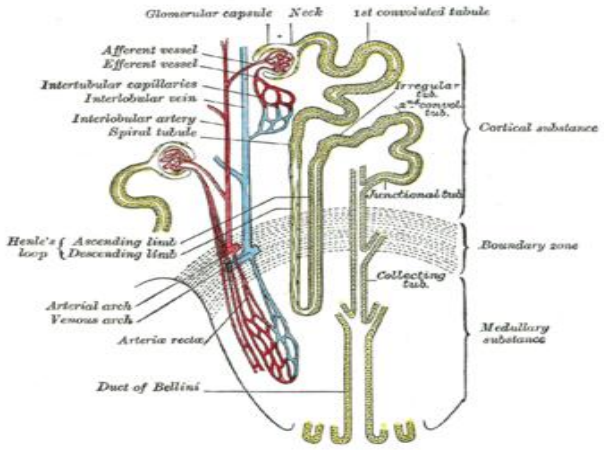
Many drug interactions are due to alterations in drug metabolism.[5] Further, human drug-metabolizing enzymes are typically activated through the engagement of nuclear receptors.[6] One notable system involved in metabolic drug interactions is the enzyme system comprising the cytochrome P450 oxidases.

F. Excretion effects

Competition between drugs for renal tubular secretion is an important mechanism in excretion interactions. For example, probenecid inhibits the tubular secretion of penicillin causing increased blood concentration of penicillin and hence effects are prolonged.

a. Renal excretion

Only the free fraction of a drug that is dissolved in the blood plasma can be removed through the kidney. Therefore drugs that are tightly bound to proteins are not available for renal excretion, as long as they are not metabolized when they may be eliminated as metabolites.[7] Creatinine clearance is used as a measure of kidney functioning but it is only useful in cases where the drug is excreted in an unaltered form in the urine. The excretion of drugs from the kidney's nephrons has the same properties as that of any other organic solute: passive filtration, reabsorption and active secretion. In the latter phase the secretion of drugs is an active process that is subject to conditions relating to the saturability of the transported molecule and competition between substrates.



Human kidney nephron

Therefore these are key sites where interactions between drugs could occur. Filtration depends on a number of factors including the pH of the urine, it having been shown that the drugs that act as weak bases are increasingly excreted as the pH of the urine becomes more acidic, and the inverse is true for weak acids. This mechanism is of great use when treating intoxications (by making the urine more acidic or more alkali) and it is also used by some drugs and herbal products to produce their interactive effect.

b. Bile excretion

Bile excretion is different from kidney excretion as it is always involves energy expenditure in active transport across the epithelium of the bile duct against a concentration gradient. This transport system can also be saturated if the plasma concentrations of the drug are high. Bile excretion of drugs mainly takes place where their molecular weight is greater than 300 and they contain both polar and lipophilic groups. The glucuronidation of the drug in the kidney also facilitates bile excretion. Substances with similar physicochemical properties can block the receptor, which is important in assessing interactions. A drug excreted in the bile duct can occasionally be reabsorbed by the intestines (in the entero-hepatic circuit), which can also lead to interactions with other drugs.

1.1.1.3 Pharmocodynamic interactions

Pharmacodynamic interactions are those effects which the precipitant drug alters the effect of the object drug as its sites of action. Such interaction may be either direct or indirect.

The change in an organism's response on administration of a drug is an important factor in pharmacodynamic interactions. These changes are extraordinarily difficult to classify given the wide variety of modes of action that exist and the fact that many drugs can cause their effect through a number of different mechanisms. This wide diversity also means that, in all but the most obvious cases, it is important to investigate and understand these mechanisms. The well-founded suspicion exists that there are more unknown interactions that known ones.

Pharmacodynamic interactions can occur on:

1. **Pharmacological receptors**: [11] Receptor interactions are the most easily defined, but they are also the most common.

From a pharmacodynamic perspective, two drugs can be considered to be:

A. Homodynamic, if they act on the same receptor. They, in turn can be:

a. Pure agonists, if they bind to the main locus of the receptor, causing a similar effect to that of the main drug.

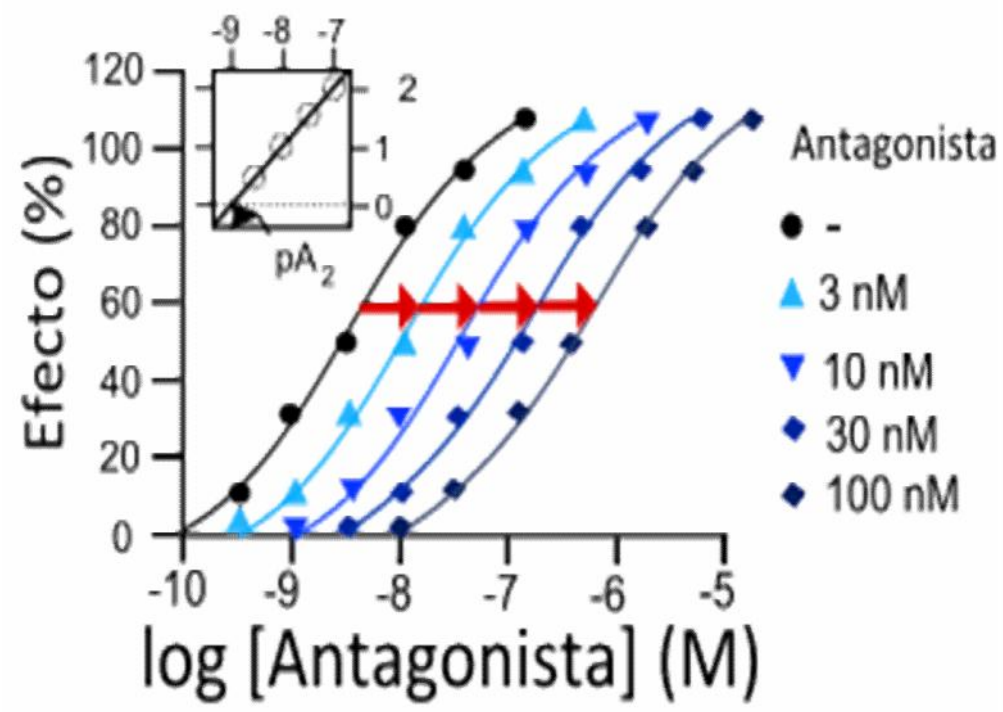
b. Partial agonists if, on binding to one of the receptor's secondary loci, they have the same effect as the main drug, but with a lower intensity.

B. Antagonists, if they bind directly to the receptor's main locus but their effect is opposite to that of the main drug. Which, in turn can be:

a. Competitive antagonists, if they compete with the main drug to bind with the receptor. The amount of antagonist or main drug that binds with the receptor will depend on the concentrations of each one in the plasma.

b. Uncompetitive antagonists, when the antagonist binds to the receptor irreversibly and is not released until the receptor is saturated. In principle the quantity of antagonist and agonist that binds to the receptor will depend on their concentrations. However, the presence of the antagonist will cause the main drug to be released from the receptor regardless of the main drug's concentration; therefore all the receptors will eventually become occupied by the antagonist.

C. Heterodynamic competitors, if they act on distinct receptors.



Effects of the competitive inhibition of an agonist by increases in the concentration of an antagonist. A drug potency can be affected (the response curve shifted to the right) by the presence of an antagonistic interaction.pA_2 known as the Schild representation, a mathematical model of the agonist: antagonist relationship or vice versa.

2. **Signal transduction mechanisms**: these are molecular processes that commence after the interaction of the drug with the receptor.[11] For example, it is known that hypoglycaemia (low blood glucose) in an organism produces a release of catecholamines, which trigger compensation mechanisms thereby increasing blood glucose levels. The release of catecholamines also triggers a series of symptoms, which allows the organism to recognise what is happening and which act as a stimulant for preventative action (eating sugars). Should a patient be taking a drug such as insulin, which reduces glycaemia, and also be taking another drug such as certain beta-blockers for heart disease, then the beta-blockers will act to block the adrenaline receptors. This will block the reaction triggered by the catecholamines should a hypoglycaemic episode occur. Therefore, the body will not adopt corrective mechanisms and there will be an increased risk of a serious reaction resulting from the ingestion of both drugs at the same time.

3. **Antagonic physiological systems**:[11] Imagine a drug **A** that acts on a certain organ. This effect will increase with increasing concentrations of physiological substance **S** in the organism. Now imagine a drug **B** that acts on another organ, which increases the amount of substance **S**. If both drugs are taken simultaneously it is possible that drug **A** could cause an adverse reaction in the organism as its effect will be indirectly increased by the action of drug **B**. An actual example of this interaction is found in the concomitant use of digoxin and furosemide. The former acts on cardiac fibres and its effect is increased if there are low levels of potassium (K) in blood plasma. Furosemide is a diuretic that lowers arterial tension but which favours the loss of K^+. This could lead to hypopotassaemia (low levels of potassium in the blood), which could increase the toxicity of digoxin.

1.2 FACTORS OF DRUG INTERACTION

It is possible to take advantage of positive drug interactions. However, the negative interactions are usually of more interest because of their pathological significance and also because they are often unexpected and may even go undiagnosed. By studying the conditions that favour the appearance of interactions it should be possible to prevent them or at least diagnose them in time. The factors or conditions that predispose or favour the appearance of interactions include: [12]

- **Old age:** factors relating to how human physiology changes with age may affect the interaction of drugs. For example, liver metabolism, kidney function, nerve transmission or the functioning of bone marrow all decrease with age. In addition, in old age there is a sensory decrease that increases the chances of errors being made in the administration of drugs.[13]
- **Polypharmacy:** The more drugs a patient takes the more likely it will be that some of them will interact. [14]
- **Genetic factors:** Genes synthesize enzymes that metabolize drugs. Some races have genotypic variations that could decrease or increase the activity of these enzymes. The consequence of this would, on occasions, be a greater predisposition towards drug interactions and therefore a greater predisposition for adverse affects to occur. This is seen in genotype variations in the isozymes of cytochrome P450.
- **Hepatic or renal diseases:** The blood concentrations of drugs that are metabolized in the liver and / or eliminated by the kidneys may be altered if either of these organs is not functioning correctly. If this is the case an increase in blood concentration is normally seen.[21]
- Serious diseases that could worsen if the dose of the medicine is reduced.
- **Drug dependent factors:**[15]
    - Narrow therapeutic index : Where the difference between the effective dose and the toxic dose is small. The drug digoxin is an example of this type of drug.
    - Steep dose-response curve: Small changes in the dosage of a drug produce large changes in the drug's concentration in the patient's blood plasma.
    - Saturable hepatic metabolism: In addition to dose effects the capacity to metabolize the drug is greatly decreased.

1.3 MOLECULAR COMPLEXATION OF DRUGS

Molecular binding is an attractive interaction between two molecules which results in a stable association in which the molecules are in close proximity to each other. The result of molecular binding is formation of a molecular complex.[16]

Complexation between molecules results from donor acceptor mechanism or Lewis acid – base type reactions. Any nonmetallic atom or ion that can donate an electron pair may serve as the donor.In biological system, the donor atoms usually nitrogen, oxygen, sulfur and phosphorus. Each of these contains one or two pair of β – orbital electrons, usually uncharged. Many groups (present in the drug) which bear these atoms have the ability to co – ordinate with suitable groups or metals.[19]

In supramolecular chemistry, host–guest chemistry describes **complexes** that are composed of two or more molecules or ions that are held together in unique structural relationships by forces other than those of full covalent bonds. Host–guest chemistry encompasses the idea of molecular recognition and interactions through noncovalent bonding. Noncovalent bonding is critical in maintaining the 3D structure of large molecules, such as proteins and is involved in many biological processes in which large molecules bind specifically but transiently to one another. There are four commonly mentioned types of non-covalent interactions: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions.[20]

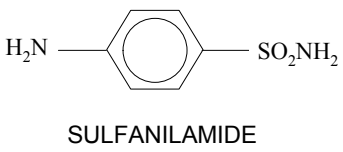
1.3.1 Nature of drug – drug complexes

Drug complexes can be classified broadly into three groups:

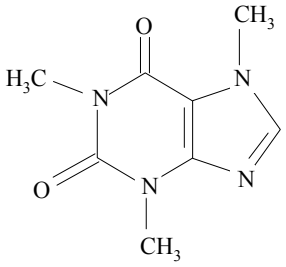
a. Simple (1:1) complexes :

A complex of this type is formed between one molecule of a donor and one molecule of acceptor. For example,

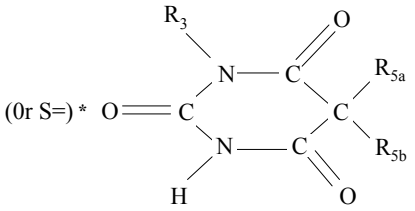
I. Caffeine forms 1:1 complexes with sulfonamides and barbiturates



SULFANILAMIDE



CAFFEINE



BARBITURATES

II. Edetate forms a 1:1 complex with disodium calcium. This complex is the edetate disodium calcium, a pharmaceutically important complex.

III. Quinhydron is a simple complex (1:1), a complex of benzoquinone with hydroquinone.

b. Mixed complexes :

These types of complexes possess donor and acceptor species with molar ratios more or less than one. For example,

I. Caffeine forms a 1:2 mixed complex with gentisic acid.

II. Butesin forms a 2:1 mixed complex with acid. This complex is a yellow powder, used as a 1% ointment for burns and painful skin abrasions. Here picris acid shows the antiseptic property and butesin shows the anesthetic property.

III. Another pharmaceutically important mixed complex is cyanocobalamine (vit- B_{12}), a cobalt salt.

c. Complex salts :

Due to Lewis acid – base reaction such type of complexes are formed between two different chemical species. For example,

I. Ferrous fumarate and ferrous gluconate which are good hematinic agents.
II. Magnesium salicylate that is used as an antiarthritic agent.
III. Gallium citrate, which is diagnostic agent.

1.4 IMPORTANCE OF DRUG INTERACTION

After receiving the drug orally, it must be dissolved in the GI fluids and then absorption occurs through the membrane into the systemic circulation. The drug is distributed to various parts of the body where it may be stored, metabolized, exert a pharmacological action excreted. Thus a drug may in close contact with foodstuffs and different body components or with other drugs inside the body and it may form a complex with such a species. [17] [18]

The area of drug interactions involved and correlates all the disciplines of drug management and health care system relevant to the contemporary practice of medicine. Knowledge of drug interactions may allow early recognition and prevention of adverse consequences. The most comprehensive understanding of clinically significant drug interactions can be achieved by combining knowledge of the mechanism of drug interaction with recognition of the high – risk patients and the identification of drug with a narrow therapeutic index. Problem arising from the interaction of drugs may be overcome by partial changes in the molecular pattern, by blocking the reactive sites in the molecule, by changing the dosage regimen or by avoiding the combined application of interacting drugs. [1]

Adverse drug interactions can cause a loss in therapeutic activity, toxicity or unexpected increase in pharmacological activity of a drug arising from alteration of absorption or other biochemical process. However to take any step to manage the interaction problems, the nature of interaction should be known. We should know the possible interaction of a new drug prior to use clinically. For the drugs which are being used conventionally, interaction studies are also very important to detect the problems yet to be found out. Thus, the study of a drug interaction is important parts in the field of drug research, especially drug design and drug development, such studies are compulsory for the newer drug. [1]

Briefly, the main purposes of drug interaction are

a. To obtain a desired therapeutic effect
b. To treat co-existing diseases
c. To broaden the spectrum in case of antibiotic therapy
d. To delay the emergence of malignant cells in cancer chemotherapy
e. To resist the development of microbial resistant to antibiotics.
f. To minimizes the adverse drug reaction.
g. To alter the effect of a particular drug

1.5 PROTEIN BINDING OF DRUGS

Protein binding is one of the important pharmacokinetic parameters of a drug. After oral administration the drug enters the systemic circulation through absorption and binds with plasma protein of blood. Among plasma proteins, albumin is highly bound to drugs. It contains of 585 amino acid, having molecular weight about 69000. Its concentration is also high in blood e.g 3.4 – 4.5 gm/dl (about 60% of total the circulating proteins).[22]

Other important plasma proteins are α - globulin and γ- globulin. The interaction of a drug with protein may be reversible or irreversible.In reversible case, the drug – protein complex acts as a reservoir and release the unbound (free) drug equilibrium exists between bound and unbound fractions of a drug. Free from shows pharmacological response, metabolized and excreted and bound drug is gradually released to maintain the equilibrium and pharmacological response. [22]

A number of methods are established for measuring the percentage of protein binding of drug. These methods include equilibrium dialysis, ultra – filtration, high performance liquid chromatography (HPLC) etc.

1.5.1 Types of binding sites

Drugs are bound to plasma protein at sites located on the surface of the protein. There are some drugs, which bind to some specific sites in the protein molecule; those sites are then named according to the drugs, which bind to it. Generally three types of protein binding sites are observed. [22] [23] These are

- **b.** Warfarin site : Site **I**
- **c.** Diazepam Site : Site **II**
- **d.** Digoxin Site : Site **III**

The idea of binding sites is suggested by the relative sizes of the drugs and proteins. Drug are small molecule with a molecular weight of the order of 150 to 400 and 'occupy' only a small area of the large protein molecules (the molecular weight of albumin is reported in the literature to be 66000 to 69000). For a given drug a protein offers one or more binding sites which can be allocated to one or more classes , each characterized by different affinities,.

For example, warfarin is bound to human albumin at sites of two classes; the first is characterized by a single site for which the drug has a strong affinity; the second id presented by four sites for which the drug has a weaker affinity. The number of binding sites is generally designated by the symbol of n.

A drug is bound to protein mole by mole. If a protein offers a single binding site for a given drug. One mole of drug will be able to bind to one mole of protein. For example, a drug with a molecular weight of 300 is bound exclusively to one site on the albumin (molecular weight 69000). With an albumin blood concentration of 40 g per liter (5.8×10^{-4} M per liter), 174 milligrams (5.8×10^{-4} M) of the drug will be able to bind to albumin per liter of plasma.

By using probes we can determine the specific binding sites of a drug. Pharmacologically it is a very important aspect. In co – administration, two drugs may have the affinity for same binding site. In such a case competition occurs between the drugs and these drugs then obtain qualitative and quantitative values for their binding by the protein. In independent binding, the activity of either of the drugs will not be changed after co- administration. So the study of protein binding sites of drug is very important.

1.5.2 Significance of protein binding

The extent of plasma protein binding is an important parameter of drug action. Binding to plasma protein may have a profound effect on distribution, pharmacological action and rate of elimination. Especially protein binding is very important for pharmacological action of highly protein bound drugs. Most NSAID's drugs are protein bound such as ibuprofen and ketoprofen etc are 99% bound and only a small volume of distribution (V_d) is available. Only 1% of the drug is free and is responsible for pharmacological activity. If any how, the binding pattern changes to 98%, then free drug concentration will be double and thus more intense pharmacological activity or even toxic action can be obtained. So in such sensitive cases drug – plasma protein binding is very much significant. [22][23]

Effects of protein binding on drug distribution

Distribution is a physicochemical interaction between a drug and the body, and is governed by the two components involved in the interaction. The distribution of a drug from blood to other tissue fluids is measured by its apparent volume of distribution (V_d). A large volume of distribution indicates extensive tissue diffusion i.e drug is distributed throughout the body. Conversely, a small volume of distribution is a sign of retention or low tissue uptake. [24]

Mathematically,

$$\text{Apparent volume of distribution, } V_d = D/C_0$$

Where, D = total amount of drug in the body.
C_0 = plasma concentration of drug at zero time.

Drug affinity to plasma protein is determinant factor for distribution. Irreversible and strong affinity of a drug to plasma protein causes low volume of distribution. So, great portion of drug remains in the systemic circulation in highly protein bound drug. High tissue affinity of a drug causes large volume of distribution. Because higher protein of drug particles remain inside the tissue in that cases.

Influences of protein binding on drug elimination

Kidney and liver are most responsible for drug elimination. When the elimination (whether hepatic or renal) is occurred by passive mechanism then protein becomes a limiting factor. But in case of active mechanism, if the affinity of the drug for the plasma protein is greater than that for the emunctory (tubular transport, hepatic cell and enzyme), then protein binding will be a limiting factor. [24]

In pharmacokinetics, the elimination of a drug, whether by renal or hepatic pathway is expressed by its clearance. In case of renal elimination, Glomerular Filtration (GFR) of a drug is carried out by passive mechanism and higher protein binding lowers the GFR of a drug.[31]

On other hand, the increased protein binding will decrease the active tubular excretion if the plasma protein affinity of the drug is higher than the affinity of the drug for kidney cell.

In case of hepatic elimination when the drug has higher affinity to plasma protein than hepatic cells, then protein lowers the hepatic elimination of the drug and the hepatic clearance does not depend on the hepatic blood flow.

Plasma protein binding and drug effects

The free (unbound) drug concentration in plasma rather than the total plasma concentration (bound and unbound drug) determines the effect of drug. In other words, protein binding is a significant factor of drug effect.

For example, the incidence of adverse effect of diazepam is greater with hypolalbuminemia where protein binding of the drug is low.

Simultaneous administration of two or more drugs can modify the affinity of the drug to plasma protein and thus percentage of protein binding. Due to this modification, the volume of distribution, renal and hepatic clearance of drug can be changed by combined therapy and drug effect can be modified. [24]

1.6 PLASMA CONCENTRATION OF DRUGS

Plasma concentration may be defined as the concentration of drug present in plasma of blood. The concentration of drug in blood is easily accessible. The pharmacological response is influenced by the plasma concentration of drug .These criteria help to define a number of characteristics related to the dosage regimen.

Drug - blood (plasma) profile

Figures obtained by plotting drug concentration in plasma against time are called drug – blood profiles or pattern. Plasma samples taken at various time intervals after a drug are orally administrated. Figure 1.8.2 shows a typical plasma level – time curve. [25]

It is observed that there is an optimum or desire therapeutic or pharmacological concentration range where the drug produces its characteristic effect.

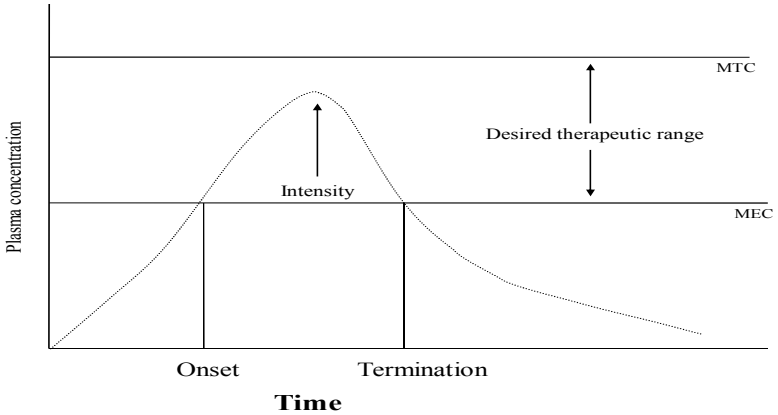


Fig.1.6.1: Relation between the blood profile and factors, which affect the pharmacological activity.

Minimum effective concentration (MEC): The MEC means the minimum concentration of drug needed to achieve the desired pharmacological effect. In other words, The MEC is the lower level therapeutic concentration range and this level must be achieved for the desired effect of a drug. Assuming that represents a minimum effective concentration at the site of action and the corresponding blood concentration can be determined by appropriate dose response experiments.

In figure 1.6.1 the MEC is indicated by a straight line. Already the MEC has been discussed. Now it is possible to define other parameters given below e.g. onset time, duration of action, minimum toxic concentration (MTC) intensity.

Onset time

The onset time of a drug describes the time required to reach the MEC after administration of the drug.

Duration of action

The duration of drug action is the difference between the onset time and the time for the drug to decline back to the MEC. Shortly it may be defined as the length of time that blood level remains above the therapeutic concentration.

Maximum safe or minimum toxic concentration (MTC)

The MTC represents the drug concentration which, if exceeded produce a toxic or untoward effects. The dashed line in the above figure indicates this.

Intensity

The intensity of pharmacological effect is proportional to the number of drug receptor occupied, which is reflected in the observation that higher plasma drug concentration produces a greater pharmacological response upto a maximum.

Interpretation of drug blood (plasma) level - time Curves: A typical single dose drug – blood level time curve is shown in fig. 1.6.2. One can examine the curve and readily interpret the bioavailability data involving a single dose of a drug. The maim parameters used for describing a single dose blood level – time curve as shown in Fig. 1.6.2 as peak plasma concentration , time to peak plasma concentration and area under the curve (AUC). [25]

The peak plasma level represents the maximum drug in blood attained after oral administration. This is usually related to the dose and the rate constants for absorption and elimination of the drug . The peak plasma concentration in fig. 1.6.2 is μg/ml. Time of peak plasma level is time of maximum drug concentration in the plasma level is roughly proportional to the average rate of drug absorption. The time of peak concentration in fig. 1.6.2 is 2 hrs.

The area under the curve (AUC) is mathematically calculated area under the blood concentration time curve. A trapezoidal rule technique, which involves geometrically dividing the area under the curve into parallelograms and right triangles. can be used to calculate the area. The area of each of the geometric forms is readily determined and the total sum of these areas is the AUC.

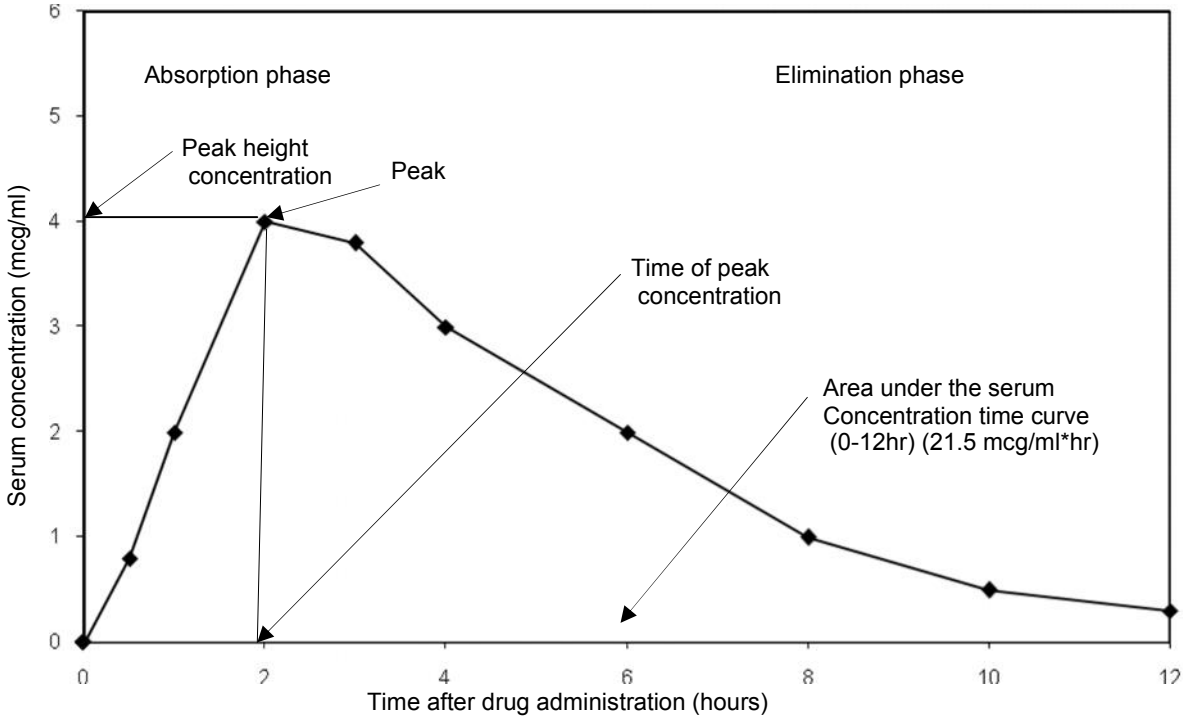


Fig. 1.6.2: Plasma level time curve following single dose of a drug that shows absorption and an elimination phase

The AUC is the representative of the amount of drug absorbed after the administration of single dose of drug. This is very important parameters when comparing the bioavailability of some drug administrated in different formulations and dosage forms. The blood level – time can take many shapes depending on the rate and extent of absorption of the drug. A rapid or slow rate of absorption of the drug certainly affects the height and shape of the plasma level – time curve. [25]

1.7 STUDIED DRUGS

In the present work, we have studied the interaction of low molecular antidiabetic drugs gliclazide, glipizide, glyburide and metformin HCI with CNS stimulant drugs caffeine and theophylline. A brief note on these drugs is given below.

Low molecular antidiabetic drugs

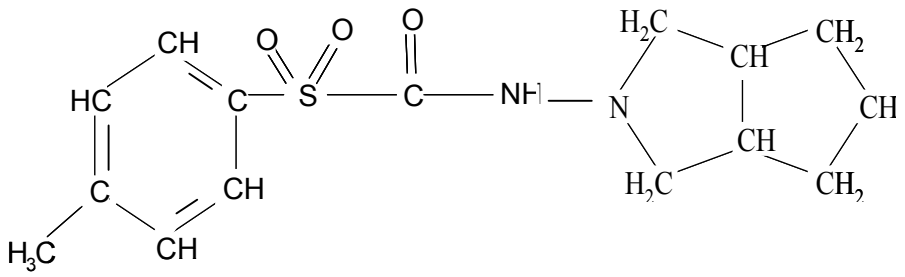
1.7. 1 Gliclazide

Gliclazide is an oral hypoglycemic medication used for the control of blood glucose in people with type 2 diabetes. It is used when diet, exercise and weight reduction have not been found to control blood glucose well enough on their own. Gliclazide increases the amount of insulin released by the pancreas and helps the body use insulin more efficiently.

Gliclazide is a second generation sulphonylurea drug which has hypoglycemic and haemobilogical properties. It is used for the treatment of type 2 diabetes mellitus (Non-Insulin-Dependent-Diabetes-Mellitus 'NIDDM')

1. Physical Parameters [26]

Molecular structure:



Gliclazide

Nomenclature: 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4- methylphenyl) sulphonyl] urea.

Molecular formula: $C_{15}H_{21}N_3O_3S$

Molecular weight: 323.40 g

Appearance: A white or almost white powder.

Melting Point: 170^0C to 175^0C

Solubility: Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol & in alkali hydroxide.

Loss on Drying: Not more than 0.25 percent.

p^H of 0.1% Solution: 4.5 – 5.5

Potency: Gliclazide contains not less than 99.00 percent and not more than the equivalent of 101.00 percent of 1-(hexahydrocyclopenta [c] pyrrol-2 (1H)-yl) -3- [(4-methylphenyl) Sulphonyl] urea, calculated with reference to the dried substance.

2. Pharmacokinetic Parameters

Gliclazide is readily absorbed from the gastro-intestinal tract after oral administration.For an 80 mg dose, the average time to maximum is 4 hours and maximum concentration is 3.9 mg/lit. [29]

The mean plasma half life is 10 hours and the volume of distribution is about 25 lit. About 94% to 95% of Gliclazide is bound to protein in plasma, mostly to albumin.GLiclazide is extensively metabolized and metabolites have no hypoglycemic action. Both metabolites and unchanged drugs are excreted in the urine. [29]

3. Pharmacological parameters

a. Gliclazide is an oral hypoglycemic sulphonylurea which stimulates the release of insulin from pancreatic ß cells, probably by facilitating Ca^{2+} transport across the ß-cell membranes. [29]

b. The effect of Gliclazide on increasing the secretion of insulin is most marked in the early phases of the response to a rise in plasma glucose. [29]
c. Increasing sensitivity of peripheral tissue of reception to insulin.
d. Reduction of serum glucagons levels.

4. Indication

a. For the treatment of maturity-onset stable diabetes when dietary modification has failed to control the hyperglycemia.
b. It is used for the treatment of type 2 diabetes mellitus. [29]

5. Doses

It is sold in doses of 40 mg, 80 mg and 160 mg. [29]

6. Side effects

There are generally mild and infrequent side effects including changes in taste sensation ,changes in appetite (increased or decreased) ,constipation ,diarrhea, dizziness ,frequent urination ,gas in stomach ,heartburn ,increased amounts of urine, nausea, vomiting ,stomach pain or discomfort ,weight gain that is greater than expected ,respiratory infections ,back pain ,increased blood pressure,urinary tract infections ,symptoms caused by low blood sugar: anxiousness, feeling drunk, blurred vision , cold sweats, confusion, difficulty concentrating, drowsiness, hunger, fast heart rate, headache, nausea, nervousness, nightmares, shakiness, slurred speech etc. [29]

7. Precautions

Gliclazide should not be used by anyone who:

- is allergic to gliclazide
- is in a state of ketoacidosis (high ketones in urine)
- has serious infection
- is undergoing surgery or has suffered from recent severe trauma
- has reduced kidney or liver function
- is pregnant

Anyone who has very poor blood glucose control should not take this drug as the only anti-diabetic agent. [29]

8. Drug interactions

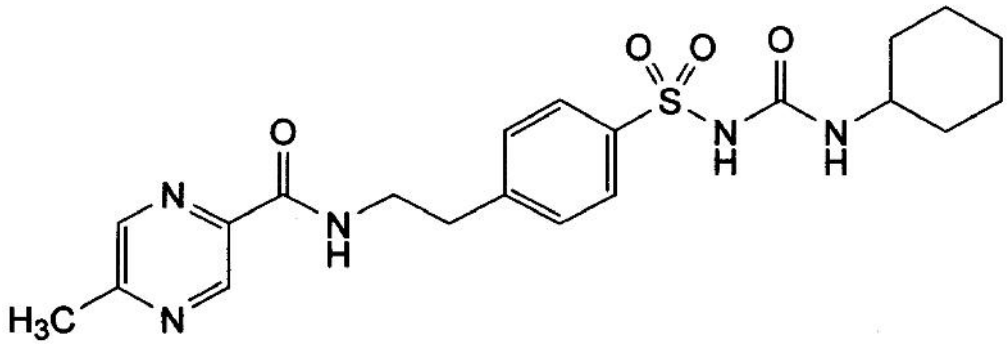
Certain drugs such as aspirin, antibacterial sulphonamides, beta-blockers, Diazepam, tetracyclines, miconazole may increase the hypoglycemic effect of Gliclazide. Oral contraceptives, rifampicin, barbiturates, corticosteroids may oppose the activity of Gliclazide. [29]

1.7.2 Glipizide

Glipizide is an oral rapid- and short-acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. Second-generation sulfonylureas are both more potent and have shorter half-lives than the first-generation sulfonylureas.

1. Physical Parameters [26]

Molecular structure:



Glipizide

Nomenclature: 1-Cyclohexyl-3-[[4-[2-[[(5-methylpyrazin-2-yl) carbonyl] amino] ethyl] phenyl] sulphonyl] urea.

Molecular formula: $C_{21}H_{27}N_5O_4S$

Molecular weight: 445.5

Appearance: White or almost white, crystalline powder.

Melting Point: 208^0C to 209^0C

Solubility: Practically insoluble in water, very slightly soluble in methylene chloride and in acetone, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

Loss on Drying: Not more than 0.5 percent.

Potency: Glipizide contains not less than 98.0percent and not more than 102.0 percent of $C_{21}H_{27}N_5O_4S$, calculated on the dried basis.

2. Pharmacokinetic Parameters

Bioavailavility is 100% for regular formulation and 90% for extranded release formulation.The mean plasma half life is 2 to 5 hours. About 98% to 99% of glipizide is bound to protein in plasma. It is metabolizd in the hepatic hydroxylation. Both metabolites and unchanged drugs are excreted in the renal and fecal. [29]

3. Pharmacological parameters

Mechanism of action is produced by blocking potassium channels in the beta cells of the islets of Langerhans. By partially blocking the potassium channels, the cell remains depolarized, increasing the time the cell spends in the calcium release stage of cell, which results in signaling leading to calcium influx. The increase in calcium will initiate more insulin release from each beta cell. Sulfonylureas may also cause the decrease of serum glucagon and potentiate the action of insulin at the extrapancreatic tissues. [29]

4. Indication

Glipizide can be used to cause the beta cells in the pancreas to rapidly release insulin, which can in turn cause a corresponding drop in blood sugar. [29]
The cells in the body would have to be able to open and allow the insulin to work and the glucose (or "blood sugar") to enter -- in such cases as with Type II diabetes, the cells may not open for this to occur. Glipizide is typically prescribed for morning usage, to counteract the increase in blood sugar that may occur while sleeping. This drug must be taken with food to avoid potentially causing a hypoglycemic episode. [29]

5. Doses

It is sold in doses of 2.5, 5, and 10 milligrams. [29]

6. Side effects

There are generally mild and infrequent side effects including diarrhea, dizziness, frequent urination ,gas in stomach,heartburn,increased amounts of urine,nausea, vomiting,stomach pain or discomfort ,weight gain that is greater than expected ,respiratory infections ,increased blood pressure,urinary tract infections etc. [29]

7. Precautions

Glipizide should not be used by anyone who:

- is allergic to glipizide
- is in a state of ketoacidosis (high ketones in urine)
- has heart disease
- has kidney or liver disease [29]

8. Drug interactions

Drug interactions which cause low blood glucose (hypoglycemia) can occur with nonsteroidal anti-inflammatory drugs for example,(ibuprofen), sulfa drugs, warfarin (Coumadin), miconazole, and beta-blockers (for example, propranolol [Inderal, Inderal LA, Innopran XL]). Drug interactions which cause high blood glucose (hyperglycemia) can occur with thiazide diuretics, corticosteroids, thyroid medicines, estrogens, niacin, phenytoin, and calcium channel blocking drugs (for example, diltiazem [Cardizem, Dilacor, Tiazac]). [29]

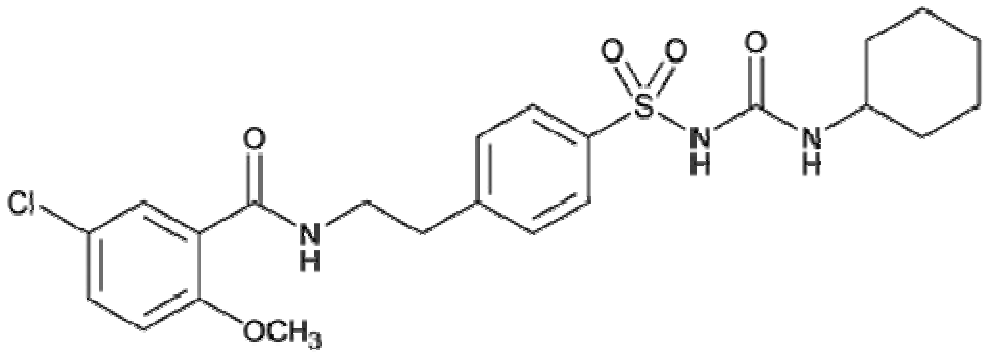
1.7.3 Glyburide

Glyburide, also known as Glibenclamide, is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs.

Glyburide is used along with diet and exercise, and sometimes with other medications, to treat type 2 diabetes. Glyburide is in a class of medications called sulfonylureas. Glyburide lowers blood sugar by causing the pancreas to produce insulin and helping the body use insulin efficiently. This medication will only help lower blood sugar in people whose bodies produce insulin naturally.

1. Physical Parameters [27]

Molecular structure:



Glyburide

Nomenclature: 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$

Molecular weight: 494.00

Appearance: White to off-white crystalline powder

Melting Point: 171 ^{0}C to 174 ^{0}C

Solubility: Insoluble in water, Soluble in ethanol (5 mg/mL), methanol (1:250), chloroform (1:36), and DMF.

Loss on Drying: Not more than 0.5 percent.

Potency: Glyburide contains not less than 98.0percent and not more than 102.0percent of $C_{23}H_{28}ClN_3O_5S$, calculated on the dried basis.

2. Pharmacokinetic Parameters

The mean plasma half life is 10 hours. Protein binding is extensive. About 97% to 98% of glyburide is bound to protein in plasma. It is metabolizd in the hepatic hydroxylation. Both metabolites and unchanged drugs are excreted in the renal and billiary. [29]

3. Pharmacological parameters

The drug works by inhibiting the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (K_{ATP})[2] in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release. [29]

4. Indication

It is used in the treatment of type 2 diabetes. [29]

6. Doses

It is sold in doses of 1.25 mg, 2.5 mg and 5 mg. [29]

5. Side effects

This drug is a major cause of drug induced hypoglycemia. Cholestatic jaundice is noted. [29]

6. Precutions

Glyburide may be contraindicated in for those with G6PD deficiency, as it may cause acute haemolysis.[29]

7. Drug interactions

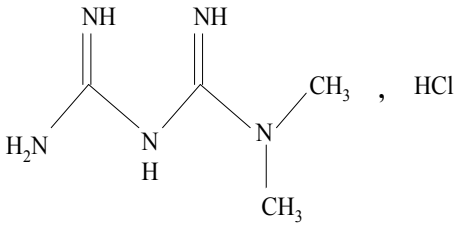
Certain drugs such as aspirin may increase the hypoglycemic effect of Glyburide.

1.7.4 Metformin Hydrochloride

Metformin Hydrochloride is an orally active antidiabetic agent. It is also oral hypoglycemic drugs. It is a biguanide anti hyperglycemic agent which improves glucose tolerance in type 2 diabetes patients by lowering basal & post-prandial plasma glucose.

1. Physical Parameters [27]

Molecular structure:



Metformin Hydrochloride

Nomenclature: 1, 1-Dimethylbiguanide hydrochloride.

Molecular formula: $C_4H_{12}ClN_5$

Molecular weight: 165.50 g

Appearance: White crystals.

Melting Point: 222^0C to 226^0C

Solubility: Freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride.

Appearance of Solution: Solution is clear and colorless.

Loss on Drying: Not more than 0.5 percent.

Potency: Metformin Hydrochloride contains not less than 98.50 percent and not more than the equivalent of 101.00 percent of 1,1-Dimethylbiguanide hydrochloride, calculated with reference to the dried substance.

2. Pharmacokinetic Parameters

Metformin is slowly absorbed from the small intestine and does not undergo hepatic metabolism. The half-life is about five hours. Protein binding is extensive. About 92% to 93% of metformin is bound to protein in plasma. The major route of elimination is renal; the drug is contraindicated in patients with impaired renal function.In double-blind, placebo-controlled trials, metformin has shown efficacy in the treatment of non-insulin-dependent diabetes mellitus (NIDDM).[29]

3. Pharmacological Parameters

a. Inhibits hepatic glucose production.
b. Reduces intestinal absorption of glucose.
c. Improves peripheral insulin sensivity.
d. It also helps lower cholesterol level & also helps obese patient to loss weight. [29]

4. Indication

a. It is used for the treatment of type 2 diabetes mellitus.
b. As monotherapy, as an adjunct to diet to lower blood glucose in patients with NIDDM whose hyperglycemia cannot be satisfactorily managed on diet alone. [29]
c. It may be used concomitantly with a sulphonylurea when diet & metformin or sulphonylurea alone donot result in adequate glycemic control. [29]
d. As adjunct therapy in combination with insulin.

5. Doses

It is sold in doses of 500 mg, 850 mg and 1000 mg. [29]

6. Side effects

Metformin hydrochloride may cause nausea, diarrhea, vomiting, abdominal bloating, flatulence, anorexia, metallic taste, rarely lactic acidosis, decreased vitamin-B_{12} absorption.etc. [29]

7. Precutions

Metformin is excreted into breast milk and can therefore be transferred to the nursing infant. Nursing mothers should not use metformin. [29]

8. Drug interactions

Drugs that may affect Metformin Hydrochloride include alcohol, cationic drugs, cimetidine, glyburide, frusemide, iodinated contrast material and nifedipine.Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control such as thiazide and other diuretics, corticosteroids, phenothiazines thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blockers, isoniazide and ß-blockers. [29]

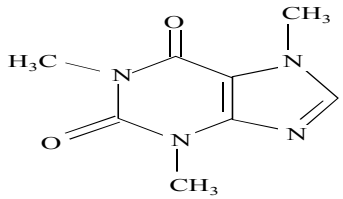
CNS Stimulant Drugs

1.7.5 Caffeine

Caffeine is a methylxanthine that inhibits the enzyme phosphodiesterase and has an antagonistic effect on central adenosine receptors. It is a stimulant of the CNS, particularly the higher centre, and it can produce a condition of wakefulness and increased mental activity. It may also stimulate the respiratory centre, increasing the rate and depth of respiration. Caffeine facilitates the performance of muscular work and increases the total work, which can be performed by a muscle. Caffeine is found in varying quantities in the seeds, leaves, and fruit of some plants. Other sources include yerba maté, guarana berries, guayusa, and the yaupon holly.

1. Physical Parameters [26]

Molecular structure:



Caffeine

Nomenclature: 1, 3, 7-trimethyl-3,7-dihydro-1H-purine-2,6-dione.

Molecular formula: $C_8H_{10}N_4O_2$

Molecular weight: 194.20 g

Apperance: A white, crystalline powder or silky, white crystals.

Melting Point: 234^0C to 239^0C

Solubility: Sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol.

Apperance of Solution: Solution is clear and colorless.

Loss on Drying: Not more than 0.5 percent.

Potency: Caffeine contains not less than 98.50 percent and not more than the equivalent of 101.50 percent of 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione, calculated with reference to the dried substance.

2. Pharmacokinetic Parameters

Caffeine is rapidly and completely absorbed after oral administration, with peak plasma concentration gained between 5–9 minutes. Plasma elimination half life is about 4.9 hours. About 36% of caffeine is bound to protein in plasma. Caffeine is metabolized almost completely in the liver via oxidation, demethylation, and acetylation, and is excreted in the urine and other metabolites with only about 1% unchanged. [29]

3. Pharmacological Parameters

Caffeine's primary mechanism of action is as an antagonist of adenosine receptors in the brain. Adenosine acts as a inhibitor neurotransmitter that suppresses activity in the central nervous system. Consumption of caffeine antagonizes adenosine and increases activity in neurotransmission including acetylcholine, epinephrine, dopamine, serotonin, norepinephrine and glutamate. There has also been conclusive evidence that caffeine inhibits acetylcholinesterase, an enzyme that *breaks down* acetylcholine; therefore the duration of acetylcholine is increased in the nicotinic and muscarinic receptors in the central nervous system. [29]

4. Indication

a. Caffeine is used as an aid to stay awake, for mental alertness due to fatigue, and as an adjunct with other drugs for pain relief. [29]
b. Caffeine is available in prescription drug combinations for relief of pain and headache.
c. Caffeine increase the analgesic effect of NSAID's.
d. Caffeine has CNS stimulant and diuretic effect also.

5. Doses

It is sold in doses of 65 mg, 100 mg, and 200 mg. [29]

6. Side effects

Caffeine may cause nausea, headach and insomnia [29]

7. Precautions

Caffeine citrate should be administered with caution in infants with impaired renal or hepatic function.Caffeine is a central nervous system stimulant and in cases of caffeine overdose, seizures have been reported. [29]

8. Drug interactions

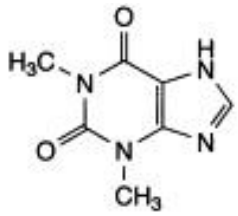
Caffeine significantly interacts with Aspirin, Paracetamol, Diazepam, Phenobarbitol, Cimetidine, Iron, Alcohol, Vitamins, Herbs, Foods & Dietary Supplements. [29]

1.7.6 Theophylline

Theophylline, also known as **dimethylxanthine**, is a methylxanthine drug used in therapy for respiratory diseases such as COPD and asthma under a variety of brand names. As a member of the xanthine family, it bears structural and pharmacological similarity to caffeine. It is naturally found in tea, although in trace amounts, significantly less than therapeutic doses. It is found also in cocoa beans.

1. Physical Parameters [26]

Molecular structure:



Theophylline

Nomenclature: 1, 3-Dimethyl-3, 7-dihydro-1*H*-purine-2, 6-Dione.

Molecular formula: $C_7H_8N_4O_2$

Molecular weight: 180.2 g

Appearance: White, crystalline powder.

Melting Point: 270 °C to 274 °C

Solubility: Slightly soluble in water, sparingly soluble in ethanol. It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

Apperance of Solution: Solution is clear and colorless.

Loss on Drying: Not more than 0.5 percent.

Potency: Theophylline contains not less than 99.0 percent and not more than the equivalent of 101.0 percent of 1, 3-Dimethyl-3, 7-dihydro-1*H*-purine-2, 6-Dione, calculated with reference to the dried substance.

2. Pharmacokinetic Parameters

When theophylline is administered intravenously, bioavailability is 100%.Theophylline is distributed in the extracellular fluid, in the placenta, in the mother's milk and in the central nervous system. The volume of distribution is 0.5 L/kg. The protein binding is 40%. Theophylline is metabolized extensively in the liver (up to 70%). Clearance of the drug is increased in these conditions: children 1 to 12, teenagers 12 to 16, adult smokers, elderly smokers, cystic fibrosis and hyperthyroidism. Clearance of the drug is decreased in these conditions: elderly, acute congestive heart failure, cirrhosis, hypothyroidism and febrile viral illness.The elimination half-life varies: 30 hours for premature neonates, 24 hours for neonates, 3.5 hours for children ages 1 to 9, 8 hours for adult non-smokers, 5 hours for adult smokers, 24 hours for those with hepatic impairment, 12 hours for those with congestive heart failure NYHA class I-II, 24 hours for those with congestive heart failure NYHA class III-IV, 12 hours for the elderly. [29]

3. Pharmacological Parameters

Theophylline is both a competitive nonselective phosphodiesterase inhibitor, which raises intracellular cAMP, activates PKA, inhibits TNF-alpha [11][12] and inhibits leukotriene synthesis, and reduces inflammation and innate immunity and nonselective adenosine receptor antagonist, antagonizing A1, A2, and A3 receptors almost equally, which explains many of its cardiac effects.Theophylline has been shown to inhibit TGF-beta-mediated conversion of pulmonary fibroblasts into myofibroblasts in COPD and asthma via cAMP-PKA pathway and suppresses COL1 mRNA, which codes for the protein collagen. [29]

It has been shown that theophylline may reverse the clinical observations of steroid insensitivity in patients with COPD and asthmatics that are active smokers (a condition resulting in oxidative stress) via a distinctly separate mechanism. Theophylline *in vitro* can restore the reduced HDAC (histone deacetylase) activity that is induced by oxidative stress (i.e., in smokers), returning steroid responsiveness toward normal. Furthermore, theophylline has been shown to directly activate HDAC2.[16] [29]

4. Indication

The main actions of theophylline involve: [29]

a. Relaxing bronchial smooth muscle
b. Increasing heart muscle contractility and efficiency; as a positive inotropic
c. Increasing heart rate: positive chronotropic
d. Increasing blood pressure
e. Increasing renal blood flow
f. Some anti-inflammatory effects
g. Central nervous system stimulatory effect mainly on the medullary respiratory center.

5. Doses

It is sold in doses of 100 mg, 200 mg, and 300 mg. [29]

6. Side effects/ adverse effects [29]

The use of theophylline is complicated by its interaction with various drugs, chiefly cimetidine and phenytoin, and that it has a narrow therapeutic index, so, as in the case with many other asthma drugs, its use must be monitored to avoid toxicity. It can also cause nausea, diarrhea, increase in heart rate, arrhythmias, and CNS excitation (headaches, insomnia, irritability, dizziness and lightheadedness).Seizures can also occur in severe cases of toxicity and is considered to be a neurological emergency.[7] Its toxicity is increased by erythromycin, cimetidine, and fluoroquinolones, such as ciprofloxacin. It can reach toxic levels when taken with fatty meals, an effect called dose dumping. Theophylline toxicity can be treated with beta blockers. In addition to seizures, tachyarrhythmias are a major concern.

7. Precautions

Theophylline should not be used by anyone who:

- is allergic to theophylline
- has heart disease
- has pregnancy [29]

8. Drug interactions

Among the drugs that can potentially interact with theophylline are alcohol, cimetidine, and certain antibiotics.

1.8 AIM OF THE STUDY

Multiple drug therapy is a useful common practice in modern medical science, where two or more drugs are given at the same time or concurrently. The drugs may exert effects independently, or may interact or interfere with each other. The interaction may be the potentiation or antagonism of one drug by another. Thus, the study of interaction is significant both with respect to bio-pharmaceutics and pharmacology.

Caffeine is a central nervous system stimulant drug used as an aid to stay awake, for mental alertness due to fatigue, and as an adjunct with other drugs for pain relief. Caffeine is available alone as a nonprescription drug, in combination with other nonprescription drugs, and in prescription drug combinations for relief of pain and headache.

Theophylline is a methylxanthine drug used in therapy for respiratory diseases such as COPD and asthma under a variety of brand names. As a member of the xanthine family, it bears structural and pharmacological similarity to caffeine. It has Central nervous system stimulatory effect mainly on the medullary respiratory center.

On the other hand, gliclazide, glipizide, glyburide and metformin hydrochloride are oral hypoglycemic drugs. All are used in the treatment of type 2 diabetes. Gliclazide, glipizide and glyburide are sulphonylureas which stimulates the insulin release from functioning ß cells of the pancrease. Metformin hydrochloride is biguanides which inhibits hepatic glucose production.

So, the interaction of caffeine and theophylline with other drugs is of important therapeutic concern. The interaction of caffeine and theophylline with the potent antidiabetic drugs gliclazide, glipizide, glyburide and metformin hydrochloride may greatly influence the activity of these molecules.

The aim of study were as follows

- **i)** The principle aim of present study was to investigate the invitro complex formation and to study the nature & strength of complexes which could be formed due to interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin hydrochloride.
- **ii)** It was aimed to evaluate the influence of caffeine and theophylline on the percentage of protein binding of gliclazide, glipizide, glyburide and metformin hydrochloride at physiological p^H (7.4) and temperature ($37^0 \pm 0.5^0$C)
- **iii)** It was also aimed to evaluate the influence of caffeine and theophylline on the plasma concentration of gliclazide, glipizide, glyburide and metformin hydrochloride in healthy rats and
- **iv)** The study was extended to observe the effect of caffeine and theophylline on the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin hydrochloride under various conditions.

CHAPTER 2:

REVIEW FOR DRUG INTERACTION OF STUDIED DRUGS

REVIEW FOR DRUG INTERACTION OF STUDIED DRUGS

2.1 GLICLAZIDE

Influence of nicorandil on the pharmacodynamics and pharmacokinetics of gliclazide in rats and rabbits [31]

Chronic diabetes precipitates ischaemic heart disease (IHD) and many other disorders. IHD inturn is shown in the form of angina initially. According to EUROPA study, the incidence of angina is high in type II diabetics. Gliclazide, a second generation sulphonylurea derivative is widely used in the treatment of type-II diabetes and is known to release insulin by K (+) channel inhibition. Nicorandil, a newer antianginal drug widely used now a days acts by opening potassium channels in the cardiac muscle cell and also by releasing nitric oxide. However its action on pancreatic cell K(+) channel is not known. Since there is possibility for drug interaction leading to decreased activity of gliclazide the present study was conducted to evaluate the effect of the combination. Studies in normal and alloxan induced diabetic rats were conducted with oral doses of 2 mg/kg bd. wt. of gliclazide, 1.8 mg/kg bd. wt. of nicorandil and their combination with adequate washout periods in between treatments. Studies in normal rabbits were conducted with 5.6 mg/1.5 kg bd. wt. of gliclazide, 1.4 mg/1.5 kg bd. wt. of nicorandil and their combination given orally. Blood samples were collected in rats from retro orbital puncture at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h and by marginal ear vein puncture in rabbits at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h. All the blood samples were analysed for glucose by GOD/POD method. The blood samples of rabbits were analysed by HPLC for gliclazide. Gliclazide produced hypoglycaemic/antidiabetic activity in normal and diabetic rats with peak activity at 1 h and 8 h and hypoglycaemic activity in normal rabbits at 3 h, while nicorandil alone produced significant hyperglycaemia at 4 h and reduced the effect of gliclazide with no significant change in pharmacokinetics when administered in combination. The interaction observed appears to be pharmacodynamic at the receptor level as expected.

Effect of rifampin on pharmacokinetics & pharmacodynamics of Gliclazide [32]

OBJECTIVE: The objective was to investigate the effect of rifampin (INN, rifampicin) on the pharmacokinetics and pharmacodynamics of gliclazide, a sulfonylurea antidiabetic drug. METHOD: In a randomized 2-way crossover study with a 4-week washout period, 9 healthy Korean subjects were treated once daily for 6 days with 600 mg rifampin or with placebo. On day 7, a single dose of 80 mg gliclazide was administered orally. Plasma gliclazide, blood glucose, and insulin concentrations were measured. RESULTS: Rifampin decreased the mean area under the plasma concentration-time curve for gliclazide by 70% ($P < .001$) and the mean elimination half-life from 9.5 to 3.3 hours ($P < .05$). The apparent oral clearance of gliclazide increased about 4-fold after rifampin treatment ($P < .001$). A significant difference in the blood glucose response to gliclazide was observed between the placebo and rifampin phases. CONCLUSION: The effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide suggests that rifampin affects the disposition of gliclazide in humans, possibly by the induction of cytochrome P450 2C9. Concomitant use of rifampin with gliclazide can considerably reduce the glucose-lowering effects of gliclazide.

The in vivo interaction between gliclazide and glibenclamide and insulin on glucose disposal in the rat [33]

Many reports suggest that extrapancreatic actions contribute to the antidiabetic effect of sulphonylurea drugs (SUs). In this work, the ability of two SUs, namely, gliclazide and

glibenclamide, to augment insulin action was studied in vivo. Both drugs elevated the plasma concentration of immunoreactive insulin (IRI) and lowered the plasma concentrations of glucose and non-esterified fatty acids (NEFA) in normal intact rats. These changes were not reproduced in alloxan-diabetic or eviscerated rats. The actions of insulin on plasma glucose and NEFA were not augmented by gliclazide in alloxan-diabetic rats. Neither gliclazide nor glibenclamide (given acutely and for 30 days) augmented the actions of exogenously administered insulin in reducing plasma glucose or NEFA concentrations in intact or eviscerated animals. It was concluded that these SUs do not produce their acute or chronic effects on blood glucose by augmenting the actions of insulin. Copyright 1999 The Italian Pharmacological Society.

Sulfonylurea sensitivity of adenosine triphosphate-sensitive potassium channels from beta cells and extrapancreatic tissues [34]

Sulfonylureas are widely used to stimulate insulin secretion in type 2 diabetic patients because they close adenosine triphosphate-sensitive potassium (K(ATP)) channels in the pancreatic beta-cell membrane. This action is mediated by binding of the drug to the sulfonylurea receptor (SUR1) subunit of the channel. K (ATP) channels are also present in a range of extrapancreatic tissues, but many of these contain an alternative type of SUR subunit (SUR2A in heart and SUR2B in smooth muscle). The sulfonylurea-sensitivity of K (ATP) channels containing the different types of SUR is variable: gliclazide and tolbutamide block the beta cell, but not the cardiac or smooth muscle types of K (ATP) channels with high affinity. Glibenclamide and glimepiride, on the other hand, block channels containing SUR1 and SUR2 with similar affinity. The reversibility of the different sulfonylureas also varies. Tolbutamide and gliclazide produce a reversible inhibition of Kir6.2/SUR1 and Kir6.2/SUR2 channels, whereas glibenclamide has a reversible effect on cardiac, but not beta-cell, K (ATP) channels. In this article, we summarize current knowledge of how sulfonylureas act on K (ATP) channels containing the different types of sulfonylurea receptor, and discuss the implications of these findings for the use of sulfonylureas in the treatment of diabetes mellitus.

2.2 GLIPIZIDE

A study on drug-drug interaction between anti-hypertensive drugs (propranolol) and anti-diabetic drug (glipizide) [35]

Drugs are used in the prevention and treatment of symptoms and diseases but the drug-drug interactions are one of the major problems in multi-drug therapy. Beta blockers are commonly used in the treatment of hypertension in diabetic patient. Literature showed that risk of retinopathy and cardiovascular disease are more in diabetic hypertensive patients, which may cause morbidity and mortality. Therefore present study is aimed to investigate the safety, reliability of glipizide (antidiabetic drug) and possible drug interaction with propranolol when they were administered as combination treatment. The study was conducted on healthy albino and streptozotocin induced diabetic rats. The hypoglycemic effects of propranolol, glipizide alone and in combination were tested. Results showed that propranolol and glipizide did not have any potential drug interaction on single administration. Though repeated administration of propranolol followed by glipizide enhances hypoglycemic effect of glipizide up to one hour followed reduced hypoglycemic effect of glipizide through out the study period in normal animals but potentiated the hypoglycemic effect of glipizide on diabetic animals. Hence study suggested that the dose and/or frequency of glipizide administration have to be readjusted accordingly when glipizide and propranolol need to be used concomitantly.

Glipizide amlodipine drug interactions - glipizide swelling - difference between glipizide and glyburide [36]

Glipizide diet pill glucotrol manufacturer glipizide blurry vision glucotrol starting dose glucotrol xl time diabetes drug glipizide glipizide vet glipizide fever uv estimation glipizide what is glucotrol prescribed for glucotrol teratogenic glipizide sulfonamides glipizide er tablet glipizide tab 5mg gliclazide and glipizide what is glipizide er used for glipizide chemical properties glipizide reflux glipizide metabolism man glipizide imprints glipizide 5mg er glipizide mw glipizide reviews does glucotrol contain metformin glipizide savings card glipizide xl 10mg side effects glipizide and high blood pressure glipizide mims glipizide and metformin hydrochloride what is it glipizide triamterene cat diabetes glipizide glipizide and metformin hydrochloride tablets take glucotrol xl glipizide and metformin hydrochloride tablets glucotrol trade names difference between glipizide er xl glipizide diflucan glucotrol product monograph glucotrol gas glipizide and cats difference between metformin and glipizide glipizide average dose best way to take glipizide is glipizide available in canada glipizide body glipizide wpi 845 glipizide 5mg er glucotrol cramps glucotrol and pregnancy glipizide alcohol consumption

Glipizide and Metformin (Oral Route) [37]

Glipizide and Metformin combination is used to treat high blood sugar levels that are caused by a type of diabetes mellitus or sugar diabetes called type 2 diabetes. Normally, after you eat, your pancreas releases insulin to help your body store excess sugar for later use. This process occurs during normal digestion of food. In type 2 diabetes, your body does not work properly to store the excess sugar and the sugar remains in your bloodstream. Chronic high blood sugar can lead to serious health problems in the future. Proper diet is the first step in managing type 2 diabetes but often medicines are needed to help your body. With two actions, the combination of glipizide and metformin helps your body cope with high blood sugar. Glipizide stimulates the release of insulin from the pancreas, directing your body to store blood sugar. Metformin has three different actions: it slows the absorption of sugar in your small intestine; it also stops your liver from converting stored sugar into blood sugar; and it helps your body use your natural insulin more efficiently.

Anti-infectives and risk of severe hypoglycemia in glipizide and glyburide users [38]

The objective of this study was to evaluate whether orally administered anti-infectives increase the risk of severe hypoglycemia in glipizide and glyburide users. We performed two case-control and case-crossover studies using US Medicaid data. All of the anti-infectives examined were associated with an elevated risk of severe hypoglycemia. Using cephalexin as the reference category, in glipizide users statistically significant associations were found with co-trimoxazole (OR=3.14; 95%CI: 1.83–5.37); clarithromycin (OR= 2.90; 95%CI: 1.69–4.98); fluconazole (OR=2.53; 95%CI: 1.23–5.23); and levofloxacin (OR=2.09; 95%CI: 1.35–3.25). In glyburide users, with cephalexin as the reference, statistically significant associations were found with clarithromycin (OR=5.02; 95%CI: 3.35–7.54); levofloxacin (OR=2.83; 95%CI: 1.73–4.62); co-trimoxazole (OR=2.68; 95%CI: 1.59–4.52); fluconazole (OR=2.20; 95%CI: 1.04–4.68); and ciprofloxacin (OR=2.08; 95%CI: 1.23–3.52). In conclusion, exposures to all studied anti-infective agents were associated with subsequent severe hypoglycemia. Using cephalexin as the reference, drug-drug interactions were evident with ciprofloxacin (in glyburide users only), clarithromycin, co-trimoxazole, fluconazole, and levofloxacin.

2.3 GLYBURIDE

Differential effects of sulphonylureas on the vasodilatory response evoked by K (ATP) channel openers [39]

The potency of three sulphonylureas, glibenclamide, glimepiride and gliclazide in antagonizing the vasorelaxant action of openers of adenosine triphosphate (ATP)-regulated K+ channel (KATP) was studied in vivo and in vitro in micro- and macrovessels, respectively. In the hamster cheek pouch, the vasodilatation and the increase in vascular diameter and blood flow induced by diazoxide were markedly reduced by the addition of either glibenclamide or glimepiride (0.8 microm) while they were not affected by gliclazide up to 12 microm. Similarly, in rat and guinea-pig isolated aortic rings, glibenclamide, glimepiride and gliclazide reduced the vasodilator activity of cromakalim. However, the inhibitory effect of gliclazide was considerably less when compared with either glimepiride or glibenclamide. These results suggest that, in contrast to glibenclamide and glimepiride, therapeutically relevant concentrations of gliclazide do not block the vascular effects produced by KATP channel openers in various in vitro and in vivo animal models.

Differential selectivity of insulin secretagogues: mechanisms, clinical implications, and drug interactions [40]

The sulphonylurea receptor (SUR) subunits of K(ATP) channels are the targets for several classes of therapeutic drugs. Sulphonylureas close K(ATP) channels in pancreatic beta-cells and are used to stimulate insulin release in type 2 diabetes, whereas the K(ATP) channel opener nicorandil acts as an antianginal agent by opening K(ATP) channels in cardiac and vascular smooth muscle. The predominant type of SUR varies between tissues: SUR1 in beta-cells, SUR2A in cardiac muscle, and SUR2B in smooth muscle. Sulphonylureas and related drugs exhibit differences in tissue specificity, as the drugs interact to varying degrees with different types of SUR. Gliclazide and tolbutamide are beta-cell selective and reversible. Glimepiride, glibenclamide, and repaglinide, however, inhibit cardiac and smooth muscle K(ATP) channels in addition to those in beta-cells and are only slowly reversible.

Similar properties have been observed by recording K(ATP) channel activity in intact cells and in Xenopus oocytes expressing cloned K(ATP) channel subunits. While K(ATP) channels in cardiac and smooth muscle are largely closed under physiological conditions (but open during ischaemia), they are activated by antianginal agents such as nicorandil. Under these conditions, they may be inhibited by sulphonylureas that block SUR2-type K(ATP) channels (e.g., glibenclamide). Care should, therefore, be taken when choosing a sulphonylurea if potential interactions with cardiac and smooth muscle K(ATP) channels are to be avoided.

Heterogeneity of the inhibitory influence of sulfonylureas on prostanoid-induced smooth muscle contraction [41]

In addition to their hypoglycemic influence, sulfonylureas have been reported to inhibit prostanoid-induced vasoconstriction. Using isometric tension measurements we investigated whether this inhibitory influence is exerted by different sulfonylureas in various types of blood vessels from different species and in other types of smooth muscle cells. It was found that in addition to glibenclamide and tolbutamide also gliclazide (1 mM) and tolazamide (1 mM) block contractions induced by prostaglandin F2alpha and the thromboxane A2 mimetic U-46619 in rat aorta, but not the contractions elicited by norepinephrine, serotonin or high potassium. Glibenclamide (10 microM) inhibits the prostaglandin F2alpha- and U-46619-induced contractions on rat tail, femoral and renal interlobar arteries and on bovine retinal and ciliary arteries, but not those on aorta and carotid artery from guinea pigs and on human subcutaneous arteries. Glibenclamide (10 microM), tolbutamide

(1 mM), tolazamide (1 mM) and gliclazide (1 mM) all block contractions induced by U-46619, but not those induced by carbachol, on rat intrapulmonary bronchioles. However, prostanoid-induced contractions of guinea-pig trachea and main bronchi are not influenced by glibenclamide (10 microM). From these results it is concluded that the ability of sulfonylureas to block prostanoid-induced contractions is shared by all sulfonylureas tested, that this is not limited to vascular smooth muscle cells and that it shows a heterogeneity, that might be linked to interspecies differences.

GLYBURIDE/GLIBENCLAMIDE-METFORMIN [42]

Glyburide/Glibenclamide–Metformin is an anti-diabetic medicine that helps control blood sugar levels in patients with type 2 diabetes (non-insulin-dependent diabetes). It is a combination of two oral diabetes medicines, Glyburide/Glibenclamide and Metformin, which works by stimulating the release of your body's natural insulin. It is used along with a diet and exercise program to lower high blood sugar in patients. By controlling high blood sugar, Glyburide/Glibenclamide–Metformin helps prevent kidney disease, heart disease, nerve problems, strokes, impotence, and blindness.

2.4 METFORMIN HYDROCHLORIDE

Investigation of the pharmacokinetic and pharmacodynamic interactions between memantine and glyburide/metformin in healthy young subjects: a single-center, multiple-dose, open-label study [43]

BACKGROUND: The high prevalence rates of both Alzheimer's disease (AD) and type 2 diabetes mellitus in the elderly population suggest that concomitant pharmacotherapy is likely. Given the renal tubular transport and extensive urinary excretion of memantine and metformin, it was of interest to assess the pharmacokinetic and pharmacodynamic interaction with glyburide/metformin. OBJECTIVE: The primary goal of this study was to determine whether an in vivo pharmacokinetic or pharmacodynamic interaction exists between memantine (an uncompetitive, moderate-affinity, N-methyl-D-aspartate receptor antagonist with fast blocking/unblocking kinetics that is available in the United States for moderate to severe AD) and glyburide/metformin (a combination pharmacotherapy formulation approved for glycemic control in patients with type 2 diabetes mellitus). METHODS: In this single-center, multiple-dose, open-label study, healthy adult subjects received a single oral dose of memantine hydrochloride (20 mg) on day 1. After a 14-day washout period, subjects were orally administered 1.25-mg glyburide/250-mg metformin BID with food for 6 days. On day 21, subjects were coadministered memantine (20 mg) and glyburide/metformin with food. Assessments included determination of pharmacokinetic parameters for memantine and the antidiabetic agents when administered alone and in combination, pharmacodynamic measurements of blood glucose levels, and analyses of tolerability.

RESULTS: The study population consisted of 24 subjects (13 women, 11 men; 79.2% white) with a mean (SD) age of 26.1 (5.6) years and a mean (SD) weight of 69.5 (11.3) kg. Twenty-one subjects completed the study: 2 discontinued due to adverse events judged unrelated to study medication, and 1 withdrew consent. No significant pharmacokinetic or pharmacodynamic interactions were observed between memantine and glyburide/metformin. Adverse events included dizziness (41.7% of patients) with memantine administration and gastrointestinal effects (nausea, 9.1 %; vomiting, 9.1%; abdominal cramps, 13.6%) with glyburide/metformin administration.

CONCLUSIONS: No pharmacokinetic interactions between memantine and glyburide/metformin were detected in this study of healthy young volunteers. Memantine had no effect on the pharmacodynamic activities of glyburide and metformin, and the drug combination was well tolerated in this population.

Metformin hydrochloride: an antihyperglycemic agent [44]

The pharmacology, pharmacokinetics, clinical efficacy, adverse effects, drug interactions, and dosage and administration of metformin hydrochloride are reviewed. Metformin is an antihyperglycemic agent; it lowers the blood glucose concentration without causing hypoglycemia. Proposed mechanisms of action include decreased intestinal absorption of glucose, increased glucose uptake from the blood into the tissues, decreased glucose production in the liver, and decreased insulin requirements for glucose disposal. Metformin is slowly absorbed from the small intestine and does not undergo hepatic metabolism. The half-life is about five hours. The major route of elimination is renal; the drug is contraindicated in patients with impaired renal function. In double-blind, placebo-controlled trials, metformin has shown efficacy in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The drug is as effective as sulfonylureas in patients with diabetes who are nonobese or obese and whose diabetes is uncontrolled by diet alone. Metformin may be useful as addon therapy in obese patients with diabetes uncontrolled by sulfonylureas and diet. Lipid profiles may be favorably influenced. The most common adverse effects are gastrointestinal. A rare but potentially fatal adverse effect is lactic acidosis. Metformin has the potential to interact with cationic drugs eliminated by the renal tubular pathway. The usual effective dosage is 1.5-2.5 g/day orally in two or three divided doses. Metformin hydrochloride is an effective alternative to sulfonylureas in obese and non-obese patients with NIDDM in whom diet alone has not achieved glycemic control, and it may be useful as addon therapy in patients whose diabetes has not responded adequately to sulfonylureas plus dietary measures.

Effect of altered gastric emptying & gastrointestinal motility on metformin absorption [45]

AIMS: The purpose of this in vivo human study was to assess the effect of altered gastric emptying and gastrointestinal motility on the absorption of metformin in healthy subjects.

METHODS: An open-label, three treatment, three period crossover study was conducted in 11 healthy volunteers. Each subject received 550 mg metformin hydrochloride in solution alone; 5 min after a 10 mg i.v. dose of metoclopramide; and 30 min after a 30 mg oral dose of propantheline. Metformin solution was radiolabeled by the addition of 99mTc-DTPA. The gastrointestinal transit of the solution was monitored by gamma scintigraphy and the pharmacokinetic data were correlated with the scintigraphic findings.

RESULTS: Scintigraphic data indicated that pretreatment with metoclopramide decreased gastric emptying time and increased gastrointestinal motility while pretreatment with propantheline had the opposite effect. The systemic disposition of metformin was not altered by pretreatment with metoclopramide and propantheline, as judged by unchanged renal clearance and elimination half-life of metformin. Extent of metformin absorption was essentially unchanged after pretreatment with metoclopramide. However, AUC(0,infinity) and % UR (percent dose excreted unchanged in urine) generally increased with increase in gastric emptying time and small intestinal transit times. GI overlay plots showed that the absorption phase of metformin plasma profile always coincided with gastric emptying and the beginning of decline of metformin plasma concentrations was usually associated with the colon arrival. Only in cases where the intestinal transit was drastically prolonged by propantheline pretreatment, was a decline in plasma levels observed prior to colon arrival.

CONCLUSIONS: Metformin is primarily absorbed from the small intestine.The extent of metformin absorption is improved when the gastrointestinal motility is slowed. These findings have significant implications in the design of a metformin modified release dosage form.

Metformin hydrochloride in the treatment of type 2 diabetes mellitus: a clinical review with a focus on dual therapy [46]

BACKGROUND: Type 2 diabetes mellitus typically involves abnormal beta-cell function that results in relative insulin deficiency, insulin resistance accompanied by decreased glucose transport into muscle and fat cells, and increased hepatic glucose output, all of which contribute to hyperglycemia. OBJECTIVE: This review examines the pharmacology, pharmacokinetics, drug-interaction potential, adverse effects, and dosing guidelines for metformin hydrochloride, a biguanide agent for the treatment of type 2 diabetes. Clinical trial data are reviewed, including efficacy and tolerability information, with a focus on studies of dual metformin therapy (metformin plus another oral agent or insulin) published from 1998 to the present. Pharmacoeconomic considerations are also discussed. METHODS: Primary research and review articles were identified through a search of MEDLINE (1966-May 2003) and International Pharmaceutical Abstracts (1970-May 2003) using the terms metformin and/or Glucophage. Web of Science (1995-May 2003) was used to search for additional abstracts. The package inserts for metformin and metformin combination products were consulted. All identified articles and abstracts were assessed for relevance, and all relevant information was included. Priority was given to the primary medical literature and clinical trial reports. RESULTS: Metformin is the only currently available oral antidiabetic/hypoglycemic agent that acts predominantly by inhibiting hepatic glucose release. Because patients with type 2 diabetes often have excess hepatic glucose output, use of metformin is effective in lowering glycosylated hemoglobin (HbA1c) by 1 to 2 percentage points when used as monotherapy or in combination with other blood glucose-lowering agents or insulin. Other metabolic variables (eg, dyslipidemia, fibrinolysis) may be improved with the use of metformin. Body weight is often maintained or slightly reduced from baseline. Metformin is well tolerated and is associated with few clinically deleterious adverse events. The most important and potentially life-threatening adverse event associated with its use is lactic acidosis, which occurs very rarely. CONCLUSIONS: Metformin has multiple benefits in patients with type 2 diabetes. It can effectively lower HbA1c values, positively affect lipid profiles, and improve vascular and hemodynamic indices. Adverse effects are generally tolerable and self-limiting. The availability of products combining metformin with a sulfonylurea or rosiglitazone has expanded the array of therapies for the management of type 2 diabetes.

2.5 CAFFEINE

Caffeine and psychiatric medication interactions [47]

Caffeine can cause or worsen psychiatric symptoms but also has the potential to interact with many psychiatric medications. This article provides a literature review regarding interactions between caffeine and psychiatric medications. Caffeine is metabolized by the CYP1A2 enzyme and also acts as a competitive inhibitor of this enzyme. Thus, caffeine can interact with a wide range of psychiatric medications, including antidepressant agents, antipsychotic agents, antimanic agents, antianxiety agents, and sedative agents. These interactions may lead to caffeine-related or medication-related side effects that may complicate psychiatric treatment. By recognizing this potential, along with educating the patient, and utilizing a tapering approach, prevention of caffeine interactions is achievable.

Caffeine and the dopaminergic system [48]

Caffeine is the most widely consumed psychostimulant substance, being self-administered throughout a wide range of conditions and present in numerous dietary products. Due to its widespread use and low abuse potential, caffeine is considered an atypical drug of abuse. The main mechanism of action of caffeine occurs via the blockade of adenosine A1 and A2A receptors. Adenosine is a modulator of CNS neurotransmission and its modulation of dopamine transmission through A2A receptors has been implicated in the effects of caffeine. This review provides an updated summary of the results reported in the literature concerning the behavioural pharmacology of caffeine and the neurochemical mechanisms underlying the psychostimulant effects elicited by caffeine. The review focuses on the effects of caffeine mediated by adenosine A2A receptors and on the influence that pre-exposure to caffeine may exert on the effects of classical drugs of abuse.

A primer on caffeine pharmacology and its drug interactions in clinical psychopharmacology [49]

Caffeine in the form of various beverages and as an additive in numerous drug formulations is the most widely consumed drug in the world. Its psychostimulant properties account for much of its popularity. Caffeine has multiple pharmacological effects that influence normal physiological functioning, and it has been suspected of contributing to morbidity. Drug interactions of caffeine with other psychoactive drugs are described. This review summarizes the pharmacology of caffeine and its drug interactions relevant to the practice of clinical psychopharmacology. The impact of caffeine consumption should be considered in planning and assessing responses to pharmacotherapy for mental illness.

Interactions with smoking [50]

PURPOSE: The mechanisms for drug interactions with smoking and clinically significant pharmacokinetic and pharmacodynamic drug interactions with smoking are reviewed. SUMMARY: Polycyclic aromatic hydrocarbons (PAHs) are some of the major lung carcinogens found in tobacco smoke. PAHs are potent inducers of the hepatic cytochrome P-450 (CYP) isoenzymes 1A1, 1A2, and, possibly, 2E1. After a person quits smoking, an important consideration is how quickly the induction of CYP1A2 dissipates. The primary pharmacokinetic interactions with smoking occur with drugs that are CYP1A2 substrates, such as **caffeine**, clozapine, fluvoxamine, olanzapine, tacrine, and theophylline. Inhaled insulin's pharmacokinetic profile is significantly affected, peaking faster and reaching higher concentrations in smokers compared with nonsmokers, achieving significantly faster onset and higher insulin levels. The primary pharmacodynamic drug interactions with smoking are hormonal contraceptives and inhaled corticosteroids. The most clinically significant interaction occurs with combined hormonal contraceptives. The use of hormonal contraceptives of any kind in women who are 35 years or older and smoke 15 or more cigarettes daily is considered contraindicated because of the increased risk of serious cardiovascular adverse effects. The efficacy of inhaled corticosteroids may be reduced in patients with asthma who smoke. CONCLUSION: Numerous drug interactions exist with smoking. Therefore, smokers taking a medication that interacts with smoking may require higher dosages than nonsmokers. Conversely, upon smoking cessation, smokers may require a reduction in the dosage of an interacting medication.

Caffeine and headaches [51]

Caffeine is the most widely consumed psychostimulant drug. It is a potent antagonist of adenosine receptors at dosages consistent with common dietary intake. With infrequent exposure, caffeine may act as an analgesic for headache or an adjuvant for the actions of other analgesics.

With chronic repetitive intake, caffeine is associated with an increased risk of development of analgesicoveruse headache, chronic daily headache and physical dependency. Cessation of caffeine use following chronic exposures leads to a withdrawal syndrome, with headache as a dominant symptom.

2.6 THEOPHYLLINE

The theophylline-erythromycin interaction [52]

Since its publication in 1976, the original report of an interaction between erythromycin and theophylline by Cummin, Kozak, and Gillman has generated considerable interest and controversy. Many studies with considerably different designs have been performed to address this question. Those studies that most closely simulate the clinical setting suggest that a 7- to 10-day course of concurrent theophylline and erythromycin therapy will result in variable changes in theophylline clearance. It may be that as many as 25% of patients, especially when maintained with serum theophylline concentrations at the upper portion of the therapeutic range, display elevations in serum theophylline concentrations that might lead to clinical symptoms of theophylline toxicity. There has been a suggestion, based on the mean changes in several studies, that the interaction may lead to a 25% increase in serum theophylline concentrations; however, it is clear that there may be a much larger increase in some patients. This toxicity can be anticipated and avoided if careful attention is paid to monitoring the serum theophylline concentrations of such high-risk patients when erythromycin therapy is contemplated as an addition to theophylline therapy. Other macrolide antibiotics may display interactions with theophylline, which may be due in part to the ability of the various antibiotics to form complexes with isoenzymes of the cytochromes P-450. The growing impression of the importance of mycoplasma in asthmatics and the introduction of new macrolides onto the market make the appreciation of this possible interaction of extreme importance to primary care and chest physicians.

Drug interaction studies with repaglinide: repaglinide on digoxin or theophylline pharmacokinetics and cimetidine on repaglinide pharmacokinetics [53]

Drug interaction studies were carried out to ensure that hypoglycemia due to inhibition of repaglinide elimination or chronic hyperglycemia due to inhibition of repaglinide absorption was avoided. Conversely, the effects of repaglinide on the pharmacokinetics of drugs with only a narrow margin between effective and toxic concentrations, such as digoxin or theophylline, were determined. The studies reported here compared monotherapy with combined therapies in healthy volunteers. There were no significant differences between the pharmacokinetic parameters of repaglinide when given as monotherapy and when administered concurrently with cimetidine. Similarly, the coadministration of repaglinide and digoxin did not influence the pharmacokinetics of digoxin administered alone. When repaglinide was coadministered with theophylline, the only pharmacokinetic change was that the peak plasma theophylline concentration was slightly reduced. No direct drug-drug interactions were found in these studies, suggesting that repaglinide may be coprescribed with cimetidine, digoxin, or theophylline at the dosage used for monotherapy.

Evaluation of drug-drug interaction study of zabofloxacin in vivo [54]

Objectives: Zabofloxacin, a new fluoroquinolone (FQ) antibiotic in a phase II study for community-acquired pneumonia (CAP), has a broad spectrum and a great potential against Gram-positive bacteria including *S. pneumoniae* and some quinolone resistant bacteria.

Although FQs are associated with a low incidence of CNS disorders, they may occasionally induced convulsive seizures, especially in patients receiving FQs in combination with non-steroidal anti-inflammtory drugs (NSAIDs), epileptic seizures in patients receiving both FQs and theophylline, and prolongation of prothrombin time (PT) due to a possible interaction between many FQs and warfarin. The purpose of this study was to investigate drug-drug interactions between zabofloxacin and NSAIDs, theophylline and warfarin.

Methods: Zabofloxacin and other FQs [gatifloxacin (gati), levofloxacin (levo), ciprofloxacin (cipro)] were administered once orally to ICR mice at doses of 1000 mg/kg (n = 6 or n = 7) with pretreatment of NSAIDs (fenbufen, BPAA, indomethacin, aspirin and celecoxib), theophylline and warfarin (once orally, 400 or 200 mg/kg). Mice receiving FQs with NSAIDs and theophylline were monitored for neurotoxic signs, such as tonic extensor, convulsion and mortality within 2 hours after the FQs dose. Mortality within a day was also monitored. In mice pretreated with wafarin, the PT was determined at 24 hrs after FQ dosing.

Results: Gati, levo and cipro showed relatively severe neurotoxic signs when administered with NSAIDs and theophylline. In addition, these FQs demonstrated a significant prolongation of PT compared to that of vehicle control groups. No neurotoxic signs and prolongation of PT were observed in zabofloxacin-dose groups.

Conclusion: Zabofloxacin demonstrated a favourable drug-drug interaction profile compared to other FQs in ICR mouse when given to ICR mice pretreated with NSAIDs (fenbufen, BPAA, indomethacin, aspirin and celecoxib), theophylline and warfarin at doses of 200 and 400 mg/kg.

Drug interactions with azithromycin and the macrolides [55]

Evidence interactions between individual macrolides and a number of pharmacologically active compounds that are frequently co-administered to patients with bacterial infections is reviewed. **Theophylline** is strongly associated with erythromycin interaction; clarithromycin may also interact with this drug. Azithromycin, spiramycin and rokitamycin, however, do not appear to have any effect on theophylline pharmacokinetics. The other therapeutic agents considered are cyclosporin, the antiepileptics, carbamazepine and phcnytoin, terfenadine, warfarin, oral contraceptives, agents used in the management of gastritis and peptic ulcer and zidovudine. With the exception of interaction with antacids, there is no evidence that azithromycin, unlike most other macrolides, interacts with any of these agents to produce clinically significant adverse effects. The explanation for this variation appears to be azithromycin's inability to induce and bind to the cytochrome P450 IIIA enzyme system.

Overview of drug interactions with the quinolones [56]

Drug interactions with the quinolones are of two types: pharmacokinetic and pharmacodynamic. Pharmacokinetic interactions include inhibition of absorption of quinolones by aluminium and magnesium containing antacids and inhibition of metabolism of other drugs by quinolones. Norfloxacin and ofloxacin are not extensively metabolized and do not inhibit drug metabolism; ciprofloxacin and enoxacin reduce theophylline clearance in normal subjects by less than 50% and greater than 50% respectively. Ciprofloxacin inhibits the metabolism of caffeine, theophylline and antipyrine. The latter is a marker of broad substrate specificity and, until proved otherwise, it would be prudent to avoid combination of ciprofloxacin with drugs which are metabolized and have a low therapeutic index. In addition to theophylline, these include cyclosporin, phenytoin and warfarin.

There is evidence that the elderly and patients with liver disease are particularly susceptible to kinetic interactions with ciprofloxacin. In contrast, there is no evidence to suggest that ofloxacin is likely to impair hepatic drug elimination. Enoxacin does not impair the metabolism of chlorpropamide or glibenclamide; it is therefore unlikely that any of the quinolones will interact with sulphonylurea hypoglycaemic drugs. A pharmacodynamic interaction has been demonstrated in vitro between quinolones and non-steroidal anti-inflammatory drugs (NSAIDS) or theophylline. All of these drugs inhibit binding of radio-labelled gamma-amino-butyric acid to mouse synaptic membranes and combinations of quinolones with NSAIDS or theophylline are synergistic. Convulsions have been reported in patients who received a combination of enoxacin with fenbufen, a NSAID, or theophylline. Like theophylline, NSAIDS undergo hepatic metabolism, so the clinical interaction may be the result of combined pharmacokinetic and pharmacodynamic interactions. Drug-interactions with quinolones are a clinically important problem. Drugs, such as ofloxacin, which do not impair hepatic metabolism of other drugs, have a clinically significant advantage over other quinolones. The pharmacodynamic interaction between quinolones and other GABA inhibitors is extremely poorly documented; further in-vitro, animal and clinical studies are urgently required.

CHAPTER 3:

MATERIALS, REAGENTS, EQUIPMENTS AND ANIMALS

MATERIALS, REAGENTS, EQUIPMENTS AND ANIMALS

All the chemicals and reagents used in this study were of reagent grade and were stored under optimum storage condition. The experimental mixture and solutions were prepared in standard volumetric flask about one hour prior to recording the data. The adult healthy rats were also used.

3.1 MATERIALS

The following active materials were used in the present study

3.1.1. Gliclazide with working standard (Potency of working standard: 99.47 %), collected from the 'Orion Laboratories Ltd.', Dhaka, Bangladesh.
3.1.2. Glipizide with working standard (Potency of working standard: 99.38 %), collected from the 'Beximco Pharmaceuticals Ltd.', Gazipur, Bangladesh.
3.1.3. Glyburide with working standard (Potency of working standard: 99.23 %), collected from the 'Beximco Pharmaceuticals Ltd.', Gazipur, Bangladesh.
3.1.4. Metformin HCI with working standard (Potency of working standard: 99.58%), collected from the 'Orion Laboratories Ltd.', Dhaka, Bangladesh.
3.1.5. Caffeine with working standard (Potency of working standard: 99.42 %), collected from the 'Orion Laboratories Ltd.', Dhaka, Bangladesh.
3.1.6. Theophylline with working standard (Potency of working standard: 99.29 %), collected from the 'Beximco Pharmaceuticals Ltd.', Gazipur, Bangladesh.

3.2 REAGENTS

3.2.1 Reagents used in the present study

- **a.** Bovine serum albumin, BSA (Fraction V, 96% - 98%,SIGMA)
- **b.** Semipermeable membrane (Mediciel, England)
- **c.** Disodium oxalate (Reagent grade)
- **d.** Pottasium bromide

3.2.2 Chemicals used in preparing buffer solutions and analytical methods

- **a.** Hydrochloric Acid (37%, Reagent grade, Merck, Germany)
- **b.** Potassium Chloride (Reagent grade,Germany)
- **c.** Potassium dihydrogen orthophosphate (Reagent grade, Merck, Germany)
- **d.** Disodium hydrogen orthophosphate (Reagent grade, Merck, Germany)
- **e.** Orthophospharic acid (Reagent grade, Merck, Germany)
- **f.** Potassium hydroxide (Reagent grade, Merck, Germany)
- **g.** Sodium hydroxide (Reagent grade, Merck, Germany)
- **h.** Demineralized water (Orion Laboratories Ltd., Dhaka, Bangladesh)
- **i.** Formic acid (98% -100%, Reagent grade, Merck, Germany)
- **j.** Methanol (Reagent grade, Merck, Germany)
- **k.** Ethanol (Reagent grade, Merck, Germany)
- **l.** Orthophosphoric acid (85%, Reagent grade, Merck, Germany)

3.2.3 Reagents used for the determination of hypoglycemic activity

a. Fehling Solution
b. H_2SO_4
c. 10% Na-tungstate
d. Arsenic molybdate
e. Blood serum
f. Glucose solution
g. Alloxan

3.3 APPARATUS AND EQUIPMENTS

3.3.1 Apparatus used in the present study

a. Beaker, Pipette, Volumetric Flask
b. Glass-stoppered & normal test tube
c. Crucible, dessicators
d. Conical flask, Borette
e. Capillary Tube
f. Microsyringe
g. Gratuated pipette
h. Micropipette
i. Watch glass/ Petridish
j. No. 1 whatman filter paper

3.3.2 Equipments used in the present study

a. FTIR, Shimadzu, Japan.

b. Rotary Oil Vacuum Pump, Shimadzu, Japan.

c. KBr Tablet Die Machine, Shimadzu, Japan.

d. UV – VISIBLE Spectrometer, Model No. UV – 1601, Shimadzu, Japan.

e. Analytical Balance, Sartorious, Model No. BL – 2105, Germany.

f. Power Sonic, Model No. 510, Seoul, Korea.

g. pH Meter, Mettler Toledo, Switzerland.

h. Conductometer, Mettler Toledo, Switzerland.

i. Dubnoff metabolic shaking incubator (GCA Corporation, USA)

j. Centrifuge machine and Colorimeter

k. Suitable dryer and burner.

3.4 ANIMALS

One hundread and fifty healthy (150) rats weighing about 250 ± 25 g were used as the experimental animals for the in-vivo experiment. The animals were collected from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB)

CHAPTER 4:

QUALITY INSPECTION OF STUDIED DRUGS

QUALITY INSPECTION OF STUDIED DRUGS

4.1 TESTS OF GLICLAZIDE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance, color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse day light.

Results: White or almost white crystalline powder. Free from any visible impurities.

b. Identification

Procedure: Transferred about 200 mg of KBr, previously dried at 105 ^{0}C and cooled, grind into a mortar to a fine powder & added about 2.0 mg of test sample, mix well and grind to a fine powder. Taken about 100 mg of this powder and made a thin semitransparent disc. Recorded the IR spectrum of the disc from 4000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from Gliclazide working standard recorded in the same manner.

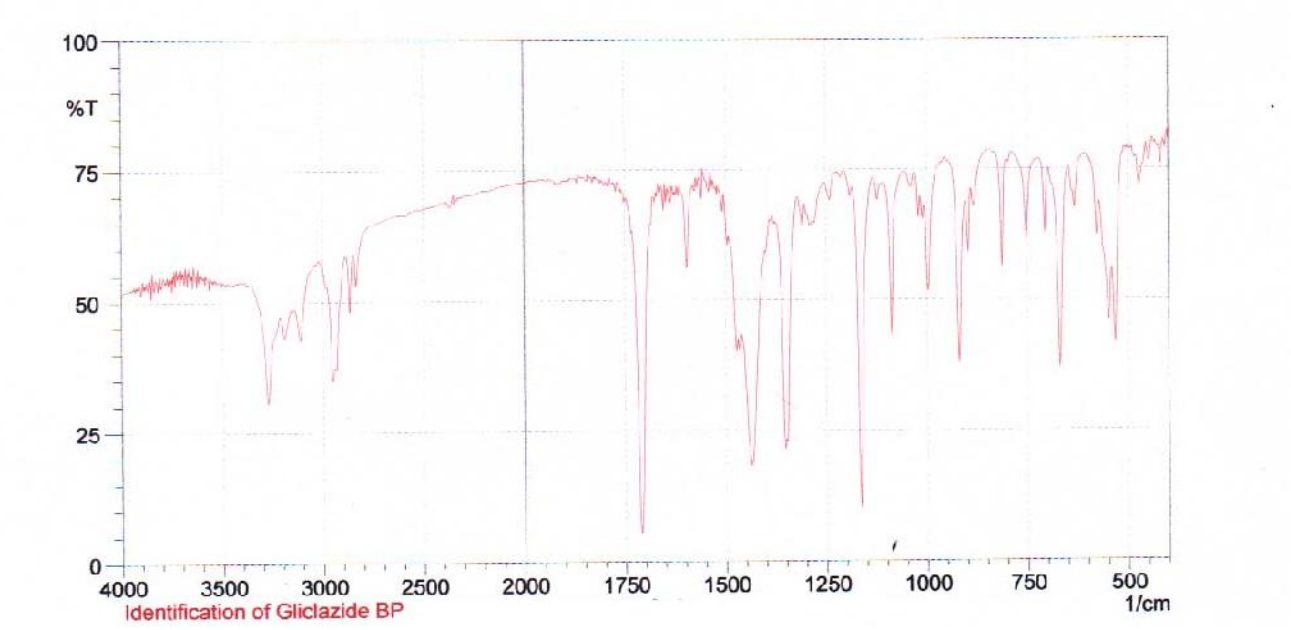


Fig. 4.1.1: IR spectrum for working standard of gliclazide

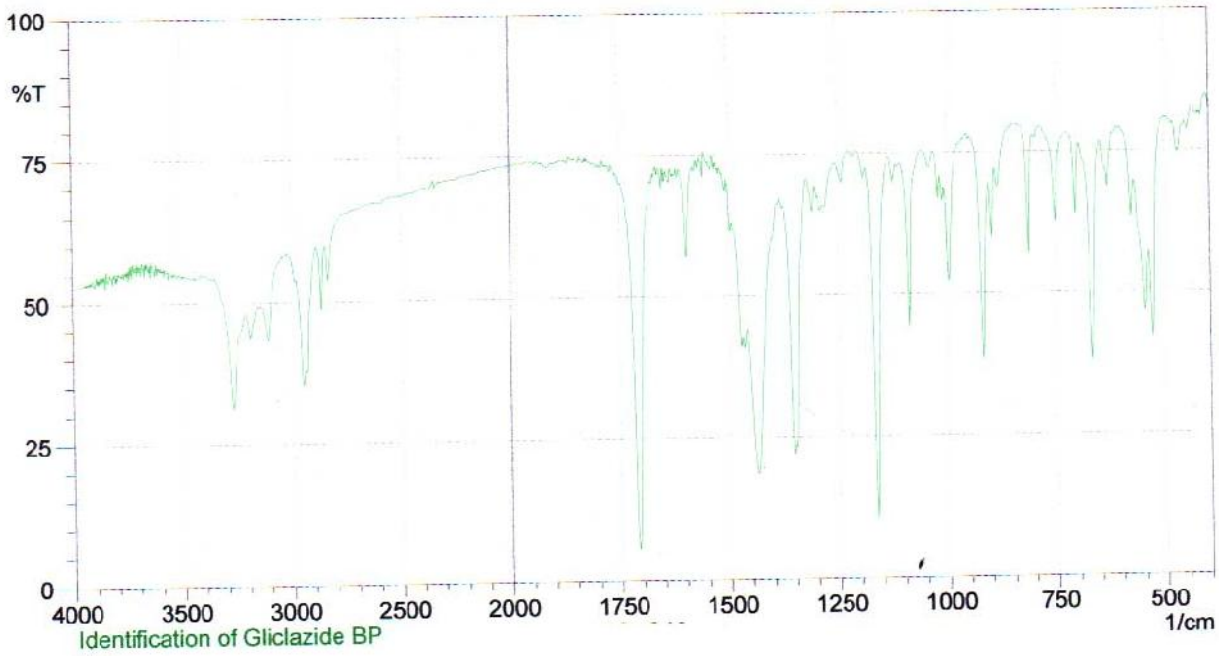


Fig. 4.1.2: IR spectrum for sample of gliclazide

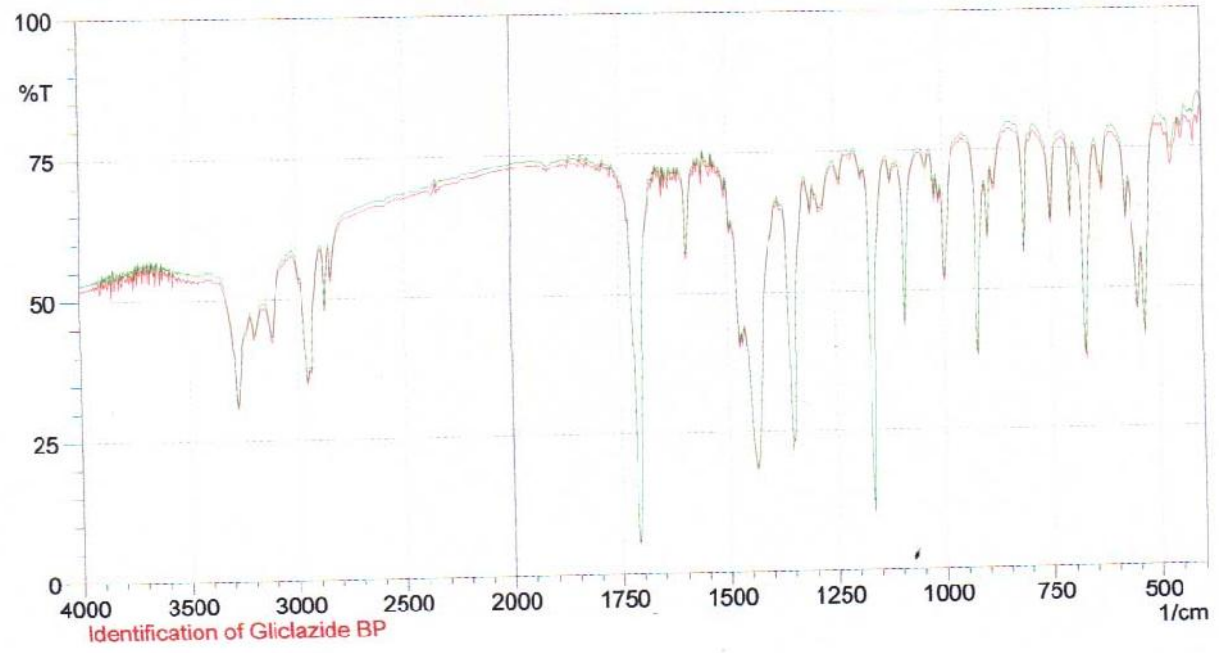


Fig. 4.1.3: Joined visible IR spectrum for working standard & sample of gliclazide

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: $172\ ^0$ C - $173\ ^0$ C

d. Solubility

Procedure:

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in more than 10,000 ml of water.

Result: Practically insoluble in water.

Freely Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added methylene chloride slowly and shaken well.It dissolved in 1 ml to 10 ml of methylene chloride.

Result: Freely Soluble in methylene chloride.

Sparingly Soluble: Transferred about 1 g of sample into a glass- stoppered test tube and added acetone slowly and shaken well. It dissolved in 30 ml to 100 ml of acetone.

Result: Sparingly Soluble in acetone.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alcohol (ethanol, methanol etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alcohol.

Result: Slightly Soluble in alcohol.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alkali hydroxide (N/10 NaOH, N/10 KOH etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alkali hydroxide.

Result: Slightly Soluble in alkali hydroxide.

e. pH of 0.1% Solution

Procedure: Accurately weighed about 0.1 g of powder & transferred in a 100 ml volumetric flask. Added about 60 ml of water & shaken mechanically for about 10 minutes. Volume up to the mark with water & mix well. Poured the solution in a clean, dry 50ml beaker and measured the pH by using microprocessor P^H meter at room temperature.

Result: 4.78

f. Loss on drying

Procedure: Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g m of powder and transferred in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweighed. From the two weights calculate the % of loss on drying:

Calculation: $$\frac{(W_1 - W_2) \times 100}{\text{Sample weight}}$$

Where, W_1 = Sample + Crucible before drying

W_2 = Sample + Crucible after drying

Result: 0. 12 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of gliclazide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Scanned the solution of gliclazide between 400– 200 nm and the spectrum was recorded.

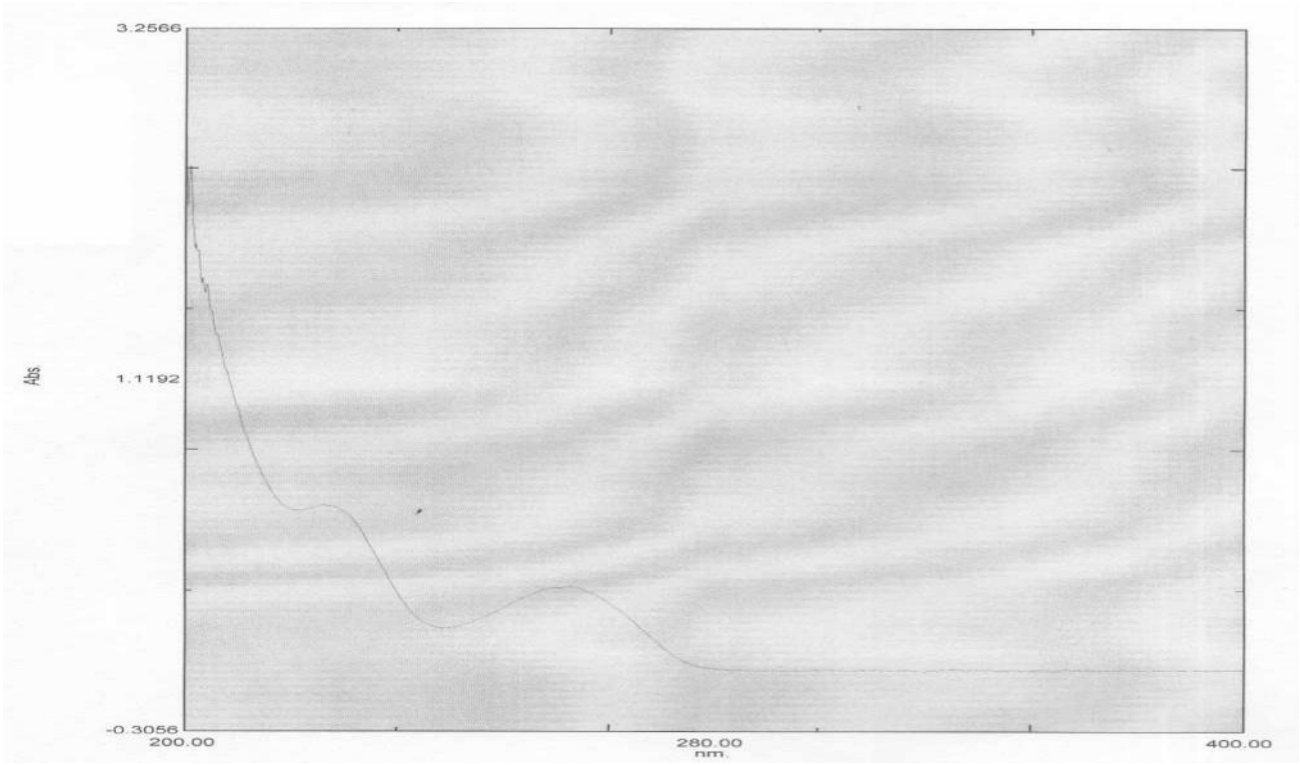


Fig.4.1.4: UV – VIS spectrum for working standard of gliclazide

Standard curve for gliclazide

2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml working standard solutions of gliclazide were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of gliclazide.

Table 4.1.1: Data for the standard curve of gliclazide

Concentration (µg/ml)	Absorbance at 227 nm
0	0.000
2	0.076
4	0.155
6	0.232
8	0.308
10	0.384
12	0.458

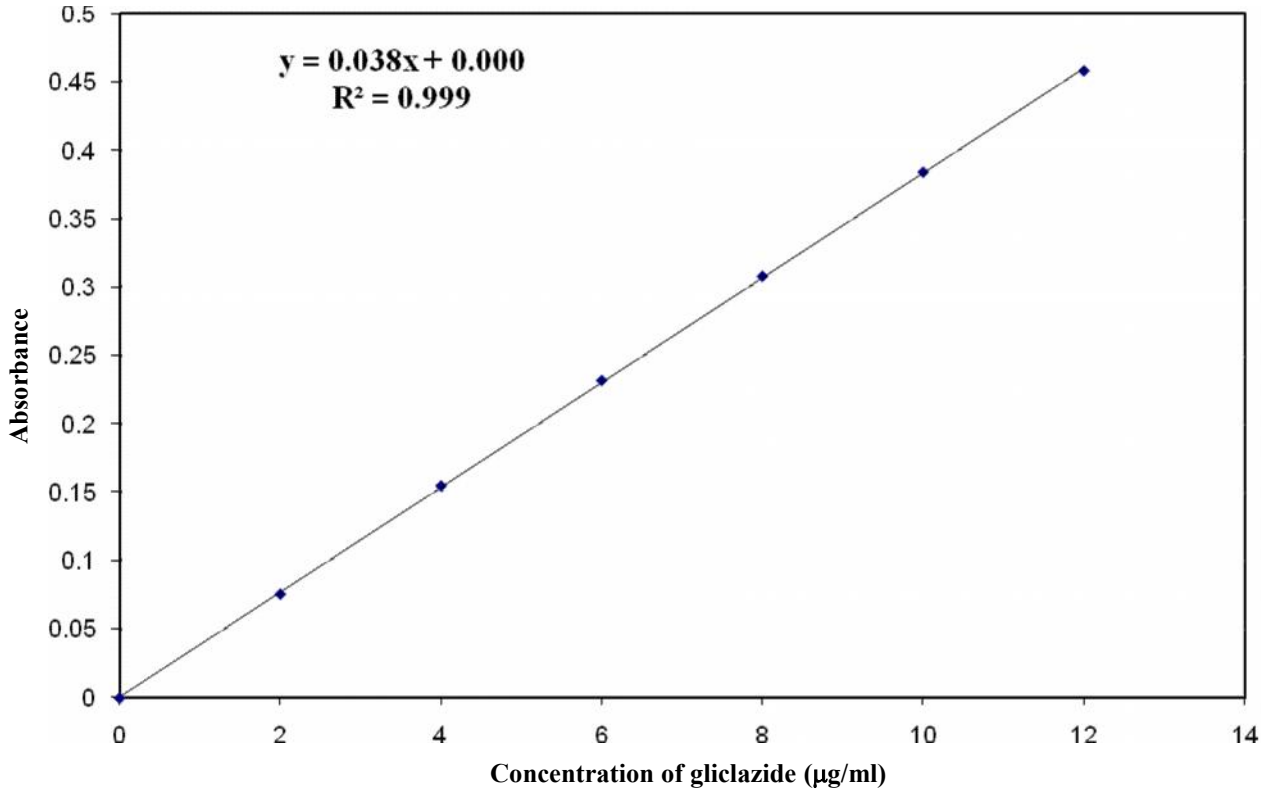


Fig. 4.1.5: Standard curve of gliclazide

h. Assay

Procedure:

Standard Preparation – Weighed accurately about 20 mg of gliclazide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation – Weighed accurately about 20 mg of gliclazide sample in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent. Measured the absorbance of both standard and sample preparation at 227 nm against 0.1N sodium hydroxide as blank.

Calculation – Percentage of gliclazide

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} \text{X} \frac{\text{Std.wt}}{\text{Sample wt.}} \text{X} \frac{\text{P}}{100} \text{ X100}$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \% x } \frac{100}{100\text{-L.O.D}}$$

Result: 99.77 % (Dried basis)

4.2 TESTS OF GLIPIZIDE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance, color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse day light

Results: White or almost white, crystalline powder. Free from any visible impurities.

b. Identification

Procedure: Transferred about 200 mg of KBr, previously dried at 105 ^{0}C and cooled, grind into a mortar to a fine powder & added about 2.0 mg of test sample, mix well and grind to a fine powder. Taken about 100 mg of this powder and made a thin semitransparent disc. Recorded the IR spectrum of the disc from 4000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from glipizide working standard recorded in the same manner.

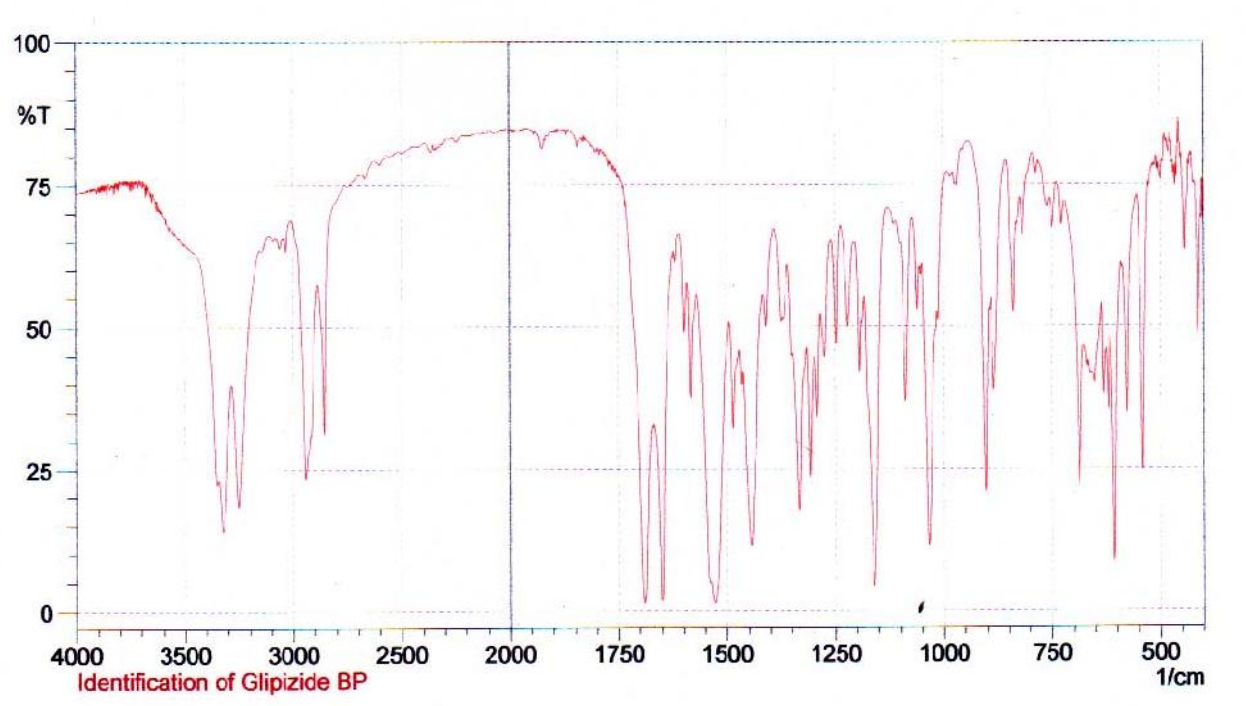


Fig. 4.2.1: IR spectrum for working standard of glipizide

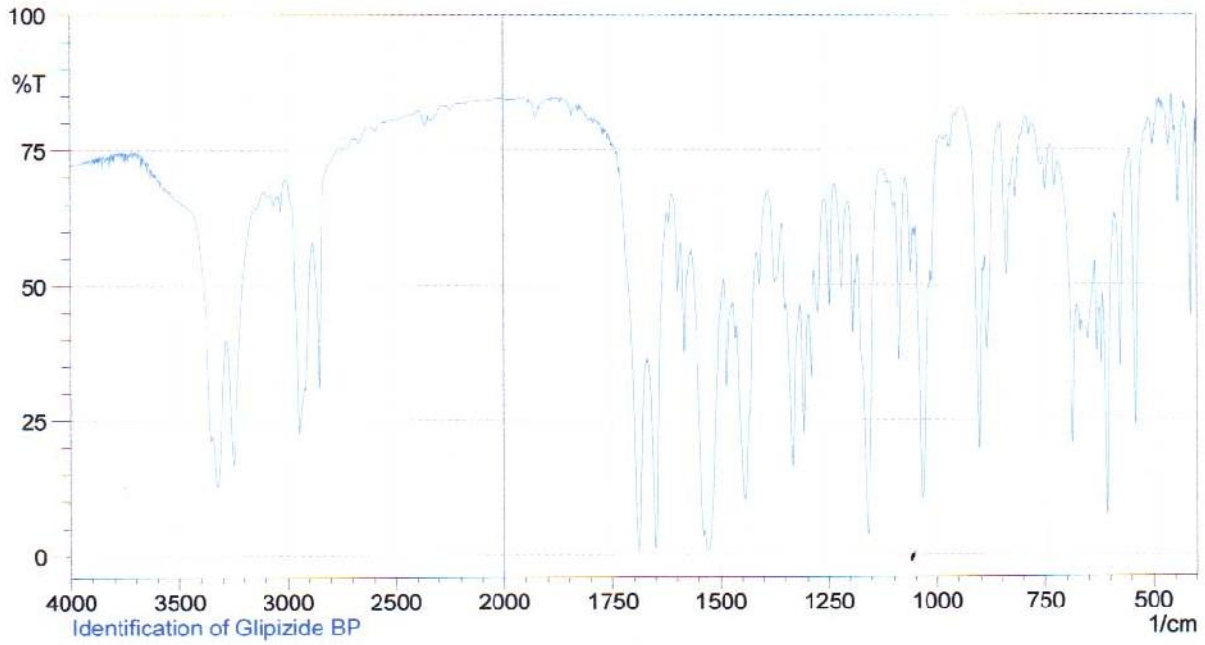


Fig. 4.2.2: IR spectrum for sample of glipizide

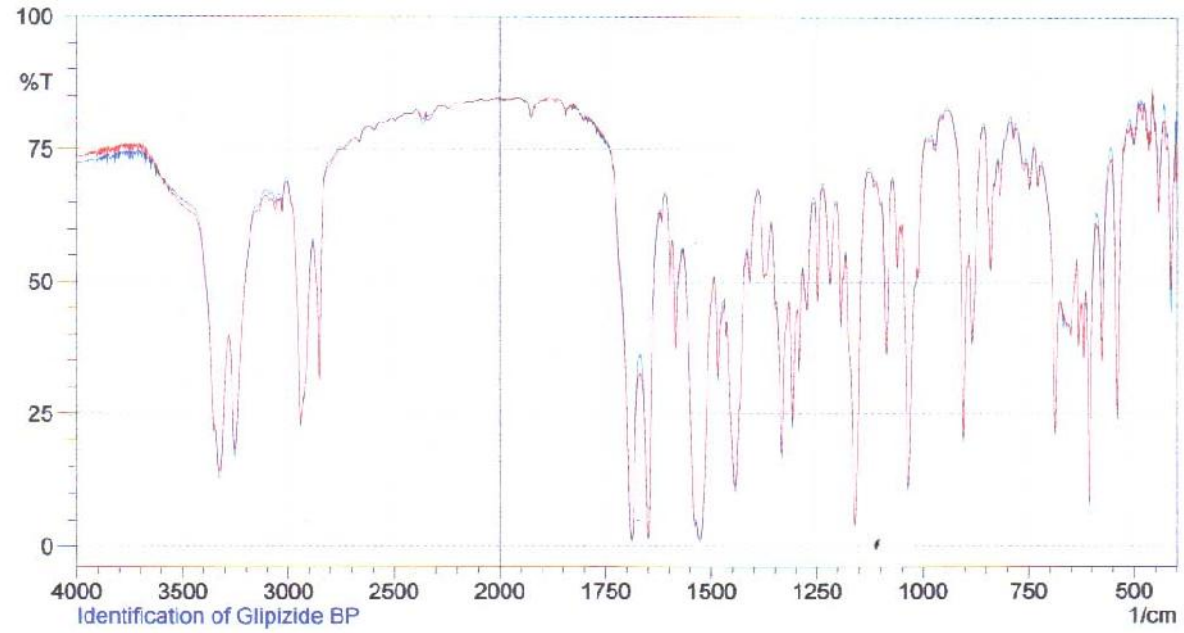


Fig. 4.2.3: Joined visible IR spectrum for working standard & sample of glipizide

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: 208^0C to 209^0C

d. Solubility

Procedure:

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in more than 10,000 ml of water.

Result: Practically insoluble in water.

Very Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added methylene chloride slowly and shaken well. It dissolved in 1000 ml to 10000 ml of methylene chloride.

Result: Very Slightly Soluble in methylene chloride.

Very Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added acetone slowly and shaken well. It dissolved in 1000 ml to 10000 ml of acetone.

Result: Very Slightly Soluble in acetone.

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added ethanol (96 per cent) slowly and shaken well. It dissolved in more than 10,000 ml of ethanol (96 per cent).

Result: Practically insoluble in ethanol (96 per cent).

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alkali hydroxide (N/10 NaOH, N/10 KOH etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alkali hydroxide.

Result: Slightly Soluble in alkali hydroxide.

e. pH of 0.1% Solution

Procedure: Accurately weighed about 0.1 g of powder & transferred in a 100 ml volumetric flask. Added about 60 ml of water & shaken mechanically for about 10 minutes. Volume up to the mark with water & mix well. Poured the solution in a clean, dry 50ml beaker and measured the pH by using microprocessor p^H meter at room temperature.

Result: 4.35

f. Loss on drying

Procedure:

Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g of powder and transferred in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweighed. From the two weights calculate the % of loss on drying:

Calculation:

$$\frac{(W1 - W_2) \times 100}{\text{Sample weight}}$$

Where, W_1 = Sample + Crucible before drying

W_2 = Sample + Crucible after drying

Result: 0.17 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of glipizide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.
Scanned the solution of glipizide between 400 – 200 nm and the spectrum was recorded.

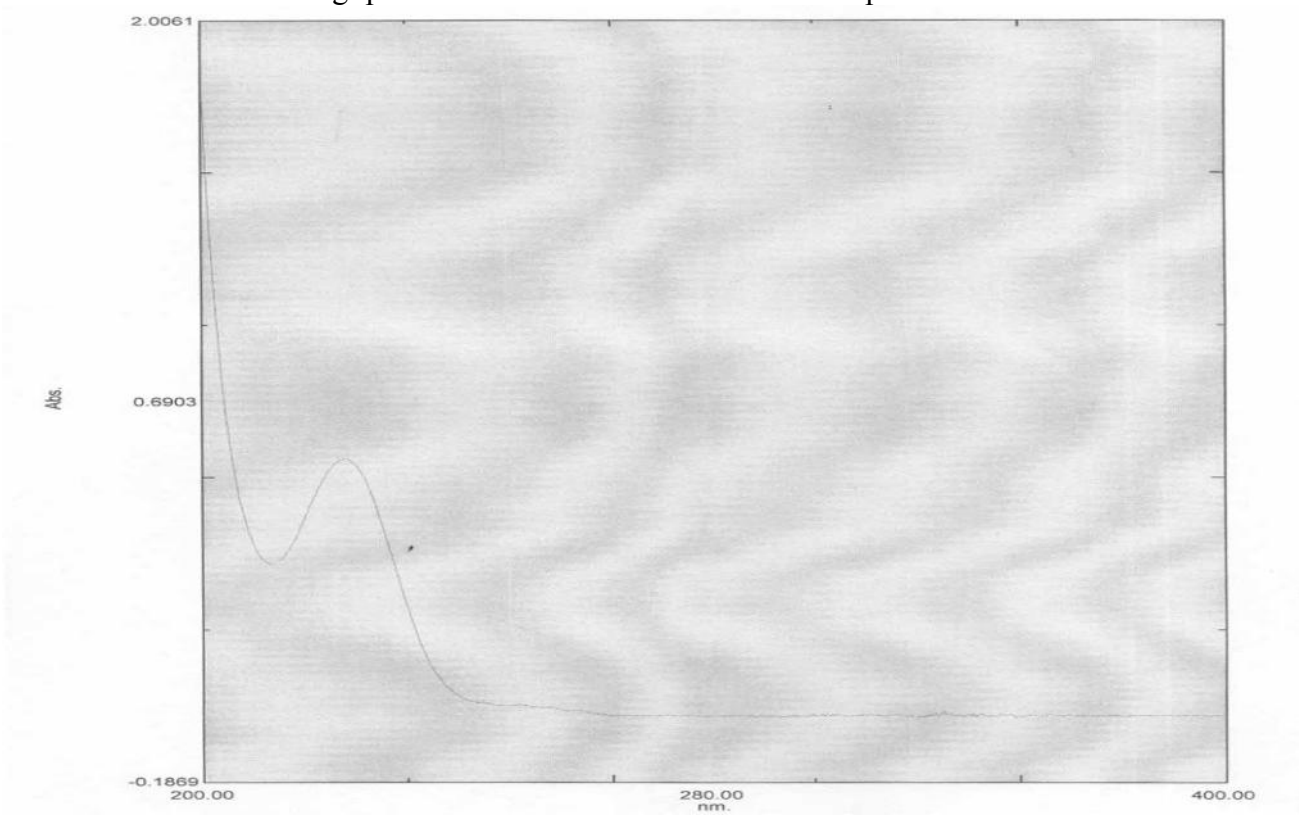


Fig.4.2.4: UV – VIS spectrum for working standard of glipizide

Standard curve of glipizide

2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml working standard solutions of glipizide were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of glipizide.

Table 4.2.1: Data for the standard curve of glipizide

Concentration (µg/ml)	Absorbance at 228 nm
0	0.000
2	0.136
4	0.269
6	0.406
8	0.533
10	0.667
12	0.794

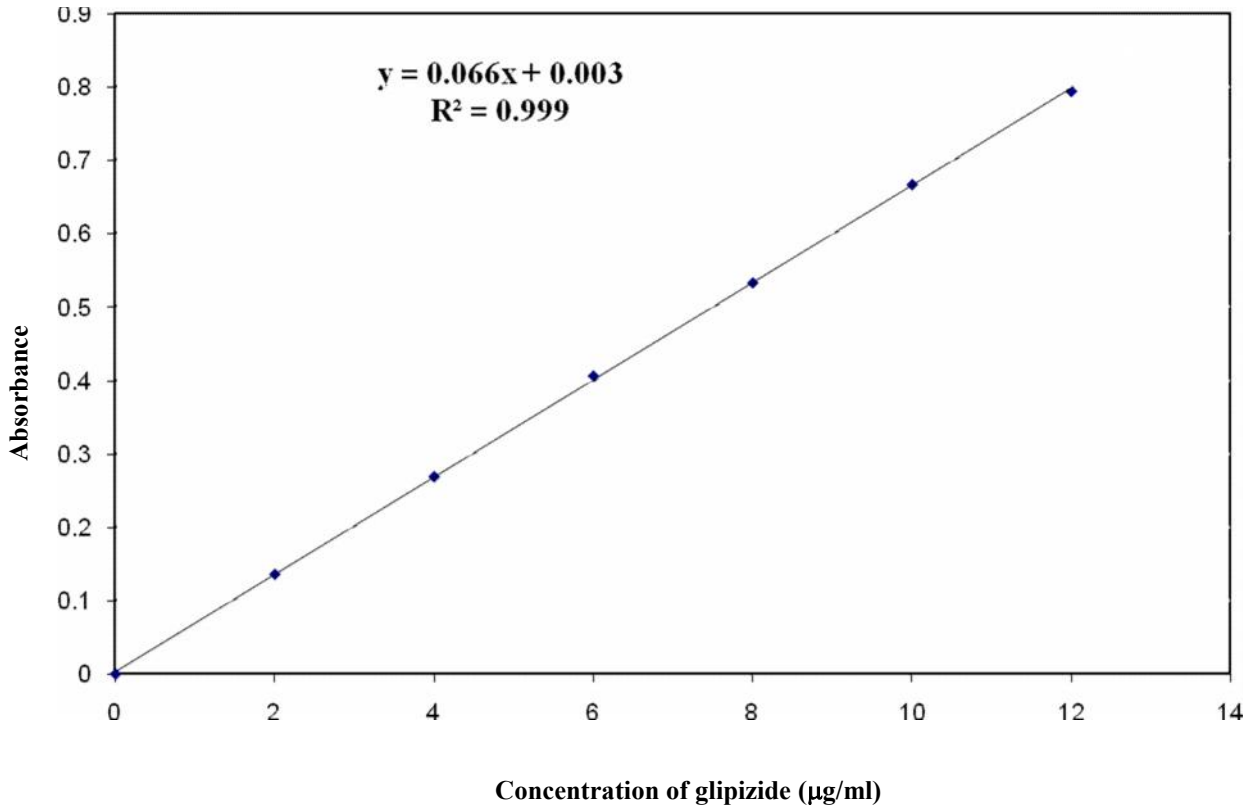


Fig. 4.2.5: Standard curve of glipizide

h. Assay

Procedure:

Standard Preparation – Weighed accurately about 20 mg of glipizide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation – Weighed accurately about 20 mg of glipizide sample in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Measured the absorbance of both standard and sample preparation at 228 nm against 0.1N sodium hydroxide as blank.

Calculation – Percentage of glipizide

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} \text{X} \frac{\text{Std.wt}}{\text{Sample wt.}} \text{X} \frac{\text{P}}{100} \text{X}100$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \%} \times \frac{100}{\text{100-L.O.D}}$$

Result: 99.27 % (Dried basis)

4.3 TESTS OF GLYBURIDE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance,color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse daylight.

Results: White to off-white crystalline powder. Free from any visible impurities.

b. Identification

Procedure: Transferred about 200 mg of KBr, previously dried at 105 ^{0}C and cooled, grind into a mortar to a fine powder & added about 2.0 mg of test sample, mix well and grind to a fine powder. Taken about 100 mg of this powder and made a thin semitransparent disc. Recorded the IR spectrum of the disc from 4000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from Glyburide working standard recorded in the same manner.

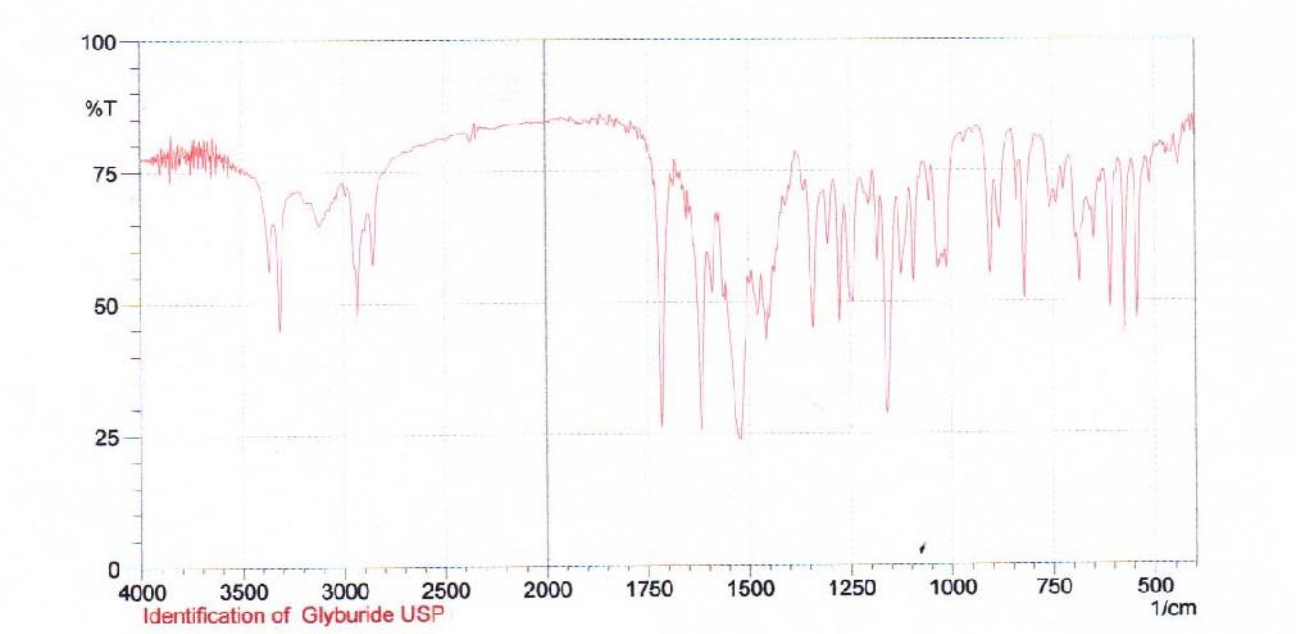


Fig. 4.3.1: IR spectrum for working standard of glyburide

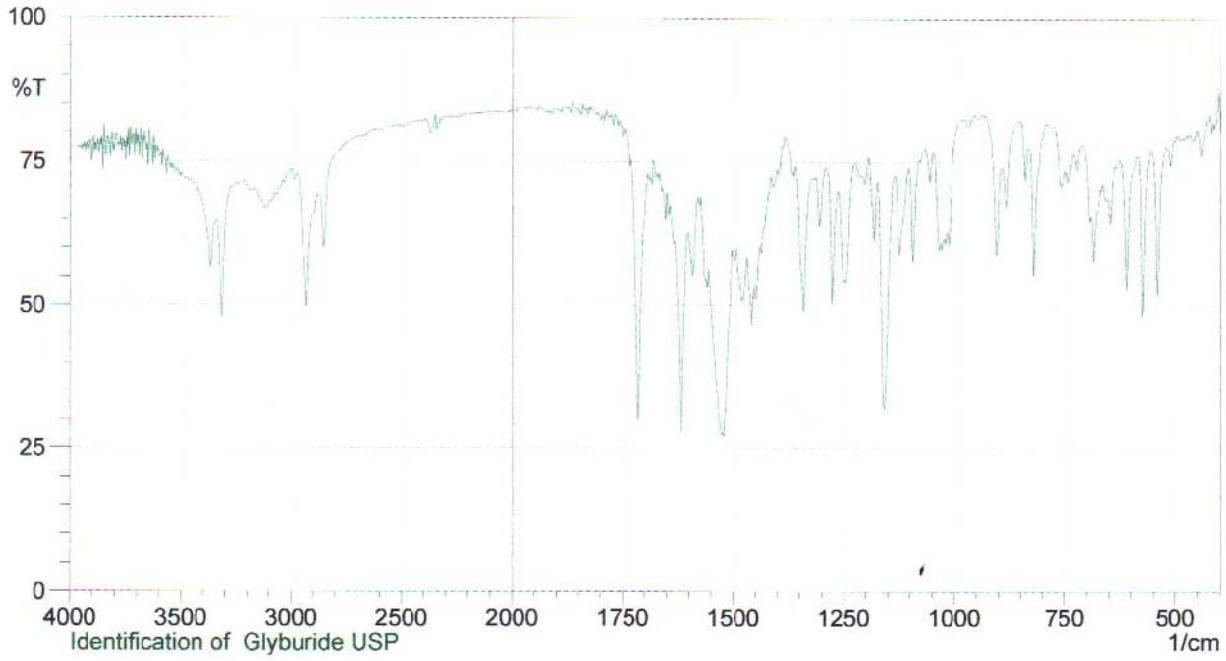


Fig. 4.3.2: IR spectrum for sample of glyburide

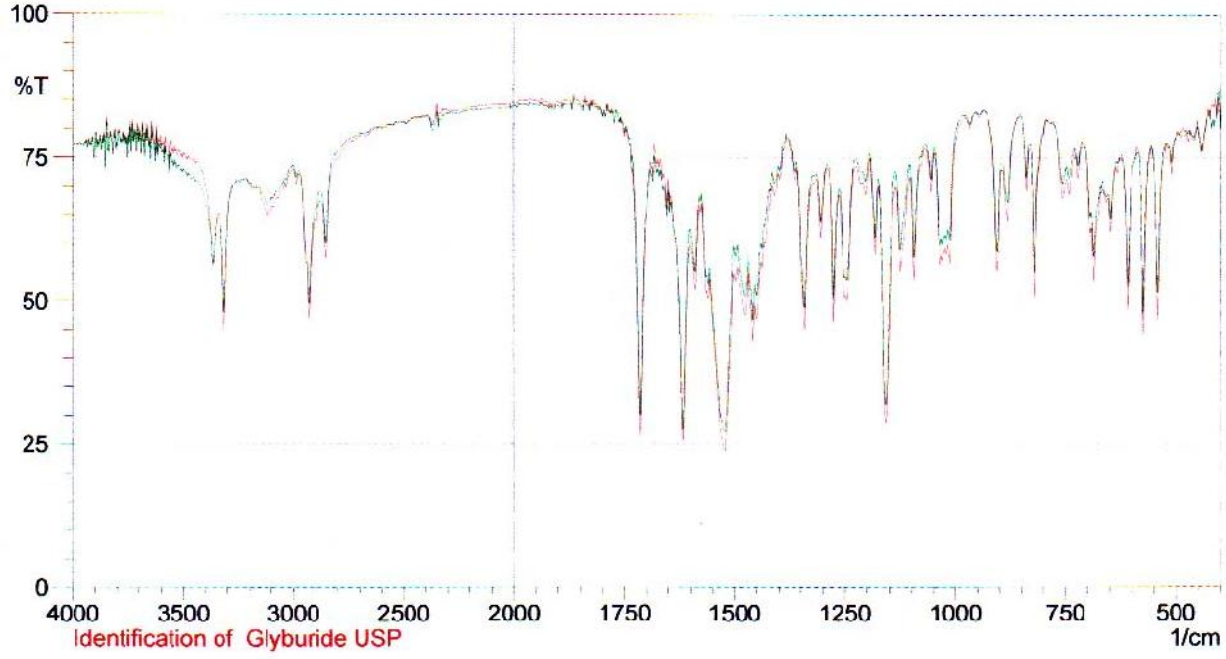


Fig. 4.3.3: Joined visible IR spectrum for working standard & sample of glyburide

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: $171\ ^{0}$ C - $173\ ^{0}$ C

d. Solubility

Procedure:

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in more than 10,000 ml of water.

Result: Practically insoluble in water.

Practically Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added ethanol slowly and shaken well.It dissolved in 100 ml to 200 ml of ethanol.

Result: Soluble in ethanol.

Practically Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added chloroform slowly and shaken well.It dissolved in 300 ml to 360 ml of chloroform.

Result: Soluble in chloroform.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alkali hydroxide (N/10 NaOH, N/10 KOH etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alkali hydroxide.

Result: Slightly Soluble in alkali hydroxide.

Practically Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added methanol slowly and shaken well.It dissolved in 200 ml to 250 ml of methanol.

Result: Soluble in methanol.

Practically Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added DMF slowly and shaken well.It dissolved in 200 ml to 250 ml of DMF.

Result: Soluble in DMF.

e. pH of 0.1% Solution

Procedure:

Accurately weighed about 0.1 g of powder & transferred in a 100 ml volumetric flask. Added about 60 ml of water & shaken mechanically for about 10 minutes. Volume up to the mark with water & mix well. Poured the solution in a clean, dry 50 ml beaker and measured the pH by using microprocessor p^{H} meter at room temperature.

Result: 3.75

f. Loss on drying

Procedure:

Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g of powder and transferred in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweighed. From the two weights calculate the % of loss on drying:

Calculation: $\frac{(W1 - W_2) \times 100}{\text{Sample weight}}$

Where, W_1 = Sample + Crucible before drying

W_2 = Sample + Crucible after drying

Result: 0.30 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of glyburide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Scanned the solution of glyburide between 400–200 nm and the spectrum was recorded.

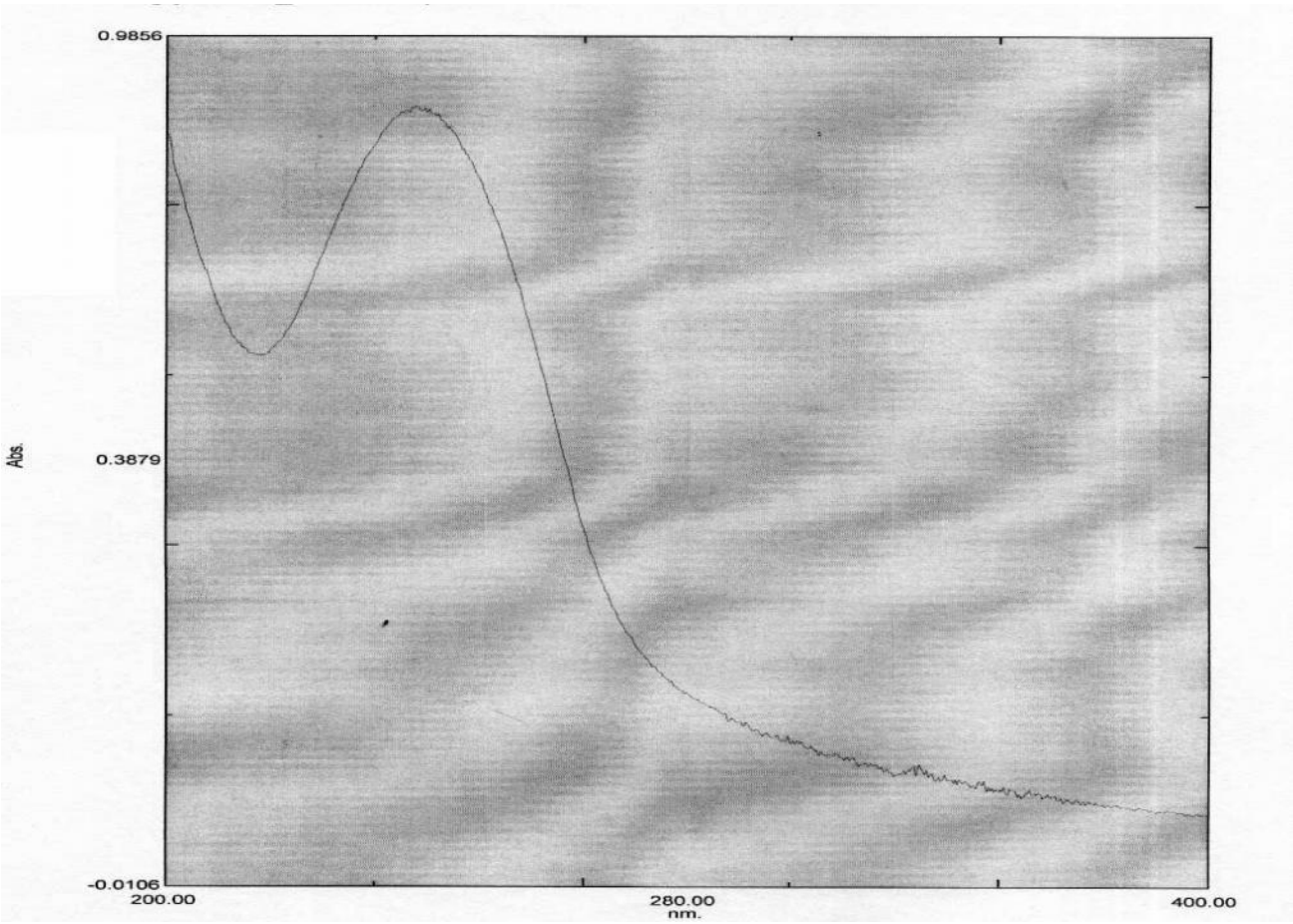


Fig.4.3.4: UV – VIS spectrum for working standard of glyburide

Standard curve of glyburide

2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml working standard solutions of glyburide were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of glyburide.

Table 4.3.1: Data for the standard curve of glyburide

Concentration (μg/ml)	Absorbance at 248 nm
0	0.000
2	0.121
4	0.228
6	0.345
8	0.455
10	0.555
12	0.669

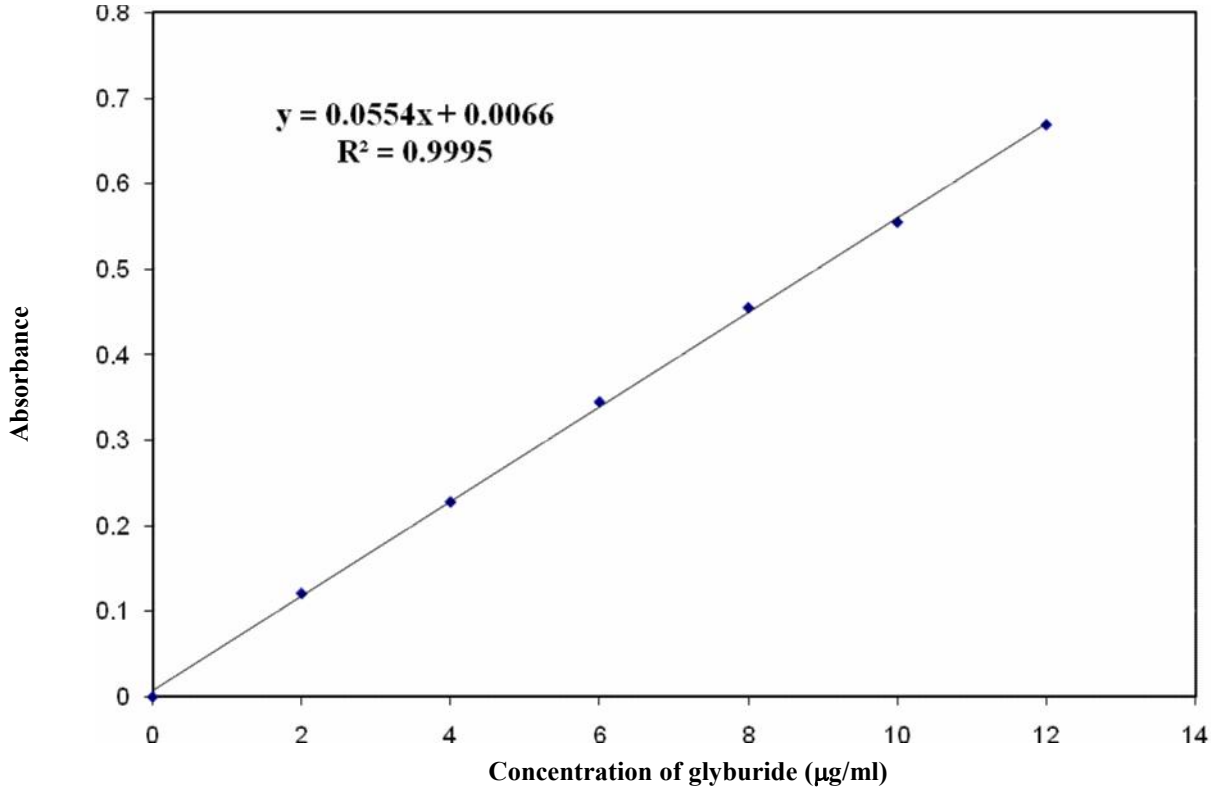


Fig. 4.3.5: Standard curve of glyburide

h. Assay

Procedure:

Standard Preparation – Weighed accurately about 20 mg of glyburide working standard in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation – Weighed accurately about 20 mg of glyburide sample in 100 ml volumetric flask, added about 70 ml of 0.1N sodium hydroxide and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Measured the absorbance of both standard and sample preparation at 248 nm against 0.1N sodium hydroxide as blank.

Calculation – Percentage of glyburide

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} X \frac{\text{Std.wt}}{\text{Sample wt.}} X \frac{P}{100} X100$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \%} \times \frac{100}{100\text{-L.O.D}}$$

Result: 99.08 % (Dried basis)

4.4 TESTS OF METFORMIN HYDROCHLORIDE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance,color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse daylight .

Results: White crystals and free from any visible impurities.

b. Identification

Procedure: Transferred about 200 mg of KBr, previously dried at 105 ^{0}C and cooled, grind into a mortar to a fine powder & added about 2.0 mg of test sample, mix well and grind to a fine powder. Taken about 100 mg of this powder and made a thin semitransparent disc. Recorded the IR spectrum of the disc from 4000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from Metformin Hydrochloride working standard recorded in the same manner.

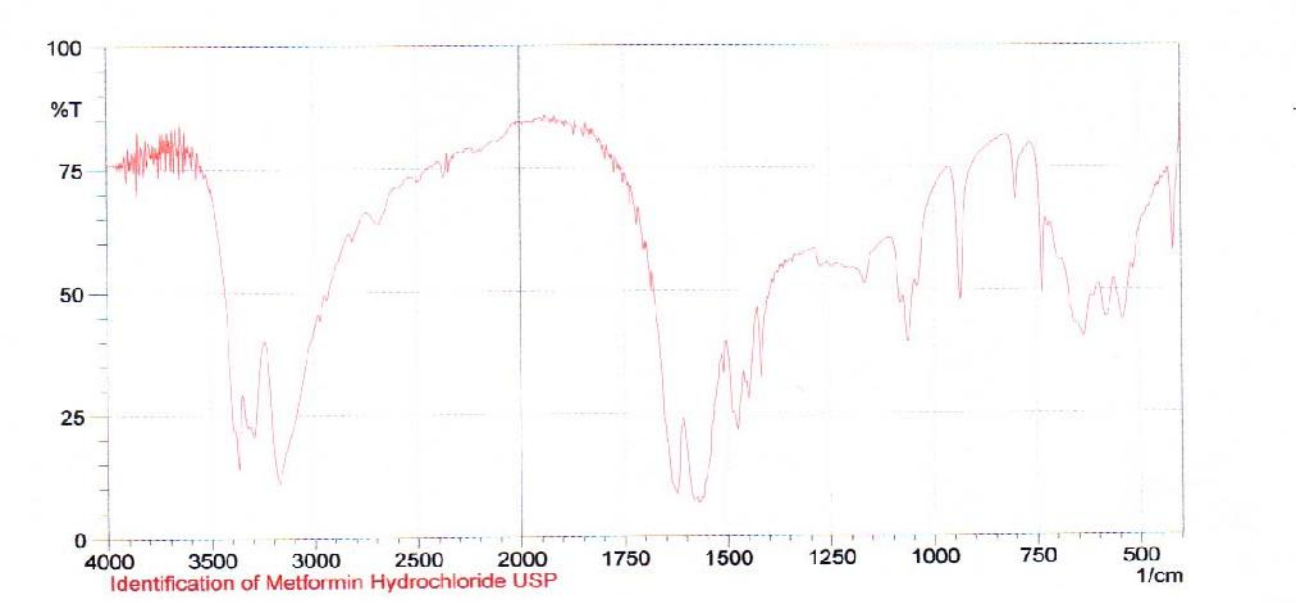


Fig. 4.4.1: IR spectrum for working standard of metformin HCI

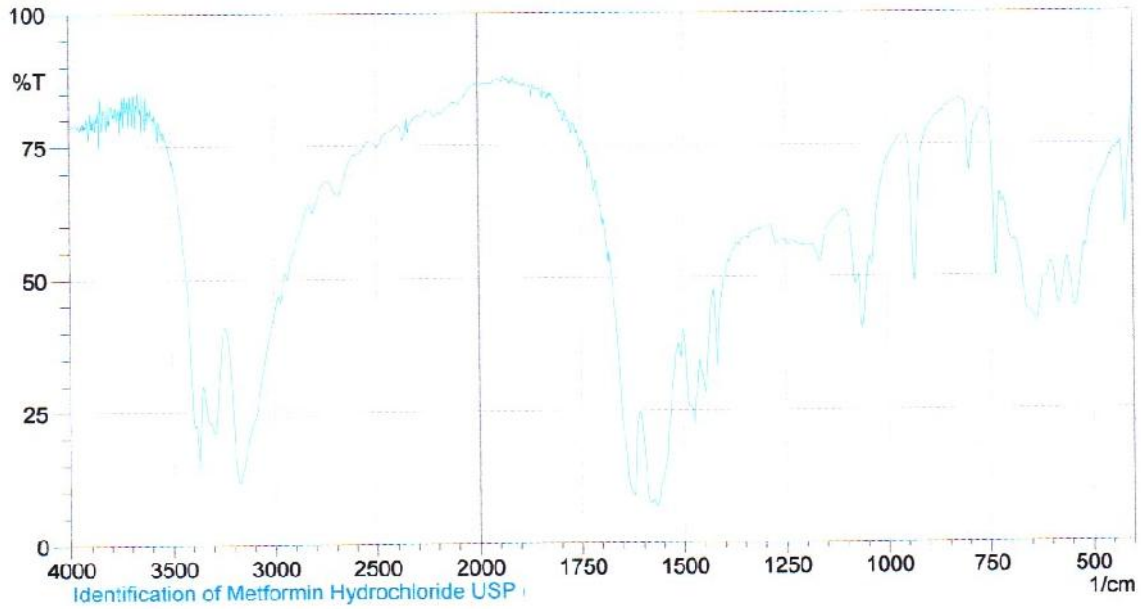


Fig. 4.4.2: IR spectrum for sample of metformin HCI

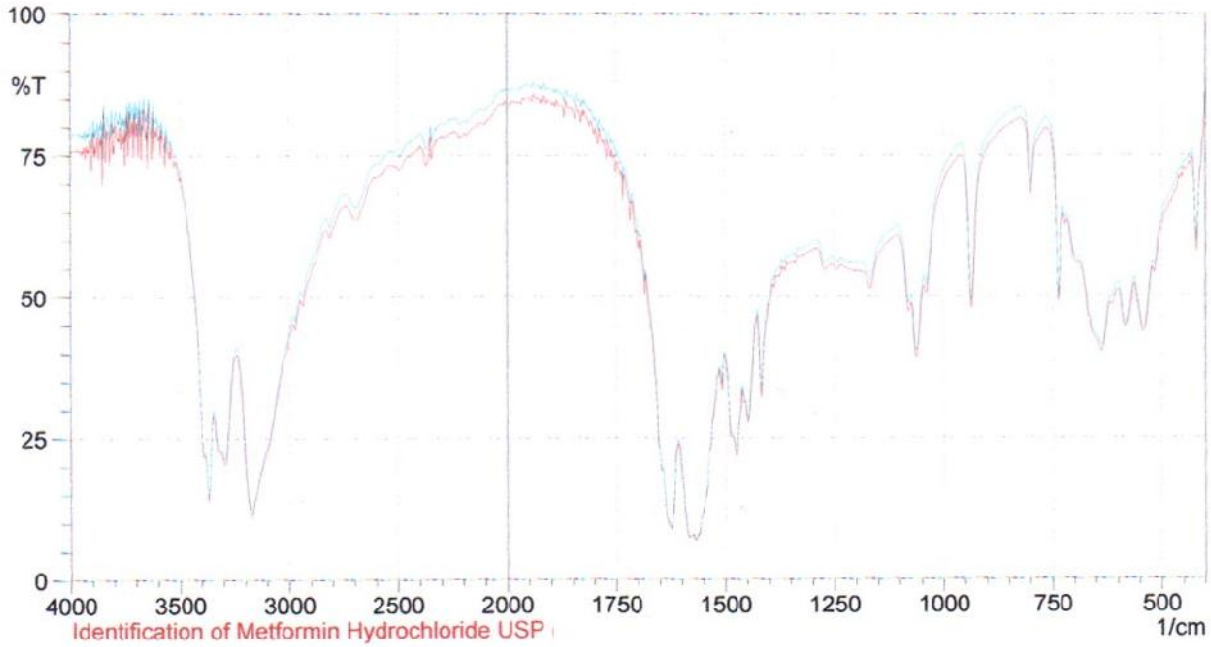


Fig.4.4.3: Joined visible IR spectrum for working standard & sample of metformin

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: $223\ ^0$ C - $225\ ^0$ C

d. Solubility

Procedure:

Freely Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in 1 ml to 10 ml of water.

Result: Freely Soluble in water.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alcohol (ethanol, methanol etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alcohol.

Result: Slightly Soluble in alcohol.

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added acetone slowly and shaken well.It dissolved in more than 10,000 ml of acetone.

Result: Practically insoluble in acetone.

Practically Insoluble: Transferred about 1 g of sample into a glass-stoppered test tube and added methylene chloride slowly and shaken well. It dissolved in more than 10,000 ml of methylene chloride.

Result: Practically insoluble in methylene chloride.

e. Apperance of Solution

Procedure: Dissolved 2.0 g of sample in water and diluted to 20 ml with the same solvent.

Result: Solution is clear & colourless

f. Loss on drying

Procedure:

Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g of powder and transfer in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweigh. From the two weights calculate the % of loss on drying:

Calculation: $\frac{(W_1 - W_2) \times 100}{\text{Sample weight}}$

Where, W_1 = Sample + Crucible before drying

W_2 = Sample + Crucible after drying

Result: 0. 32 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of Metformin HCI working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Scanned the solution of metformin HCI between 400 – 200 nm and the spectrum was recorded.

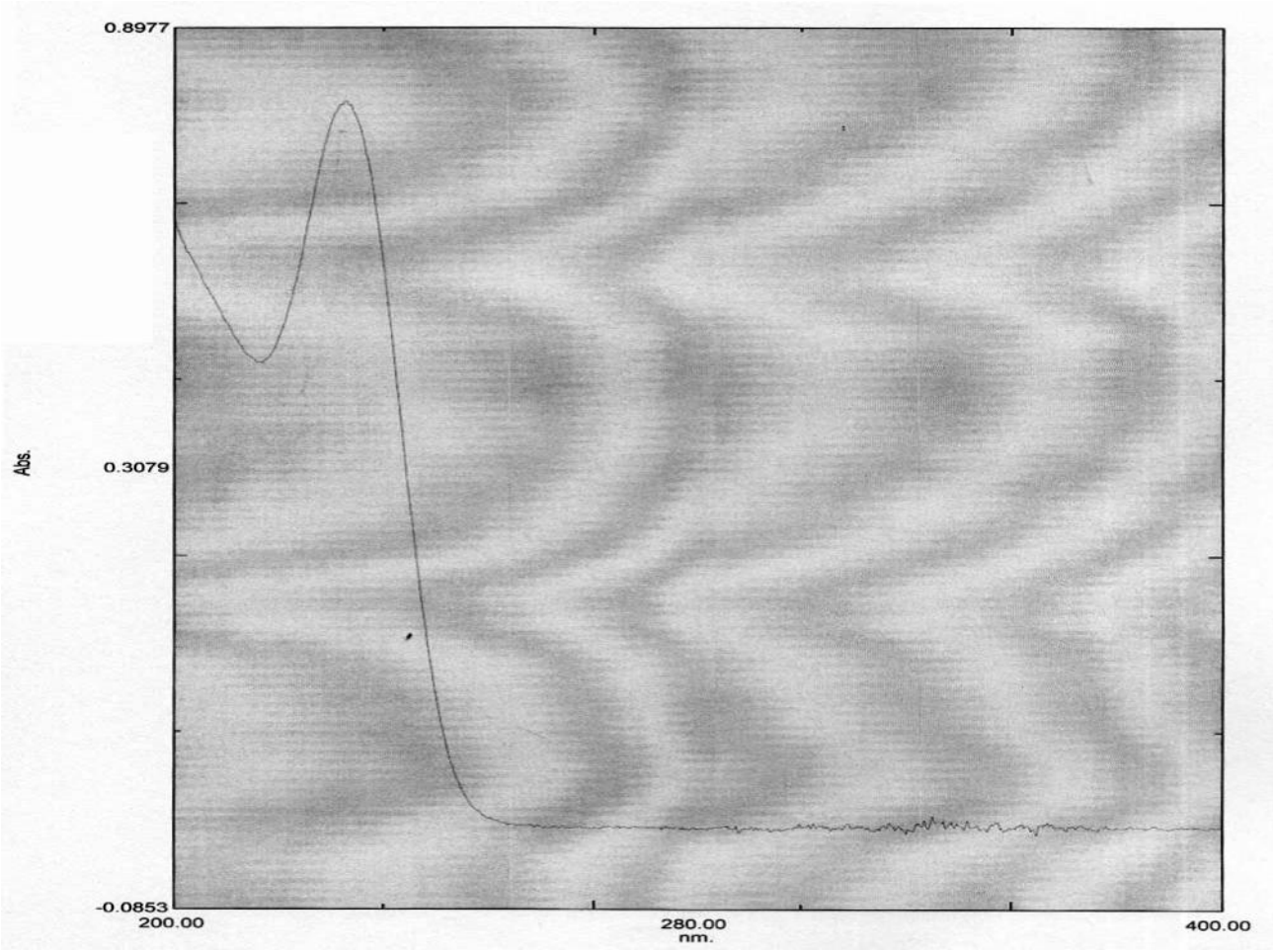


Fig.4.4.4: UV – VIS spectrum for working standard of metformin HCI

Standard curve of metformin HCI

2 μg/ml, 4 μg/ml, 6 μg/ml, 8 μg/ml, 10 μg/ml and 12 μg/ml working standard solutions of metformin HCI were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of metformin HCI.

Table 4.4.1: Data for the standard curve of metformin HCI.

Concentration (μg/ml)	Absorbance at 233 nm
0	0.000
2	0.153
4	0.322
6	0.463
8	0.623
10	0.768
12	0.923

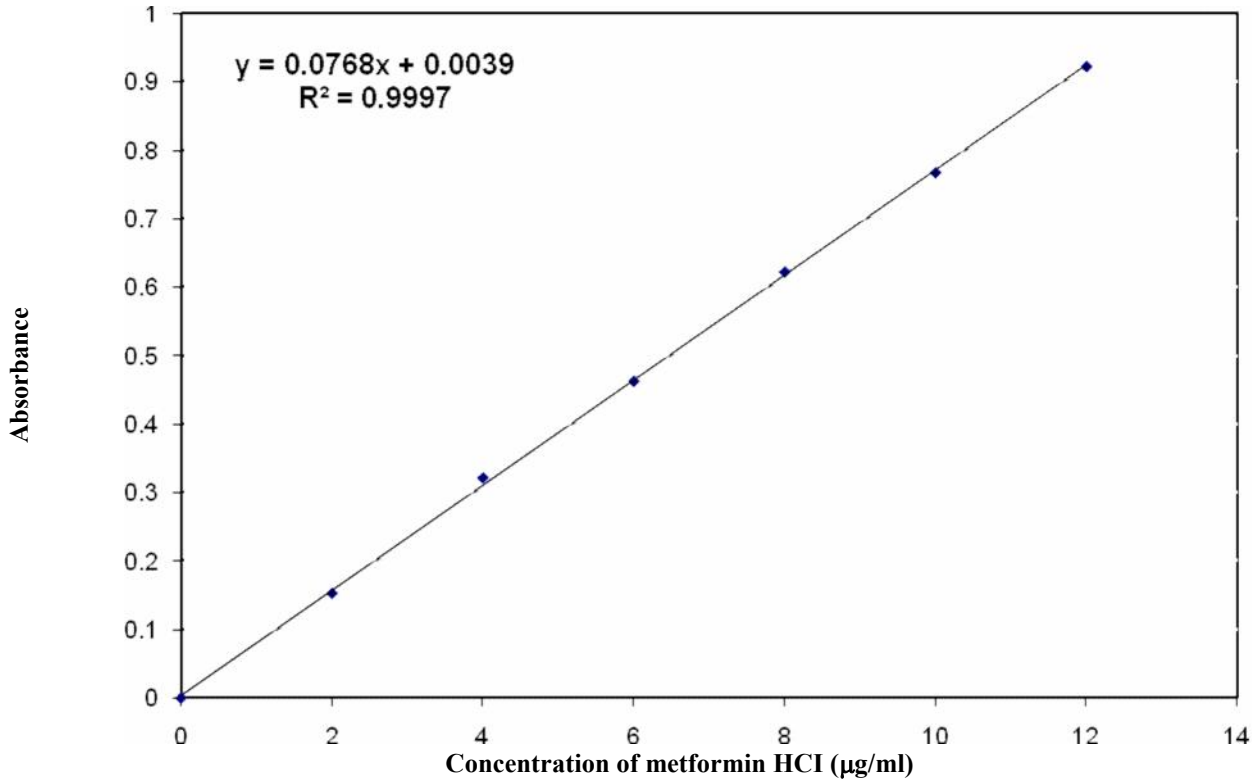


Fig. 4.4.5: Standard curve for metformin HCI

h. Assay

Procedure:

Standard Preparation- Weighed accurately about 20 mg of Metformin Hydrochloride working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation- Weighed accurately about 20 mg of Metformin Hydrochloride sample in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Measured the absorbance of both standard and sample preparation at 233 nm against water as blank.

Calculation - Percentage of Metformin HCI

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} X \frac{\text{Std.wt}}{\text{Sample wt.}} X \frac{P}{100} X100$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \%} \times \frac{100}{100\text{-L.O.D}}$$

Result: 99.88 % (Dried basis)

4.5 TESTS OF CAFFEINE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance,color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse daylight .

Results: A white, crystalline powder. Free from any visible impurities.

b. Identification

Procedure: Transferred about 200 mg of KBr, previously dried at 105^0C and cooled, grind into a mortar to a fine powder & added about 2.0 mg of test sample, mix well and grind to a fine powder. Taken about 100 mg of this powder and made a thin semitransparent disc. Recorded the IR spectrum of the disc from 4000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from Caffeine working standard recorded in the same manner.

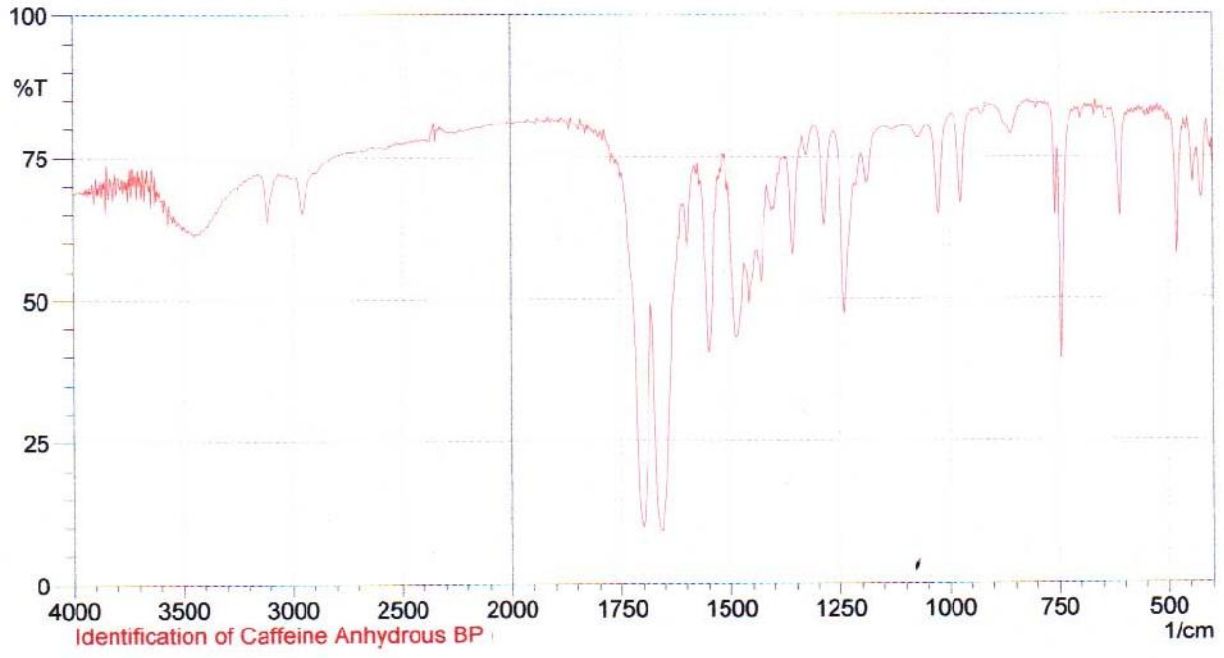


Fig. 4.5.1: IR spectrum for working standard of caffeine

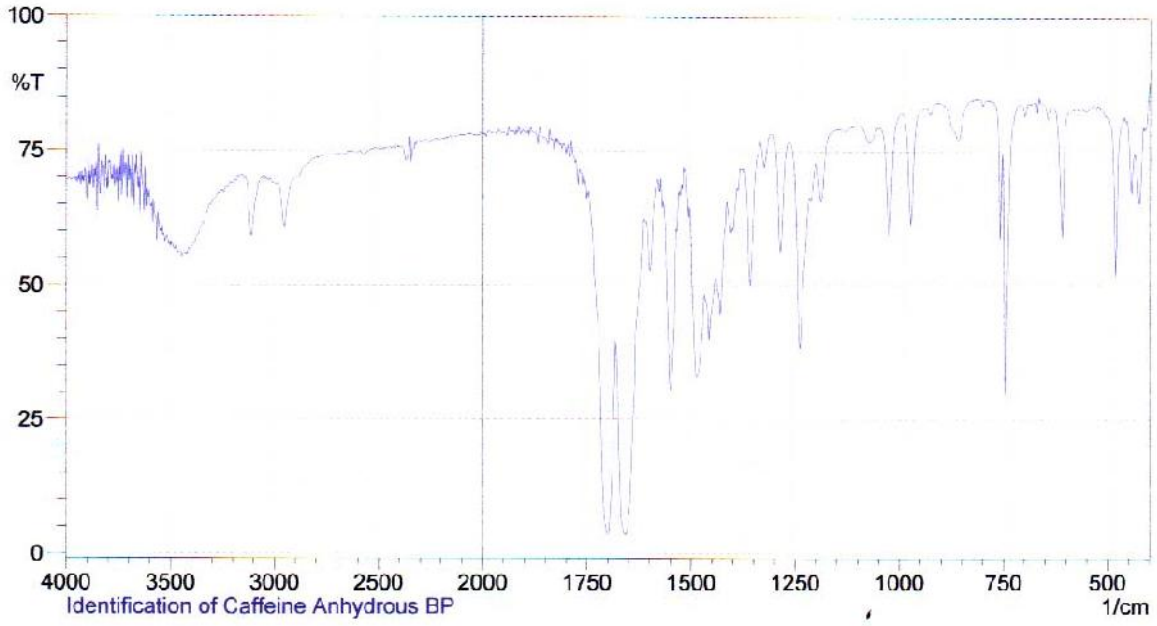


Fig. 4.5.2: IR spectrum for sample of caffeine

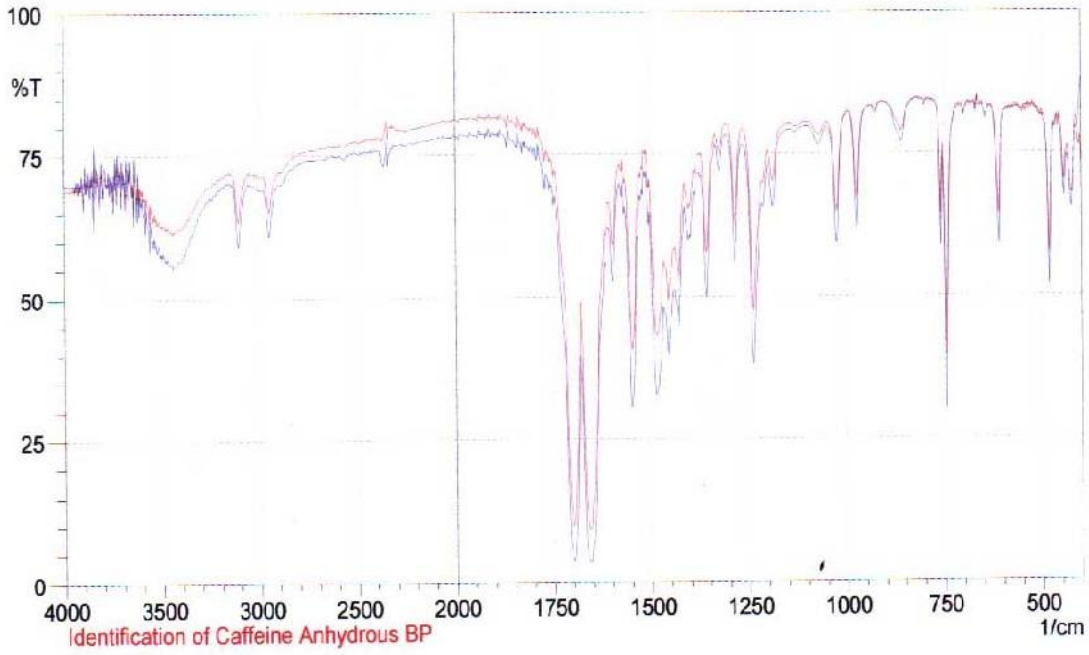


Fig. 4.5.3: Joined visible IR spectrum for working standard & sample of caffeine

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: 235^{0} C - 237^{0} C

d. Solubility

Procedure:

Sparingly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in 30 ml to 100 ml of water.

Result: Sparingly Soluble in water.

Freely Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added boiling water slowly and shaken well.It dissolved in 1 ml to 10 ml of boiling water.

Result: Freely Soluble in boiling water.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added ethanol slowly and shaken well. It dissolved in 100 ml to 1000 ml of ethanol.

Result: Slightly Soluble in ethanol.

e. Apperance of Solution

Procedure: Dissolved 0.50 g of sample with heating in 50 ml of of carbon-dioxide free water prepared from distilled water, cool and diluted to 50 ml with the same solvent.

Result: Solution is clear & colorless.

f. Loss on drying

Procedure:

Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g of powder and transfer in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweigh. From the two weights calculate the % of loss on drying:

Calculation: $\frac{(W1 - W2) \times 100}{\text{Sample weight}}$

Where, W1 = Sample + Crucible before drying

W2 = Sample + Crucible after drying

Result: 0. 35 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of Caffeine working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100ml with same solvent.

Scanned the solution of caffeine between 400 – 200 nm and the spectrum was recorded.

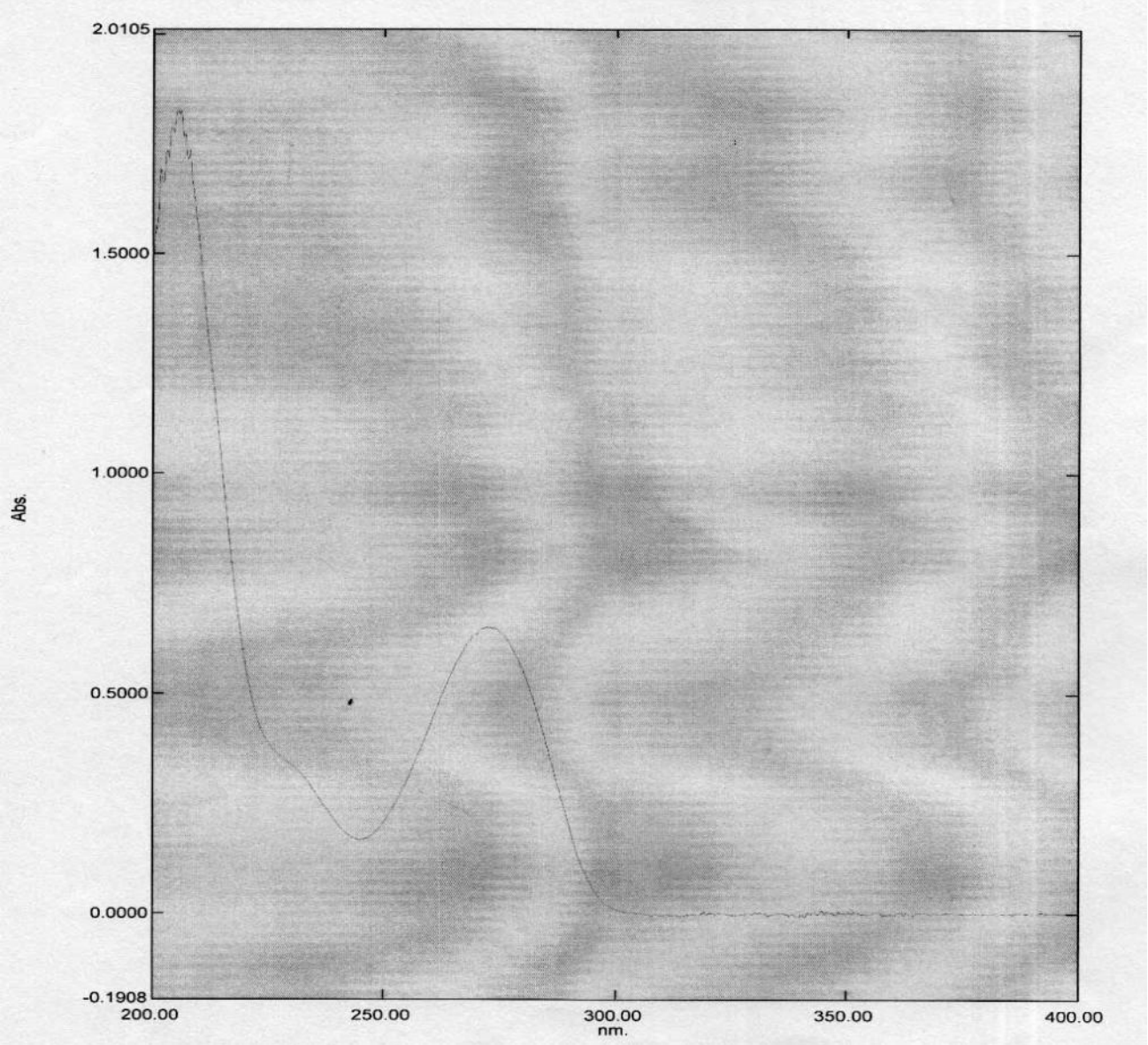


Fig.4.5.4: UV – VIS spectrum for working standard of caffeine

Standard curve of caffeine

2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml working standard solutions of caffeine were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of caffeine. [92] [93] [98]

Table 4.5.1: Data for the standard curve of caffeine

Concentration (μg/ml)	Absorbance at 273 nm
0	0.000
2	0.115
4	0.232
6	0.345
8	0.461
10	0.575
12	0.690

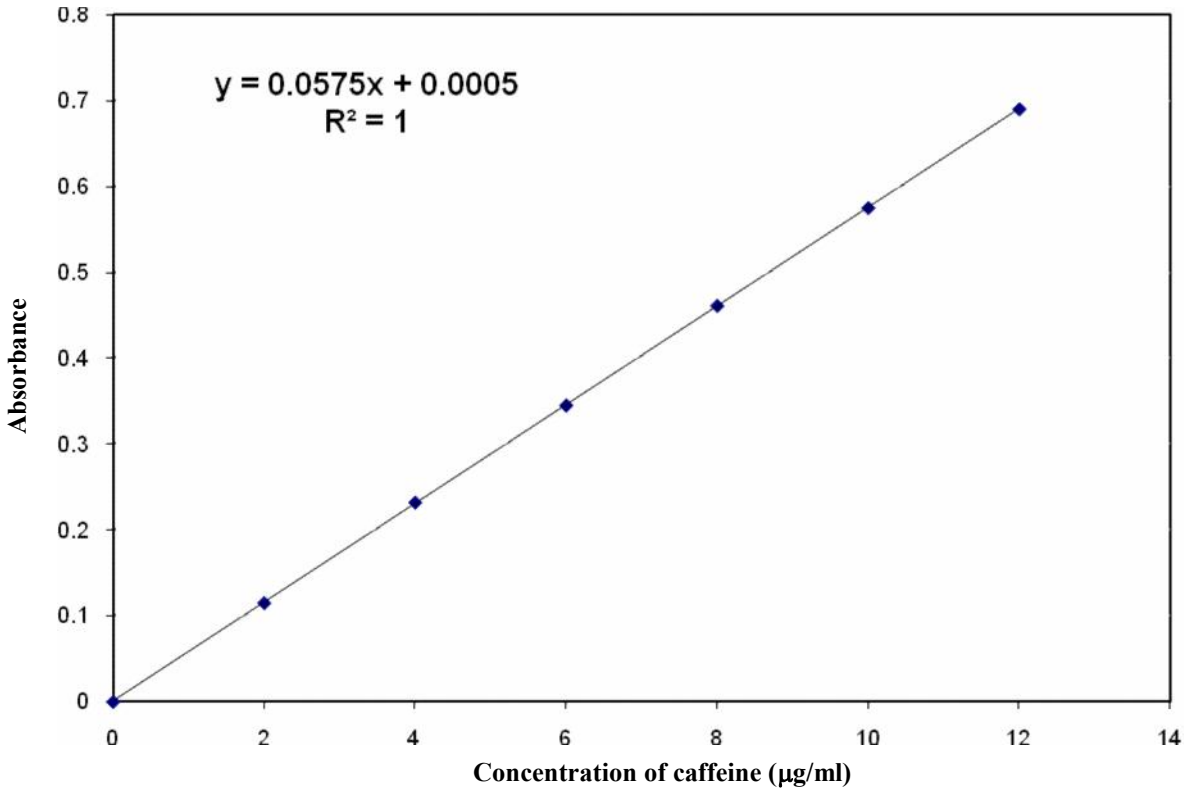


Fig. 4.5.5: Standard curve for caffeine

h. Assay

Procedure:

Standard Preparation – Weighed accurately about 20 mg of Caffeine working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation- Weighed accurately about 20 mg of Caffeine sample in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Measured the absorbance of both standard and sample preparation at 273 nm against water as blank.

Calculation - Percentage of Caffeine

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} X \frac{\text{Std.wt}}{\text{Sample wt.}} X \frac{P}{100} X100$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \%} \times \frac{100}{100\text{-L.O.D}}$$

Result: 99.72 % (Dried basis)

4.6 TESTS OF THEOPHYLLINE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance, color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse daylight .

Results: White, crystalline powder. Free from any visible impurities.

b. Identification

Procedure: Triturate a small quantity of the substance to be examined with the minimum quantity of liquid paraffin R; 5-10 mg of the substance to be examined is usually sufficient to make an adequate mull using one drop of liquid paraffin r. Compress the mull between 2 plates (Sodium Chloride Window) transparent to infrared radiation. Recorded the IR spectrum of the disc from 2000 cm-1to 400 cm-1 taking air as a blank. The IR absorption spectrum of the test sample should be concordant with the spectrum obtained from theophylline working standard recorded in the same manner.

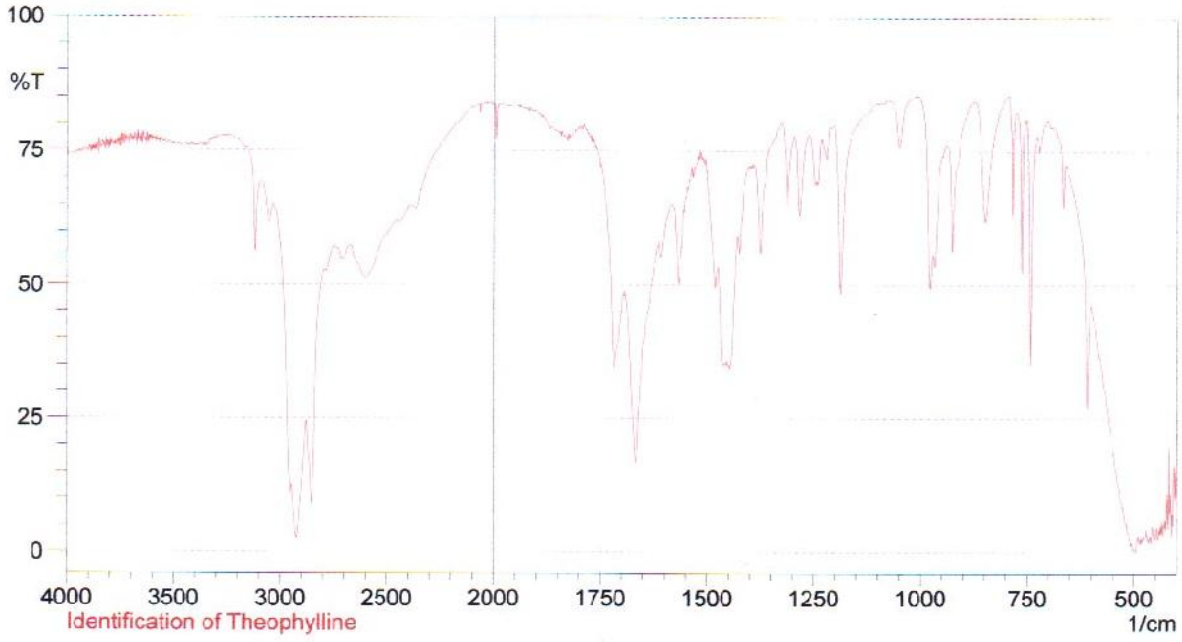


Fig. 4.6.1: IR spectrum for working standard of theophylline

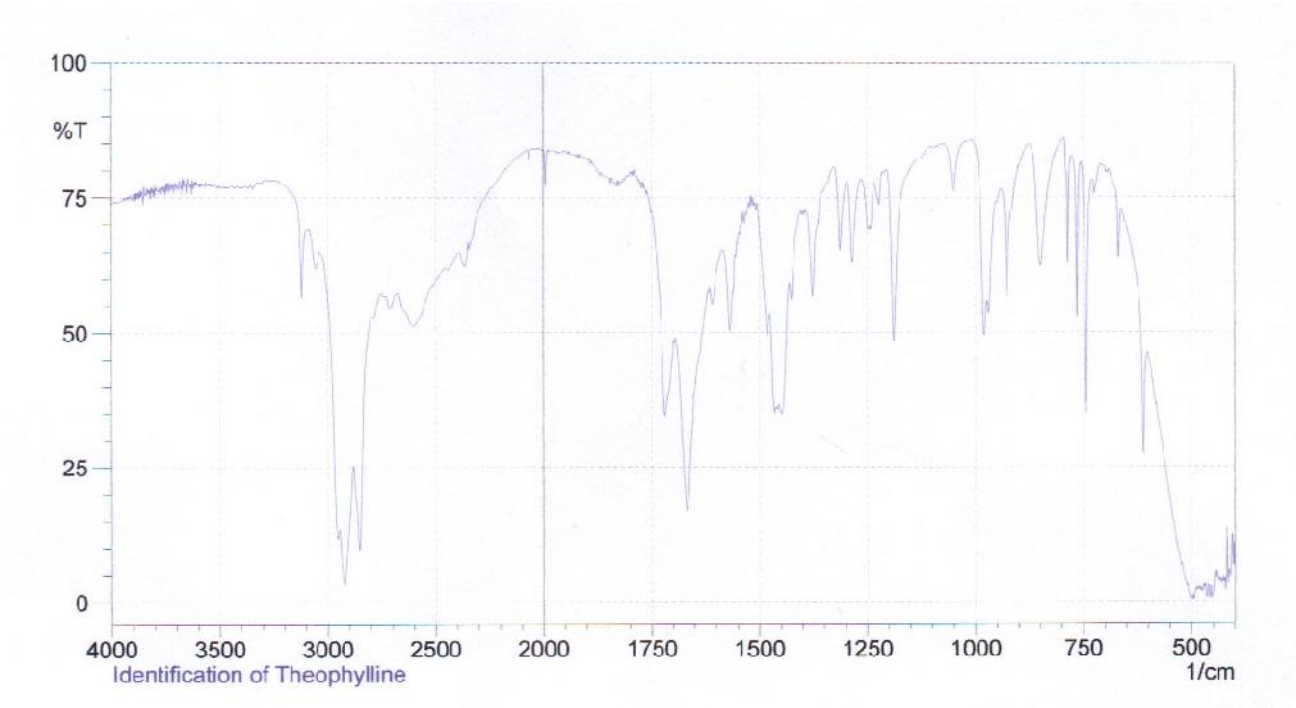


Fig. 4.6.2: IR spectrum for sample of theophylline

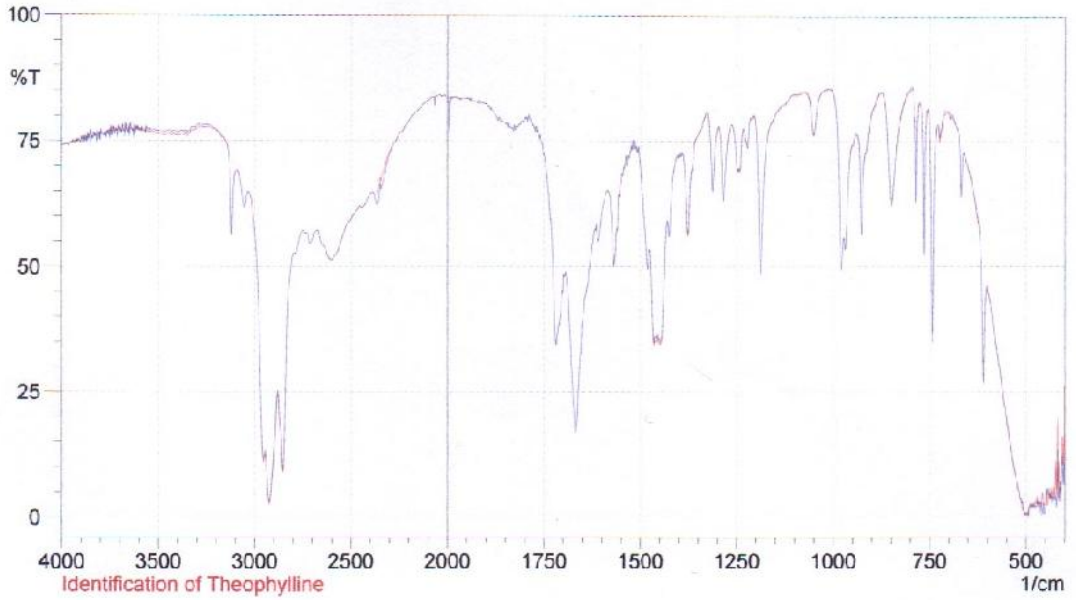


Fig. 4.6.3: Joined visible IR spectrum for working standard & sample of theophylline

Result: Sample complies with working standard.

c. Melting Point

Procedure: Transferred the sample to a dry capillary tube and pack the powder by tapping on a hard surface so to form tightly packed column about 4 to 6 mm in height. Inserted the capillary tube into the hole of melting point apparatus. Put "ON" the heater switch. Noted the temperature at which the substance start shrinking and completely liquidification occurs, which is indicated by the formation of a definite melting.

Result: 271 ^{0} C - 273 ^{0} C

d. Solubility

Procedure:

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added water slowly and shaken well. It dissolved in 100 ml to 1000 ml of water.

Result: Slightly Soluble in water.

Sparingly Soluble: Transferred about 1 g of sample into a glass- stoppered test tube and added ethanol slowly and shaken well. It dissolved in 30 ml to 100 ml of ethanol.

Result: Sparingly Soluble in ethanol.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added alkali hydroxide (N/10 NaOH, N/10 KOH etc) slowly and shaken well. It dissolved in 100 ml to 1000 ml of alkali hydroxide.

Result: Slightly Soluble in alkali hydroxide.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added ammonia slowly and shaken well. It dissolved in 100 ml to 1000 ml of ammonia.

Result: Slightly Soluble in ammonia.

Slightly Soluble: Transferred about 1 g of sample into a glass-stoppered test tube and added mineral acids slowly and shaken well. It dissolved in 100 ml to 1000 ml of mineral acids.

Result: Slightly Soluble in mineral acids.

e. Apperance of Solution

Procedure: Dissolved 0.50 g of sample with heating in 50 ml of of carbon-dioxide free water prepared from distilled water, cool and diluted to 50 ml with the same solvent.

Result: Solution is clear & colorless.

f. Loss on drying

Procedure:

Weighed a crucible that has been dried under the same conditions to be employed in the determination. Accurately weighed about 1.0 g of powder and transfer in the crucible and dry in a gravity convection oven at 105°C for 3 hrs. After drying cool the crucible in a dessicators and reweigh. From the two weights calculate the % of loss on drying:

Calculation:

$$\frac{(W1 - W2) \times 100}{\text{Sample weight}}$$

Where, W1 = Sample + Crucible before drying

W2 = Sample + Crucible after drying

Result: 0.33 %

g. Determination of wavelength for assay

Procedure: Weighed accurately about 20 mg of Caffeine working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Scanned the solution of caffeine between 400 – 200 nm and the spectrum was recorded.

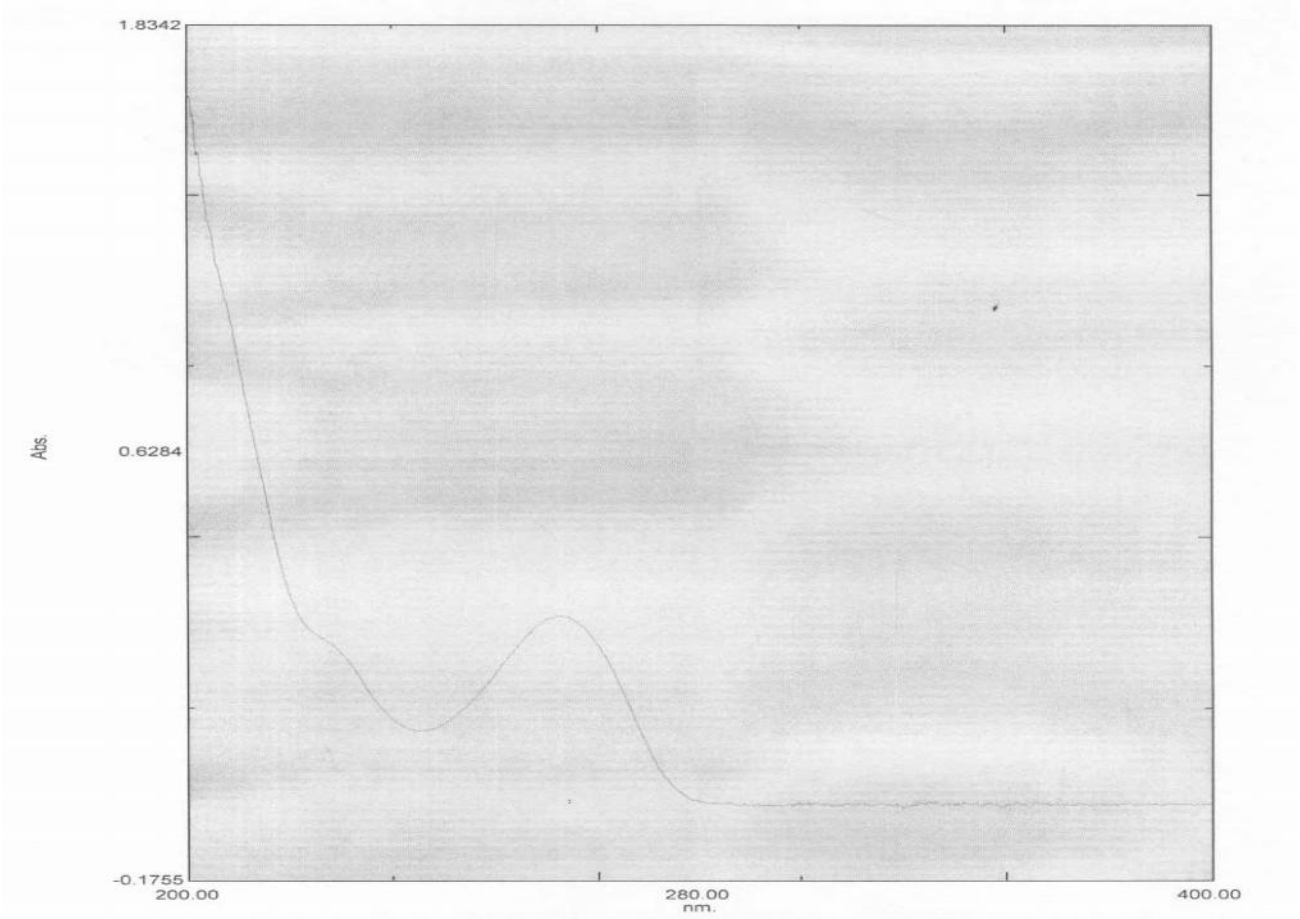


Fig.4.6.4: UV – VIS spectrum for working standard of theophylline

Standard curve of theophylline

2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml working standard solutions of caffeine were prepared. Then, a standard curve was prepared by plotting absorbance VS concentration of theophylline. [92] [93] [98]

Table 4.6.1: Data for the standard curve of theophylline

Concentration (μg/ml)	Absorbance at 276 nm
0	0.000
2	0.123
4	0.225
6	0.333
8	0.442
10	0.563
12	0.672

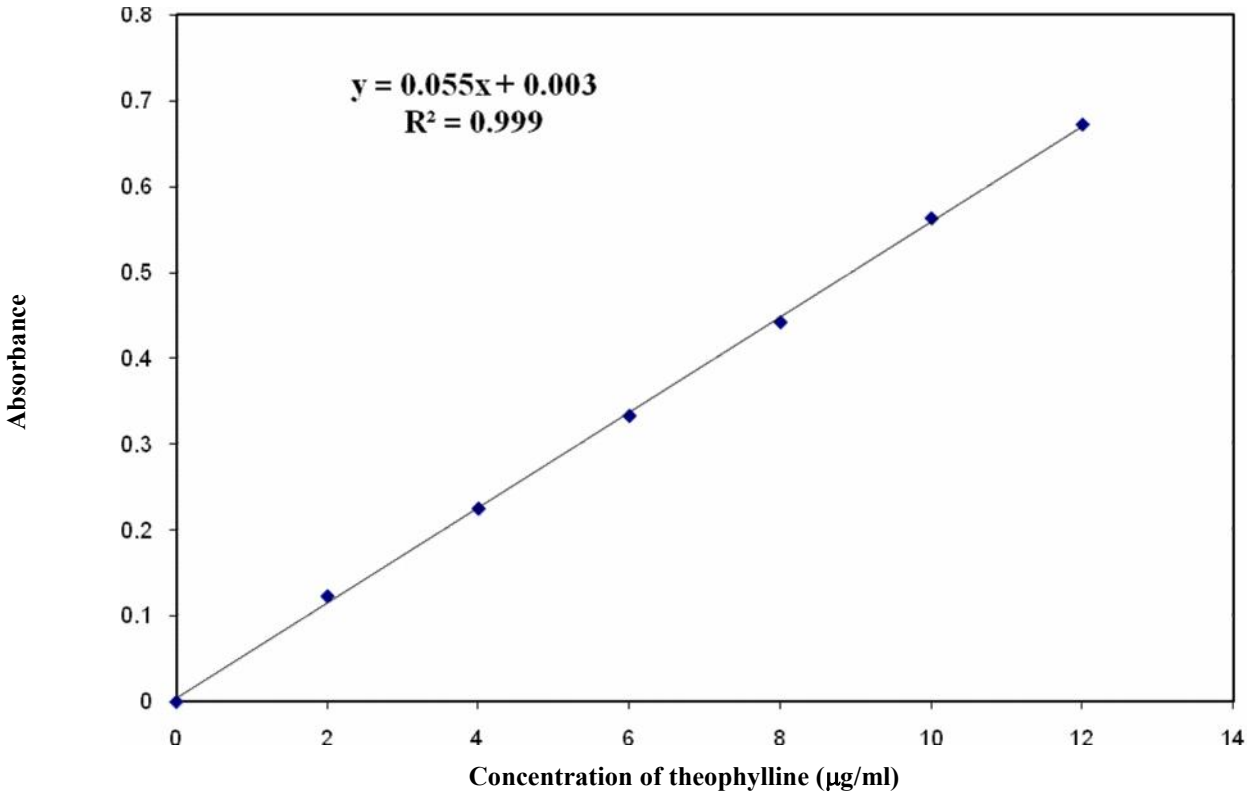


Fig. 4.6.5: Standard curve for theophylline

h. Assay

Procedure:

Standard Preparation – Weighed accurately about 20 mg of Caffeine working standard in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Test preparation- Weighed accurately about 20 mg of Caffeine sample in 100 ml volumetric flask, added about 70 ml of water and shaken vigorously for 15 minutes and volume to 100 ml with water. Diluted 5 ml of this solution to 100 ml with same solvent.

Measured the absorbance of both standard and sample preparation at 276 nm against water as blank.

Calculation - Percentage of theophylline

$$\frac{\text{Abs. of sample}}{\text{Abs. of std.}} X \frac{\text{Std.wt}}{\text{Sample wt.}} X \frac{P}{100} X100$$

Where, P = Potency of standard

$$\text{Assay (Dried basis)} = \text{Assay \%} \times \frac{100}{100\text{-L.O.D}}$$

Result: 99.52 % (Dried basis)

4.7 TESTS OF GLUCOSE

a. Appearance

Procedure: Dispensed about 1 g of powder on a watch glass or petridish having white background & observed the nature of the substance, color of the substance and any extraneous matter present (black particles, fibers etc.) in diffuse daylight .

Results: White, crystalline powder. Free from any visible impurities.

b. Determination of wavelength for glucose deternmination

Procedure: 50 µg/ml was prepared by using supplied 1% glucose solutions.Scanned the solution of glucose between 200 – 800 nm and the spectrum was recorded.

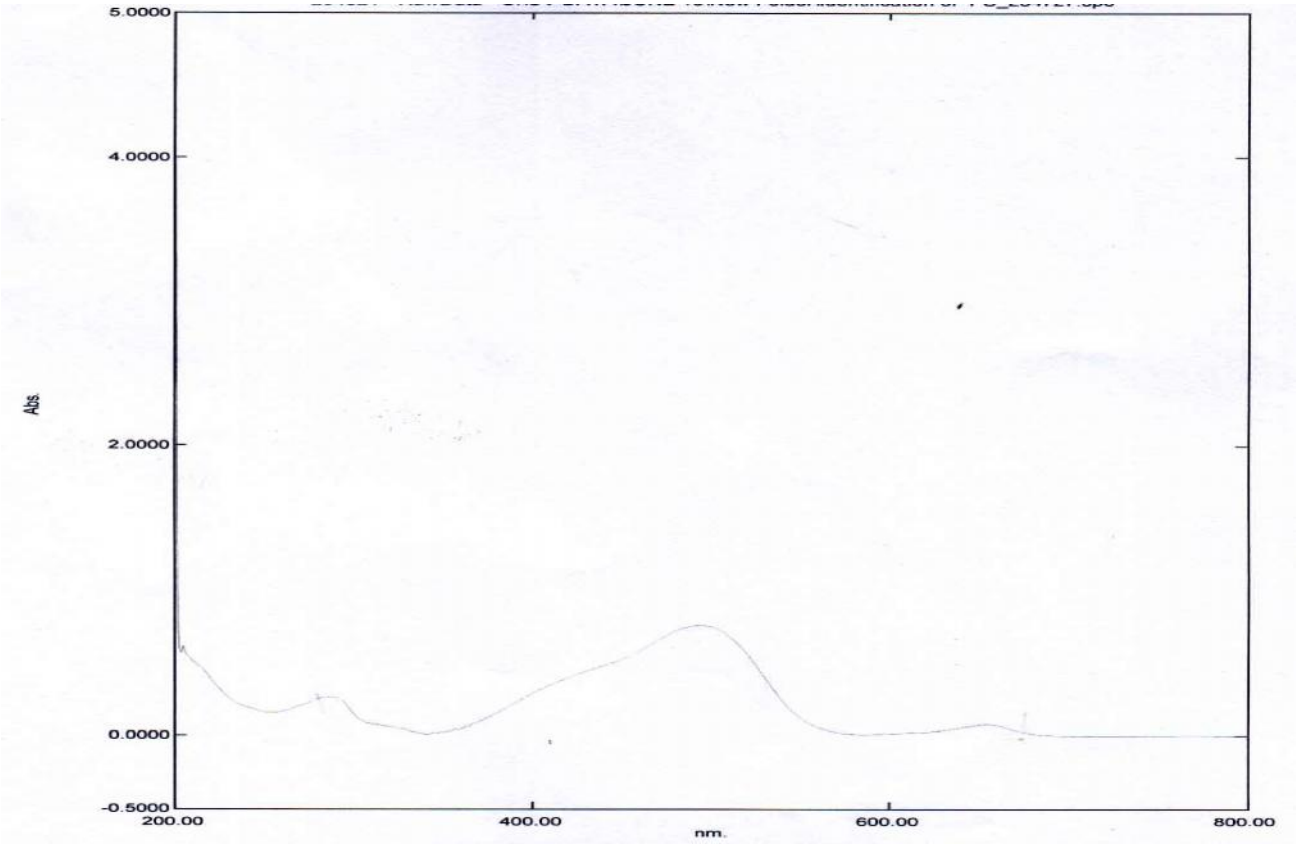


Fig. 4.7.1: UV – VIS spectrum for glucose solution

Standard curve

20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml, 100 μg/ml and 120 μg/ml glucose solutions were prepared by using supplied 1% glucose solutions. Then, a standard curve was prepared by plotting absorbance VS concentration of glucose.

Table 4.7.1: Data for the standard curve of glucose

Concentration of glucose (μg/ml)	Absorbance at 520 nm
0	0.000
20	0.023
40	0.047
60	0.069
80	0.087
100	0.109
120	0.131

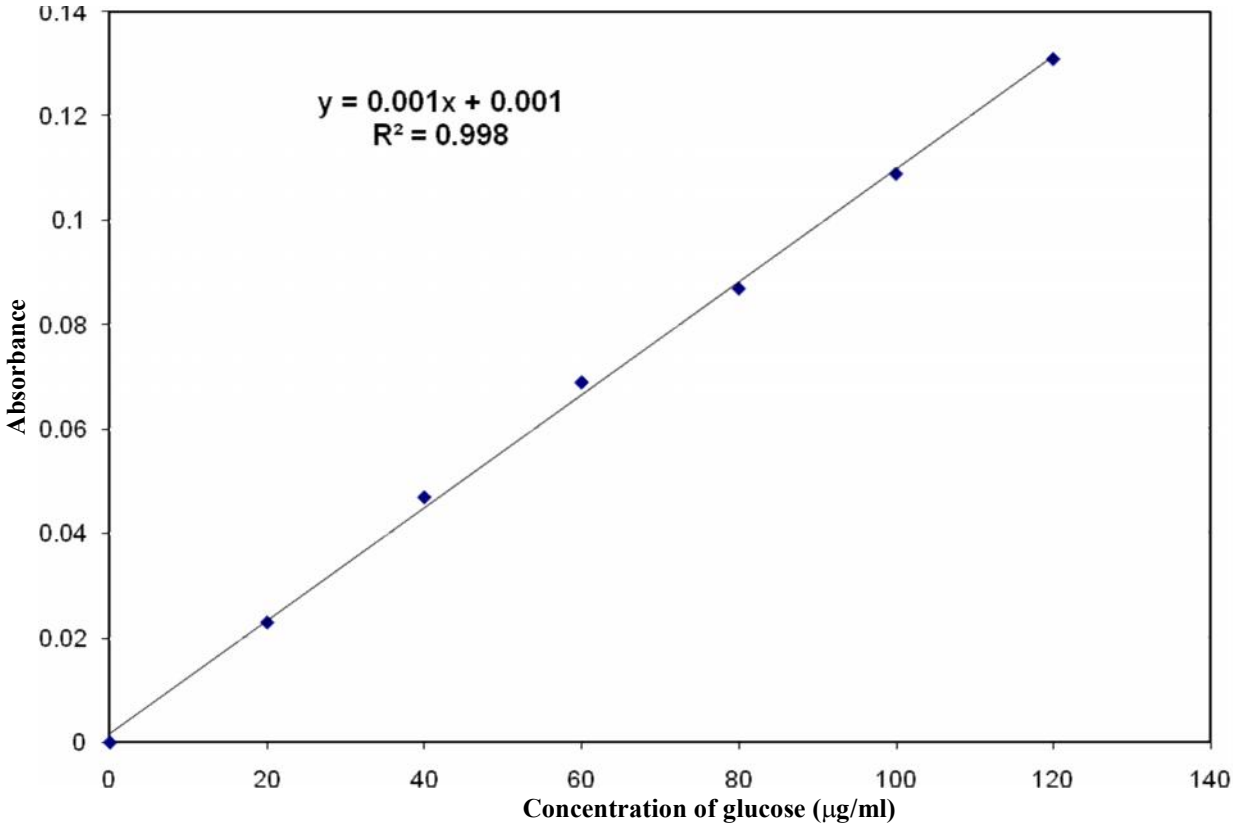


Fig. 4.7.2: Standard curve of the glucose

CHAPTER 5:

PREPARATION OF STOCK AND BUFFER SOLUTIONS

PREPARATION OF STOCK AND BUFFER SOLUTIONS

5.1 PREPARATION OF STOCK SOLUTIONS [57] [58] [59]

5.1.1 Gliclazide

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.3234 gm of gliclazide in demineralized water (slightly alkaline) and make the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.1.2 Glipizide

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.4455 gm of glipizide in demineralized water (slightly alkaline) and make the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.1.3 Glyburide

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.4940 gm of glyburide in demineralized water (slightly alkaline) and make the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.1.4 Metformin Hydrochloride

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.1656 gm of metformin hydrochloride in demineralized water and makes the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.1.5 Caffeine

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.1942 gm of caffeine in demineralized water and makes the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.1.6 Theophylline

100 ml stock solution of $1x10^{-2}$ **M** was prepared by dissolving 0.1802 gm of theophylline in demineralized water and makes the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solution.

5.2 Preparation of Bovine Serum Albumin (BSA) Solution

100 ml solution of $5x10^{-5}$ **M** was prepared by dissolving 0.3450 gm of bovine serum albumin **(M.W. 6900 gm)** in M/15 phosphate buffer having p^H 7.4 and makes the volume upto 100 ml with the same solvent.

5.3 PREPARATION OF BUFFER SOLUTIONS [26] [60] [61]

5.3.1 p^H 1.4

This buffer was prepared by dissolving 6.57 gm of potassium chloride in demineralized water and added 119.0 ml of 0.1 **M** hydrochloric acid.

Made the volume up to 1000 ml with the same solvent. Adjusted to p^H 1.4 with hydrochloric acid. 250 ml of 0.1 **M** HCI was prepared by mixing 2.25 ml of 37% HCI with demineralized water and the final volume up to 250 ml.

5.3.2 p^H 2.4

This buffer was prepared by mixing of 6.7 ml orthophosphoric acid with 50.0 ml of a 4% v/v solution of 2M sodium hydroxide and diluted to 1000.0 ml with demineralized water. Adjusted to p^H 2.4 with sodium hydroxide. 100 ml of 2 **M** NaOH was prepared by dissolving 8.0 gm of NaOH in demineralized water and the final volume up to 100 ml. 100 ml of 4% v/v solution of 2 **M** NaOH was prepared by diluting 4 ml of 2 **M** NaOH to 100 ml with demineralized water.

5.3.3 p^H 3.4

This buffer was prepared by mixing of 302.80 ml of 0.1 **M** formic acid with 95.0 ml of 0.1 **M** KOH and diluted to 1000.0 ml with demineralized water. 100 ml of 0.1 **M** KOH was prepared by dissolving 0.373 gm of KOH in demineralized water and the final volume up to 100 ml. 500 ml of 0.1 **M** formic acid was prepared by mixing 3.77 ml of 100% formic acid with demineralized water and the final volume up to 500 ml.

5.3.4 p^H 4.4

This buffer was prepared by mixing of 120.00 ml of 0.1 **M** formic acid with 99.50 ml of 0.1 **M** KOH and diluted to 1000.0 ml with demineralized water. 100 ml of 0.1 **M** KOH was prepared by dissolving 0.373 gm of KOH in demineralized water and the final volume up to 100 ml. 250 ml of 0.1 **M** formic acid was prepared by mixing 1.88 ml of 100% formic acid with demineralized water and the final volume up to 250 ml.

5.3.5 p^H 5.4

This buffer was prepared by dissolving 1.76 gm of Na_2HPO_4 and 13.61 gm of KH_2PO_4 in sufficient demineralized water to produce 1000 ml. Adjusted to p^H 5.4 with 0.05 **M** orthophosphoric acid or 2 **M** NaOH. 100 ml of 0.05 **M** orthophosphoric acid was prepared by mixing 0.337 ml of 85% orthophosphoric acid with demineralized water and the final volume up to 100 ml and 100 ml of 2 **M** NaOH was prepared by dissolving 8.0 gm of NaOH in demineralized water and the final volume up to 100 ml.

5.3.6 p^H 6.4

This buffer was prepared by mixing of 302.10 ml of 0.02 **M** KH_2PO_4 with 131.90 ml of 0.01 **M** Na_2HPO_4 and diluted to 1000.0 ml with demineralized water. 500 ml of 0.02 **M** KH_2PO_4 was prepared by dissolving 1.360 gm of KH_2PO_4 in demineralized water and the final volume up to 500 ml. 250 ml of 0.01 **M** Na_2HPO_4 was prepared by dissolving 0.350 gm of Na_2HPO_4 in demineralized water and the final volume up to 250 ml. [33]

5.3.7 p^H 7.4

This buffer was prepared by mixing of 65.40 ml of 0.02 **M** KH_2PO_4 with 289.70 ml of 0.01 **M** Na_2HPO_4 and diluted to 1000.0 ml with demineralized water.100 ml of 0.02 **M** KH_2PO_4 was prepared by dissolving 0.2722 gm of KH_2PO_4 in demineralized water and the final volume up to 100 ml. 500 ml of 0.01 **M** Na_2HPO_4 was prepared by dissolving 0.710 gm of Na_2HPO_4 in demineralized water and the final volume up to 500 ml.

CHAPTER 6:

METHODS FOR INVESTIGATION OF UDIED DRUGS

METHODS FOR INVESTIGATION OF STUDIED DRUGS

6.1 METHODS FOR DETERMINATION OF DRUG INTERACTION

6.1.1 Infrared Spectroscopy Method

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer.

Fourier transform infrared spectroscopy (**FTIR**) [62] is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range.

In this procedure, the infrared region of the electromagnetic spectrum of caffeine, theophylline, gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures in solid state were compared with those of each interacting species. The concentrations of the samples were kept at very dilute levels in each case and the measurements were made using FTIR automatic recording instrument with a constant temperature and humidity. The spectra were compared with the pure sample in each combination.

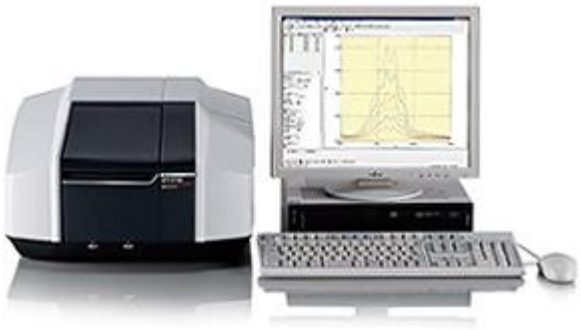
In this method, IR spectrum are recorded from 4000 cm-1 to 400 cm-1 taking air as a blank for each drug of caffeine, theophylline, gliclazide, glipizide, glyburide and metformin by semitransparent disc and FTIR.

Observed the characteristics of the recorded IR spectrum of caffeine, theophylline, gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures in the same manner.

6.1.2 UV-Visible Spectrophotometric Method

Spectrophotometer is a valuable tool for elucidating the composition of complex ions in solution and for determining their formation constants. The power of the technique lies in the fact that quantitative absorption measurements can be performed without disturbing the equilibria under consideration. Although most Spectrophotometric studies of complexes involve systems in which a reactant or a product absorbs, non-absorbing systems can also be investigated successfully.

In this procedure, the ultraviolet absorption characteristics of caffeine, theophylline, gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures in solution (p^H 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4)[64] were compared with those of each interacting species. The concentrations of the samples were kept at very dilute levels in each case and the measurements were made using an UV – VIS automatic recording instrument with a constant temperature cell compartment and automatic recording unit. [57] [58] [59]



The stock solutions of the samples were diluted to appropriate levels, mixed buffer at the desired p^H and the spectra were recorded between 400 – 200 nm. The spectra were compared with the pure sample in each combination. [61]

6.1.3 Continuous Variation Method (Job's Method)

The method of continuous variation or Job's method is used to determine the stoichiometry of a binding event. In this method, the total molar concentration of the two binding partners (e.g. a protein and ligand or a metal and a ligand) are held constant, but their mole fractions are varied.

In the method of continuous variations, cation & ligand solutions with identical analytical concentrations are mixed in such a way that the total volume and the total moles of reactants in each mixture is constant but the mole ratio of reactants varies systematically (for example, 1:9, 8:2, 7:3 and so forth). [63]

Solution of different concentrations of caffeine, theophylline, gliclazide, glipizide, glyburide and metformin were prepared and the absorbance of each solution is then measured and corrected for any absorbance the mixture might exhibit if no reaction had occurred.The continuous-variation plots were prepared by plotting corrected absorbance against the volume fraction of one reactant.

6.1.4 Mole-Ratio Method (Yoe-Jones Method)

Mole ratio method is used to find a portion (usually in moles) of a chemical from a known portion of another. In the mole-ratio method, a series of solutions is prepared in which the analytical concentration of one reactant (usually the cation) is held constant while that of the other is varied. [63]

Solution of different concentrations of caffeine, theophylline, gliclazide, glipizide, glyburide and metformin were prepared individually and a plot of absorbance versus mole ratio of the reactants is than prepared. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained.

6.1.5 Conductometric Method

Conductometric titration is a type of titration in which the electrolytic conductivity of the reaction mixture is continuously monitored as one reactant is added.Conductometric titrations were carried out to detect the complexation of each caffeine and theophylline with gliclazide, glipizide, glyburide and metformin as well as to find the molar ratios of the interacting species to the drug molecule in the complex.[64] 40 ml of 0.001**M** solution of each caffeine and theophylline were taken in different 100 ml beaker and were titrated individually with gradual addition of 0.01**M** solution of each gliclazide, glipizide, glyburide and metformin from a burette. Reversely 40 ml of 0.001**M** solution of each gliclazide, glipizide, glyburide and metformin were titrated with gradual addition of 0.01**M** solution of each caffeine and theophylline under similar conditions.

The conductance values (ms) were plotted versus molar ratios between the two species in the system. The titration curves showed break at the points of possible interaction. All the titrations were performed with solutions adjusted to p^H 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 using a p^H meter. [64]

6.1.6 Ardon's Spectrophotometric Method

Ardon's method is used to confirm the complexation. In this method, concentrations of drugs are varied while keeping the concentrations of the ligand fixed. [65] The absorbance of solutions having p^H 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 were measured at 273 nm for caffeine and at 276 nm for theophylline using UV – Visible spectrophotometer. For calculations, the Ardon's equation was used. This equation is given bellow-

$$1/ (D - E_A C) = 1/ KC (E_{com} - E_A) [B]^n + 1/ C (E_{com} - E_A)$$

Where, D = absorbance of mixture
C = molar concentration of the drug
B = molar concentration of the ligand (the drug, which is the target)
E_{com} = molar extinction co-efficient of the complex.
E_A = molar extinction co-efficient of the drug.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for 1/ (D - E_AC) was plotted versus 1/ [B] to get the straight lines. The concentration of each caffeine and theophylline were kept constant at 5 x 10^{-5}**M** (denoted by C in the equation). The 1:1 complex gave a straight line in the plots with an intercept and slope.

The stability constant of the complex was given by-

K = intercept / slope

It is mentioned that this method is only valid for the systems where 1:1 complexes are found. [64]

6.2 METHODS FOR DETERMINATION OF PROTEIN BINDING

The Equilibrium Dialysis Method

Dialysis is a simple process in which small solutes diffuse from a high concentration solution to a low concentration solution across a semipermeable membrane until equilibrium is reached.

Equilibrium dialysis is one of the methods used for the determination of protein binding of any compound.[23] This method was developed by E.Singlas (1987) which consists in dialyzing the unbound fraction of a compound contained in a protein (bovine serum albumin) solution through a semipermeable membrane.

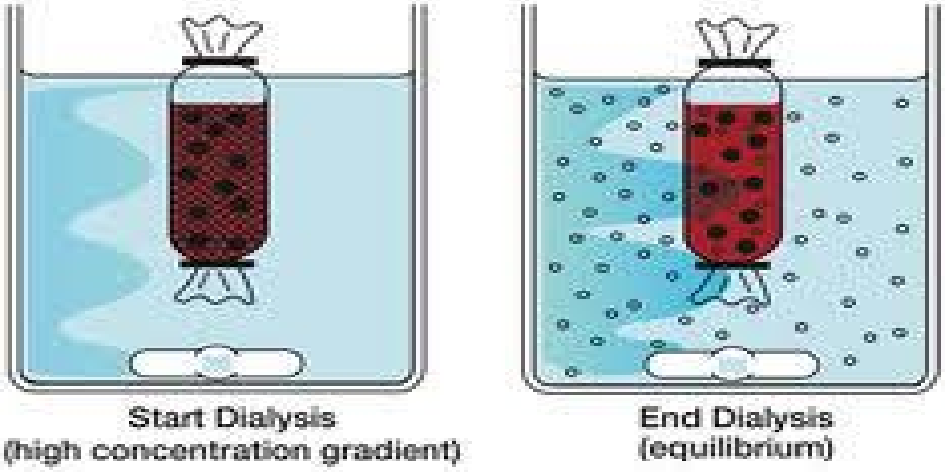
In this study, this method was used for the determination of protein binding of gliclazide, glipizide, glyburide & metformin and their 1:1 mixtures with caffeine & theophylline. [58]

Firstly, dialysis membrane was activated and then dialysis was performed.

Activation Procedure

Procedure for activation of dialysis membrane is given below-

- **a.** The dialysis membrane were cut into 12 cm pieces look like a bag (also called dialysis bag) and immersed in boiling 1**M** NaHCO3 solution for about 1 hr to make sure that the inside of the bag was washed as well as outside and the process was repeated once.
- **b.** Then these bags were immersed in boiling demineralized water for about 1 hr with intermittent change of the water making sure that all the anions and cations are washed out. Then these were well washed with demineralized water.
- **c.** Then these were immersed in M/15 phosphate buffer having p^H 7.4 [60] at about 70^0C for 1 hr. The process was repeated once.
- **d.** Finally, these were rinsed with demineralized water and stored in a refrigerator with the same buffer.



Dialysis procedure

a. The activated membrane were filled individually with bovine serum albumin (BSA) solution with different concentrations of gliclazide, glipizide, glyburide and metformin or their 1:1 mixtures with caffeine & theophylline, keeping the total volume 3 ml.

b. Then, these were immersed in a fixed amount (25 ml) of M/15 phosphate buffer having p^H 7.4 in a 100 ml conical flask.

c. Conical flasks were shaken gently at $37^0 \pm 0.5^0$C for about 8 hrs in a Dubnoff metabolic shaking incubator.

d. After completion of dialysis, the absorbance of buffer (outside the membrane) was measured individually at 227 nm, at 228 nm, at 248 nm and at 233 nm incase of gliclazide, glipizide, glyburide and metformin alone and their 1:1 mixtures with caffeine and theophylline respectively using the UV-VIS recording spectrophotometer.

Diagrammatically, the equilibrium dialysis process may be shown as-

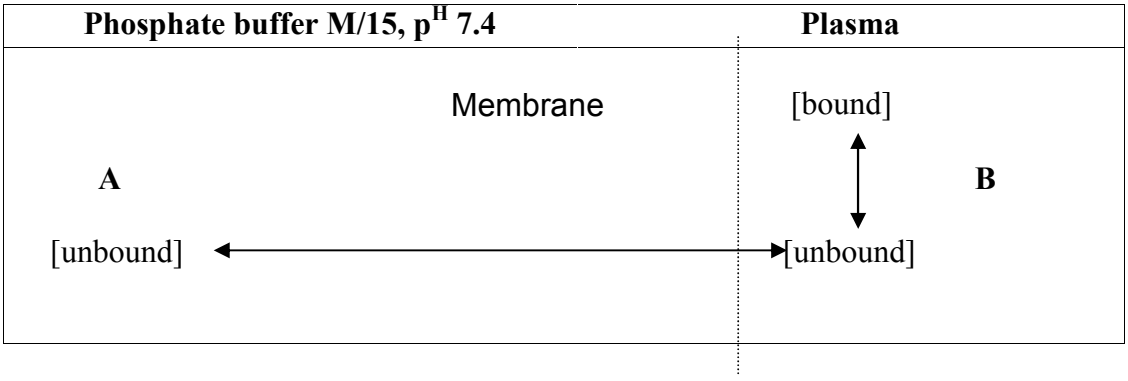


Fig 6.2.1: The equilibrium dialysis process

In the above figure, the dashed or aero lines represents the membrane which allow only small molecules (drug or drug-drug complex) to diffuse through it from the compartment **A** to the compartment **B** containing the buffer. The compartment **A** contains the drug or drug-drug complex and bovine serum albumin. At equilibrium condition, the concentration of unbound drug becomes equal in the two compartments. The concentrations of the drug in the buffer compartment **A** represent the unbound drug, and that in plasma compartment **B** represents the sum of the bound and unbound drugs.

Preparation of standard curve

For the spectrophotometric determination of drug concentration into the buffer compartment **A**, a standard curve was used (shown in the fig. 6.2.2 - fig.6.2.5).

To prepare the standard curve, a treated **M/15** phosphate buffer (p^H 7.4) was used. Solution of different concentrations of (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 **M x 10^5**) of each gliclazide, glipizide, glyburide and metformin were prepared individually in this buffer and standard curve was prepared for each by plotting absorbance (measured at 227 nm, at 228 nm, at 248 nm and at 233 nm incase of gliclazide, glipizide, glyburide and metformin respectively) against concentrations.

Table 6.2.1: Data for the standard curve of gliclazide for determination of protein binding

Concentration of drug in buffer M x 10^5	Absorbance at 227 nm
0.0	0.000
1.0	0.178
2.0	0.372
3.0	0.573
4.0	0.755
5.0	0.975
6.0	1.175
7.0	1.401
8.0	1.578
9.0	1.779

N.B: The treated buffer was obtained by allowing the equilibrium dialysis of the semi permeable fragments of plasma from the inside of the dialysis membrane to the **M/15** phosphate buffer (p^H 7.4).

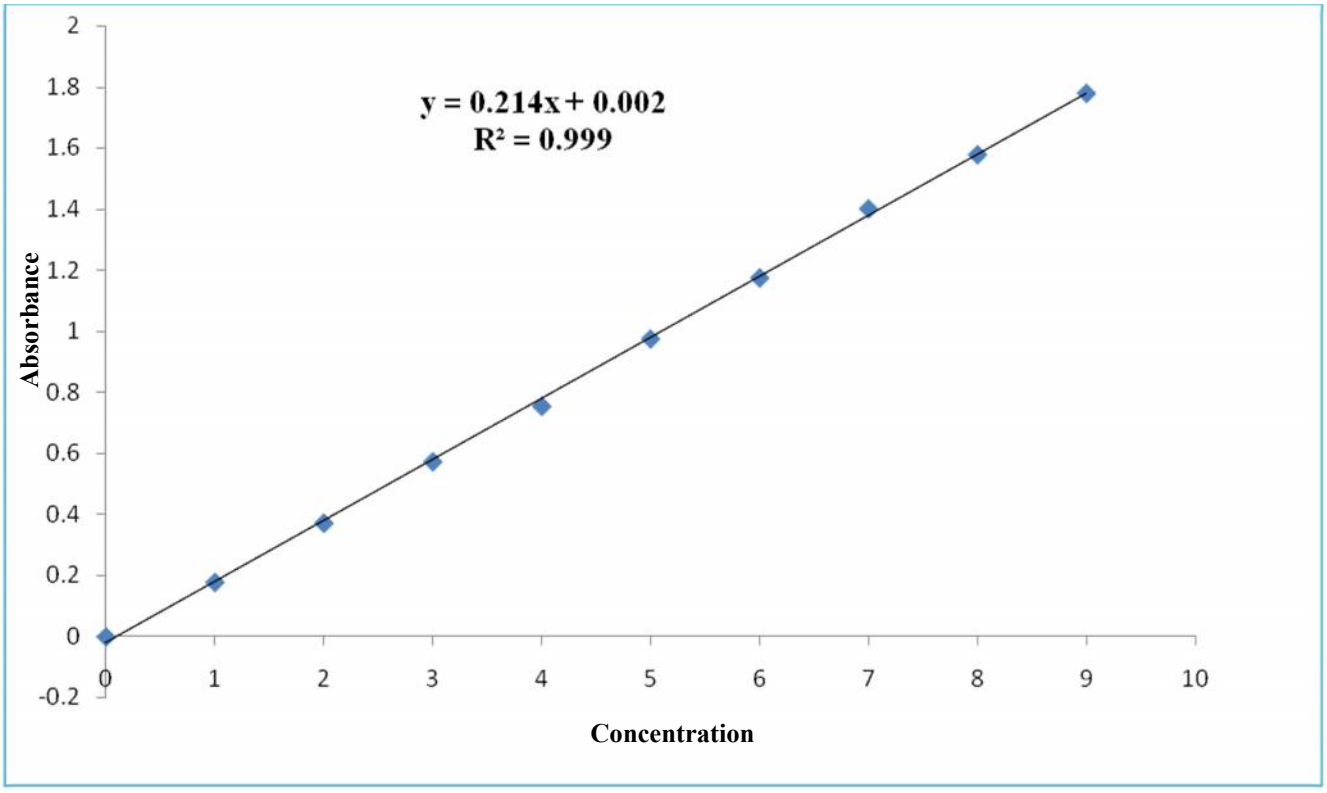


Fig. 6.2.2: Standard curve for gliclazide for determination of protein binding

Table 6.2.2: Data for the standard curve of glipizide for determination of protein binding
Chapter 6

Concentration of drug in buffer M x 10^5	Absorbance at 228 nm
0.0	0.000
1.0	0.221
2.0	0.426
3.0	0.595
4.0	0.817
5.0	0.993
6.0	1.158
7.0	1.344
8.0	1.555
9.0	1.723

N.B: The treated buffer was obtained by allowing the equilibrium dialysis of the semi permeable fragments of plasma from the inside of the dialysis membrane to the **M/15** phosphate buffer (p^H 7.4).

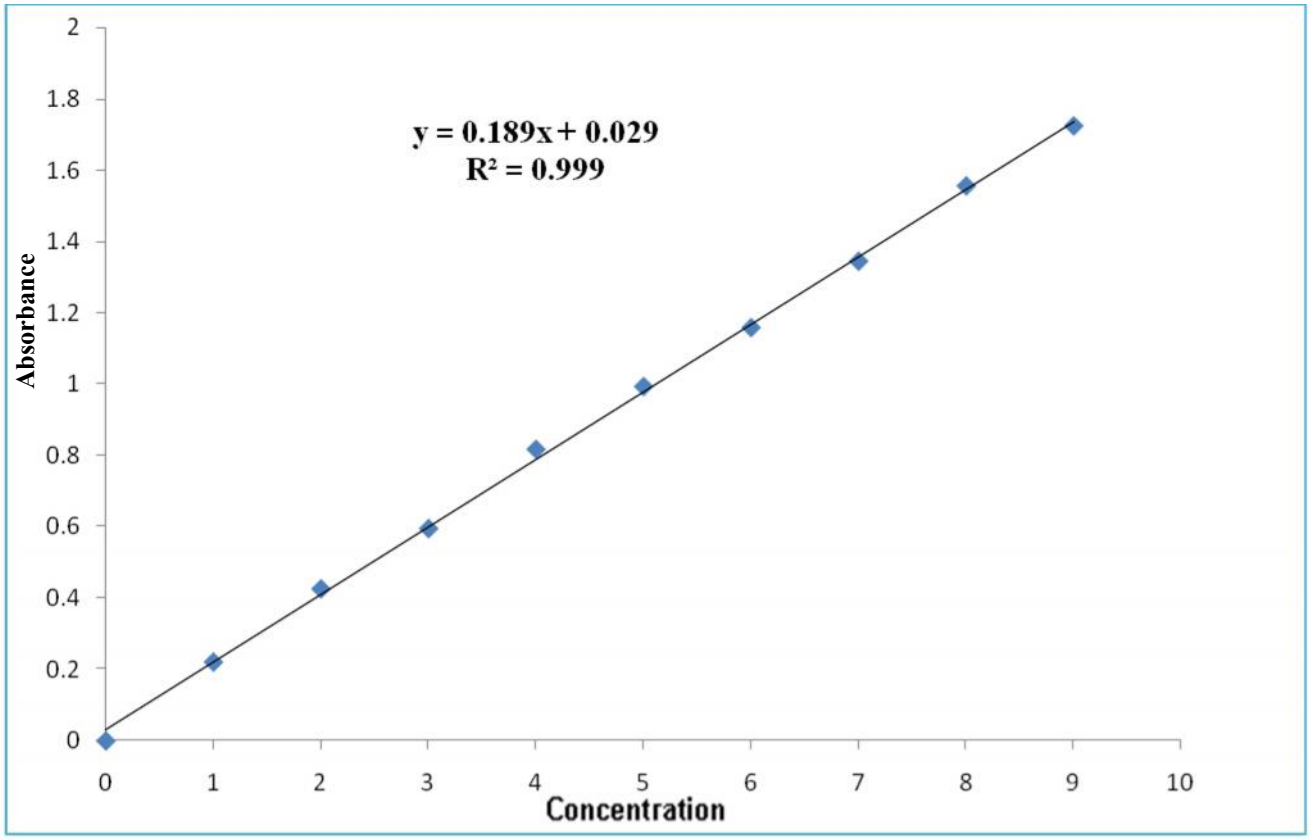


Fig. 6.2.3: Standard curve for glipizide for determination of protein binding

Table 6.2.3: Data for the standard curve of glyburide for determination of protein binding

ion of drug in buffer M x 10^5	Absorbance at 248 nm
0.0	0.000
1.0	0.203
2.0	0.355
3.0	0.528
4.0	0.726
5.0	0.889
6.0	1.053
7.0	1.238
8.0	1.451
9.0	1.635

N.B: The treated buffer was obtained by allowing the equilibrium dialysis of the semi permeable fragments of plasma from the inside of the dialysis membrane to the **M/15** phosphate buffer (p^H 7.4).

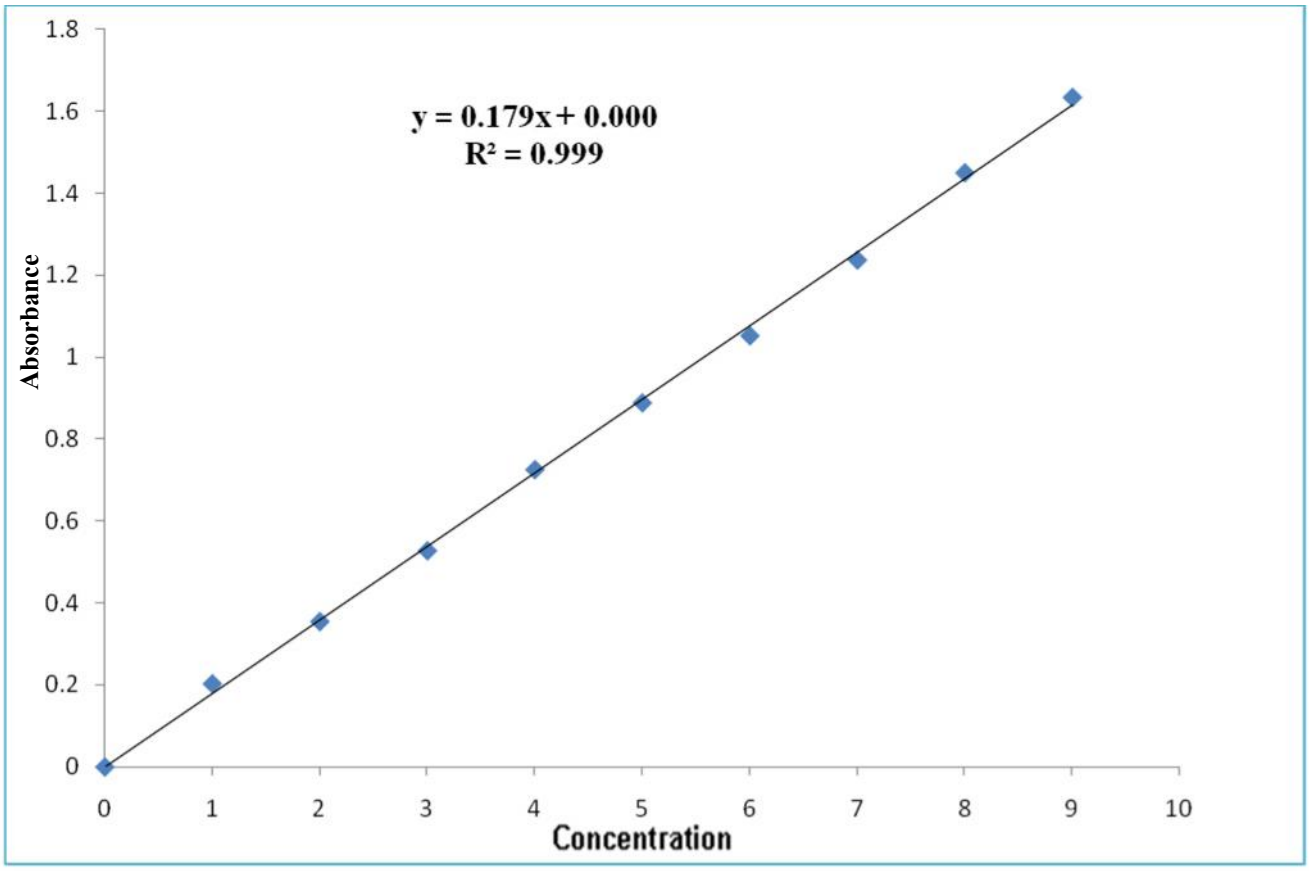


Fig. 6.2.4: Standard curve for glyburide for determination of protein binding

Table 6.2.4: Data for the standard curve of metformin for determination of protein binding

Concentration of drug in buffer M x 10^5	Absorbance at 233 nm
0.0	0.000
1.0	0.235
2.0	0.529
3.0	0.725
4.0	0.928
5.0	1.185
6.0	1.351
7.0	1.536
8.0	1.748
9.0	1.955

N.B: The treated buffer was obtained by allowing the equilibrium dialysis of the semi permeable fragments of plasma from the inside of the dialysis membrane to the **M/15** phosphate buffer (p^H 7.4).

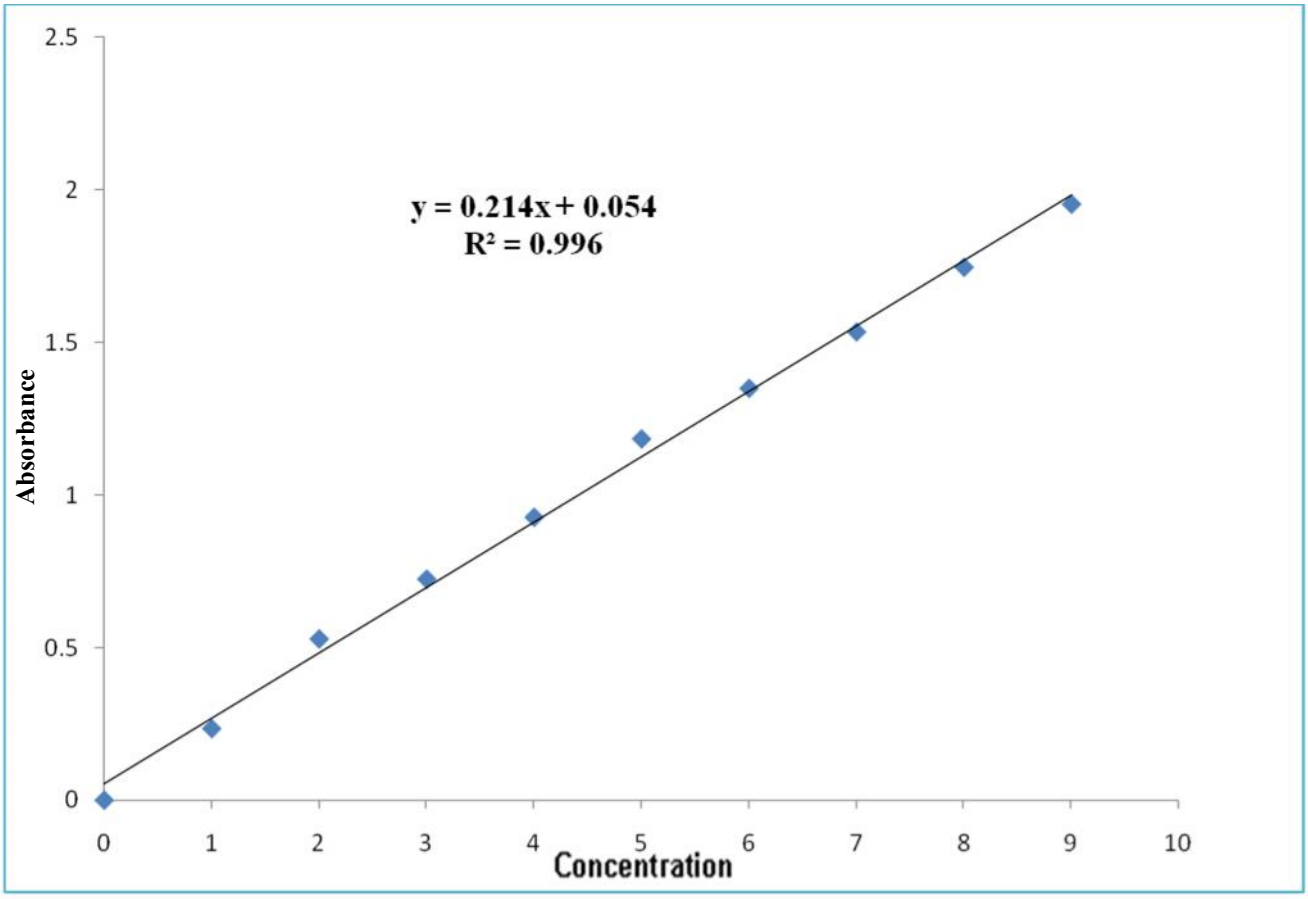


Fig. 6.2.3: Standard curve for metformin for determination of protein binding

Calculation of percentage of protein binding

Chapter 6

ount of drug was given into plasma compartment (dialysis bag). Then, concentration of drug present in the buffer (outside of this compartment) after equilibrium was measured. This measurement gave the total amount of drug that remains in the dialysis bag. Thus, we can get the sum of free drug and plasma bound drug at equilibrium.

The percentage of protein binding (F) of the drug is calculated using the following equation,

$$F = \frac{[\text{Bound drug}]}{[\text{Unbound drug}]} \times 100$$

$$= \frac{[B] - [A]}{[A]} \times 100$$

Where, [A] = Molar conc. of drug in buffer compartment

[B] = Molar conc. of drug in plasma compartment which found by subtracting [A] from the initial conc. of the drug (amount of drug in plasma after equilibrium).

By measuring the percentage of protein binding of giliclazide, glipizide, glyburide, metformin and their 1:1 mixture with caffeine and theophylline; a comparative picture was obtained shown in the figure.

Calculation of number of binding sites and the affinity constant

In the present study, number of binding sites and affinity constants of giliclazide, glipizide, glyburide, metformin and their 1:1 mixture with caffeine and theophylline were calculated by Scatchard method. **[23] [58]**

In this method, a curve was produced by plotting r/ [D] Vs r, where r is the ratio between the molar concentration of the bound drug and the molar concentration of protein i.e.

$$r = \frac{[B] - [A]}{[Pt]}$$

and D is the concentration of the unbound drug i.e. [A].

The curve thus obtained called Scatchard plot. The Scatchard plot when extrapolated on Y axis, gave an intercept nK, the intersection on X-axis representing n and the slope of line AB being k. Here, k is the affinity constant and n is the number of binding sites of protein binding.

6.3 METHODS FOR DETERMINATION OF PLASMA CONCENTRATION

Chapter 6 **nt by the UV-Visible Spectrophotometric Method**

This experiment was performed to evaluate the effect of caffeine and theophylline on serum concentration of gliclazide, glipizide, glyburide and metformin. The UV-Visible Spectrophometric method was used for such determination after oral single administration of gliclazide, glipizide, glyburide & metformin alone and with caffeine & theophylline in rat.

Experimental Rat

Procedure of in-vivo experiment

To determine the plasma concentration of gliclazide, glipizide, glyburide and metformin, plasma samples were taken at variuos time intervals after a drug is orally administered.

6.3.1 Oral administration of drug in rat

Plasma samples were taken at variuos time intervals after a drug is orally administered. 52 adult rats of 250 ± 25 g body weight were used. They were kept rest for 7 days with normal diet. These rats were divided into 13 groups each having 4 (four), marked as I, II, IIIA, IIIB, IV, VA, VB, VI, VIIA, VIIB, VIII, IXA, IXB and here group-I was used as control.

Group I: Group-I was used as the control group.

Group II: As a dose of 1.60 mg/kg of gliclazide was administrated by orogastric tube in each rat.

Group IIIA: 1:1 mixture of 1.60 mg/kg of gliclazide with 1.30 mg/kg of caffeine was administrated by orogastric tube in each rat.

Group IIIB: 1:1 mixture of 1.60 mg/kg of gliclazide with 2.00 mg/kg of theophylline was administrated by orogastric tube in each rat.

Group IV: As a dose of 0.20 mg/kg of glipizide was administrated by orogastric tube in each rat.

Group VA: 1:1 mixture of 0.20 mg/kg of glipizide with 1.30 mg/kg of caffeine was administrated by orogastric tube in each rat.

Group VB: 1:1 mixture of 0.20 mg/kg of glipizide with 2.00 mg/kg of theophylline was administrated by orogastric tube in each rat.

Group VI: As a dose of 0.10 mg/kg of glyburide was administrated by orogastric tube in each rat.

Group VIIA: 1:1 mixture of 0.10 mg/kg of glyburide with 1.30 mg/kg of caffeine was administrated by orogastric tube in each rat.

Group VIIB: 1:1 mixture of 0.10 mg/kg of glyburide with 2.00 mg/kg of theophylline was administrated by orogastric tube in each rat.

Group VIII: As a dose of 10.00 mg/kg of metformin was administrated by orogastric tube in each rat.

Group IXA: 1:1 mixture of 10.00 mg/kg of metformin with 1.30 mg/kg of caffeine was administrated by orogastric tube in each rat.

Group IXB: 1:1 mixture of 10.00 mg/kg of metformin with 2.00 mg/kg of theophylline was administrated by orogastric tube in each rat.

They were over night fasted before drug administration. Blood samples (1ml) were collected from cutting the tip of the tail into centrifuge tubes before drug administration and at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 hr after drug administration.

All blood samples were protected from light, immediately centrifuged at 3000 rev/min for 10 minutes and the plasma samples separated into vials and kept into deep freeze up to taking the absorbance. Absorbances were measured at 227 nm, 228 nm, 248 nm and 233 nm in case of gliclazide, glipizide, glyburide and metformin respectively.[66]

6.3.2 Preparation of standard curve

For preparing the standard curves, control (1ml) samples were taken individually with 1 ml of gliclazide, glipizide, glyburide and metformin solution, which contains 20 µg/ml of gliclazide, glipizide, glyburide and metformin. These were used as stock solution of 20, 40, 60, 80, 100, 120 ng/ml respectively for each drugs. Then absorbance was taken of each solution for each drug individually. Finally absorbance values were plotted against the concentration (each of gliclazide, glipizide, glyburide and metformin solutions) and calibration curves were produced.

Table 6.3.1: Data for the calibration curve for gliclazide for determination of plasma concentration

Conc. of gliclazide in control plasma (ng/ml)	Absorbance at 227 nm
00	0.000
20	0.009
40	0.015
60	0.022
80	0.029
100	0.036
120	0.043

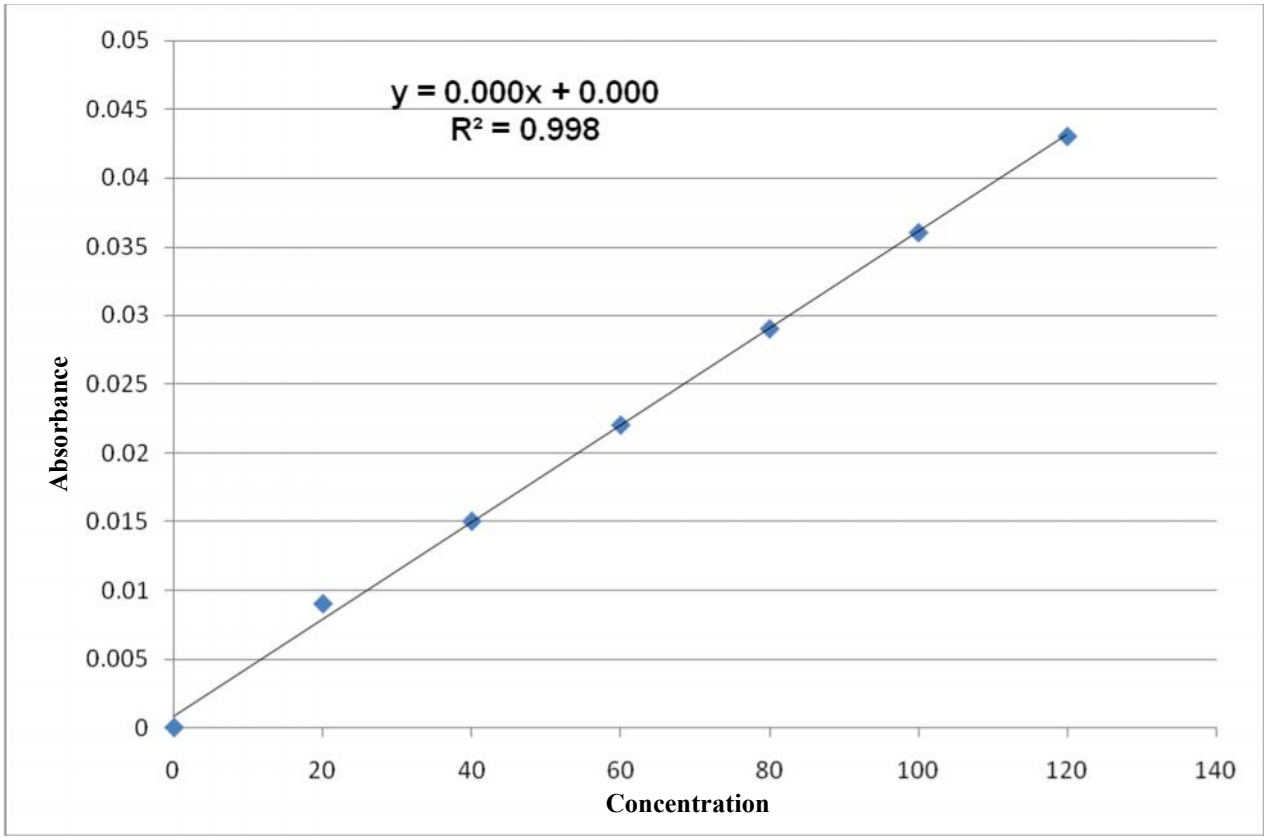


Fig. 6.3.1: Calibration curve for gliclazide for determination of plasma concentration

Table 6.3.2: Data for the calibration curve for glipizide for determination of plasma concentration

Conc. of glipizide in control plasma (ng/ml)	Absorbance at 228 nm
00	0.000
20	0.007
40	0.015
60	0.023
80	0.032
100	0.040
120	0.048

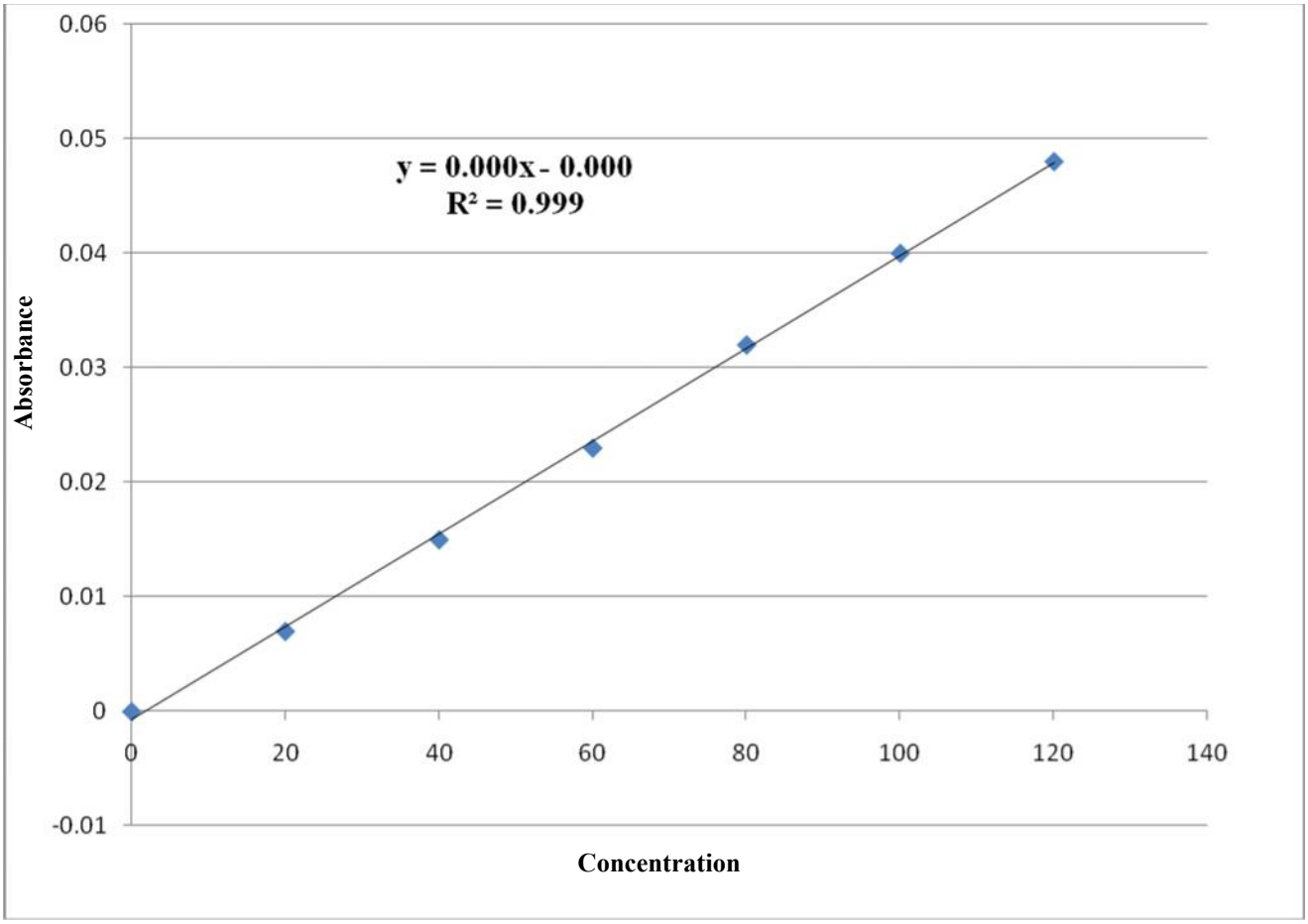


Fig. 6.3.2: Calibration curve for glipizide for determination of plasma concentration

Table 6.3.3: Data for the calibration curve for glyburide for determination of plasma concentration

Conc. of glyburide in control plasma (ng/ml)	Absorbance at 248 nm
00	0.000
20	0.005
40	0.012
60	0.020
80	0.025
100	0.033
120	0.039

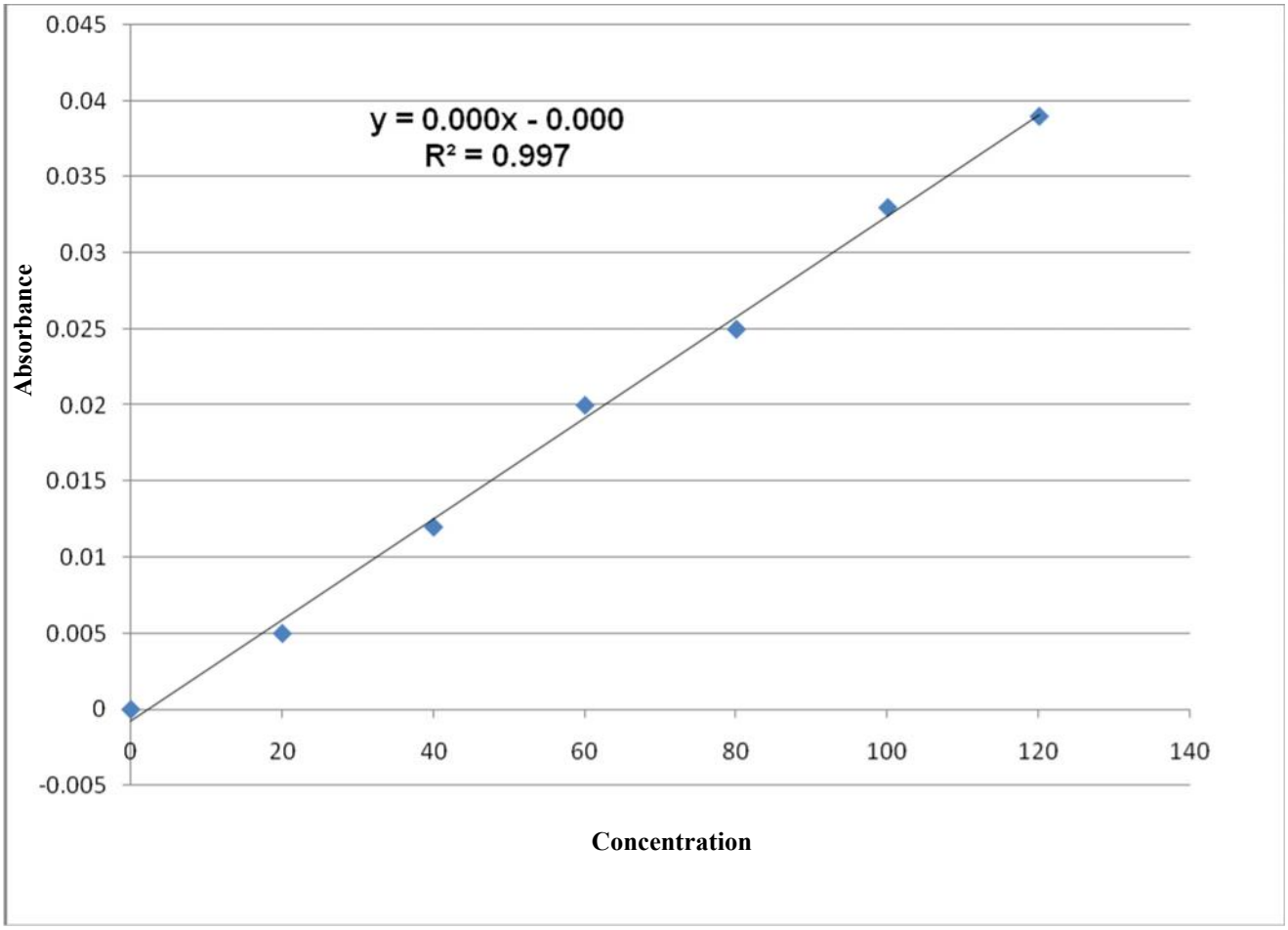


Fig. 6.3.3: Calibration curve for glyburide for determination of plasma concentration

Table 6.3.4: Data for the calibration curve for metformin for determination of plasma concentration

Conc. of metformin in control plasma (ng/ml)	Absorbance at 233 nm
00	0.000
20	0.008
40	0.018
60	0.026
80	0.035
100	0.043
120	0.051

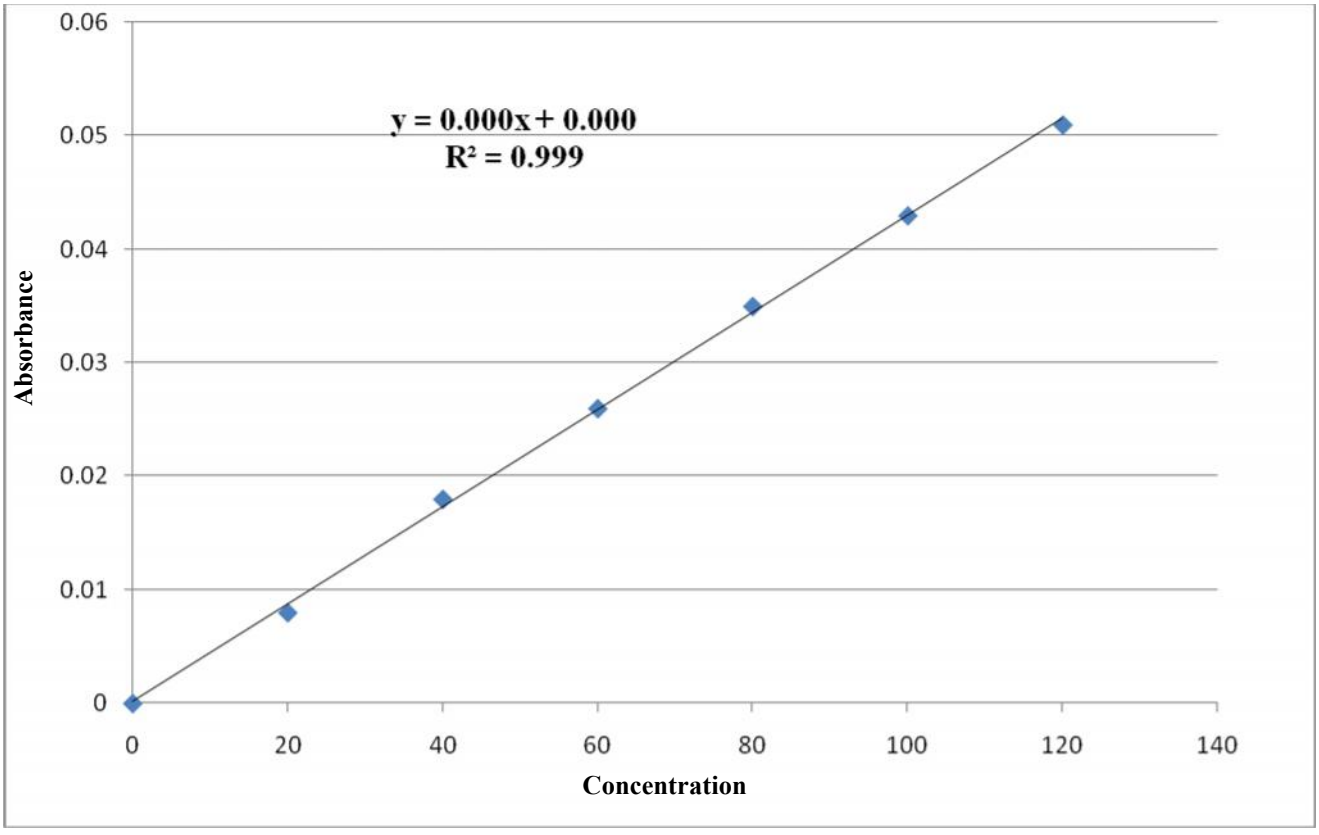


Fig. 6.3.4: Calibration curve for metformin for determination of plasma concentration

6.4. METHODS FOR THE STUDY OF THE HYOPOGLYCEMIC ACTIVITY

In-vivo experiment by the UV-Visible Spectrophometric method

To observe the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin, blood sugar level of rat was determined.[67] [68] Drugs were administered in rats and were observed for 4 weeks. Here diabetes was artificially produced in rats by administration of alloxan. The UV-Visible Spectrophotometric merhod was used for such determination after single administration of each gliclazide, glipizide, glyburide and metformin alone and with caffeine and theophylline in rat.

Diabetic Rat

Procedure of in-vivo experiment

To determine the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin, plasma samples were taken at variuos time intervals after a drug is administered.

6.4.1 Administration of drug in rat

70 adult rats aged 2 - 3 months, each weighing 250 ± 25 g and both sexes were used in the in-vivo experiment. [69] [70] [71] They were divided into 14 groups each having 5 (marked as I, II, III, IVA, IVB, V, VIA, VIB,VII, VIIIA, VIIIB, IX, XA and XB). Here Group-I was the control group. Drugs were administered in rats and were observed for four weeks. Diabetes was induced in rats by administration of alloxan.

Group I: Each rat received 2 ml of intraperitoneal injection of normal saline for three consecutive days.

Group II: Each rat received 2 ml of intraperitoneal injection of alloxan [82] in a dose of 40 mg/kg body weight for three consecutive days.

Group III: They received alloxan injection as in group II. On the fourth day (after induction of diabetes as indicated by hyperglycemia) each rat received gliclazide solution as a dose of 1.60 mg/kg body weights.

Group IVA: They received alloxan injection as in group II. On the fourth day (after induction of diabetes as indicated by hyperglycemia) each rat received a mixture of gliclazide & caffeine solution as a dose of 1.60 mg/kg and 1.30 mg/kg body weights respectively.

Group IVB: They received alloxan injection as in group II. On the fourth day (after induction of diabetes as indicated by hyperglycemia) each rat received a mixture of gliclazide & theophylline solution as a dose of 1.60 mg/kg and 2.00 mg/kg body weights respectively.

Group V: As above, they received alloxan injection and on the fourth day each rat received glipizide solution as a dose of 0.20 mg/kg body weights.

Group VIA: As above, they received alloxan injection and on the fourth each rat received a mixture of glipizide & caffeine solution as a dose of 0.20 mg/kg and 1.30 mg/kg body weights respectively.

Group VIB: As above, they received alloxan injection and on the fourth each rat received a mixture of glipizide & theophylline solution as a dose of 0.20 mg/kg and 2.00 mg/kg body weights respectively.

Group VII: They also received alloxan injection.On the fourth day each rat received glyburide solution as a dose of 0.10 mg/kg body weights.

Group VIIIA: As above, they also received alloxan injection and a mixture of a mixture of glyburide & caffeine solution as a dose of 0.10 mg/kg and 1.30 mg/kg body weights respectively.

Group VIIIB: As above, they also received alloxan injection and a mixture of a mixture of glyburide & theophylline solution as a dose of 0.10 mg/kg and 2.00 mg/kg body weights respectively.

Group IX: They also received alloxan injection and on the fourth day (after induction of diabetes as indicated by hyperglycemia) each rat received metformin solution as a dose of 10 mg/kg body weights.

Group XA: As above, they also received alloxan injection and a mixture of metformin & caffeine solution as a dose of 10 mg/kg and 1.30 mg/kg body weights respectively.

Group XB: As above, they also received alloxan injection and a mixture of metformin & theophylline solution as a dose of 10 mg/kg and 2.00 mg/kg body weights respectively.

In all experiment cases, blood was collected after overnight fasting for estimation of sugar. Blood samples (1ml) were collected from the cutting the tip of the tail into centrifuge tubes.

6.4.2. Estimation of blood sugar level in rat

(1) Required test tubes were taken and marked in various numbers for each group.

(**2)** Blood was collected by syringe in a small glass tube containing disodium oxalate and shaken the tube to mix the blood with disodium oxalate to inhibit the coagulation.

(3) In each tube 0.5 ml of blood and 8.5 ml demineralized water was taken. 0.5 ml of 10% Na–tungstate and 0.5 ml 2/3N H_2SO_4 were mixed properly for complete precipitation of protein.

(4) To sediment the precipitated proteins and to obtain supernatant fluid centrifuge the mixture at a maximum speed for 5 minutes.

(5) 1 ml of supernatant fluid was taken by 1 ml pipette into the test tubes containing 1 ml of Fehling solution (Fehling solution was prepared *in sito* by mixing 25 ml solution A and 1 ml of solution of B) and then heated in a water bath for 30 minutes . Then the solutions cooled in a beaker of cold water.

(6) 1 ml of arsenic molybdate and 22 ml of demineralized water were added and mixed well.

(7) The absorbance was measured at 520 nm and the concentration of glucose was determined by using standard curve.

6.4.3 Preparation of standard curve

20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml, 100 μg/ml and 120 μg/ml glucose solutions were prepared by using supplied 1% glucose solutions. 1 ml of each solution was taken in different marked tubes by 1 ml pipette containing 1 ml of Fehling solution (Fehling solution was prepared *in sito* by mixing 25 ml of solution A and 1 ml of solution of B) and then heated in a water bath for 30 minutes. Then the solutions were cooled in a beaker of cold water and 1 ml of arsenic molybdate and 22 ml of demineralized water were added and mixed well. Then, the absorbance was measured at 520 nm and a standard curve was prepared by plotting absorbance VS concentration of glucose.

Table 6.4.1: Data for the standard curve for the estimation of blood sugar level

Specimen concentration (μg/ml)	Absorbance at 520 nm
0	0.000
20	0.021
40	0.044
60	0.067
80	0.090
100	0.112
120	0.135

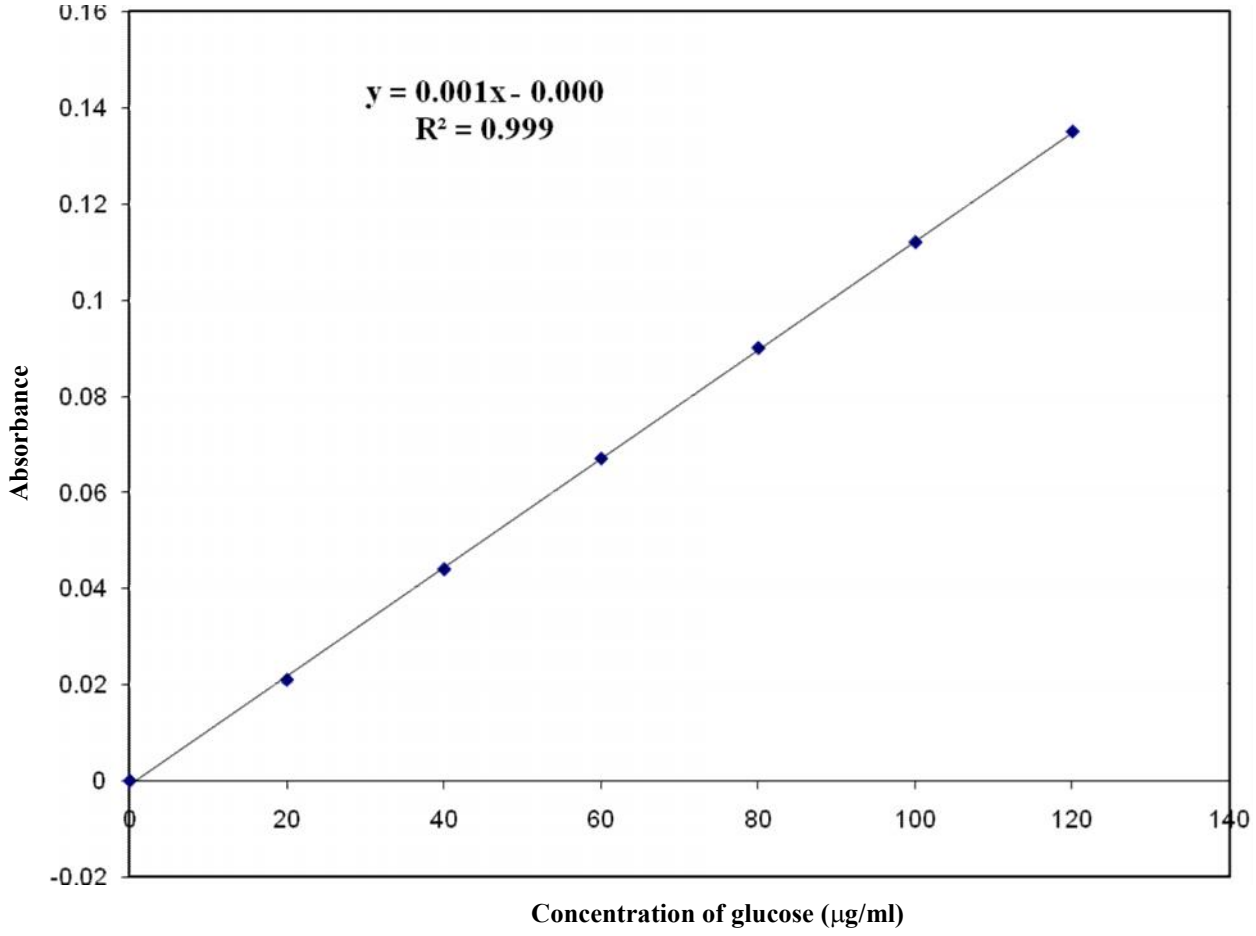


Fig. 6.4.1: Standard curve for the estimation of blood sugar level

CHAPTER 7:

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

In the present investigation, the interaction of caffeine & theophylline with gliclazide, glipizide, glyburide and metformin have been studied by different methods. The infrared spectra characteristics, the ultraviolet absorption spectral characteristics, continuous variation analysis, mole-ratio analysis, conductometric titration analysis and Ardon's spectrophotometric analysis reveal the formation of complexes. The effects of caffeine and theophylline on the protein binding characteristics of gliclazide, glipizide, glyburide and metformin have been explained by equilibrium dialysis method.The in-vivo experiment shows that the influence of caffeine and theophylline on plasma concentration of gliclazide, glipizide, glyburide and metformin. The effect of caffeine and theophylline on the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin has also been studied. [57] [58] [59]

Statistical Analysis

The results of all experiments were expressed as Mean ± SEM values for each experiment. Differences in mean values between experimental groups were analyzed by unpaired't' test. A probability value less than 0.05 ($p<0.05$) was defined to be significant. [95]

7.1 IN-VITRO STUDY ON THE INTERACTION OF CAFFEINE AND THEOPHYLLINE WITH GLICLAZIDE, GLIPIZIDE, GLYBURIDE AND METFORMIN

7.1.1 OBSERVATION OF INFRARED SPECTRA FOR INITIAL INTERACTION BY IR SPECTROSCOPY METHOD

In this procedure, the infrared region of the electromagnetic spectrum of caffeine, theophylline, gliclazide, glipizide, glyburide, metformin and their 1:1 mixtures in solid state were compared with those of each interacting species. The concentrations of the samples were kept at very dilute levels in each case and the measurements were made using FTIR automatic recording instrument with a constant temperature and humidity. The spectra were compared with the pure sample in each combination.

In this method, IR spectrums were recorded from 4000 cm-1 to 400 cm-1 taking air as a blank for the drug of caffeine, theophylline and their 1:1 mixture with gliclazide, glipizide, glyburide and metformin, by semitransparent disc and by FTIR.

Observed the characteristics of the recorded IR spectrums of caffeine & theophylline alone and their 1:1 mixture with gliclazide, glipizide, glyburide and metformin in the same manner.The recorded IR spectrum of caffeine and theophylline varied with the spectrum of gliclazide, glipizide, glyburide and metformin recorded in the same manner.

The spectra of target molecules alone and the mixture of (1:1) of caffeine and theophylline with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of caffeine and theophylline with the drugs that alter the absorption intensities.

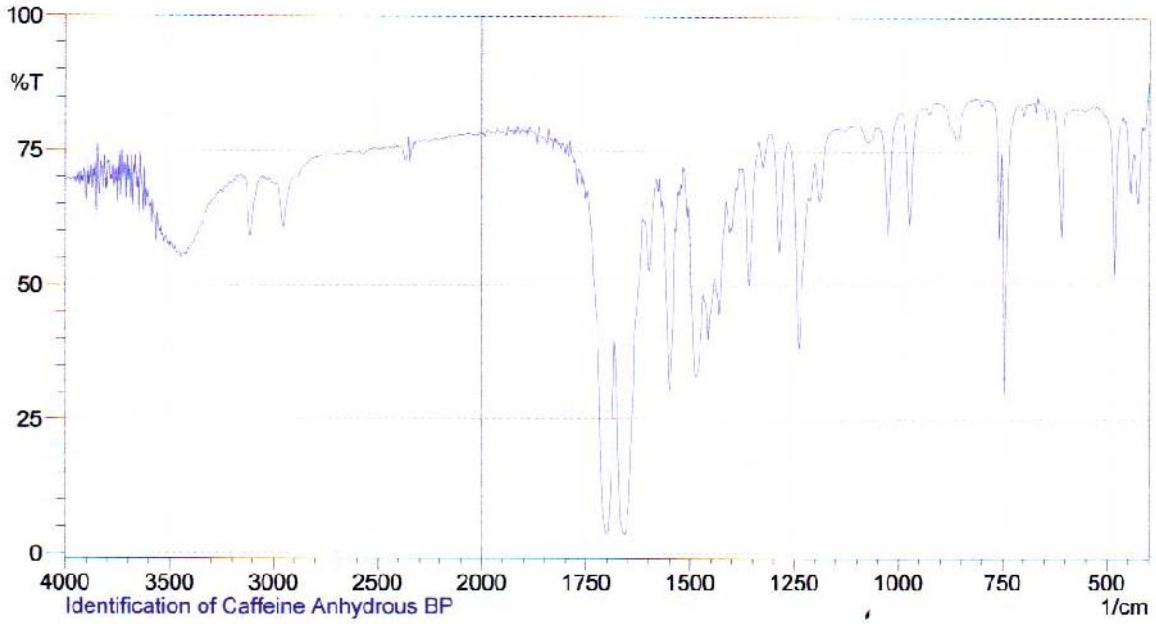


Fig. 7.1.1.1: IR spectrum of Caffeine Anhydrous BP alone

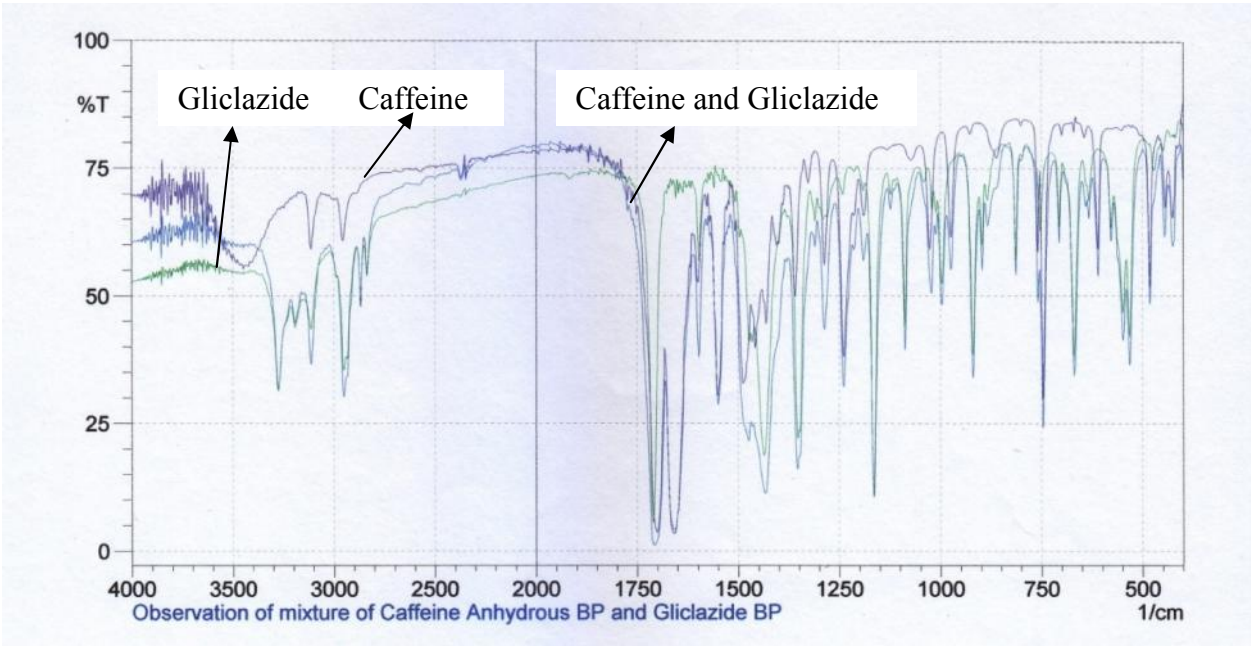


Fig. 7.1.1.2: Observation of mixture of Caffeine Anhydrous BP and Gliclazide BP

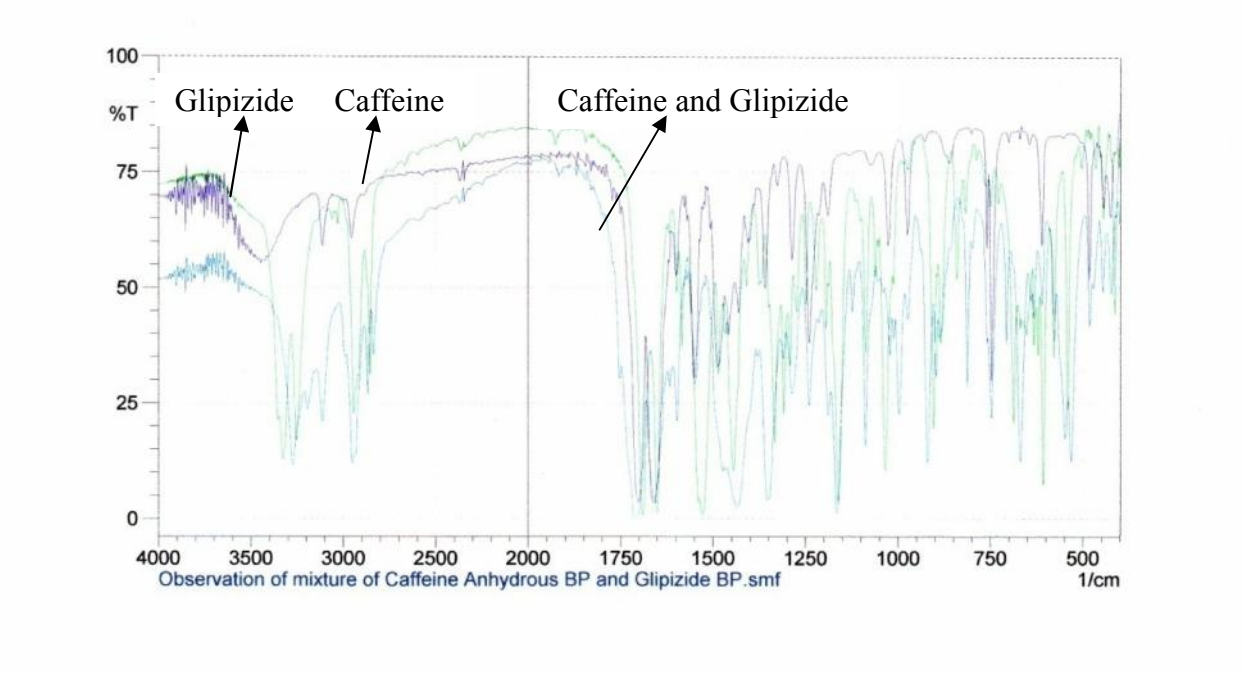


Fig. 7.1.1.3: Observation of mixture of Caffeine Anhydrous BP and Glipizide BP

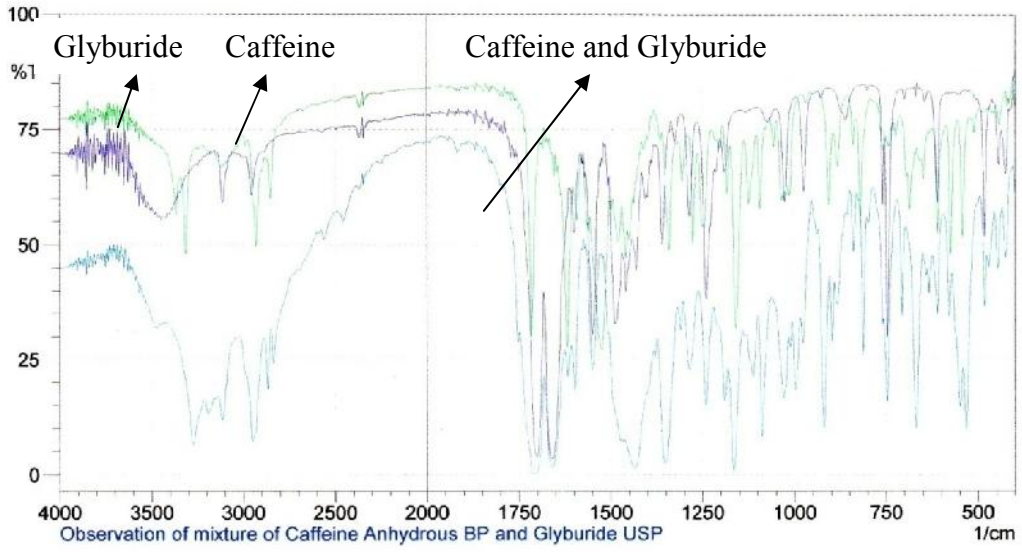


Fig. 7.1.1.4: Observation of mixture of Caffeine Anhydrous BP and Glyburide USP

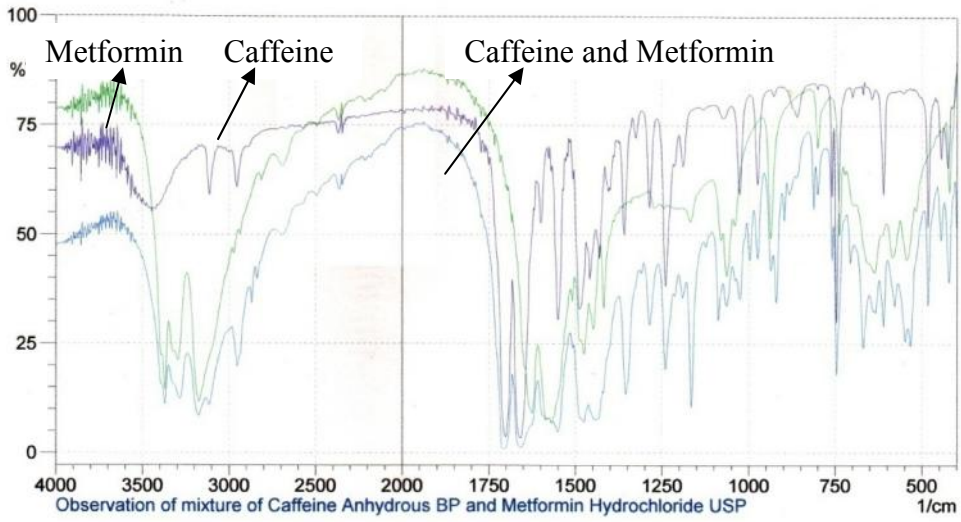


Fig. 7.1.1.5: Observation of mixture of Caffeine Anhydrous BP and Metformin Hydrochloride USP

From above spectrum, we infer that the recorded IR spectrums of each mixture of caffeine-gliclazide, caffeine-glipizide, caffeine-glyburide and caffeine-metformin recorded in the same manner were greatly influenced of each other.

From above spectrum, we also infer that the spectra of target molecules alone and the mixture of (1:1) of caffeine with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities including some shifts in the absorption maxima. This may be due to interaction of caffeine with the drugs that alter the absorption intensities**.**

It can be inferred that the molecular species of caffeine when separately mixed with gliclazide, glipizide, glyburide and metformin showed some changes in absorption characteristics of these drug molecules including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction of drugs.

So, it can be infered that the infrared spectra characteristics of caffeine with gliclazide, glipizide, glyburide or metformin reveal the initial interaction of each interacting species.

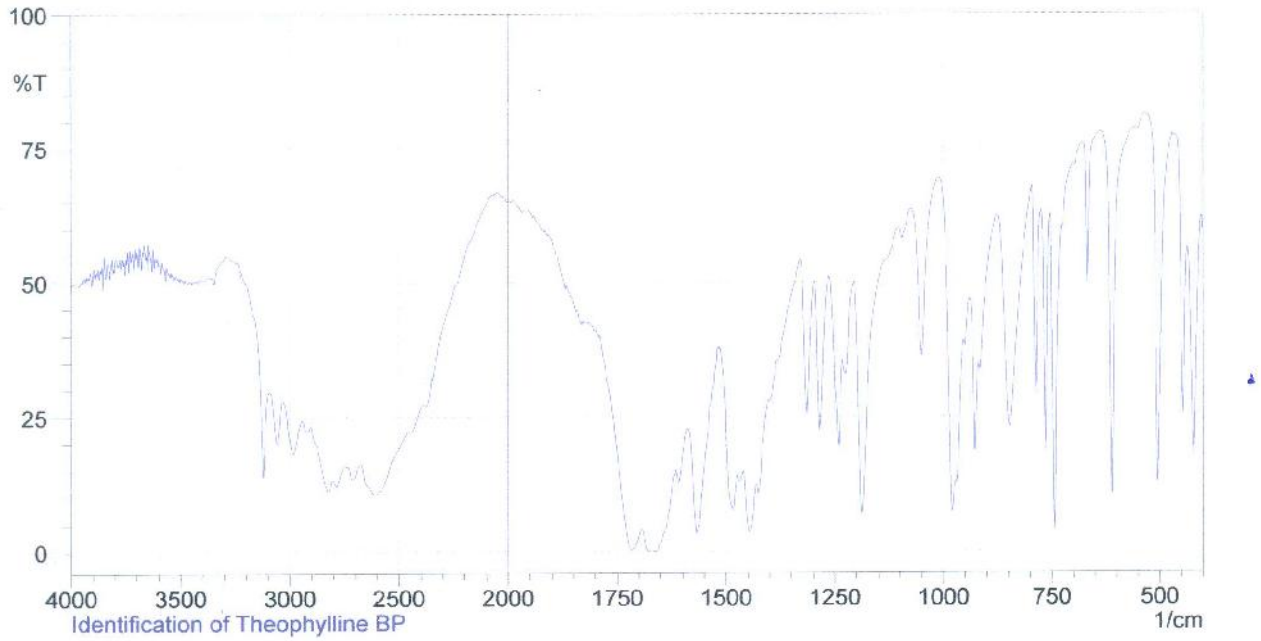


Fig. 7.1.1.6: IR spectrum of Theophylline BP alone

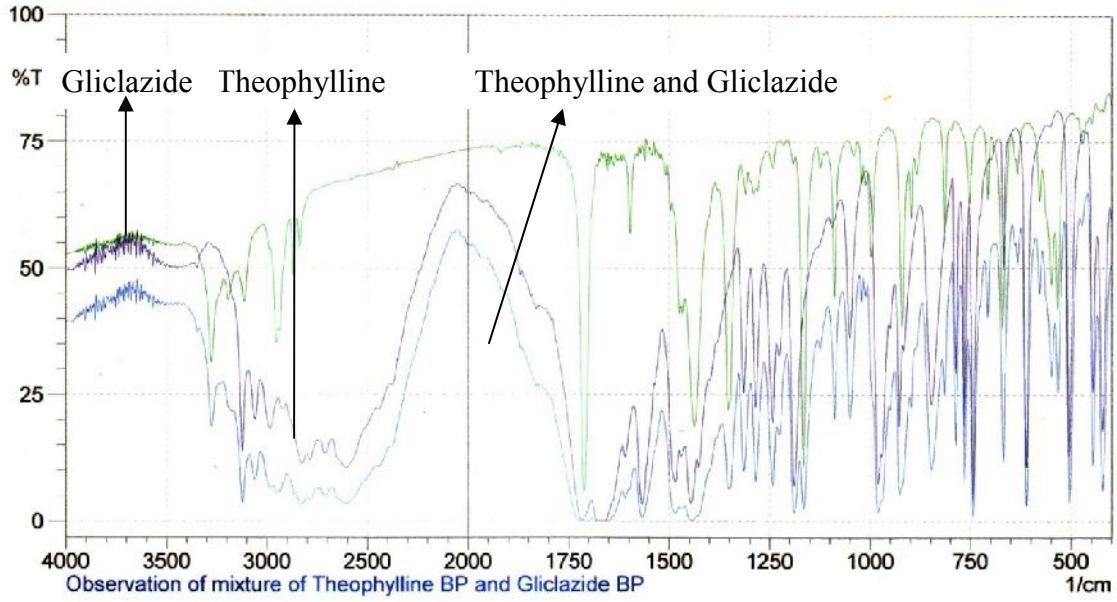


Fig. 7.1.1.7: Observation of mixture of Theophylline BP and Gliclazide BP

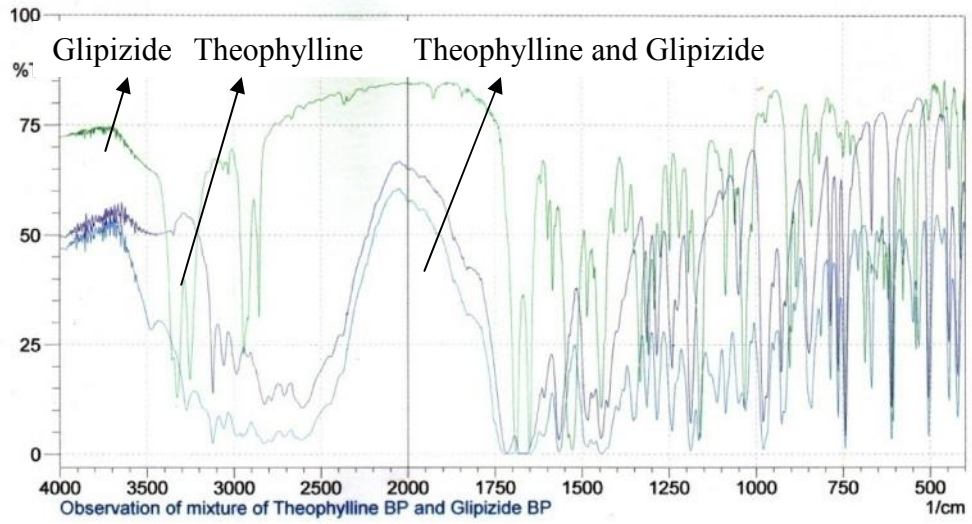


Fig. 7.1.1.8: Observation of mixture of Theophylline BP and Glipizide BP

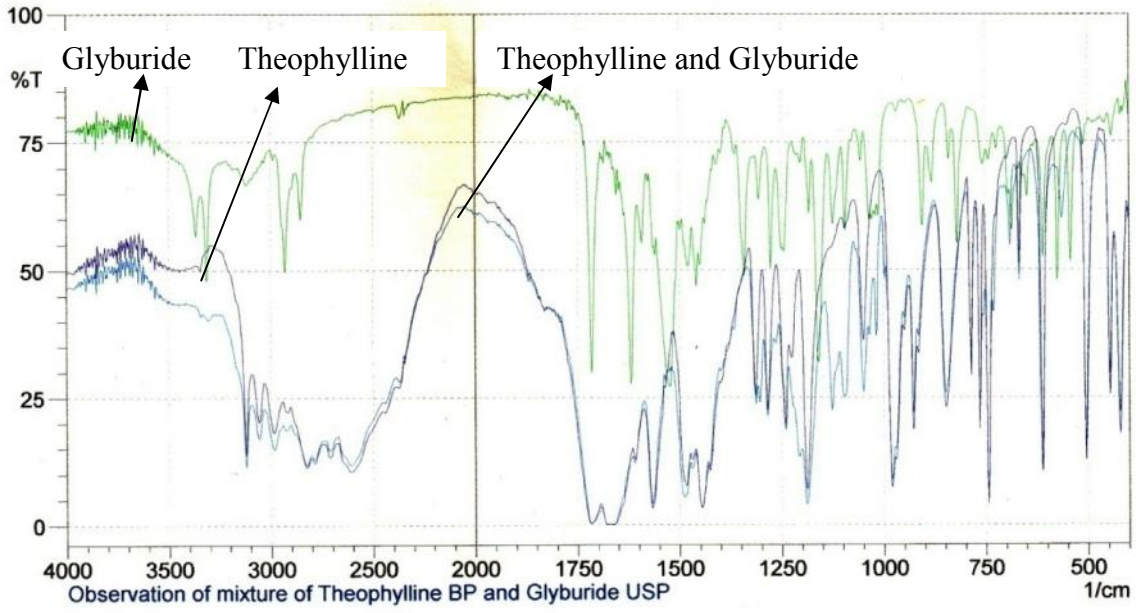


Fig. 7.1.1.9: Observation of mixture of Theophylline BP and Glyburide USP

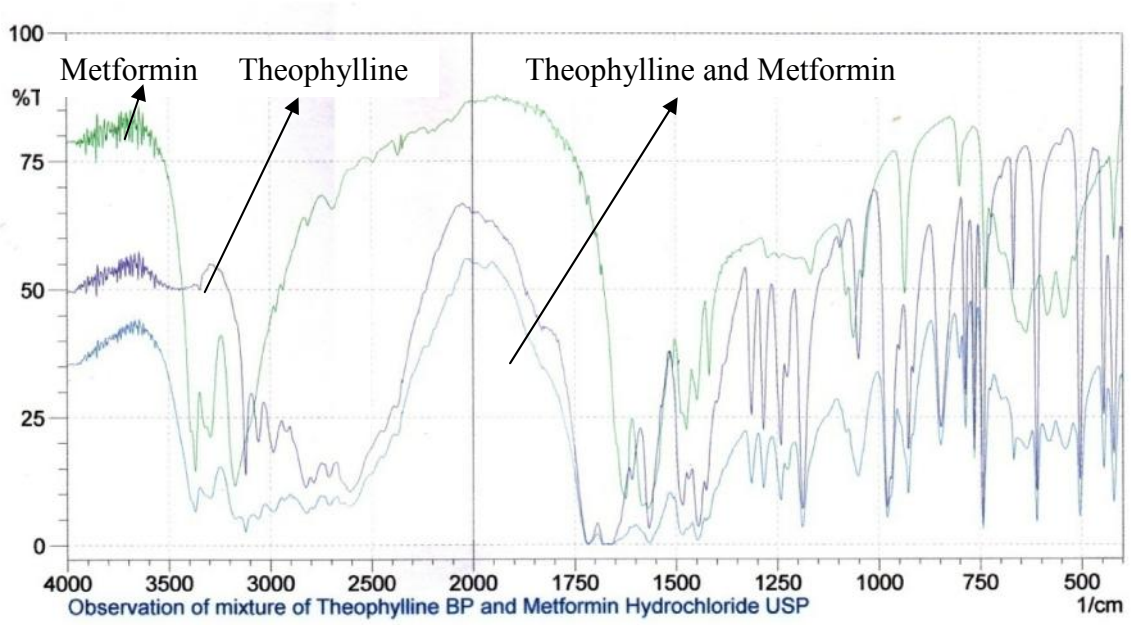


Fig. 7.1.1.10: Observation of mixture of Theophylline BP and Metformin Hydrochloride USP

From above spectrum, we infer that the recorded IR spectrums of each mixture of theophylline-gliclazide, theophylline-glipizide, theophylline-glyburide, theophylline-metformin recorded in the same manner were greatly influenced of each other.

From above spectrum, we also infer that the spectra of target molecules alone and the mixture of (1:1) of theophylline with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of theophylline with the drugs that alter the absorption intensities.

It can be infered that the molecular species of theophylline when separately mixed with gliclazide, glipizide, glyburide and metformin showed some changes in absorption characteristics of these drug molecules including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction of drugs.

So, it can be infered that the infrared spectra characteristics of theophylline with gliclazide, glipizide, glyburide or metformin reveal the initial interaction of each interacting species.

7.1.2 OBSERVATION OF ABSORPTION SPECTA FOR INITIAL INTERACTION BY UV-VISIBLE SPECTRPHOTOMETRIC METHOD

In the present study, the UV absorption of the drug and drug-drug interaction mixtures was measured at 200-400 nm. In this procedure, the ultraviolet absorption characteristics of caffeine, theophylline, gliclazide, glipizide, glyburide & metformin and their 1:1 mixtures in solution (pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4) were compared with those of each interacting species.

Each of the drugs studied shows absorption in UV-VIS region. The molecular species of gliclazide, glipizide, glyburide and metformin when separately mixed with caffeine & theophylline showed some changes in absorption characteristics of these drug molecules including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction of drugs. [57] [58] [59]

Matsui et al. (1975) reported that a "host-guest" complexation results in alteration of spectral patterns.

Gur Yanova et al. (1975) observed that alteration of absorption intensities occurs in case of donor-acceptor complexation.

The concentrations of the samples were kept at very dilute levels in each case and the measurements were made using an UV – VIS automatic recording instrument with a constant temperature cell compartment and automatic recording unit. The stock solutions of the samples were diluted to appropriate levels, mixed buffer at the desired pH and the spectra were recorded between 400 – 200 nm. The spectra were compared with the pure sample in each combination.

The spectra of target molecules alone and the mixture of (1:1) of each caffeine and theophylline with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of caffeine and theophylline with the drugs that alter the absorption intensities as donor-acceptor complexation occurs.

This finding was similar to that of ''the evaluation of *in vitro* interaction of diltiazem hydrochloride with iron (II) in the aqueous media'' [72] and ''the evaluation of *in vitro* interaction of nifidipine hydrochloride with copper (II) in the aqueous media'' [73]

The spectra of caffeine alone at different pHs showed a sharp absorption maximum at 273 nm (fig.7.1.2.1). The intensities of these peaks vary with pH. 1:1 mixtures of caffeine with the other molecular species showed noticeable changes in the absorption intensities due to interaction (fig.7.1.2.2-7.1.2.29).

The spectra of theophylline alone at different pHs showed a sharp absorption maximum at 276 nm (fig.7.1.2.30). The intensities of these peaks vary with pH. 1:1 mixtures of theophylline with the other molecular species showed noticeable changes in the absorption intensities due to interaction (fig.7.1.2.31-7.1.2.58).

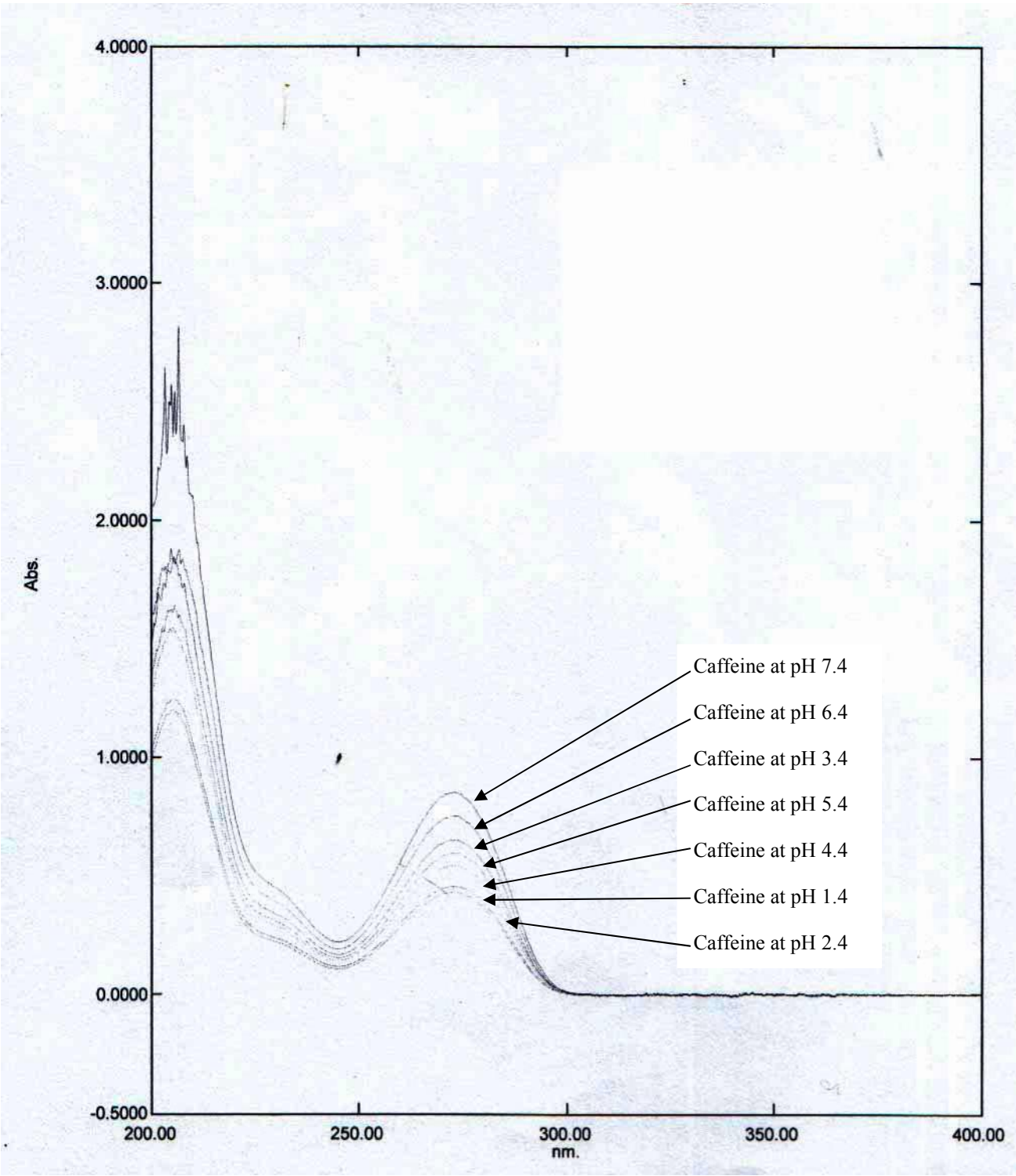


Fig. 7.1.2.1: UV spectra of Caffeine at different p^H (Conc. of Caffeine = 0.0001 M)

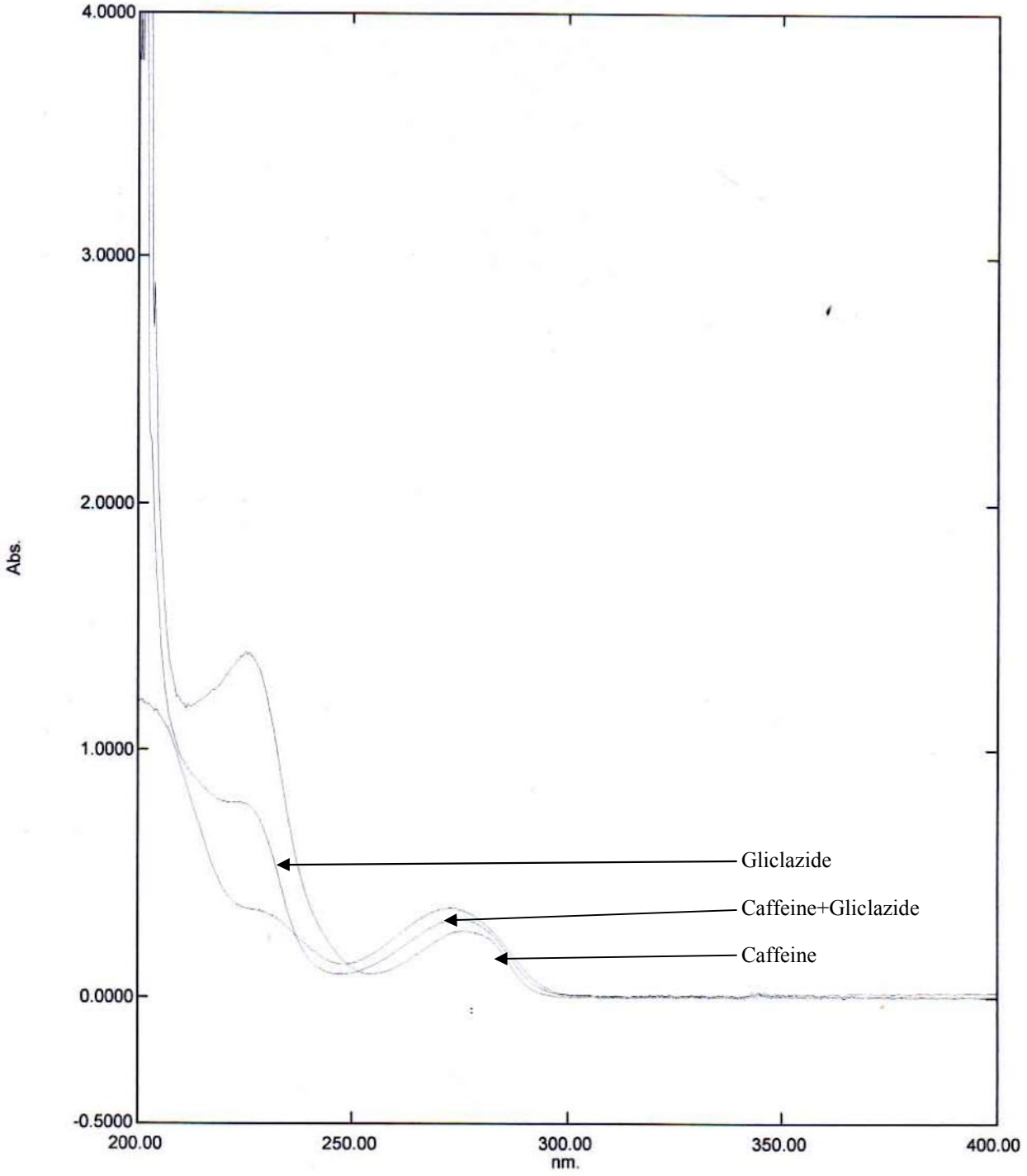


Fig.7.1.2.2: UV spectra of Caffeine – Gliclazide systems at pH 1.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

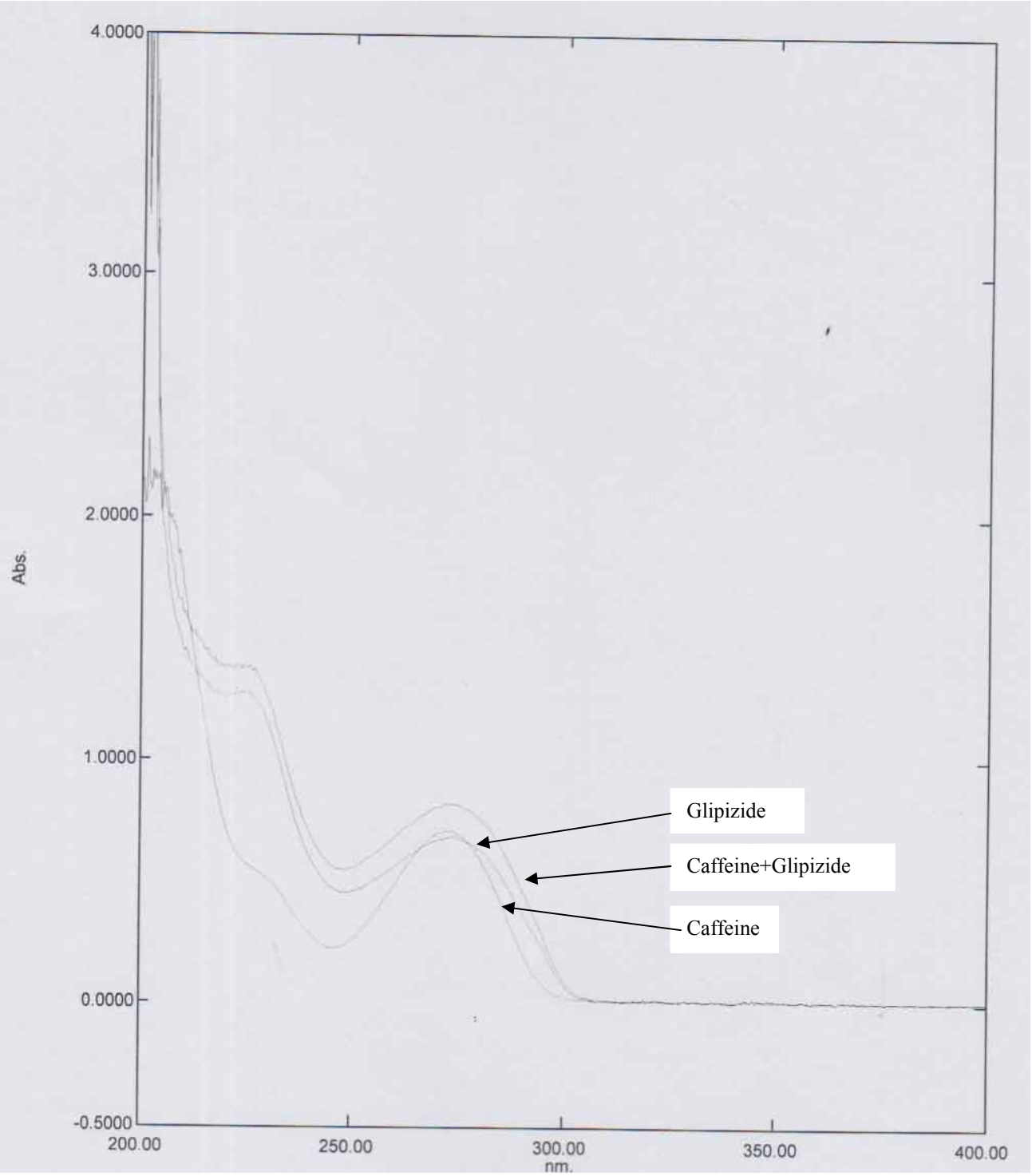


Fig.7.1.2.3: UV spectra of Caffeine – Glipizide systems at pH 1.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

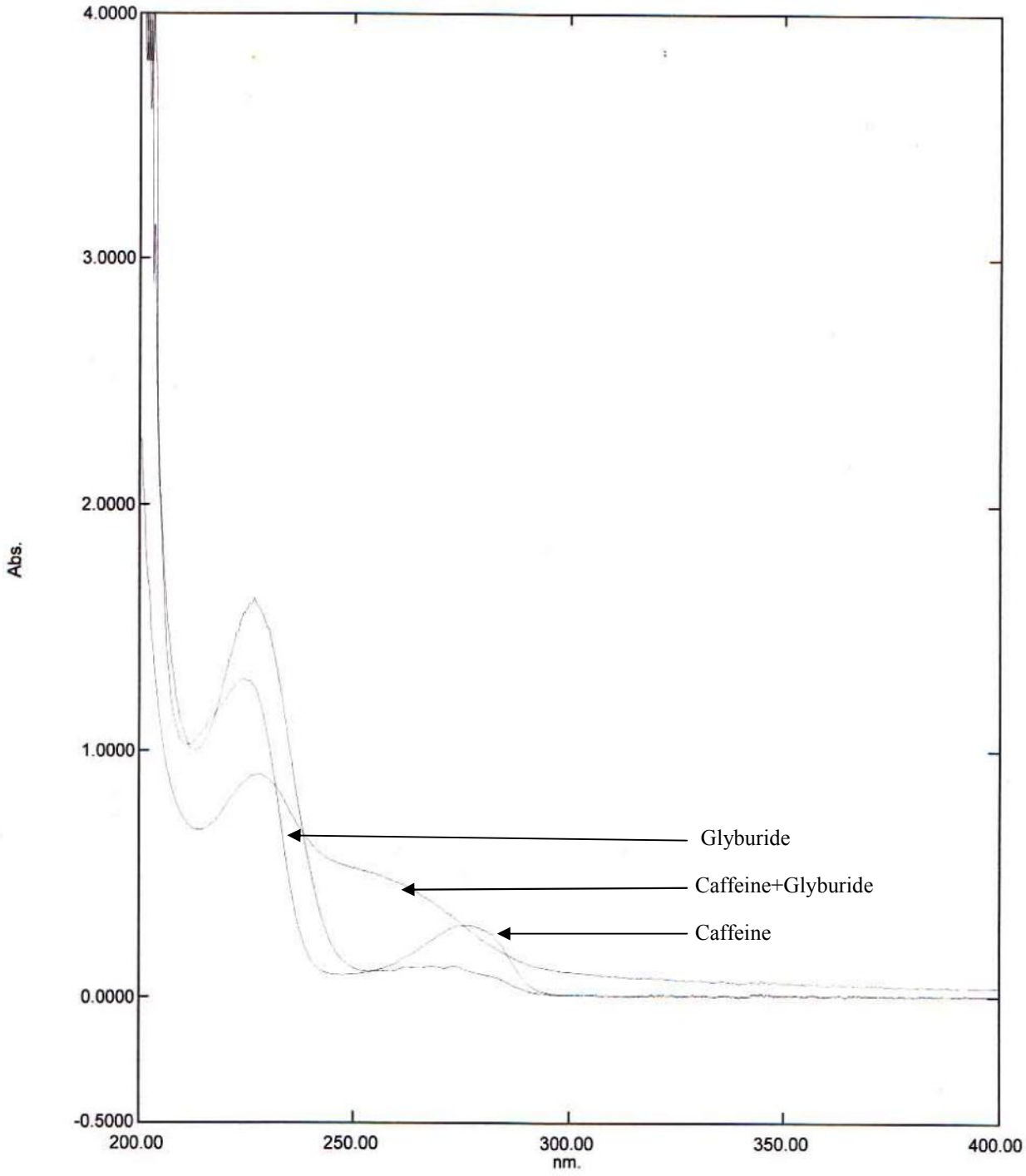


Fig.7.1.2.4: UV spectra of Caffeine – Glyburide systems at pH 1.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

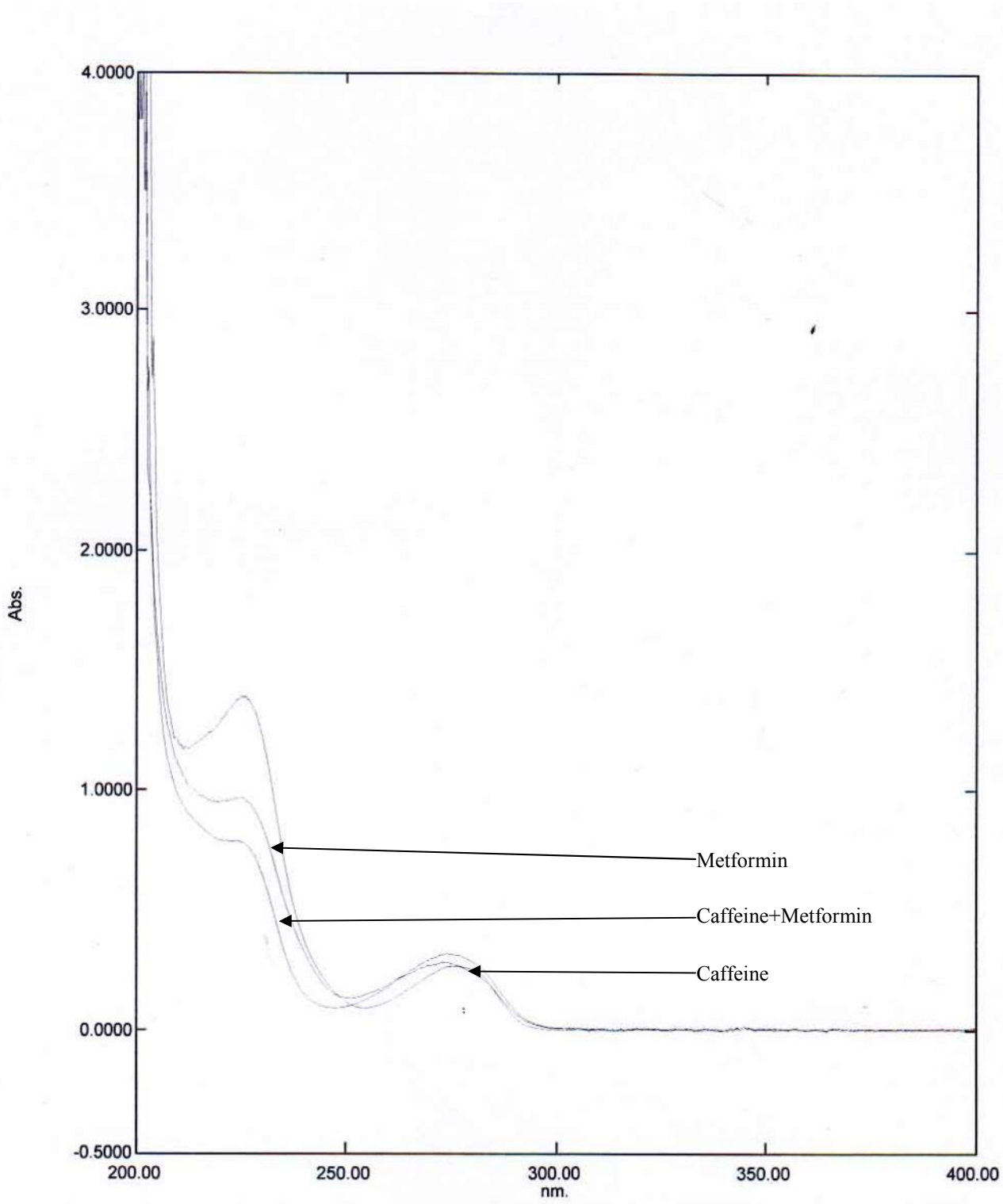


Fig.7.1.2.5: UV spectra of Caffeine – Metformin systems at pH 1.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

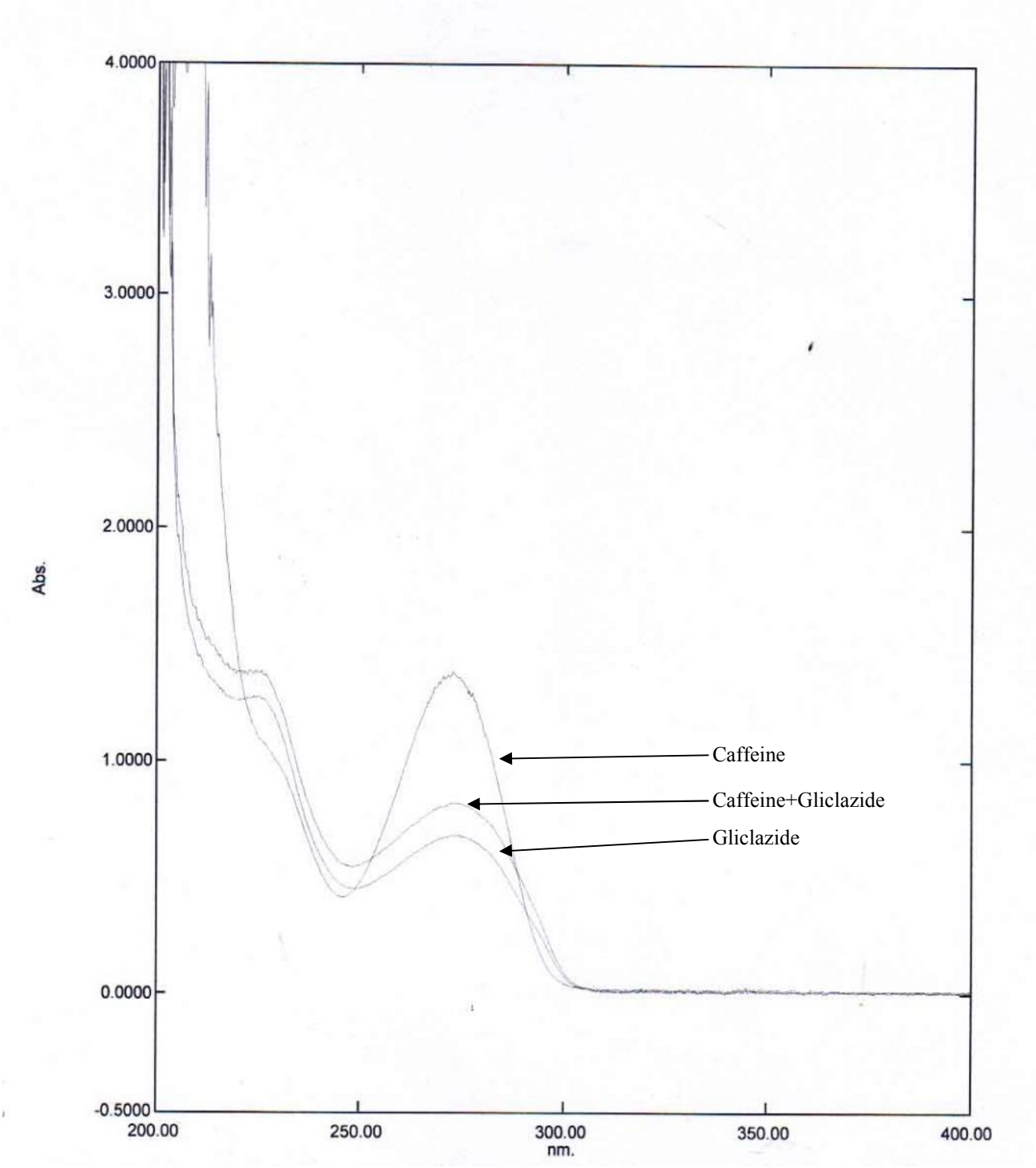


Fig.7.1.2.6: UV spectra of Caffeine – Gliclazide systems at pH 2.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

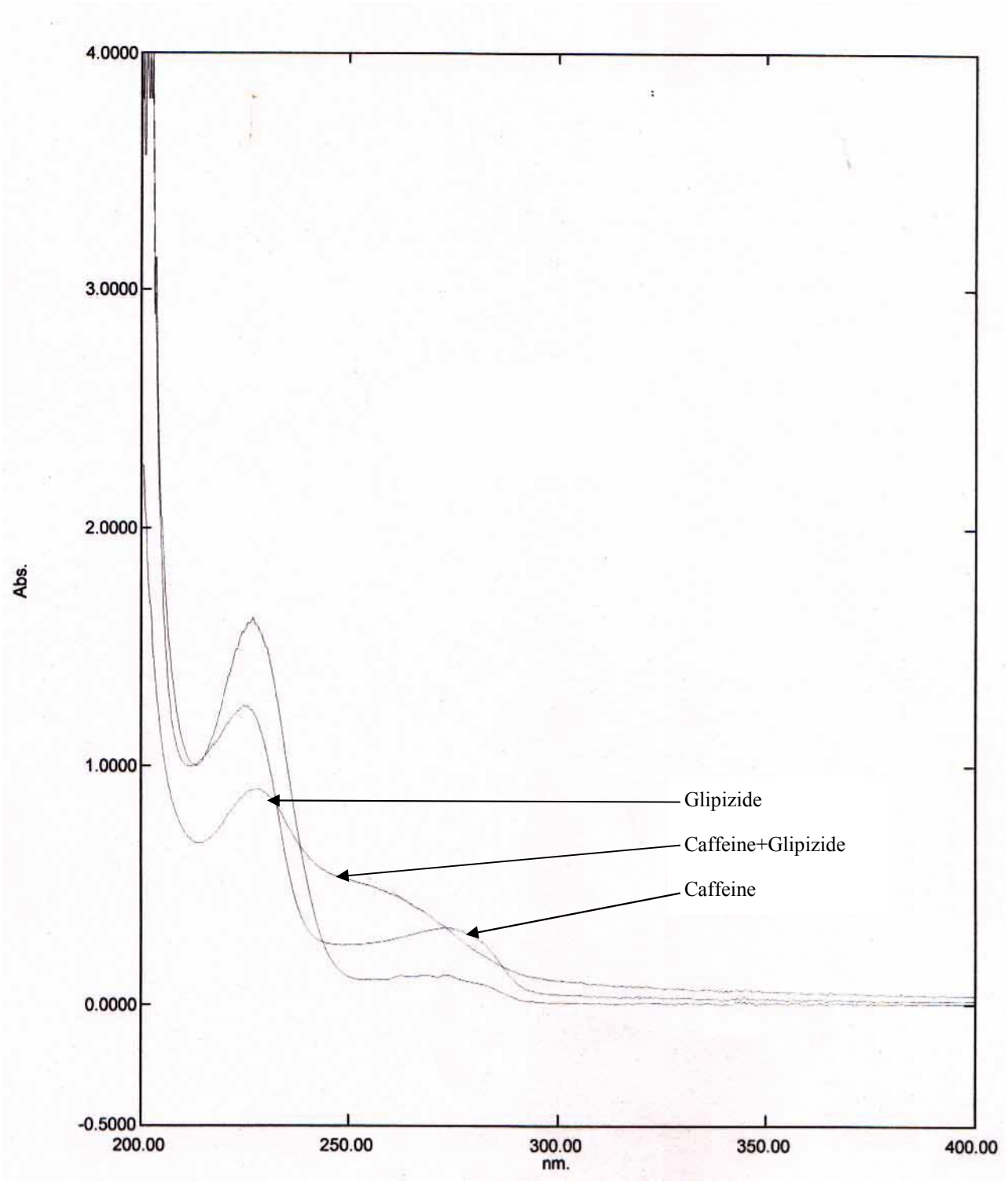


Fig.7.1.2.7: UV spectra of Caffeine – Glipizide systems at pH 2.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

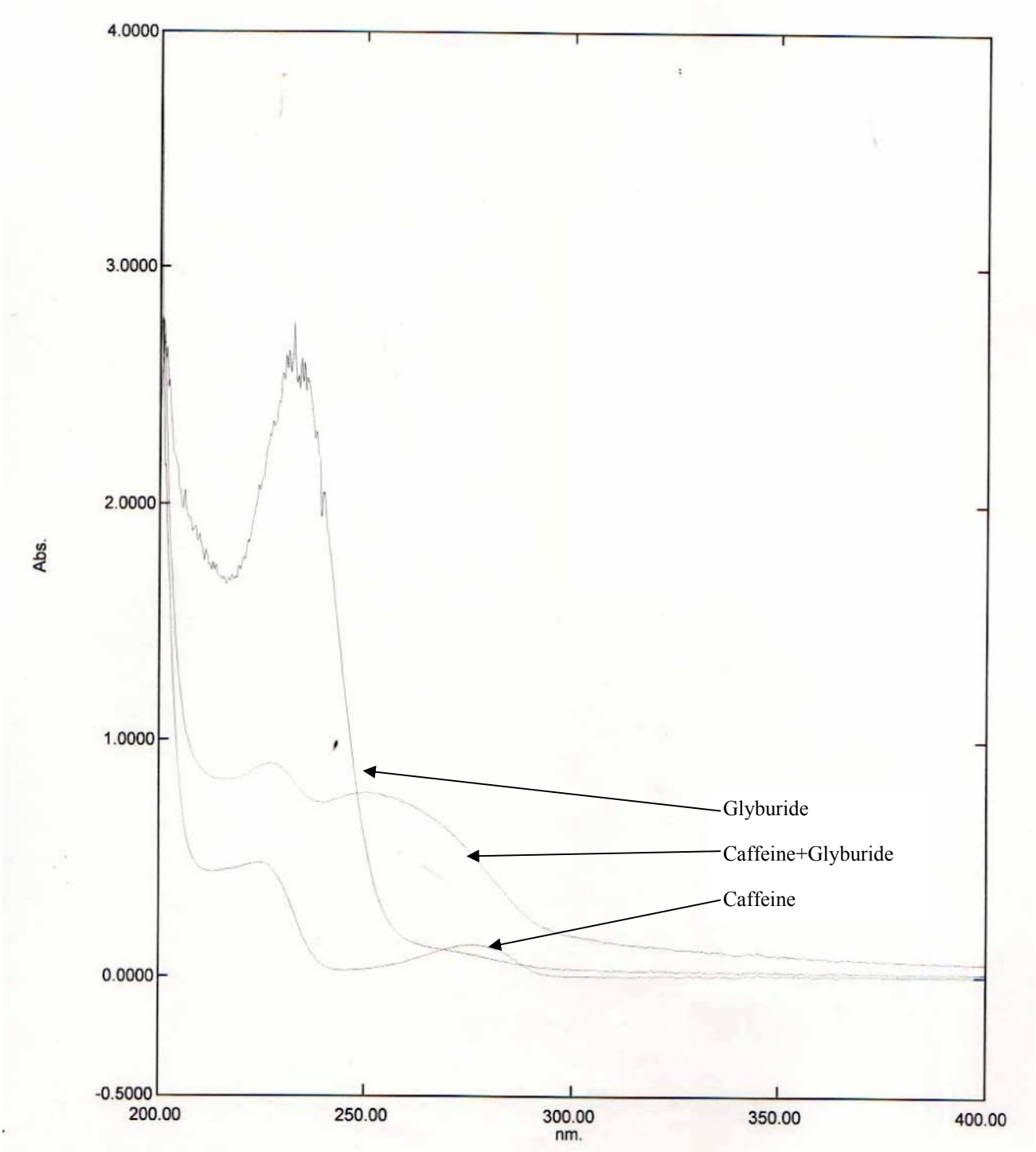


Fig.7.1.2.8: UV spectra of Caffeine – Glyburide systems at pH 2.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

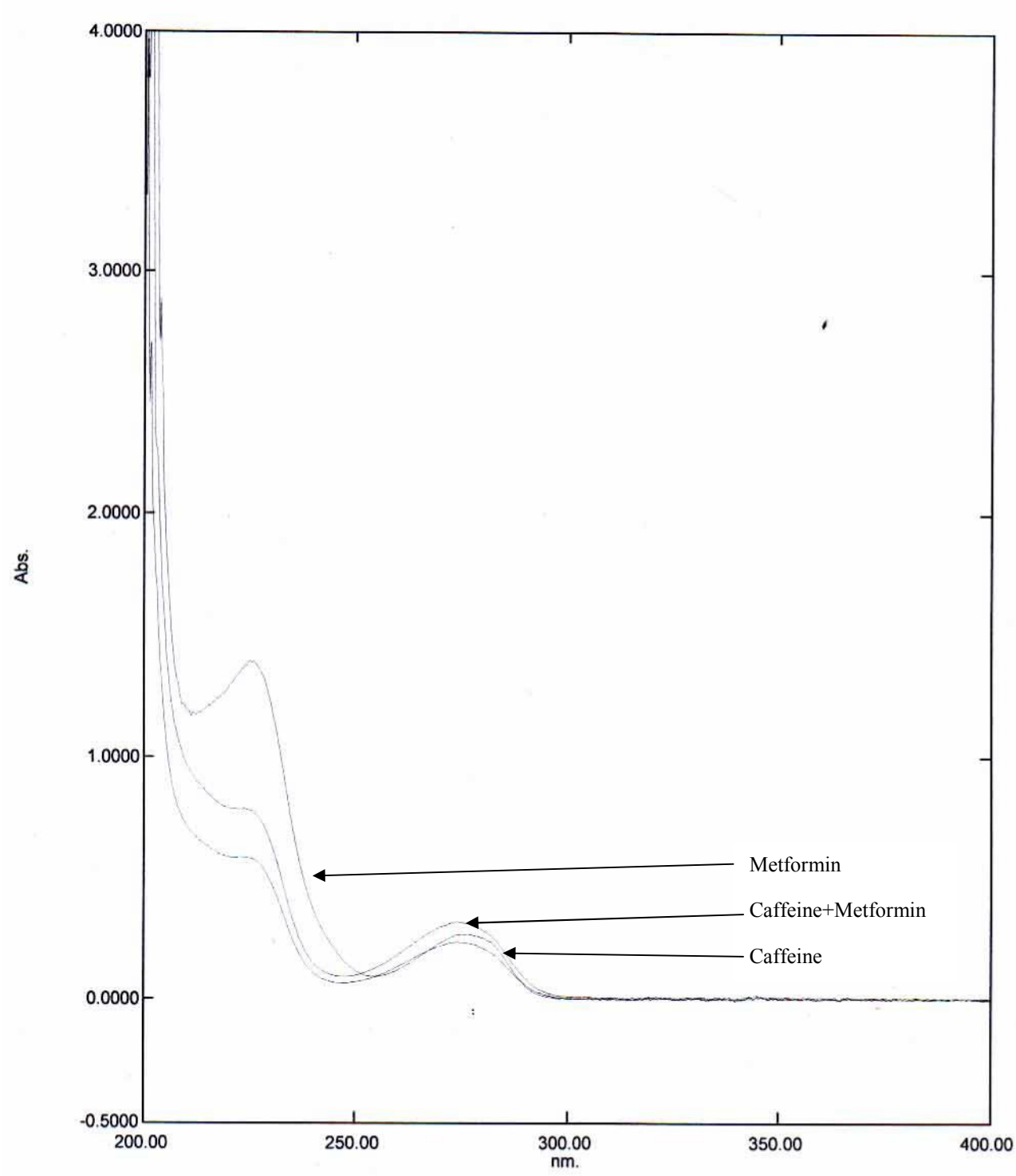


Fig.7.1.2.9: UV spectra of Caffeine – Metformin systems at pH 2.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

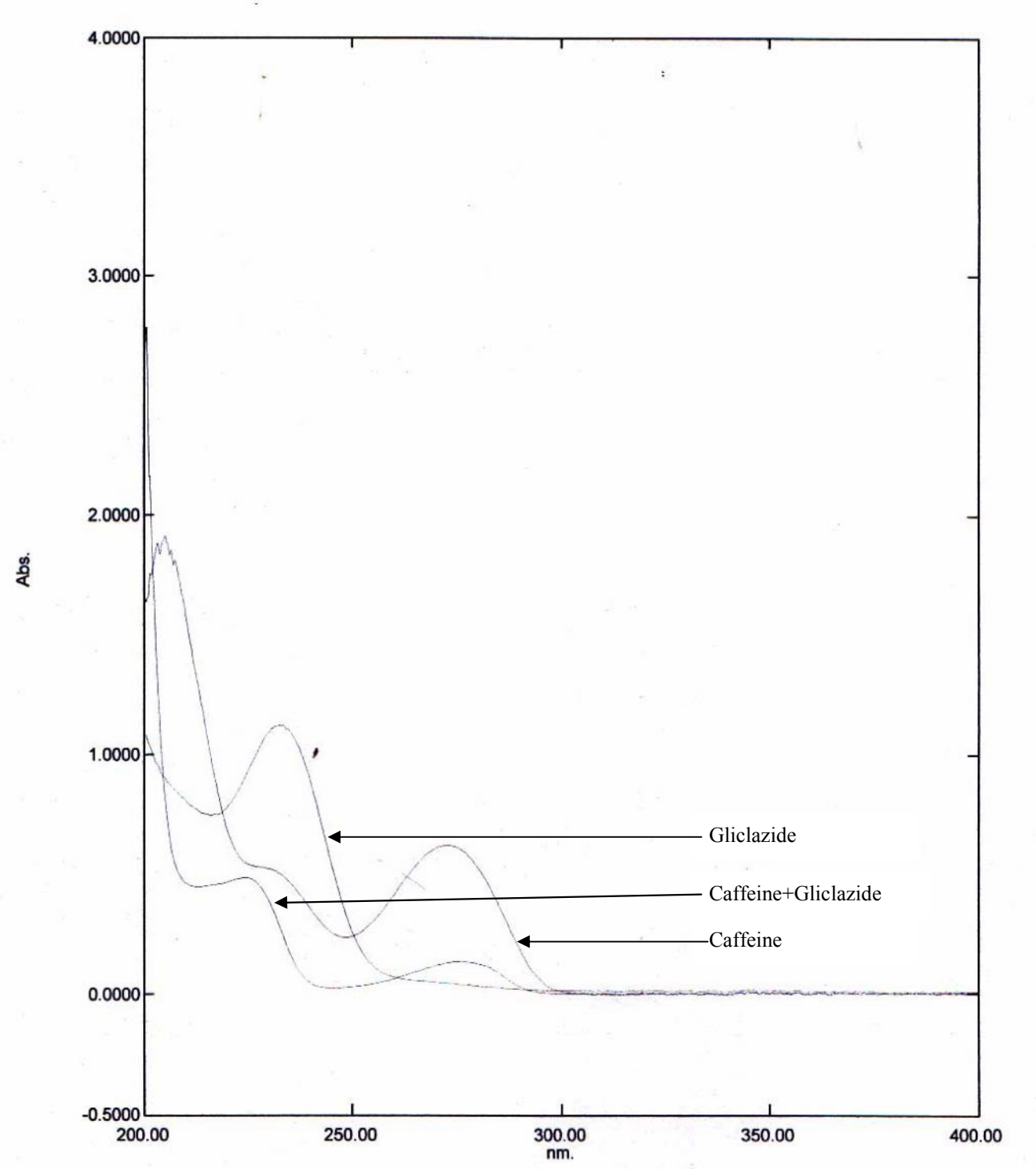


Fig.7.1.2.10: UV spectra of Caffeine – Gliclazide systems at pH 3.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

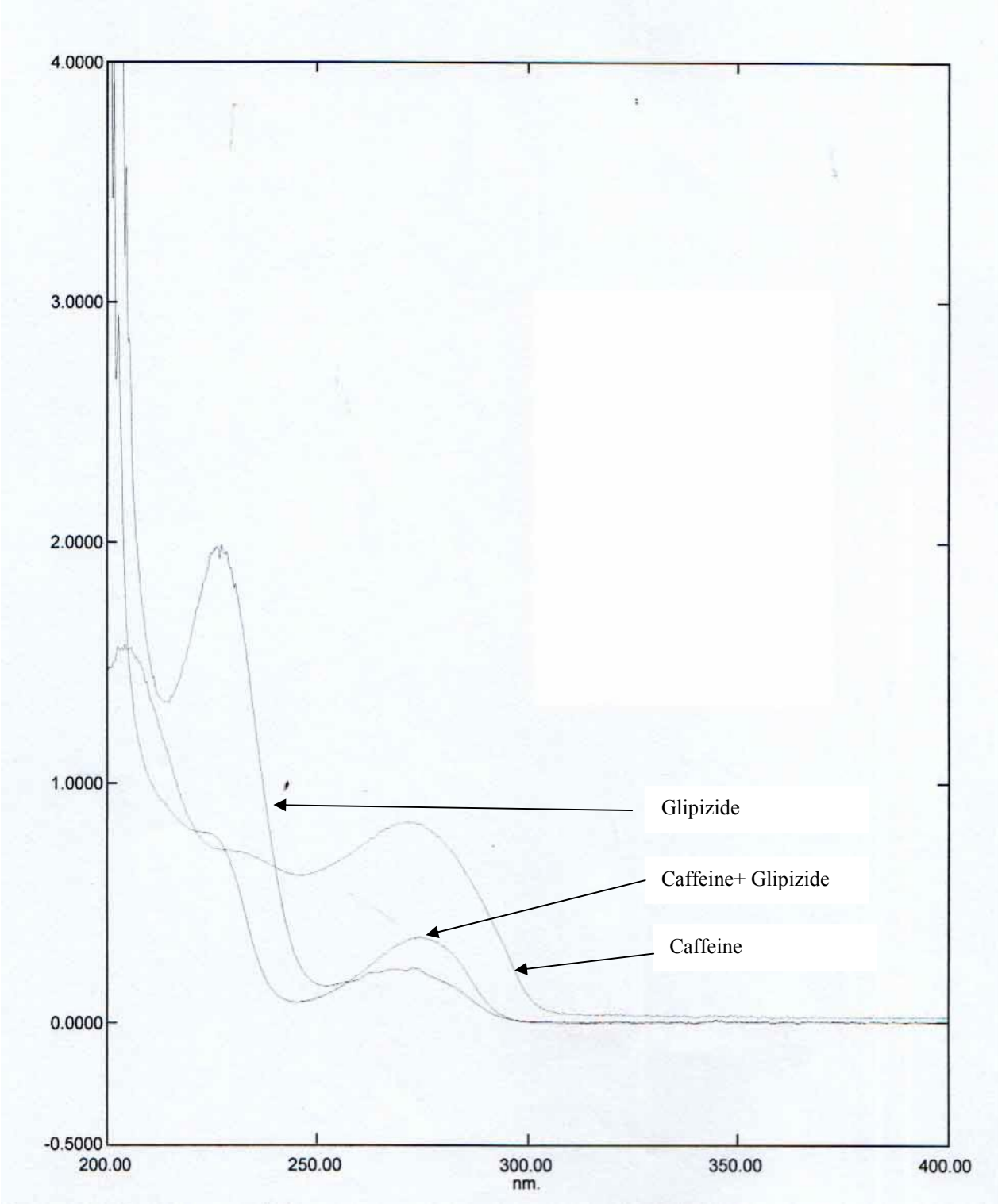


Fig.7.1.2.11: UV spectra of Caffeine – Glipizide systems at pH 3.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

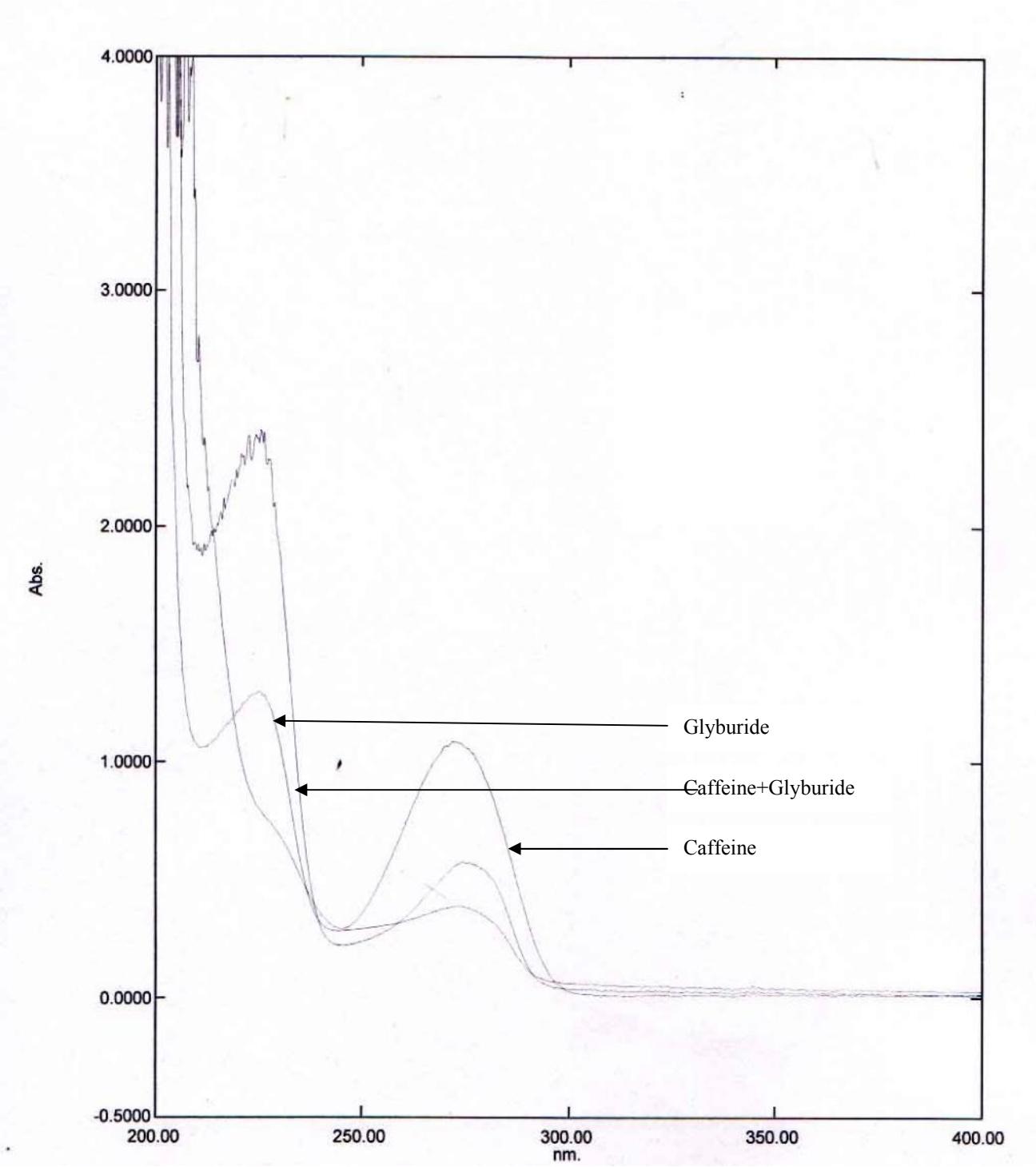


Fig.7.1.2.12: UV spectra of Caffeine – Glyburide systems at pH 3.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

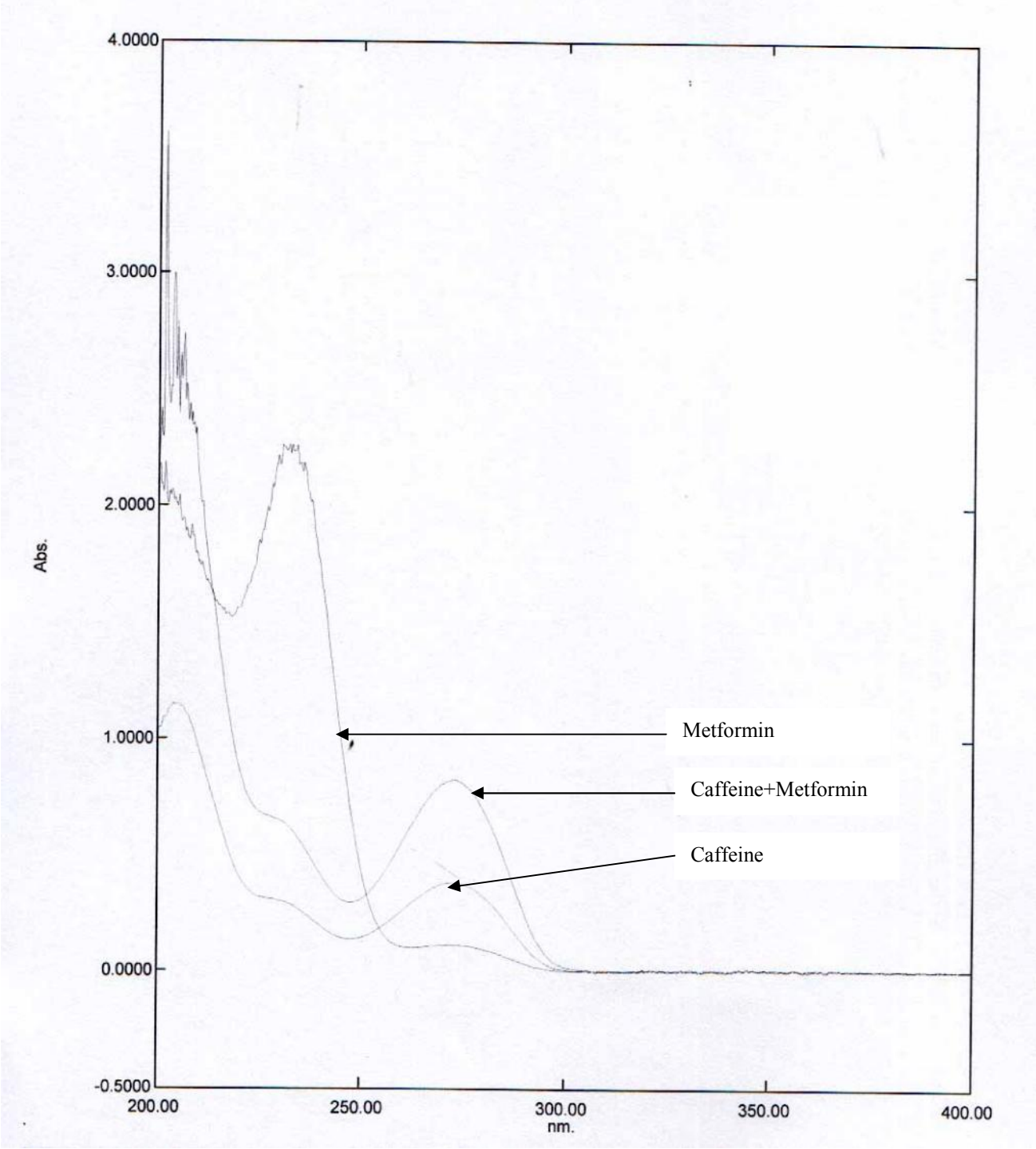


Fig.7.1.2.13: UV spectra of Caffeine – Metformin systems at pH 3.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

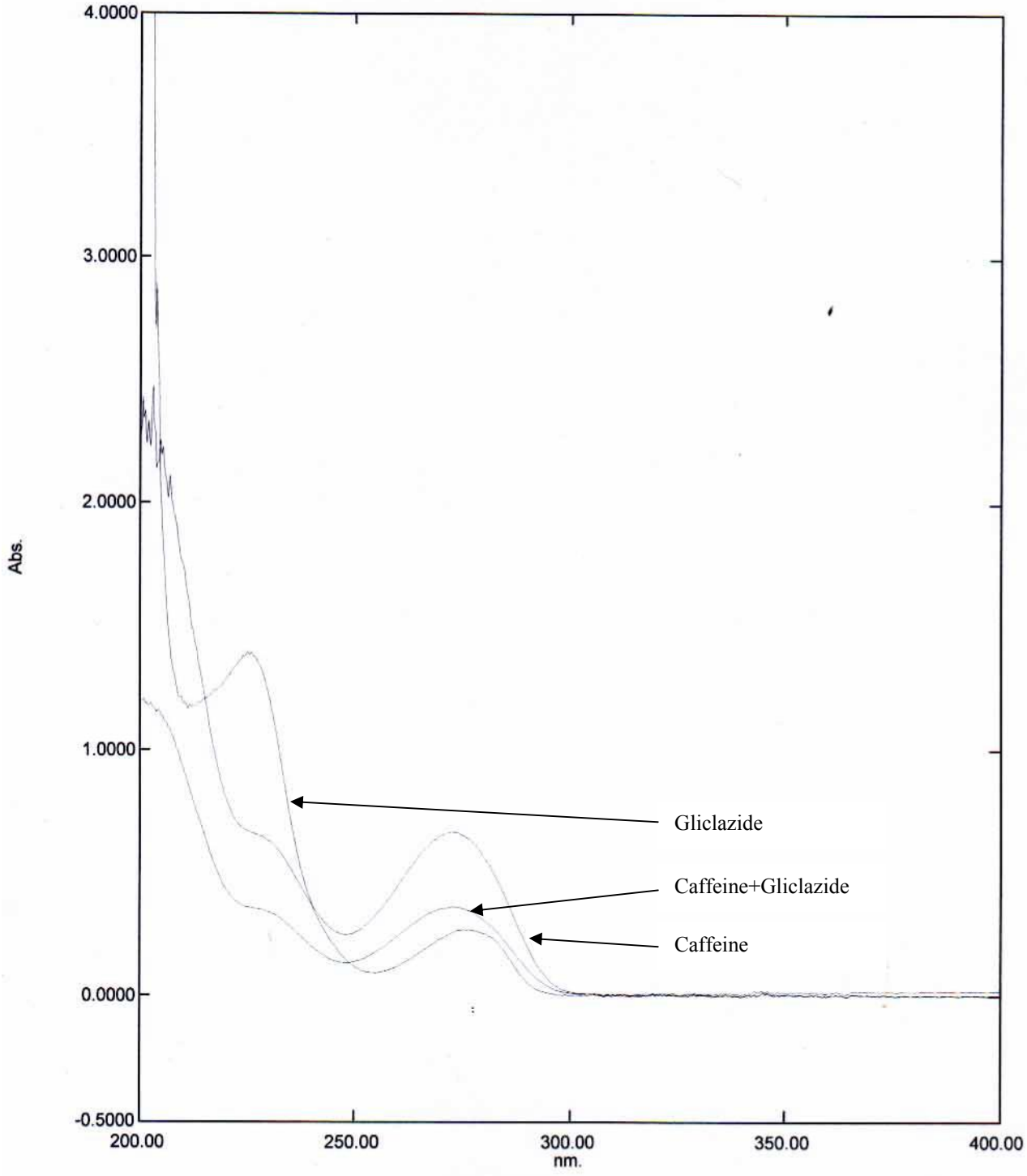


Fig.7.1.2.14: UV spectra of Caffeine – Gliclazide systems at pH 4.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

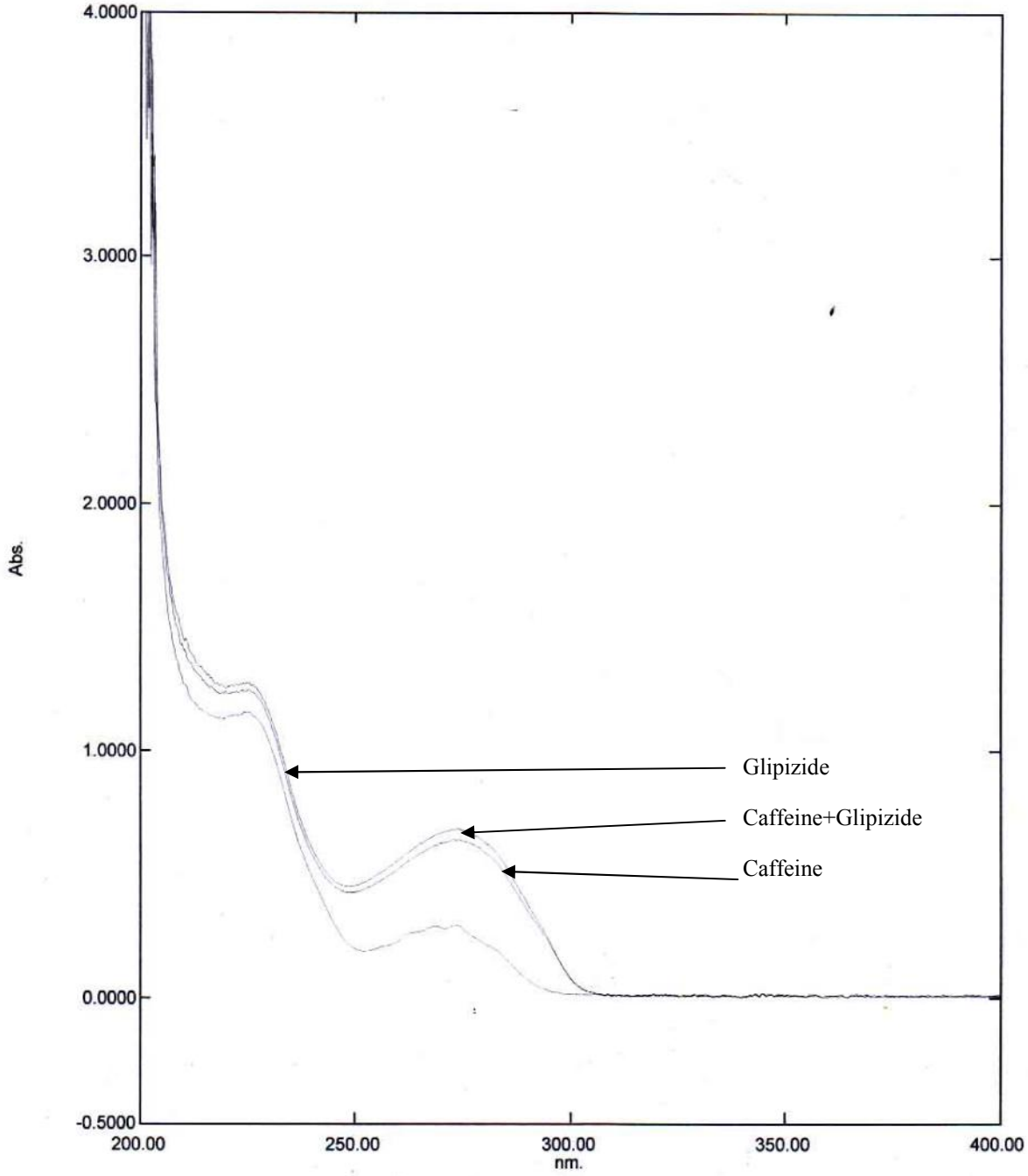


Fig.7.1.2.15: UV spectra of Caffeine – Glipizide systems at pH 4.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

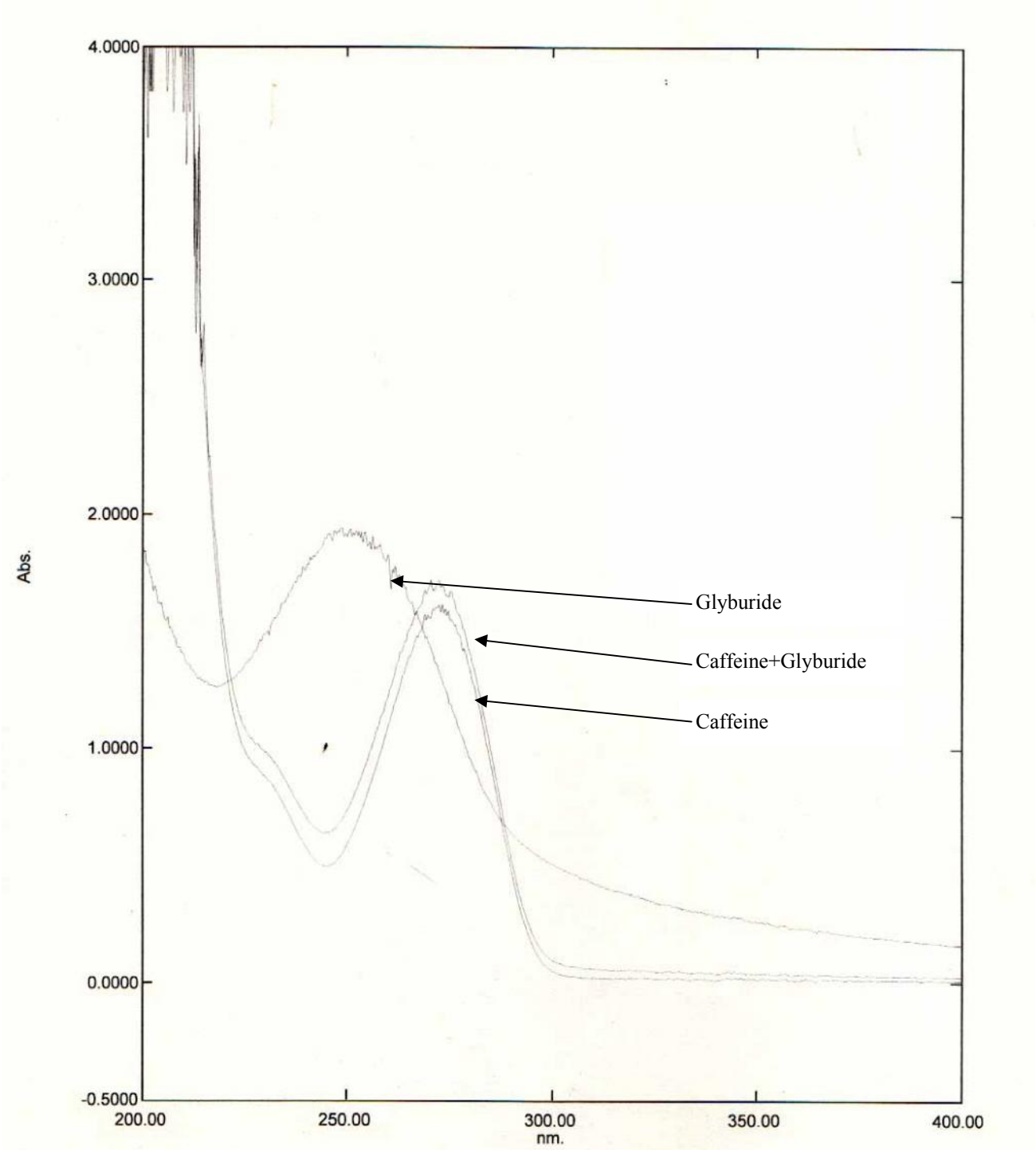


Fig.7.1.2.16: UV spectra of Caffeine – Glyburide systems at pH 4.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

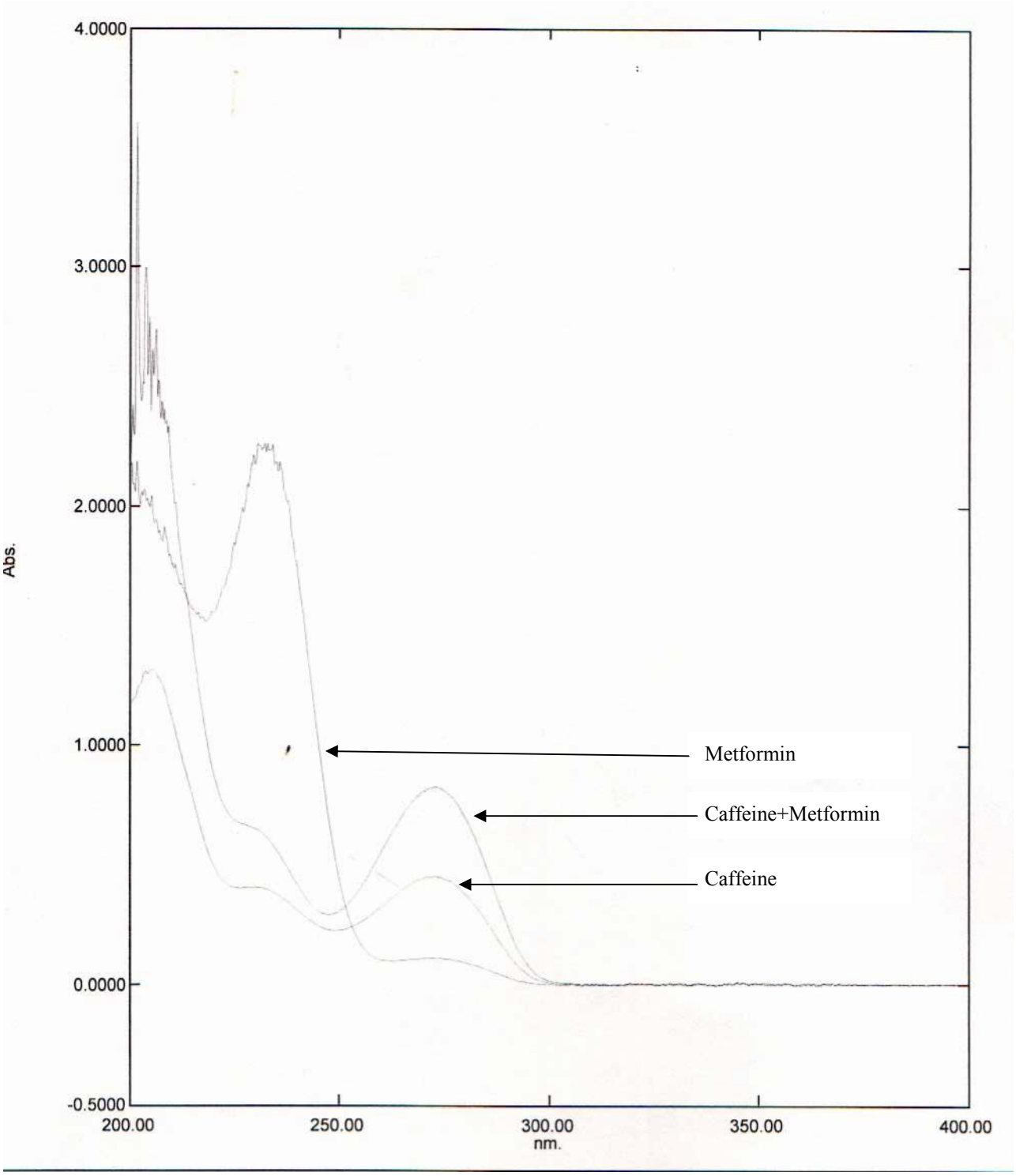


Fig.7.1.2.17: UV spectra of Caffeine – Metformin systems at pH 4.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

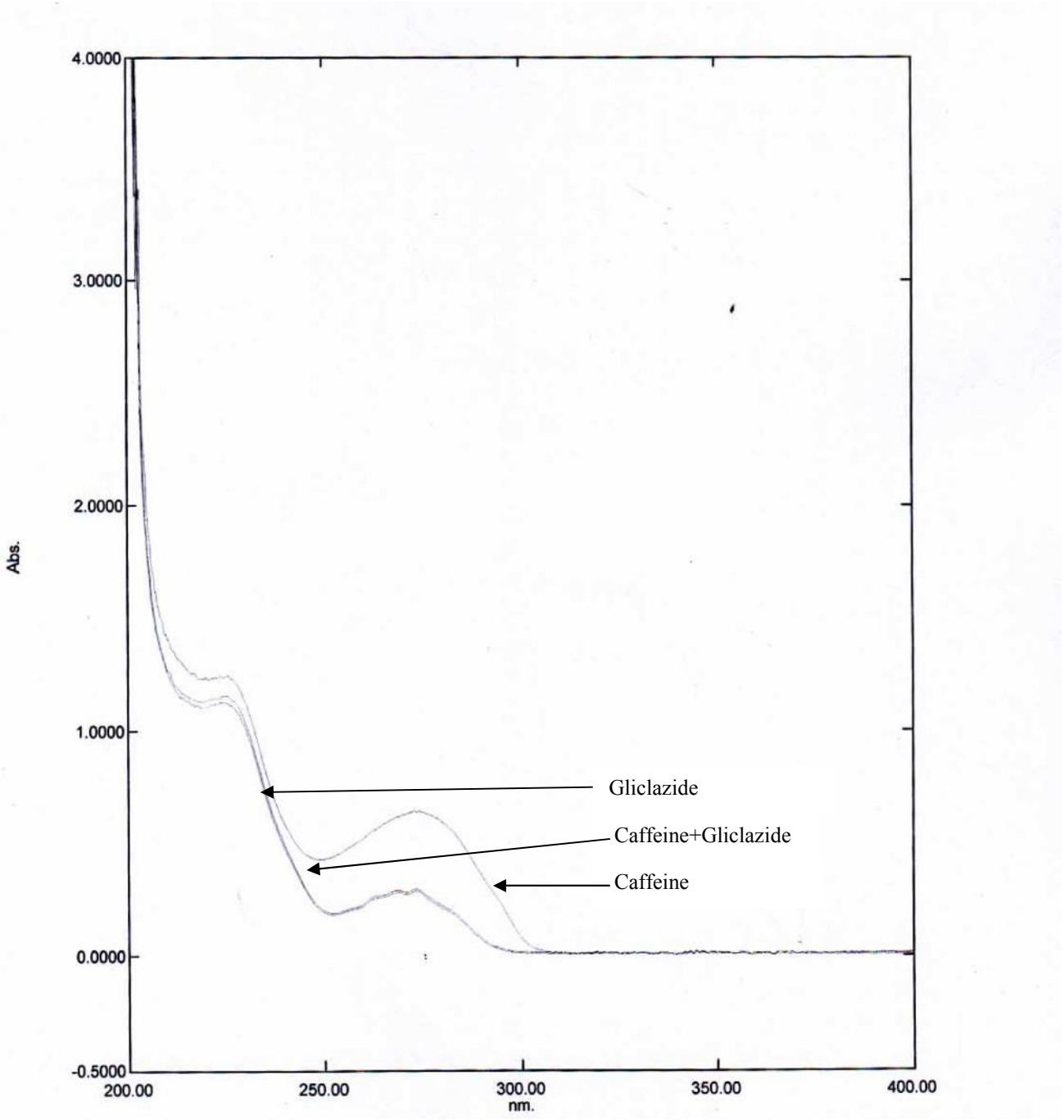


Fig.7.1.2.18: UV spectra of Caffeine – Gliclazide systems at pH 5.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

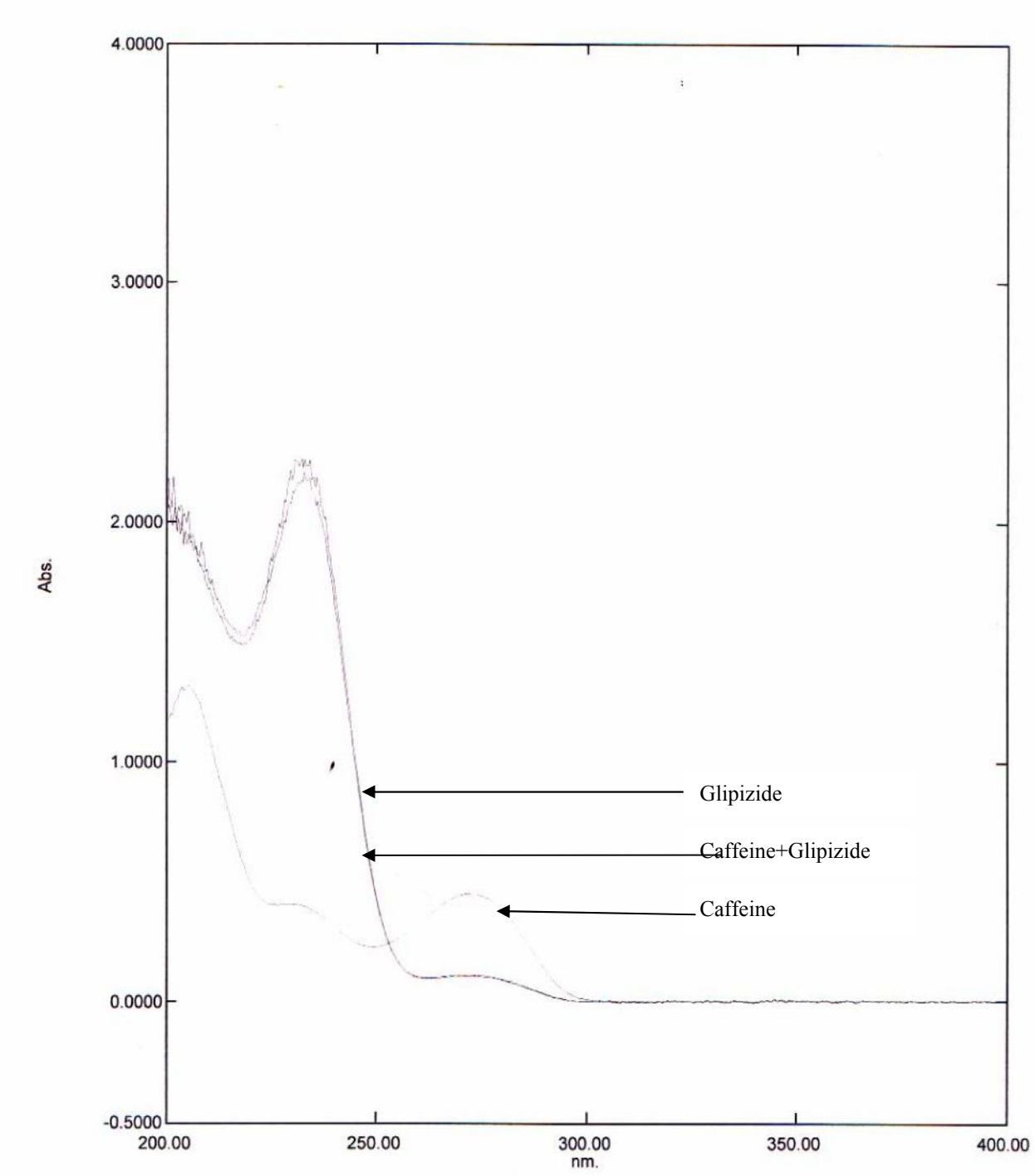


Fig.7.1.2.19: UV spectra of Caffeine – Glipizide systems at pH 5.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

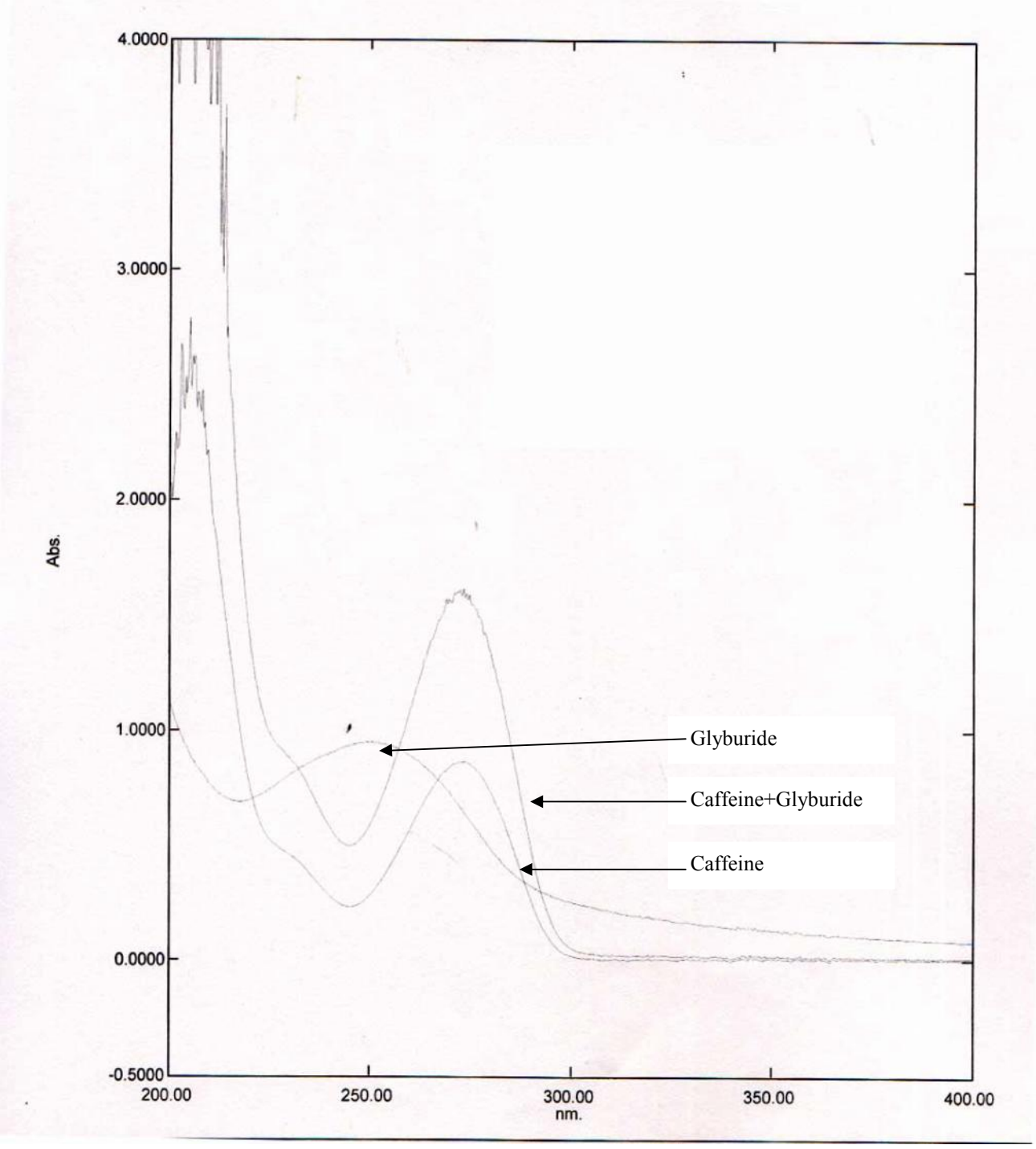


Fig.7.1.2.20: UV spectra of Caffeine – Glyburide systems at pH 5.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

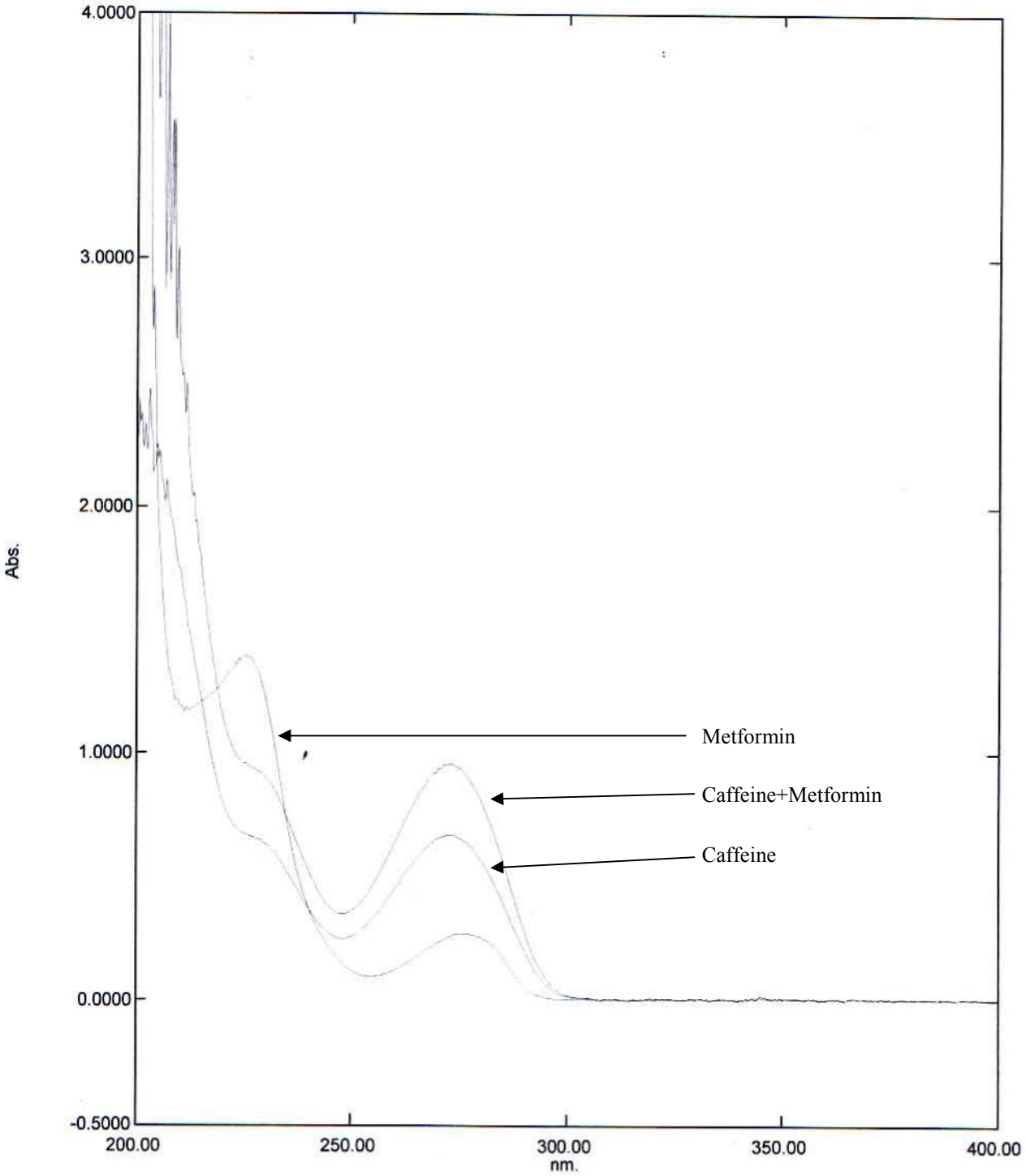


Fig.7.1.2.21: UV spectra of Caffeine – Metformin systems at pH 5.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

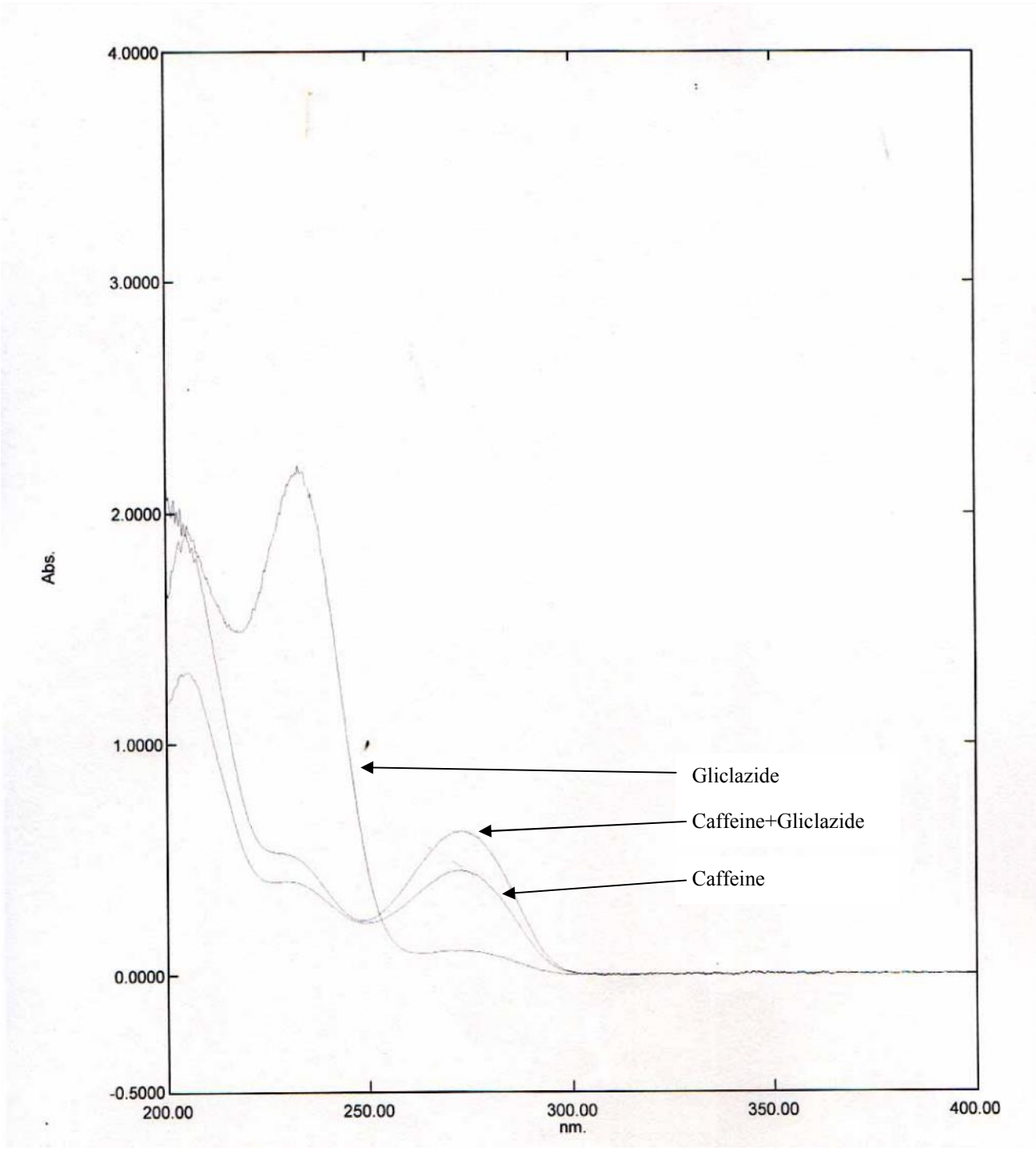


Fig.7.1.2.22: UV spectra of Caffeine – Gliclazide systems at pH 6.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

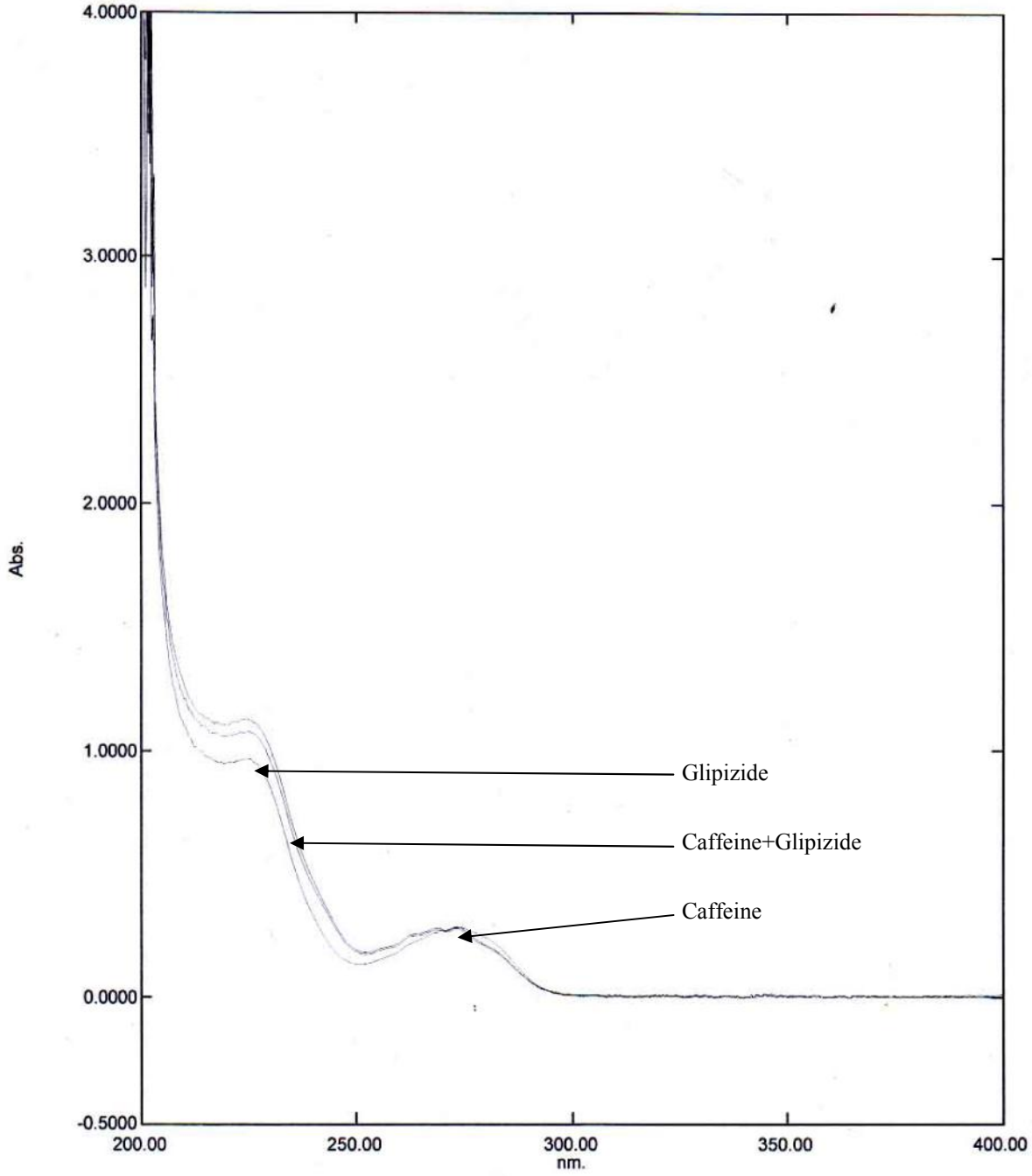


Fig.7.1.2.23: UV spectra of Caffeine – Glipizide systems at pH 6.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

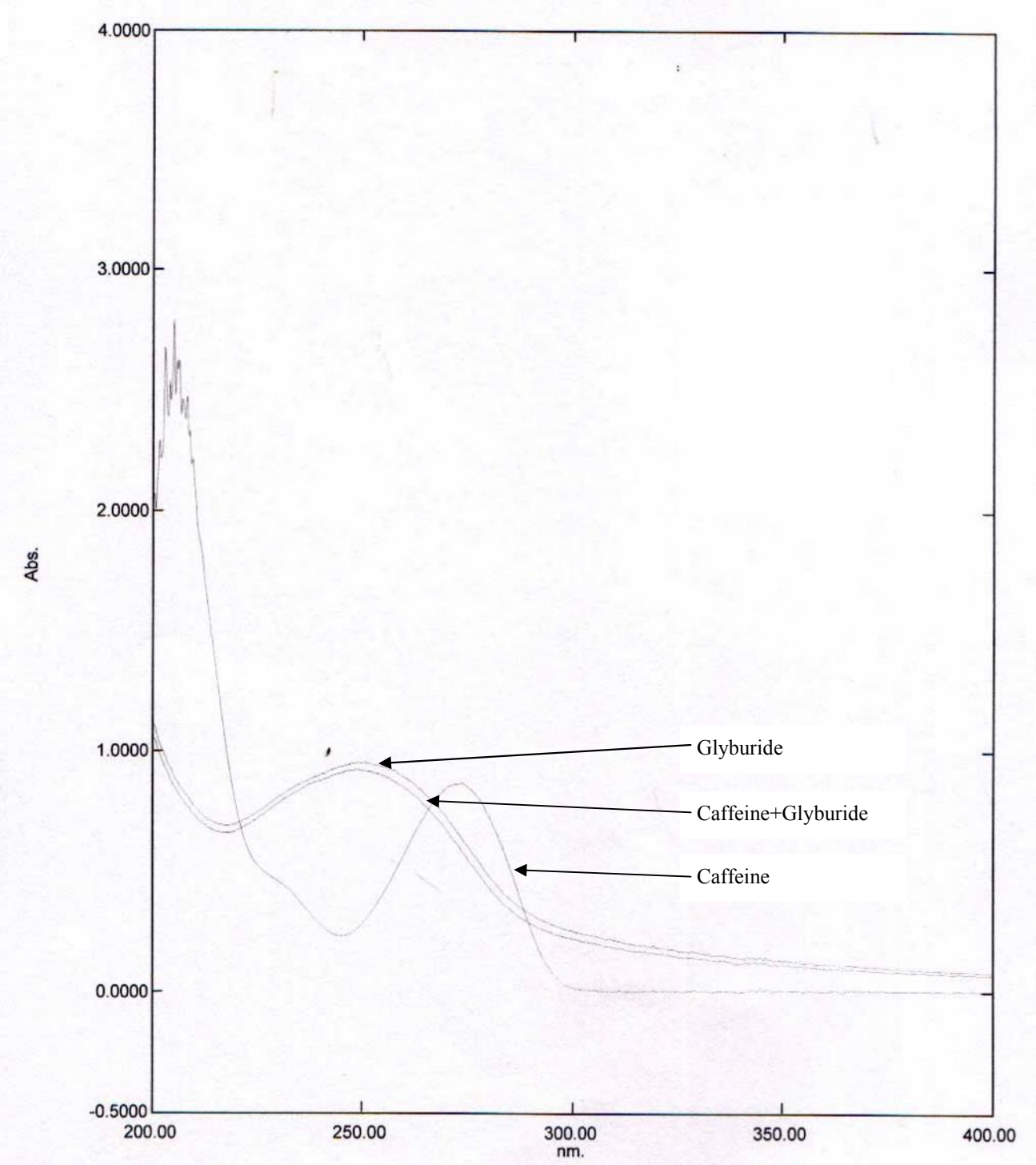


Fig.7.1.2.24: UV spectra of Caffeine – Glyburide systems at pH 6.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

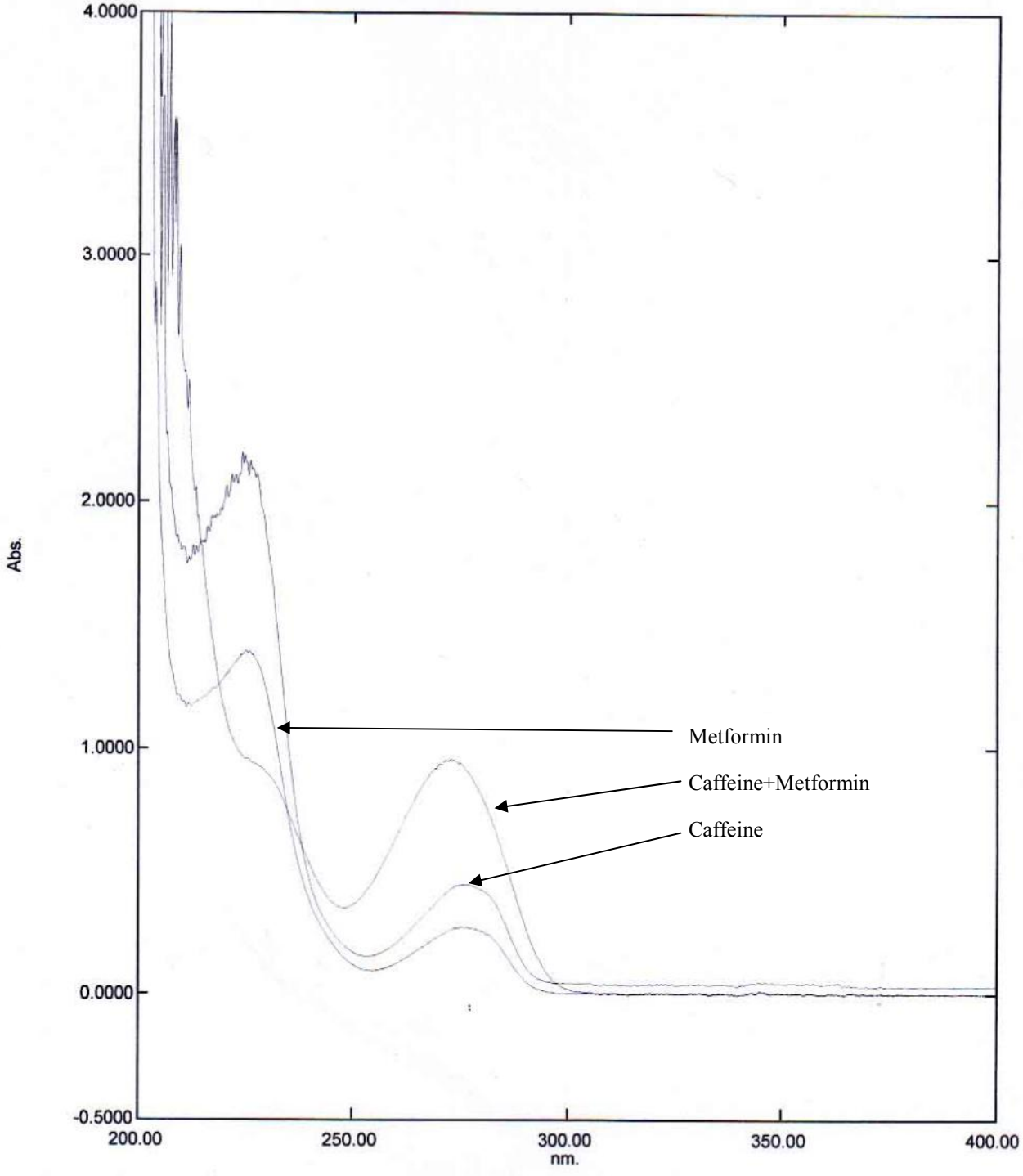


Fig.7.1.2.25: UV spectra of Caffeine – Metformin systems at pH 6.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

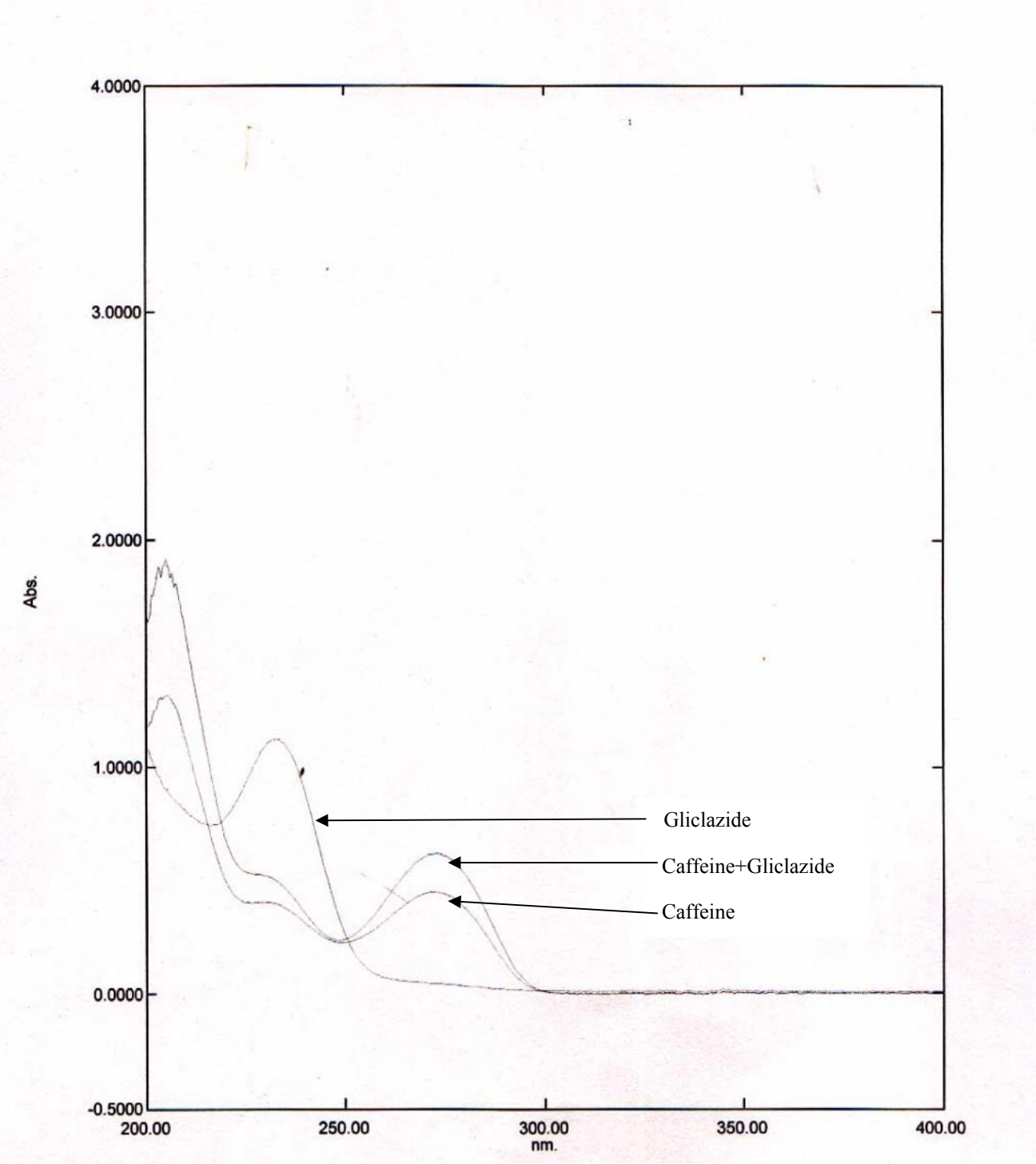


Fig.7.1.2.26: UV spectra of Caffeine – Gliclazide systems at pH 7.4 (Conc. of Caffeine = Conc. of Gliclazide = 0.0001 M)

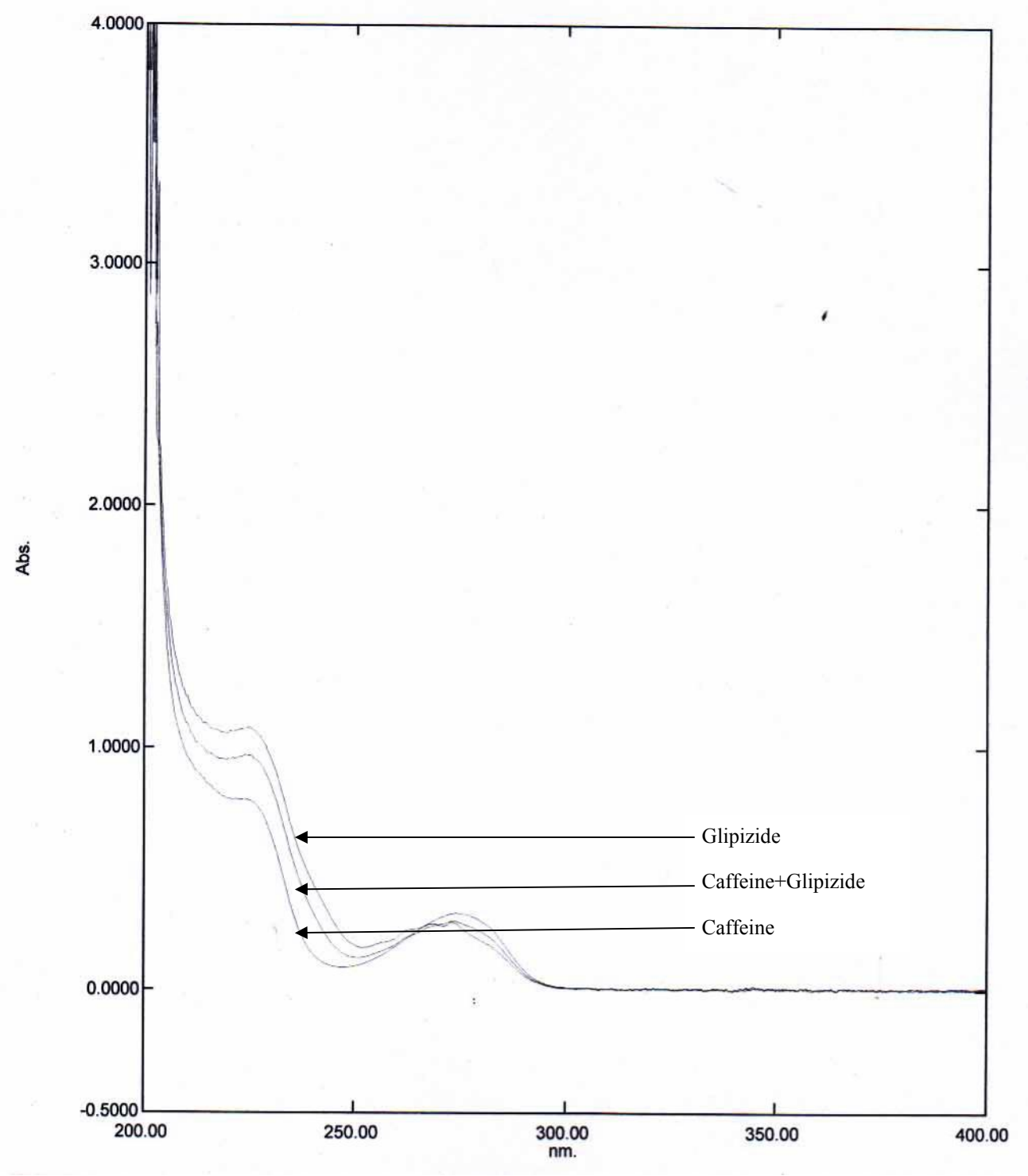


Fig.7.1.2.27: UV spectra of Caffeine – Glipizide systems at pH 7.4 (Conc. of Caffeine = Conc. of Glipizide = 0.0001 M)

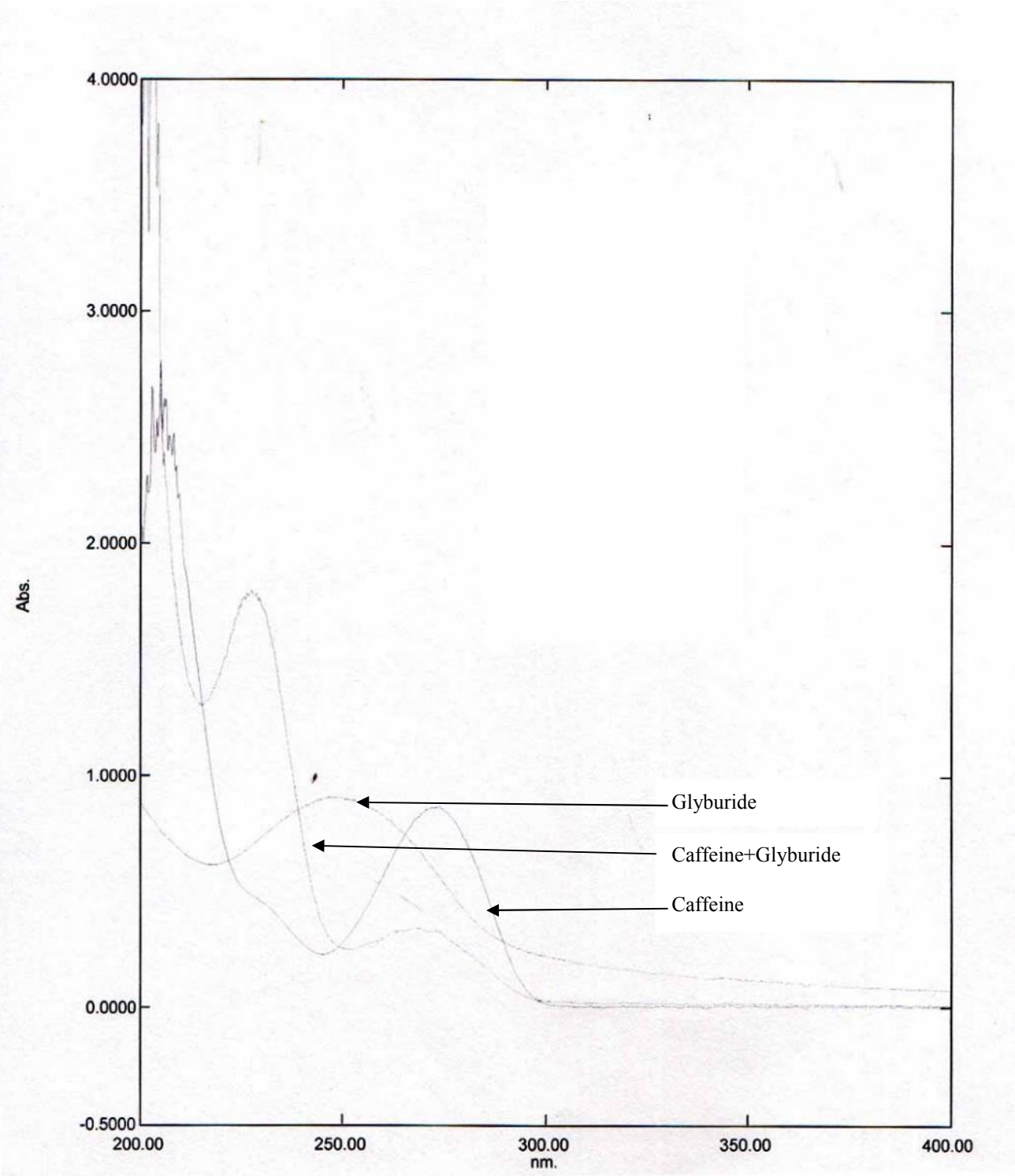


Fig.7.1.2.28: UV spectra of Caffeine – Glyburide systems at pH 7.4 (Conc. of Caffeine = Conc. of Glyburide = 0.0001 M)

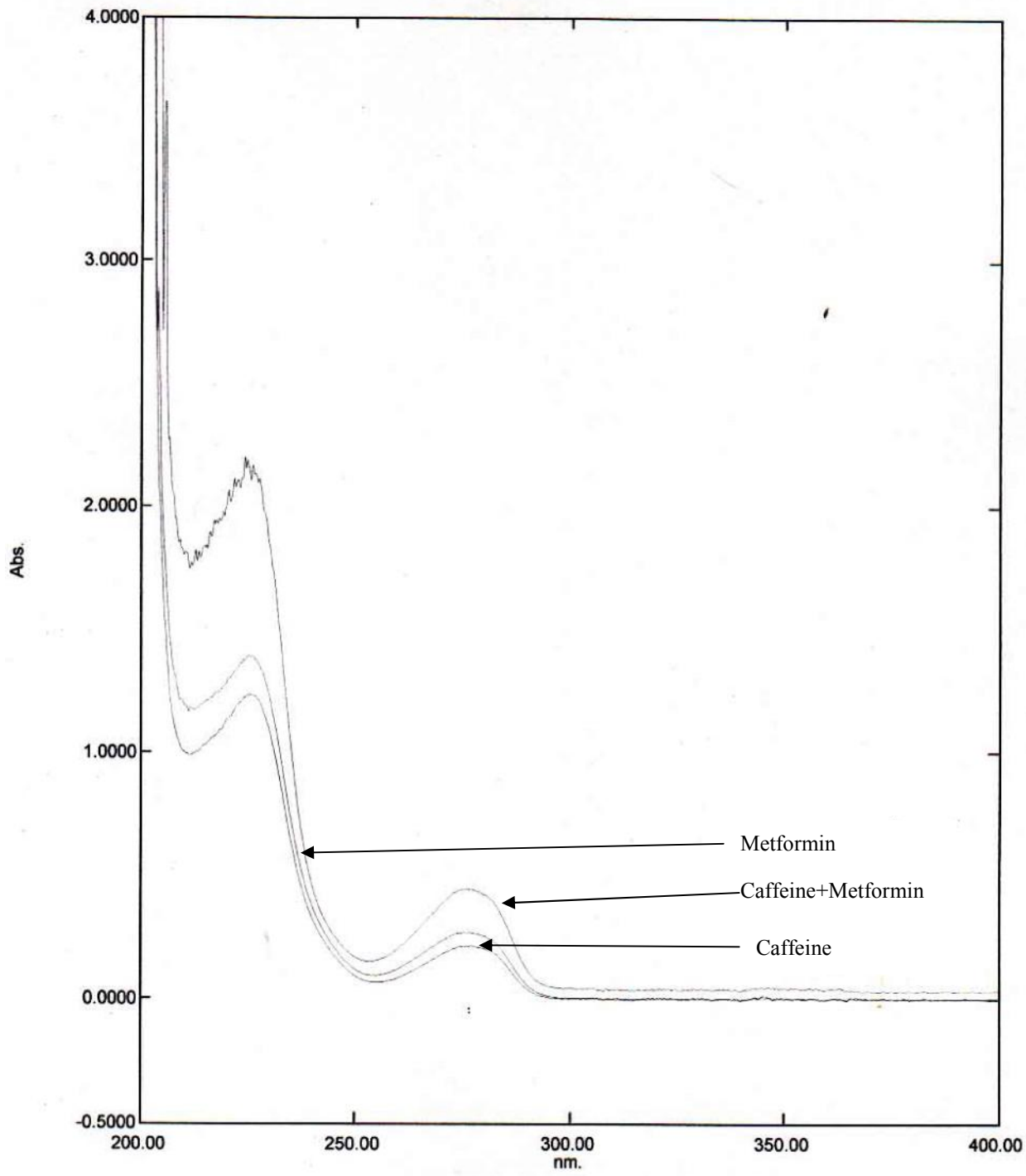


Fig.7.1.2.29: UV spectra of Caffeine – Metformin systems at pH 7.4 (Conc. of Caffeine = Conc. of Metformin = 0.0001 M)

From above spectrum, it can be inferred that the spectra of target molecules alone and the mixture (1:1) of caffeine with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of caffeine with the drugs that alter the absorption intensities as donor-acceptor complexation occurs.

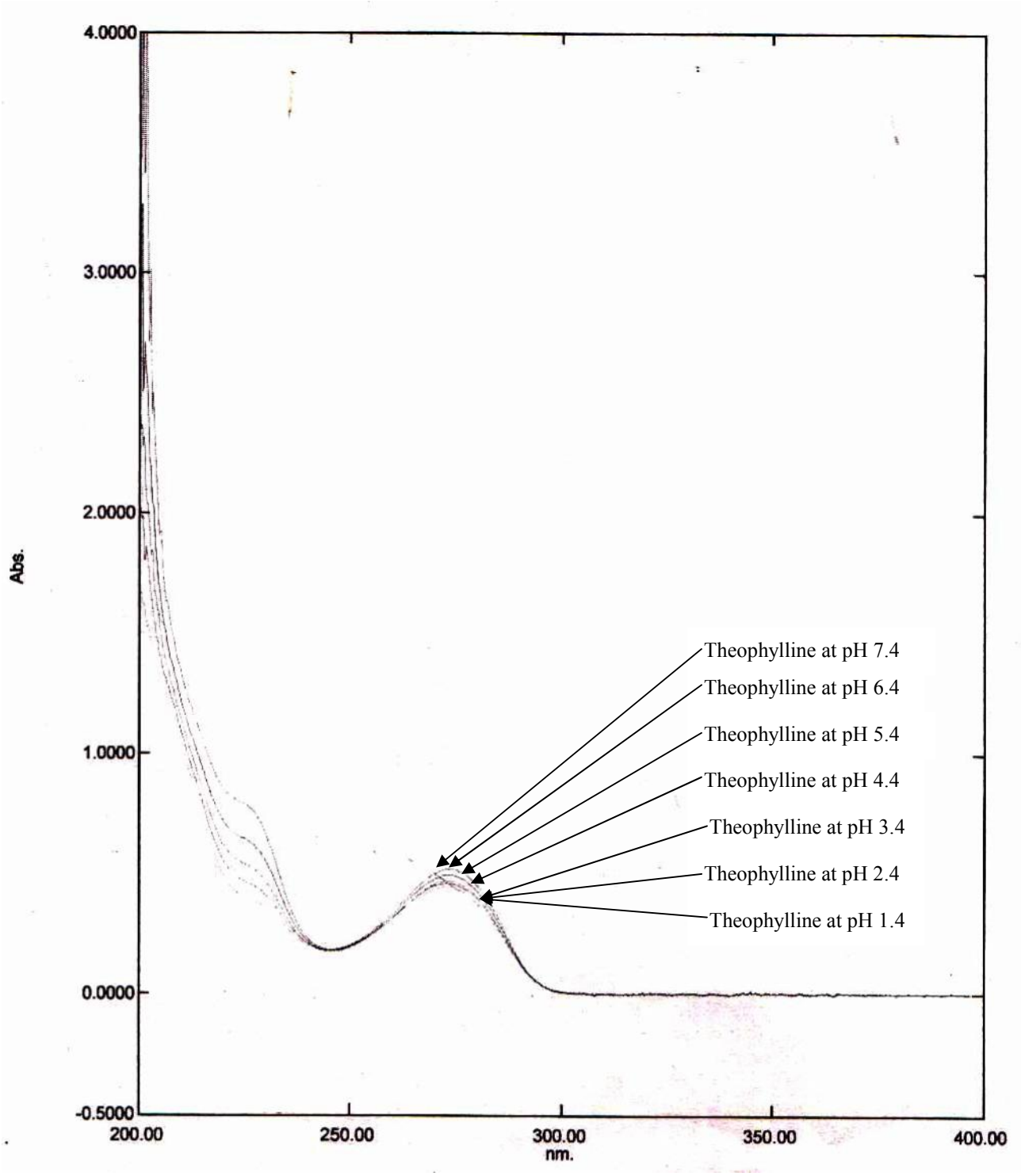


Fig.7.1.2.30: UV spectra of Theophylline at different p^H (Conc. of theophylline = 0.0001 M)

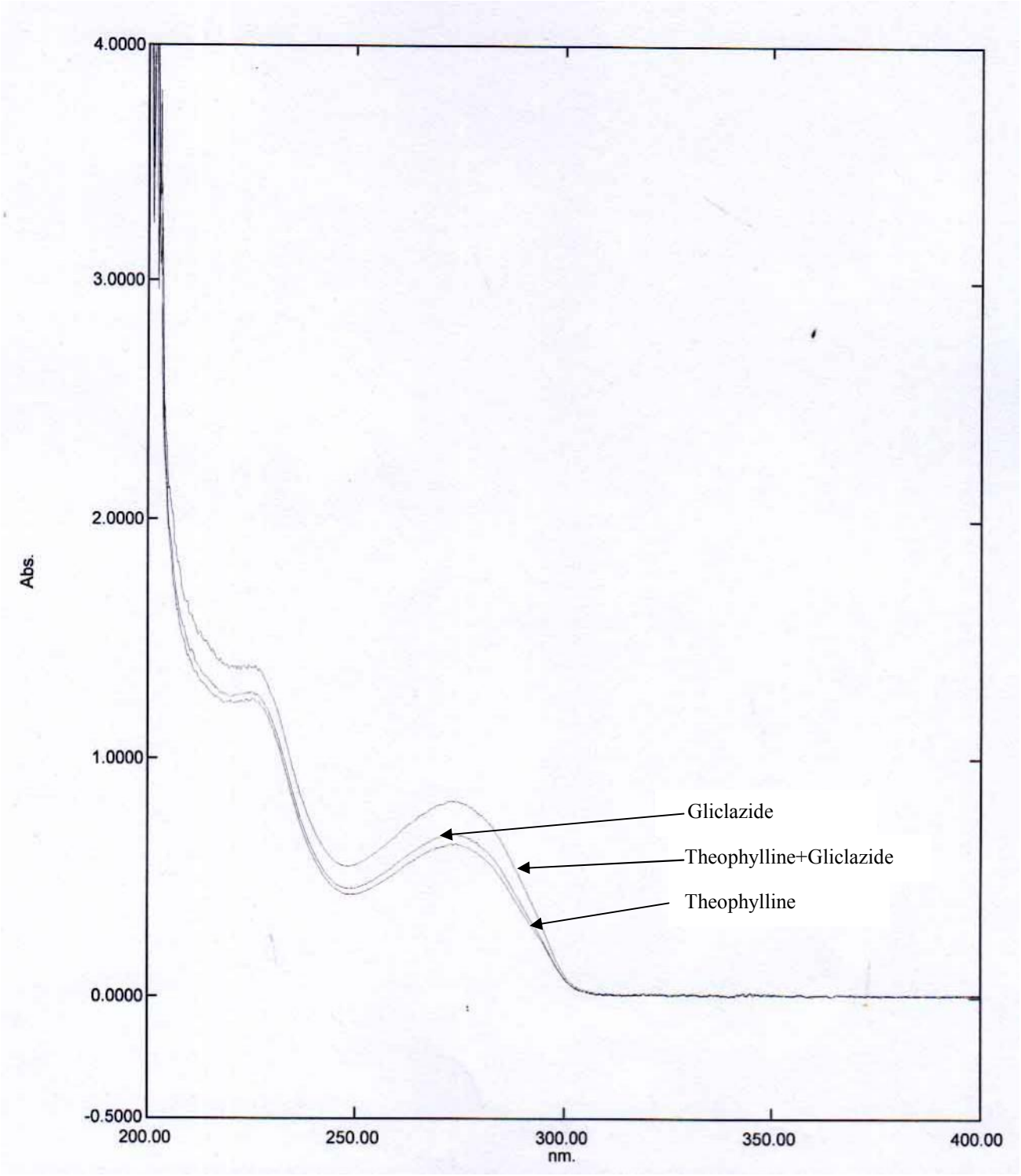


Fig.7.1.2.31: UV spectra of Theophylline – Gliclazide systems at pH 1.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

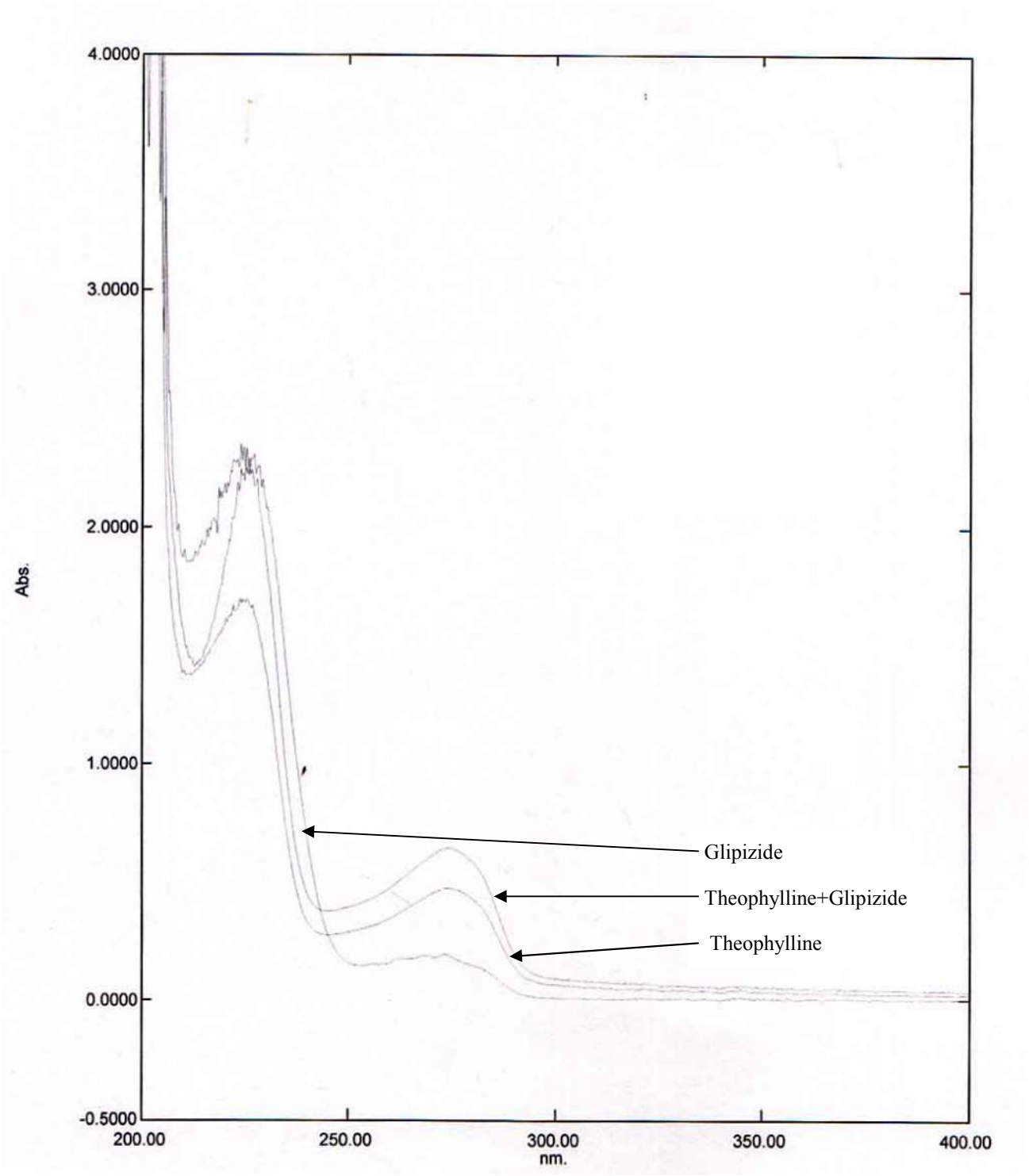


Fig.7.1.2.32: UV spectra of Theophylline – Glipizide systems at pH 1.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

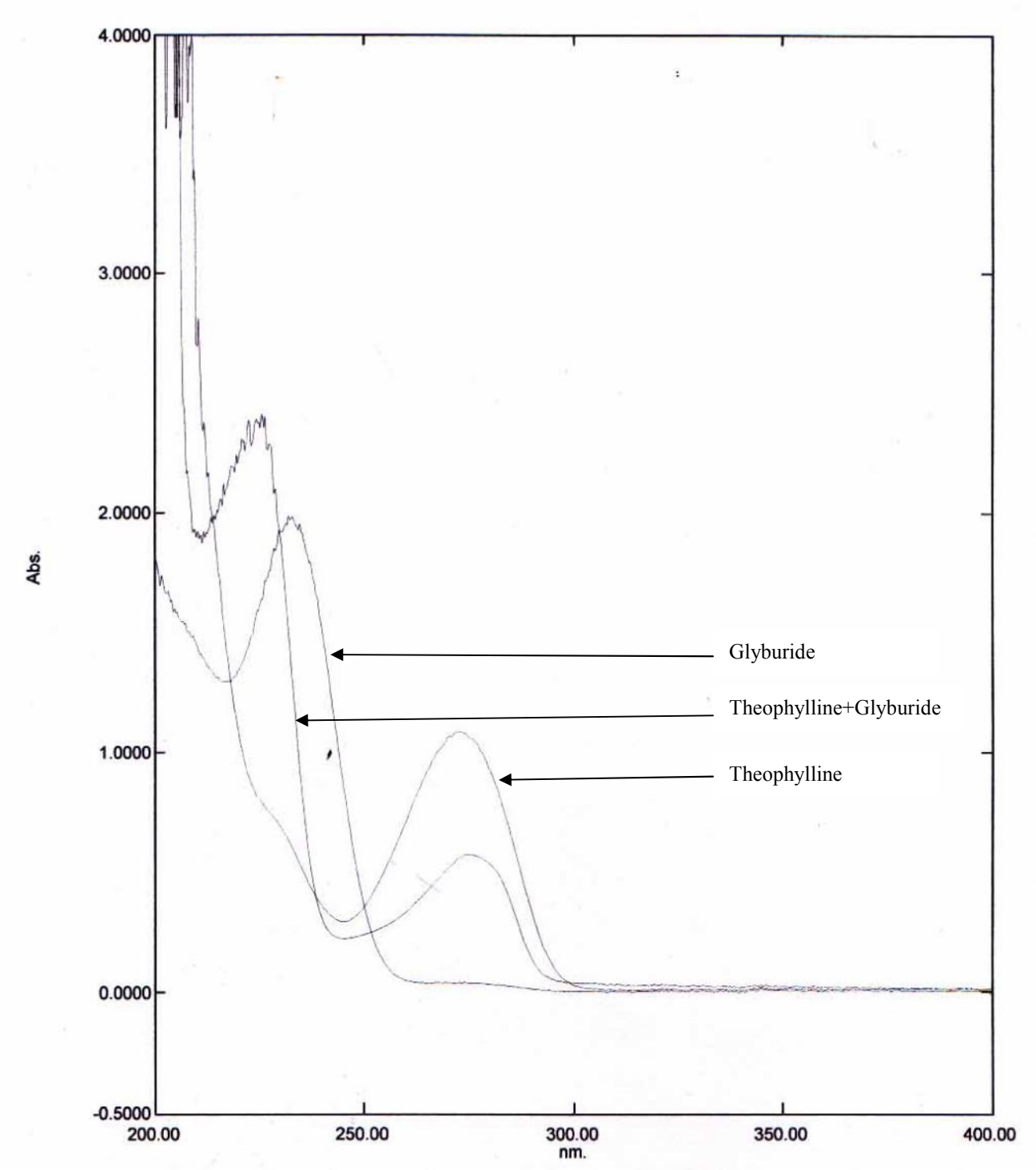


Fig.7.1.2.33: UV spectra of Theophylline – Glyburide systems at pH 1.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

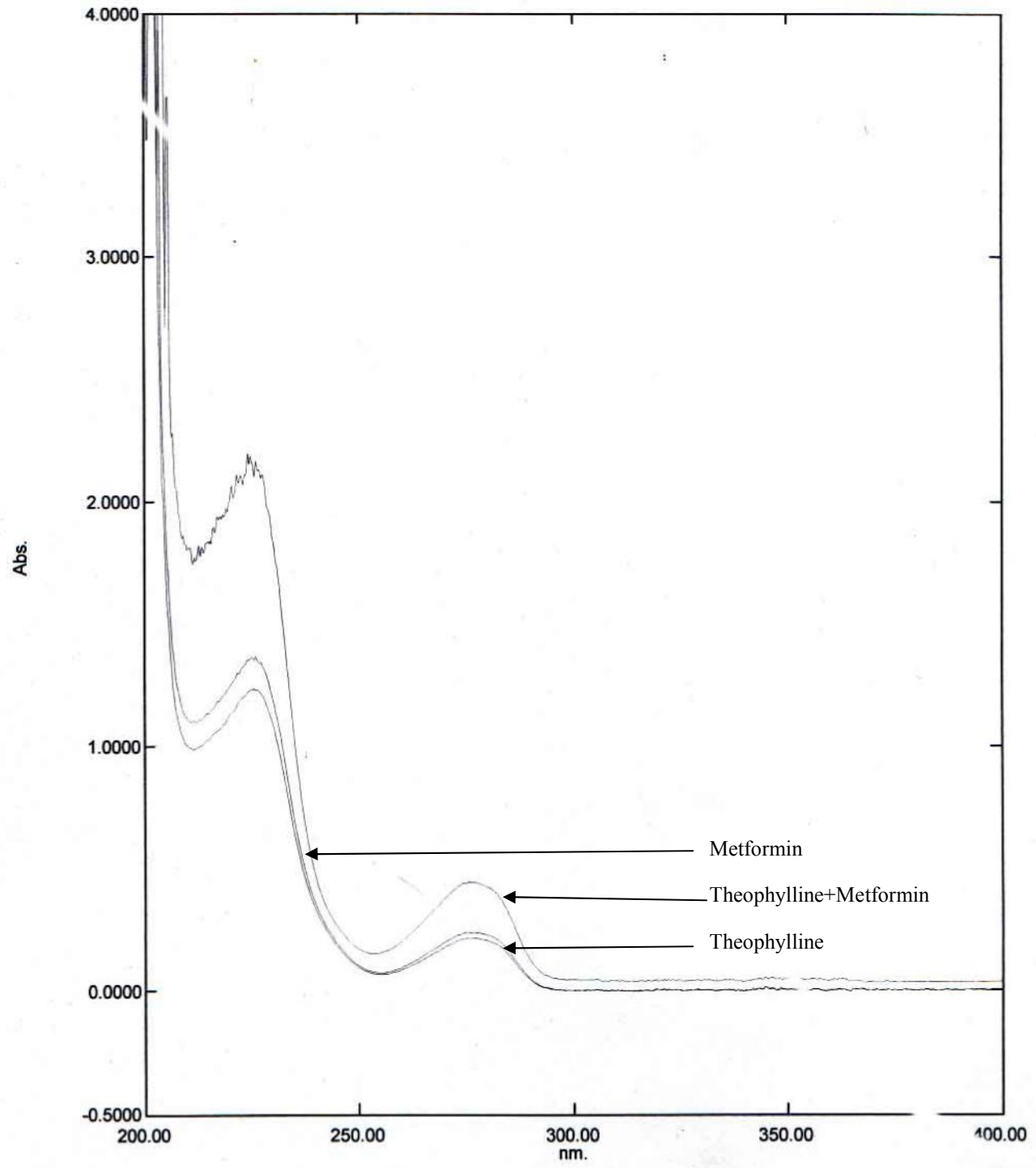


Fig.7.1.2.34: UV spectra of Theophylline – Metformin systems at pH 1.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

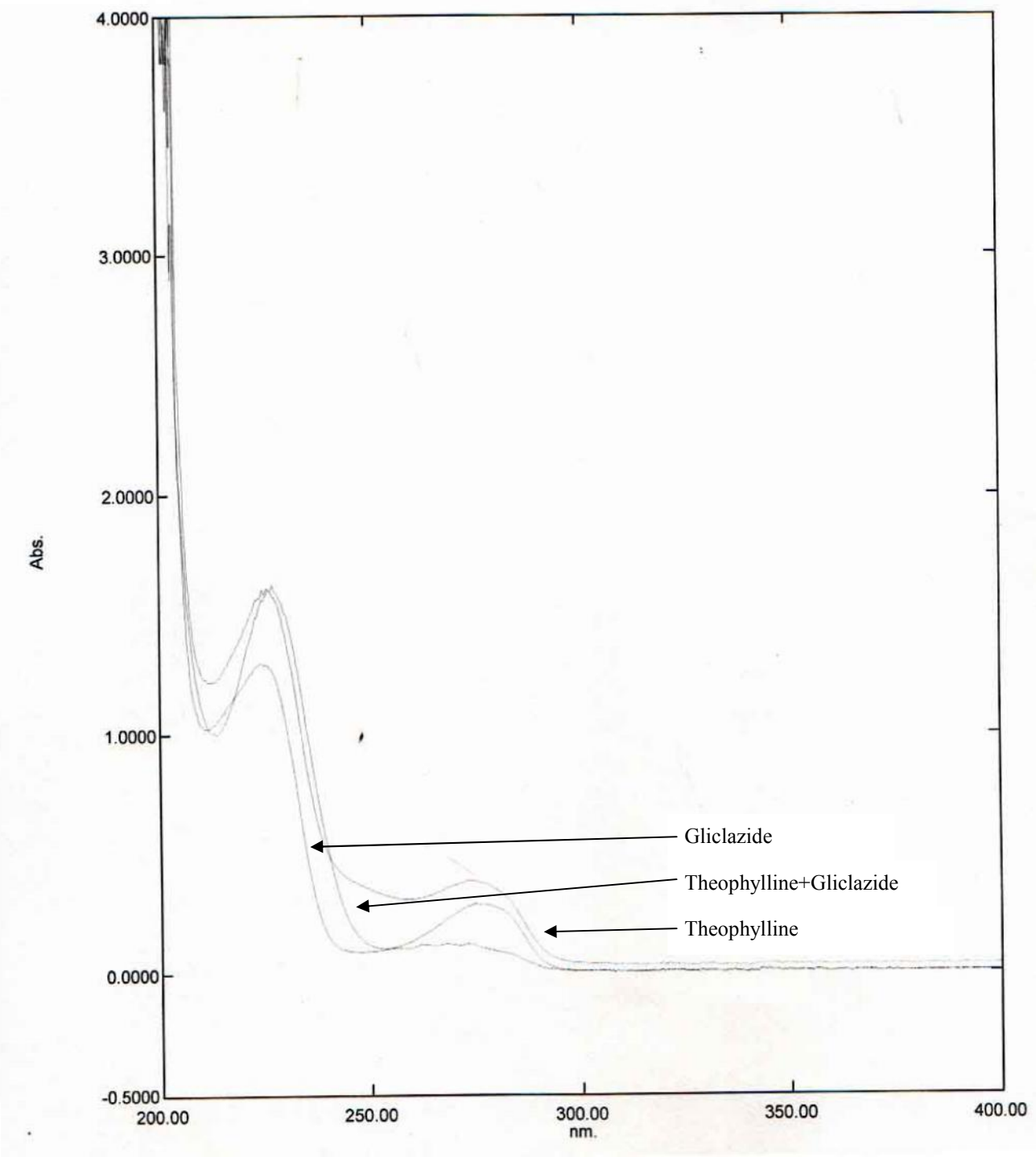


Fig.7.1.2.35: UV spectra of Theophylline – Gliclazide systems at pH 2.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

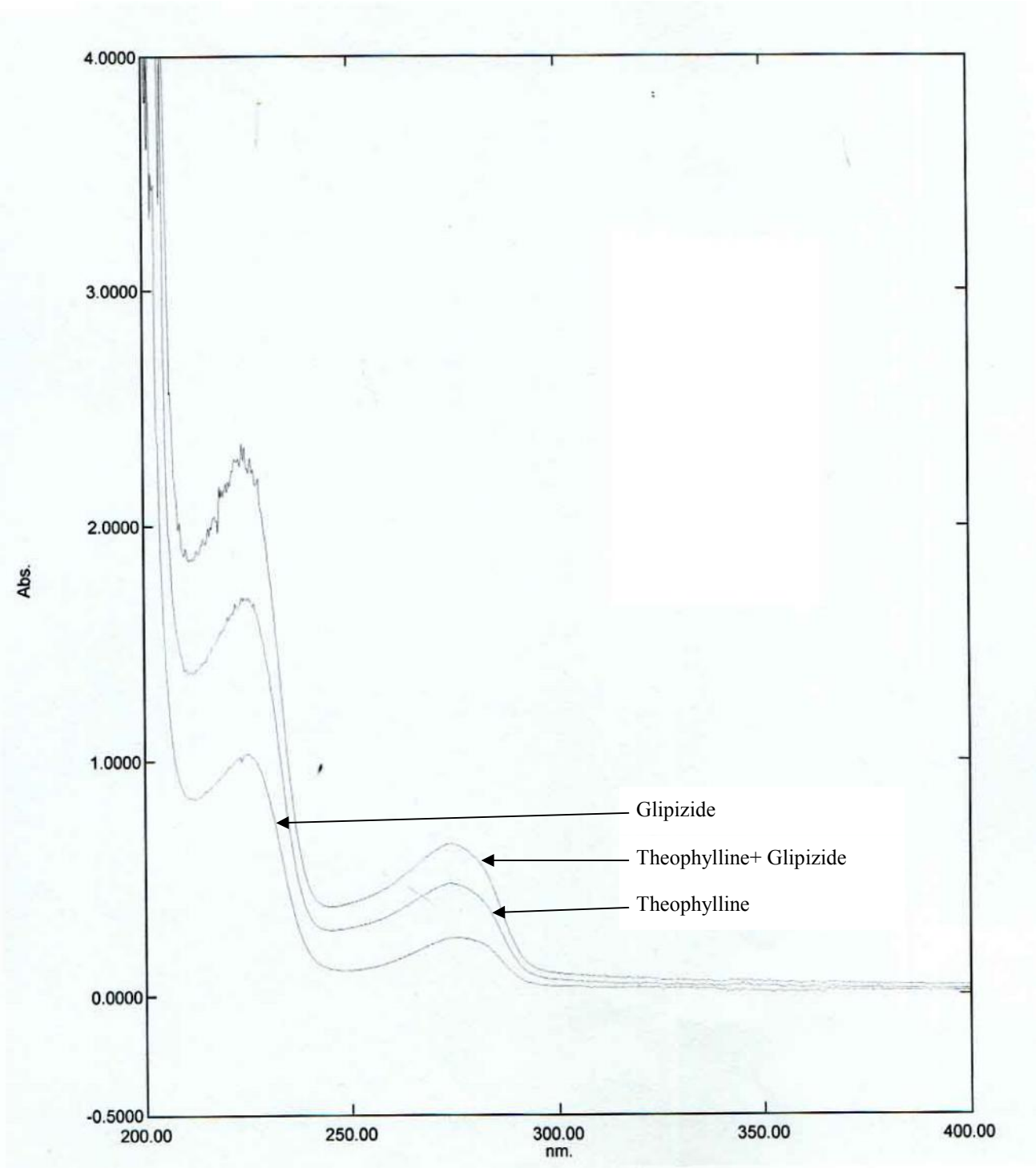


Fig.7.1.2.36: UV spectra of Theophylline – Glipizide systems at pH 2.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

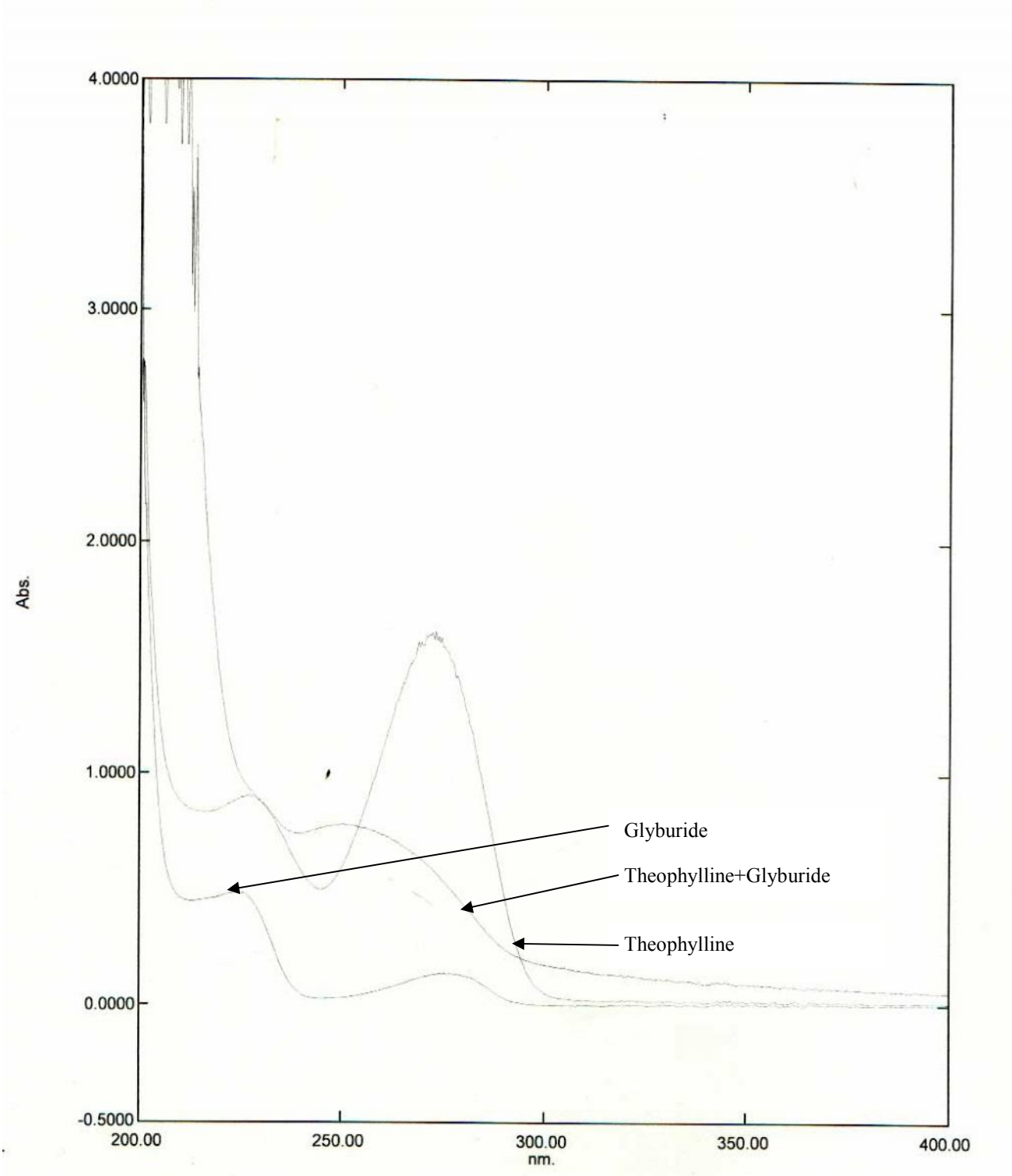


Fig.7.1.2.37: UV spectra of Theophylline – Glyburide systems at pH 2.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

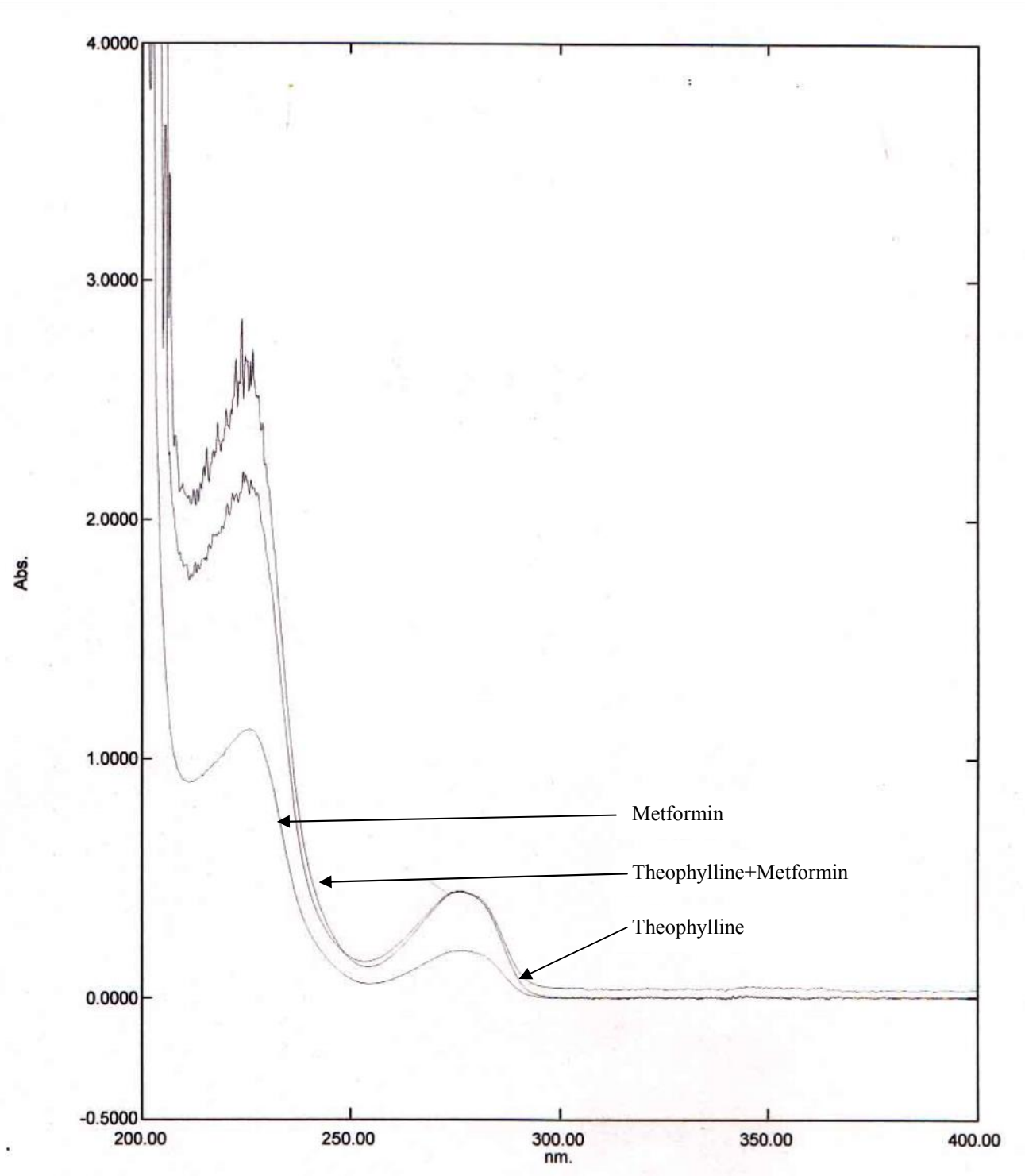


Fig.7.1.2.38: UV spectra of Theophylline – Metformin systems at pH 2.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

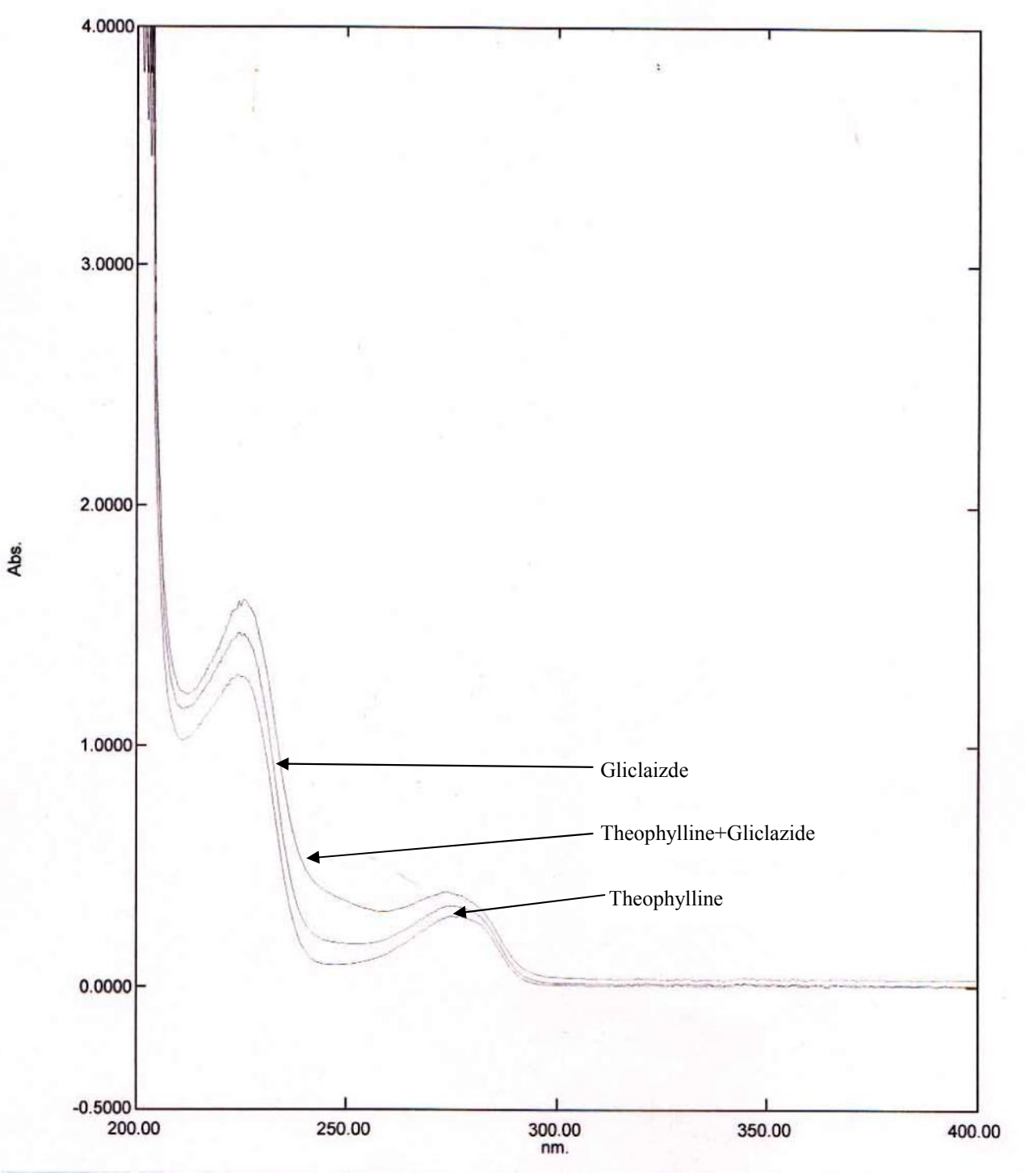


Fig.7.1.2.39: UV spectra of Theophylline – Gliclazide systems at pH 3.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

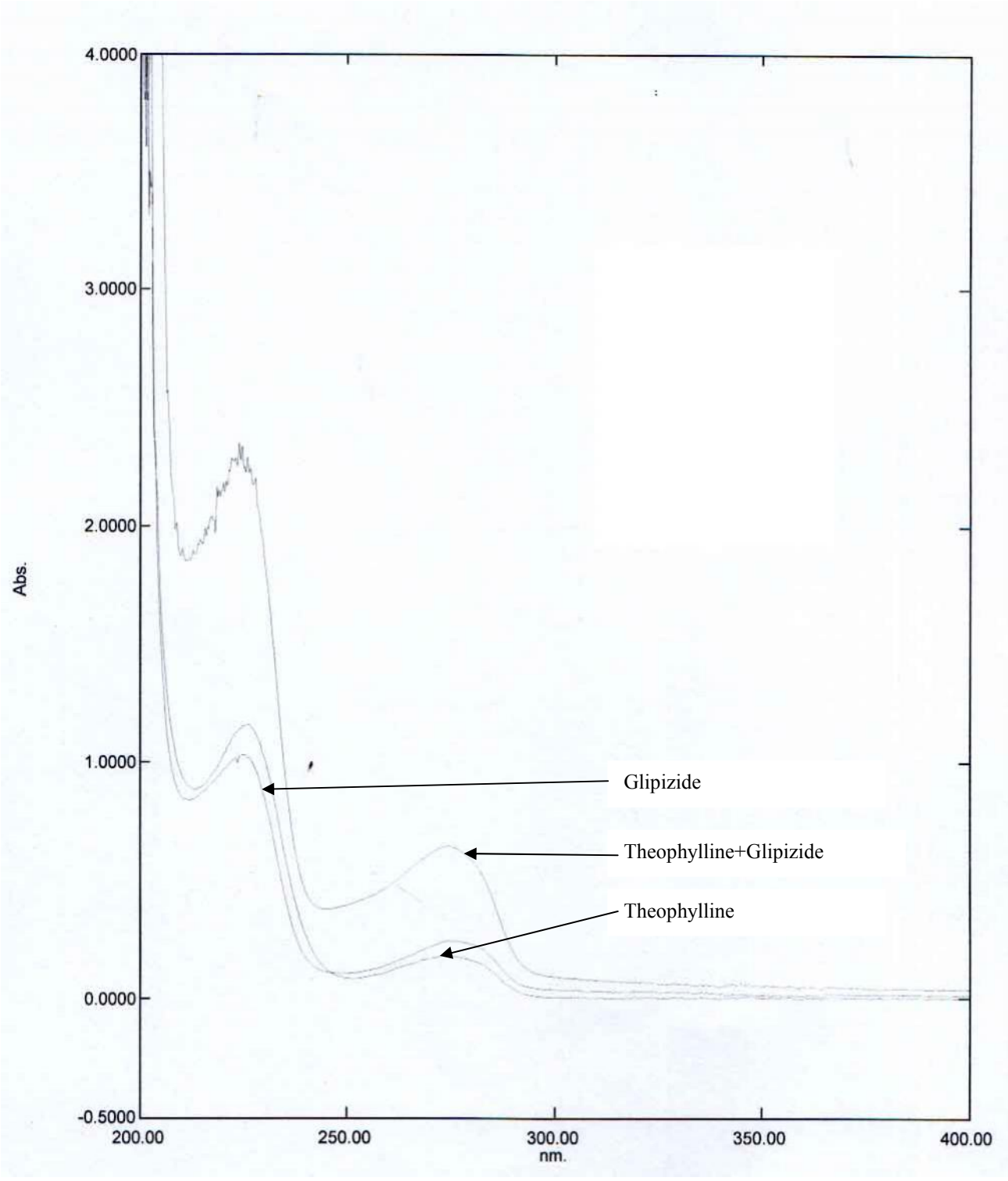


Fig.7.1.2.40: UV spectra of Theophylline – Glipizide systems at pH 3.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

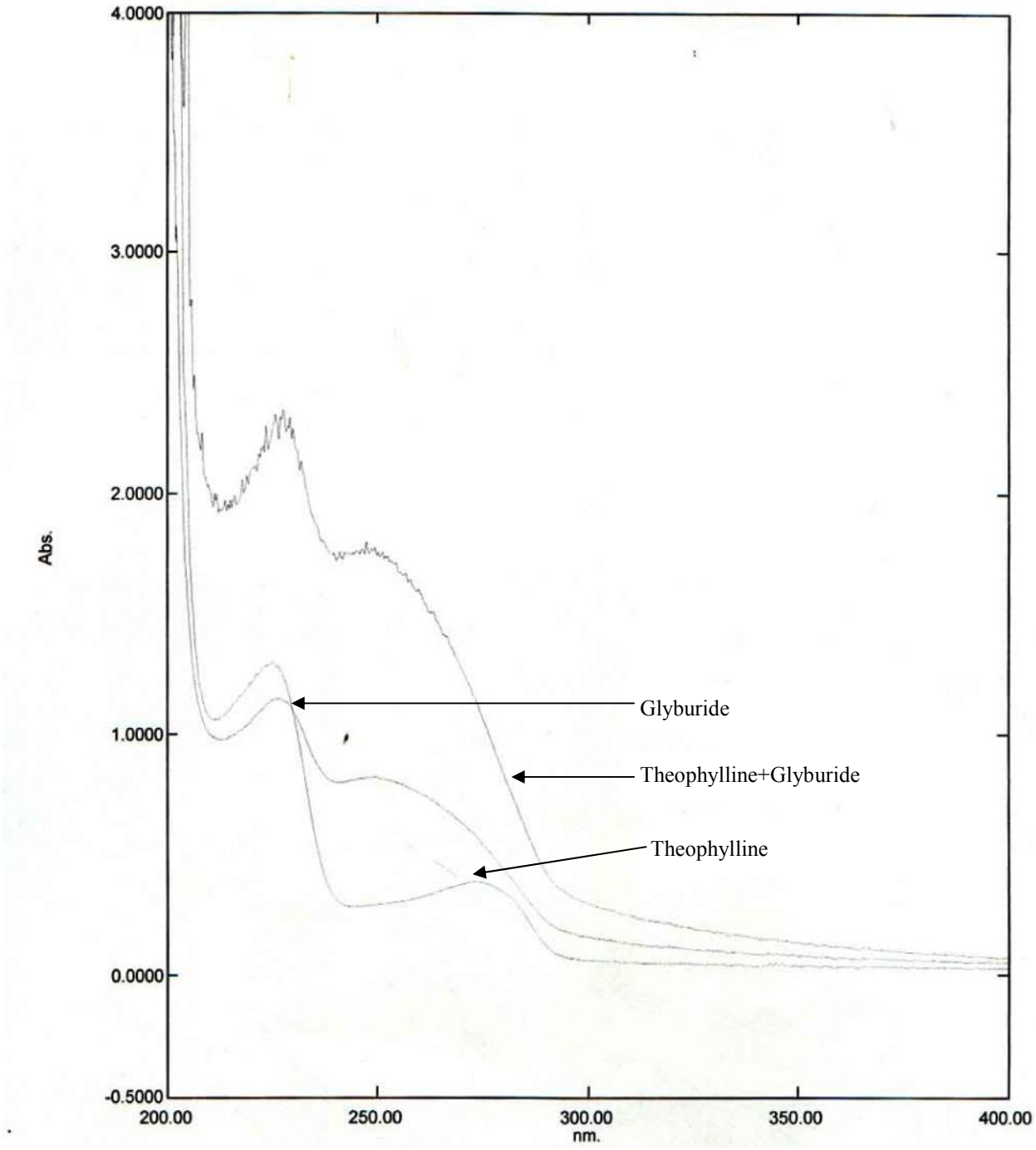


Fig.7.1.2.41: UV spectra of Theophylline – Glyburide systems at pH 3.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

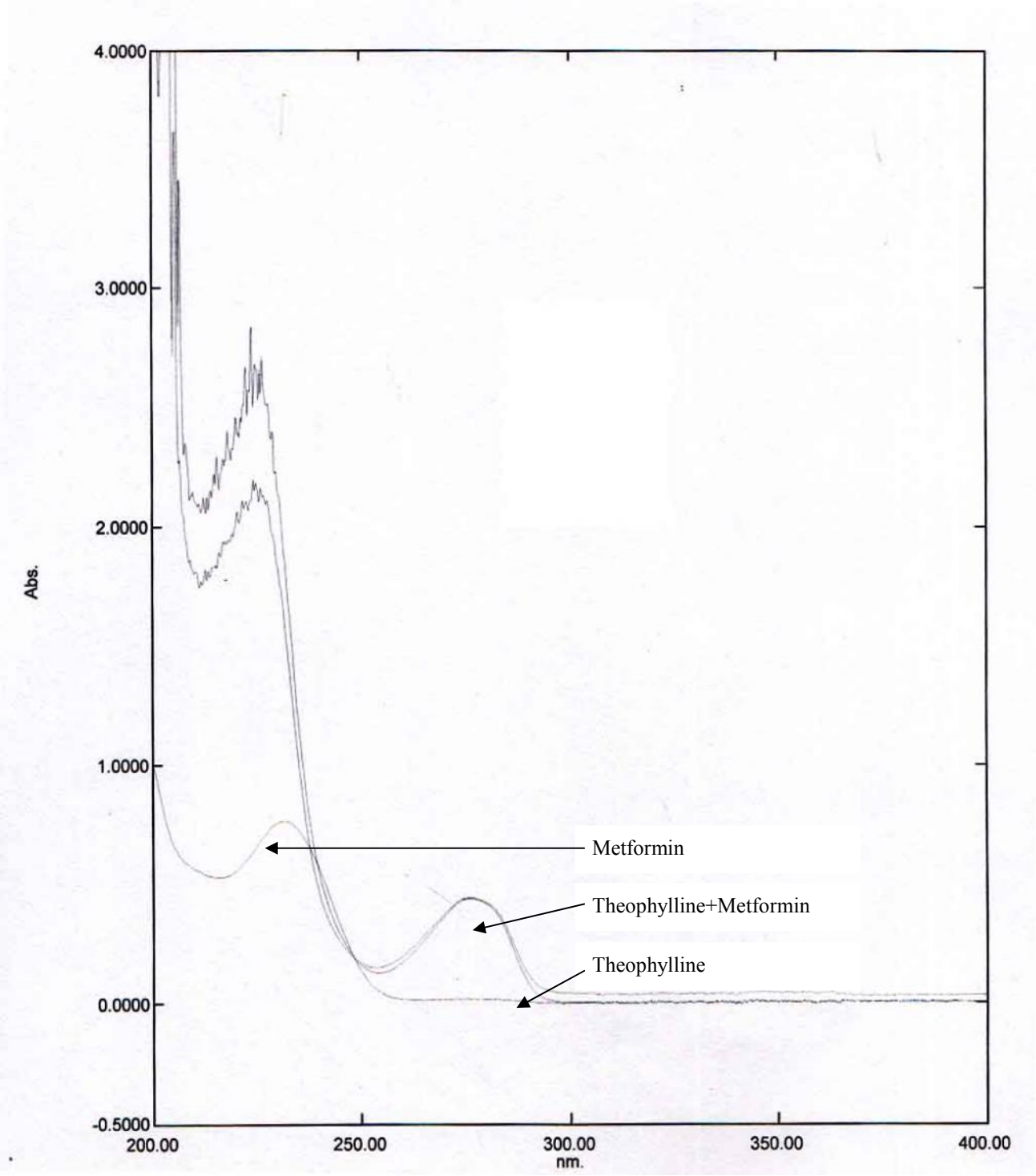


Fig.7.1.2.42: UV spectra of Theophylline – Metformin systems at pH 3.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

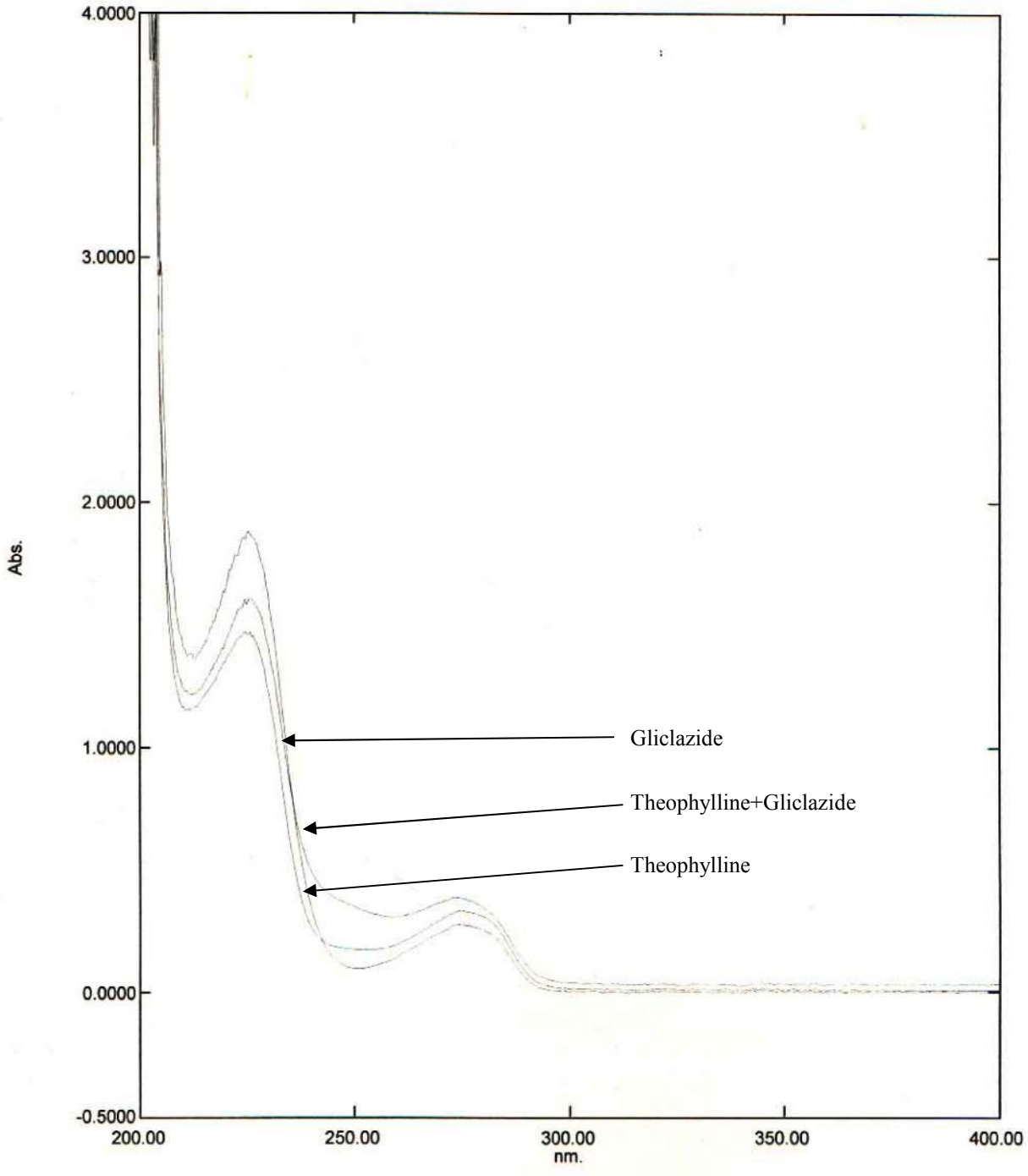


Fig.7.1.2.43: UV spectra of Theophylline – Gliclazide systems at pH 4.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

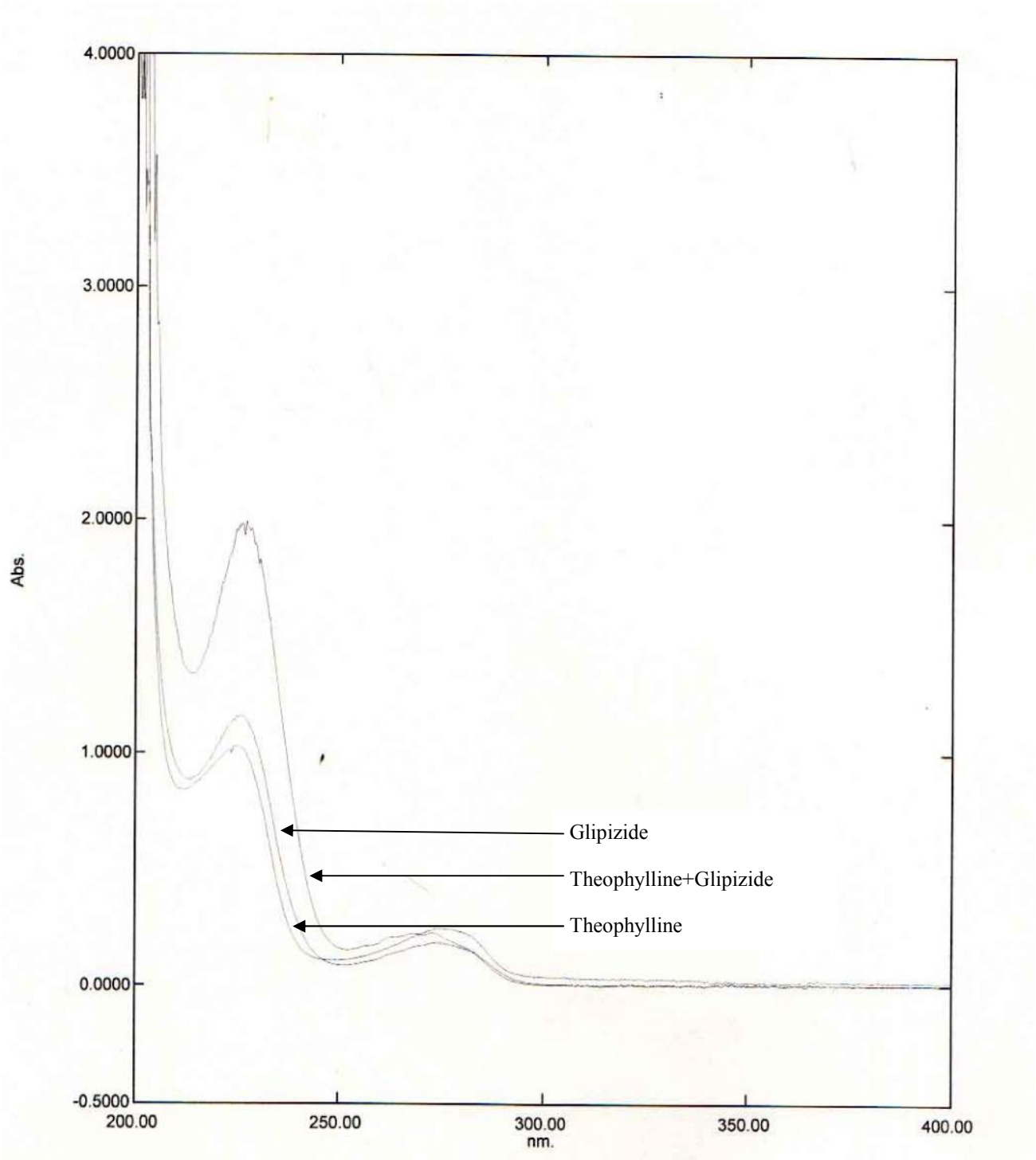


Fig.7.1.2.44: UV spectra of Theophylline – Glipizide systems at pH 4.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

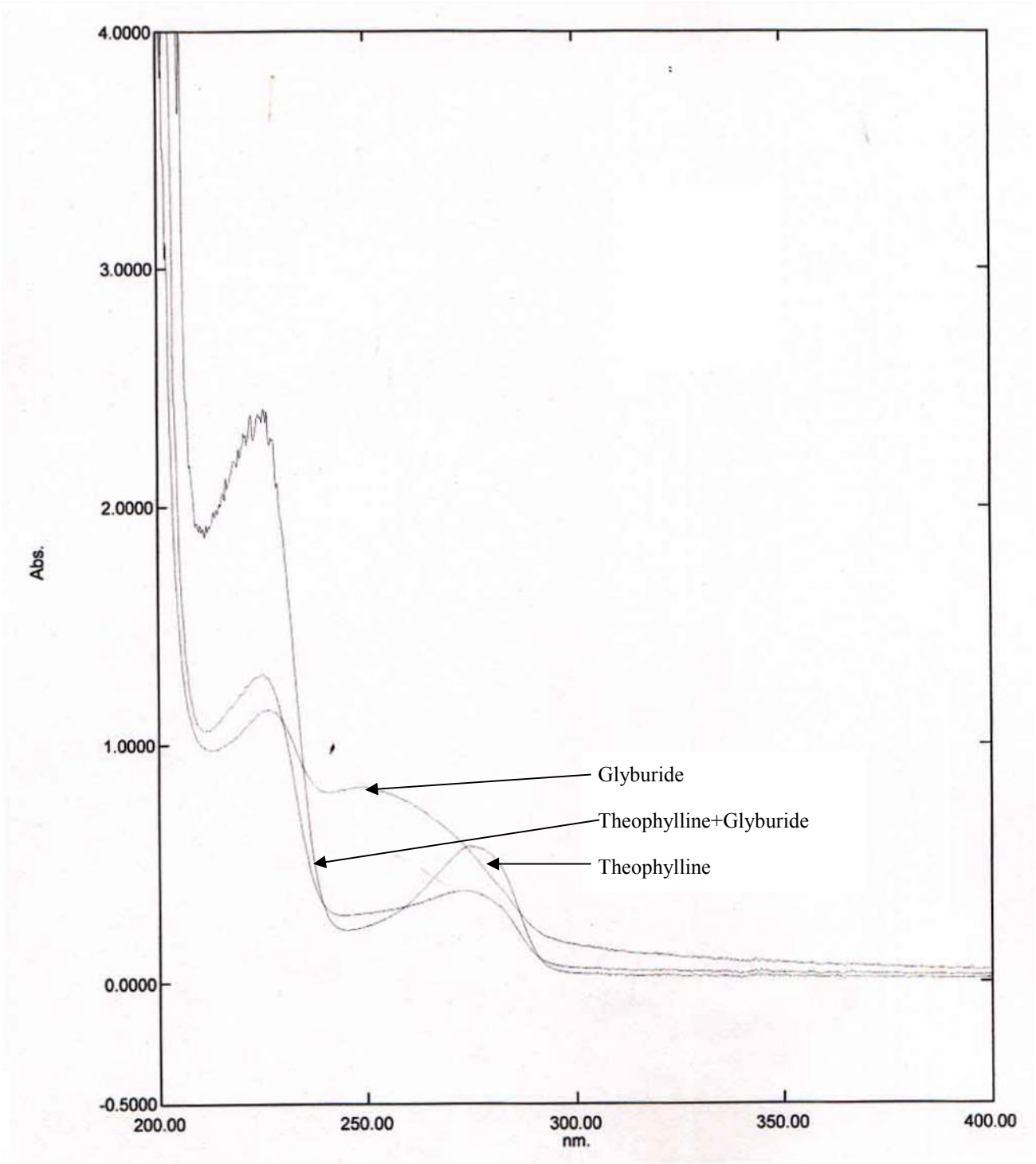


Fig.7.1.2.45: UV spectra of Theophylline – Glyburide systems at pH 4.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

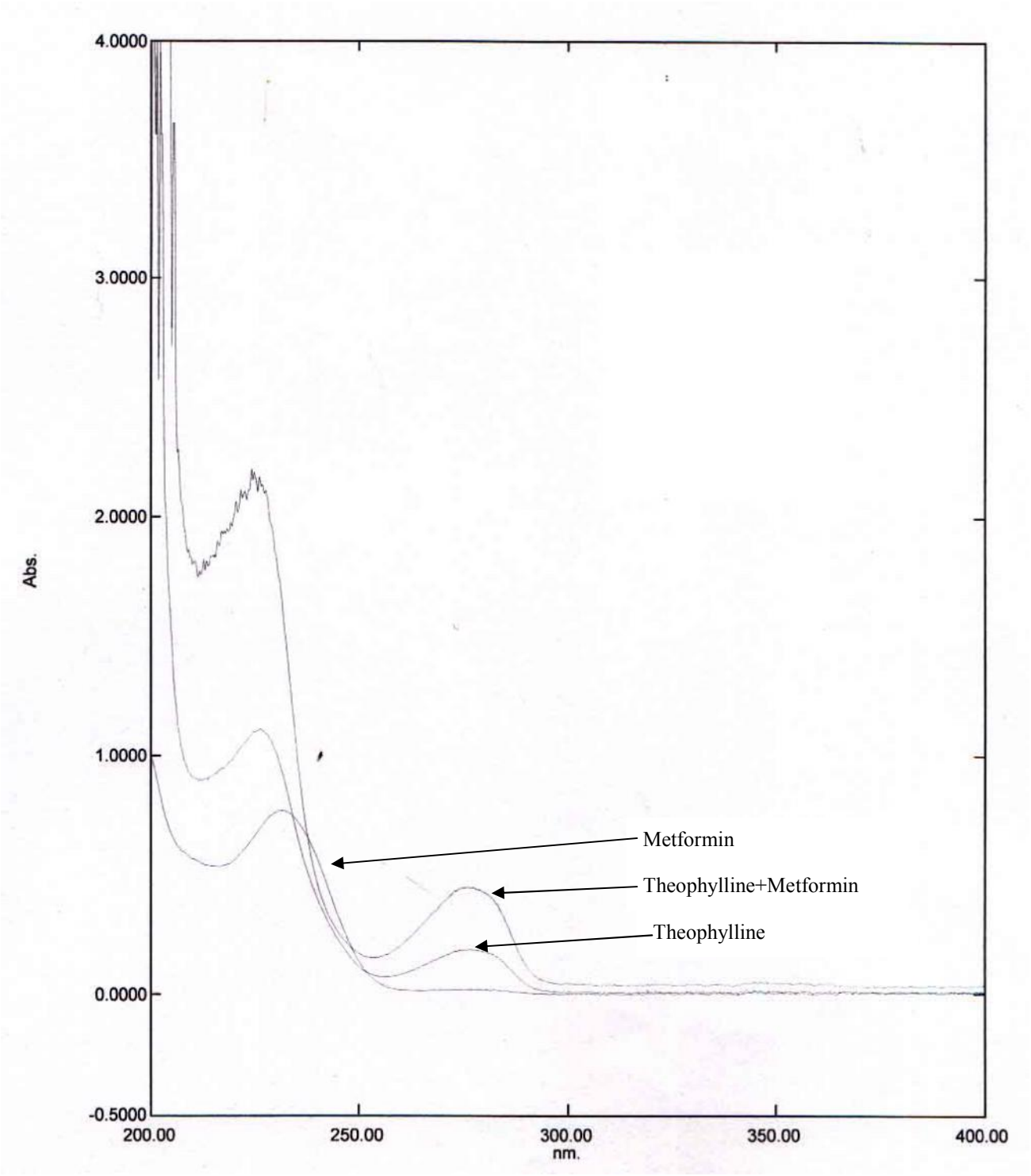


Fig.7.1.2.46: UV spectra of Theophylline – Metformin systems at pPH 4.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

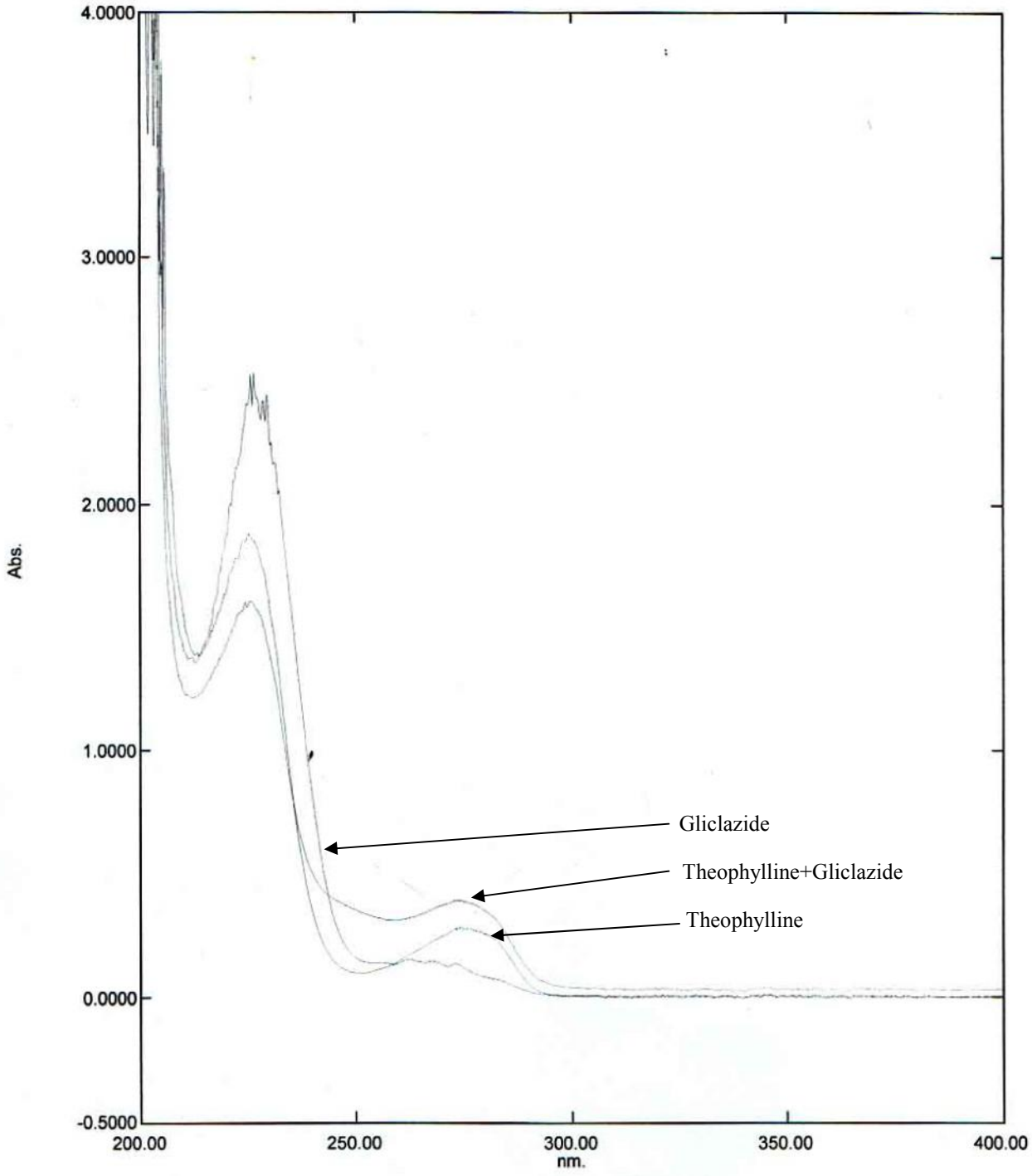


Fig.7.1.2.47: UV spectra of Theophylline – Gliclazide systems at pH 5.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

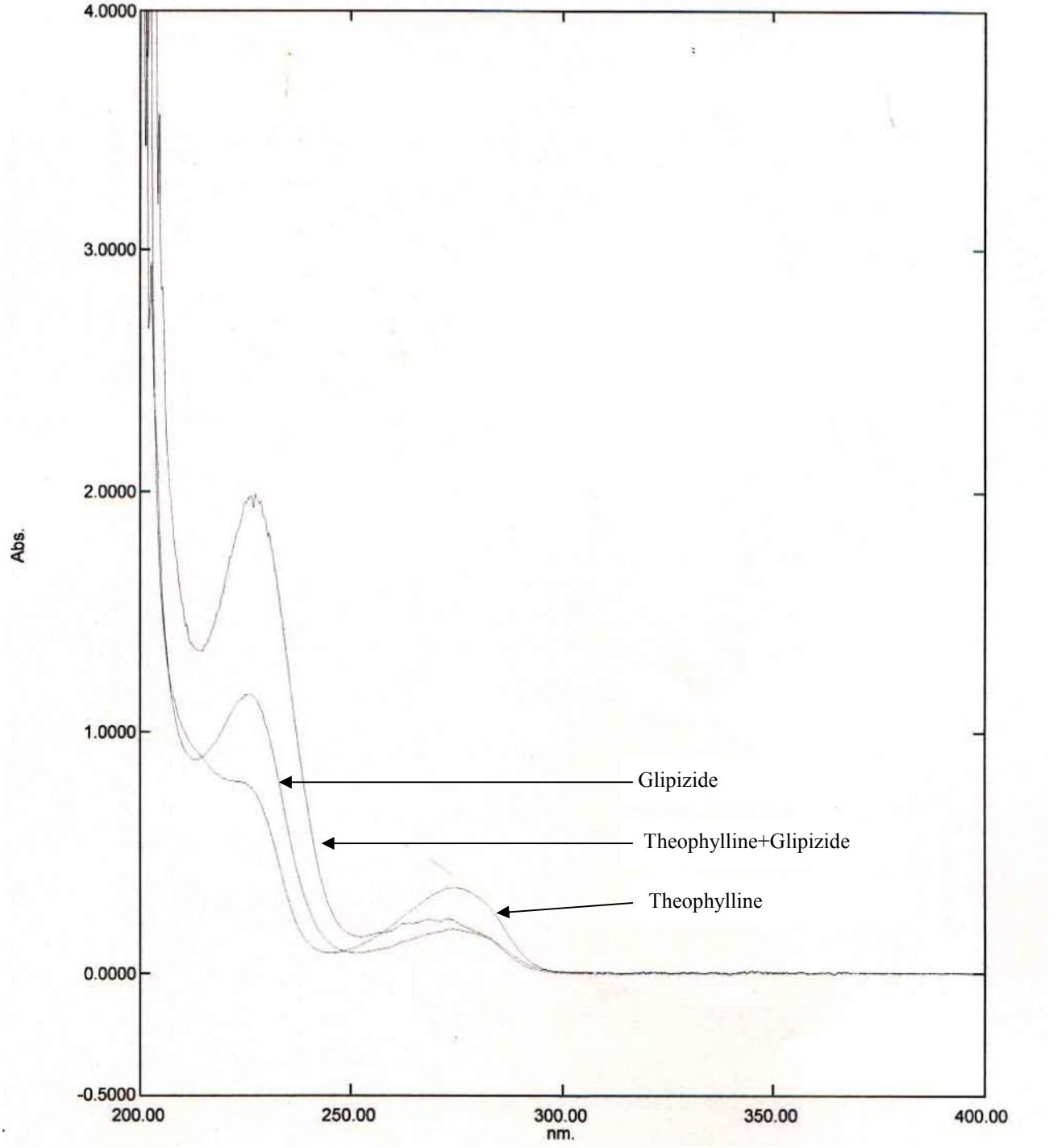


Fig.7.1.2.48: UV spectra of Theophylline – Glipizide systems at pH 5.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

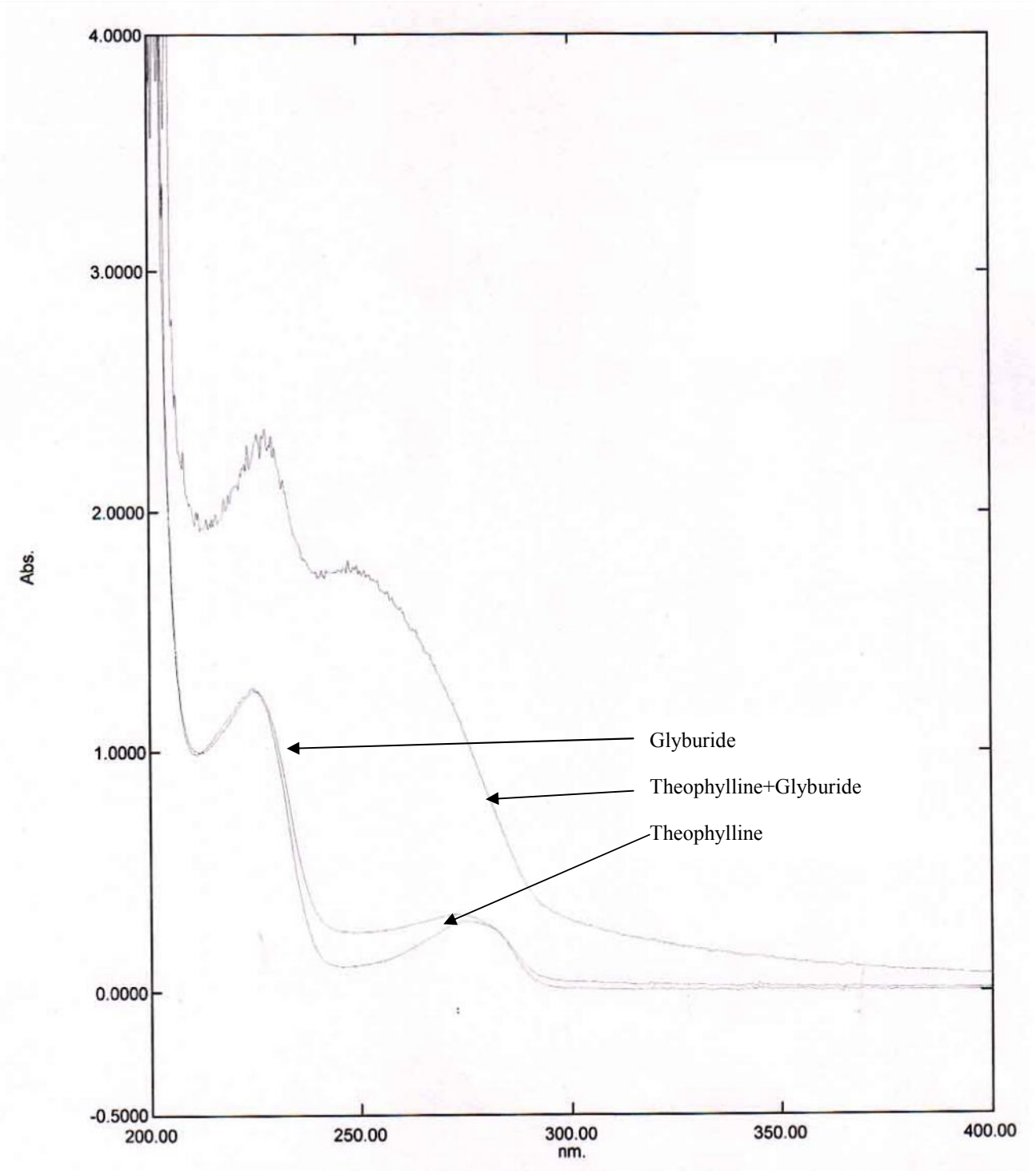


Fig.7.1.2.49: UV spectra of Theophylline – Glyburide systems at pH 5.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

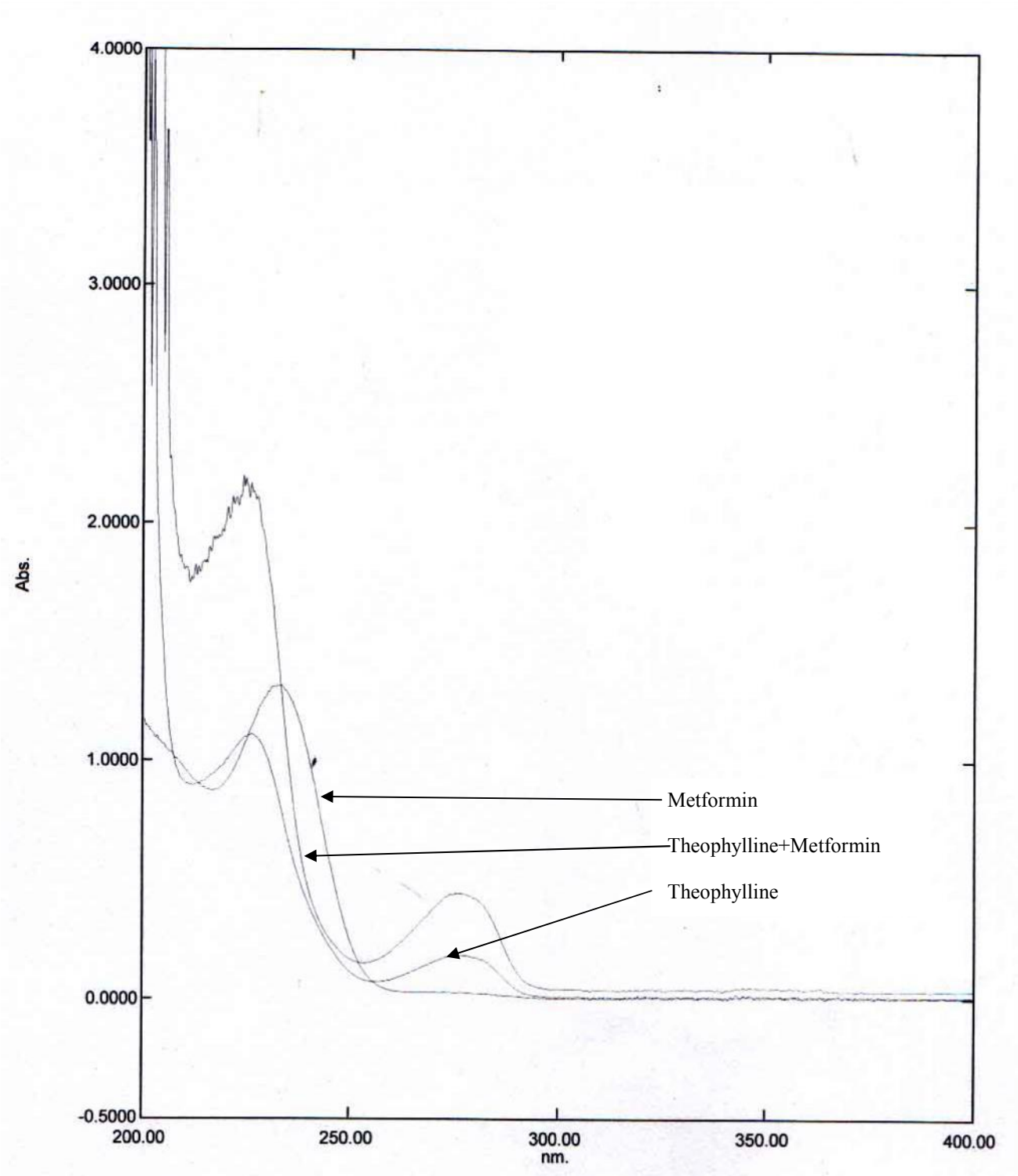


Fig.7.1.2.50: UV spectra of Theophylline – Metformin systems at pH 5.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

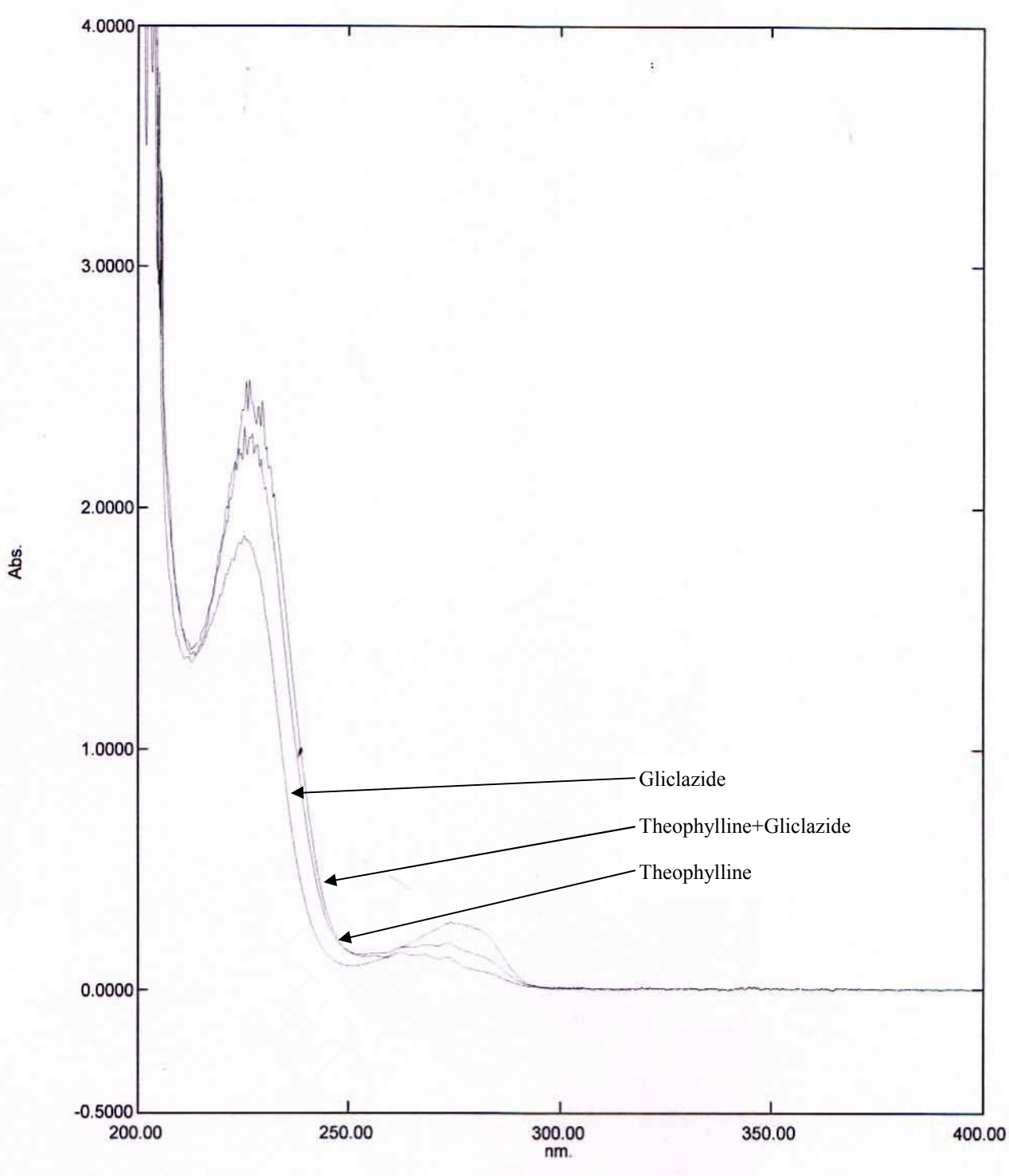


Fig.7.1.2.51: UV spectra of Theophylline – Gliclazide systems at pH 6.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

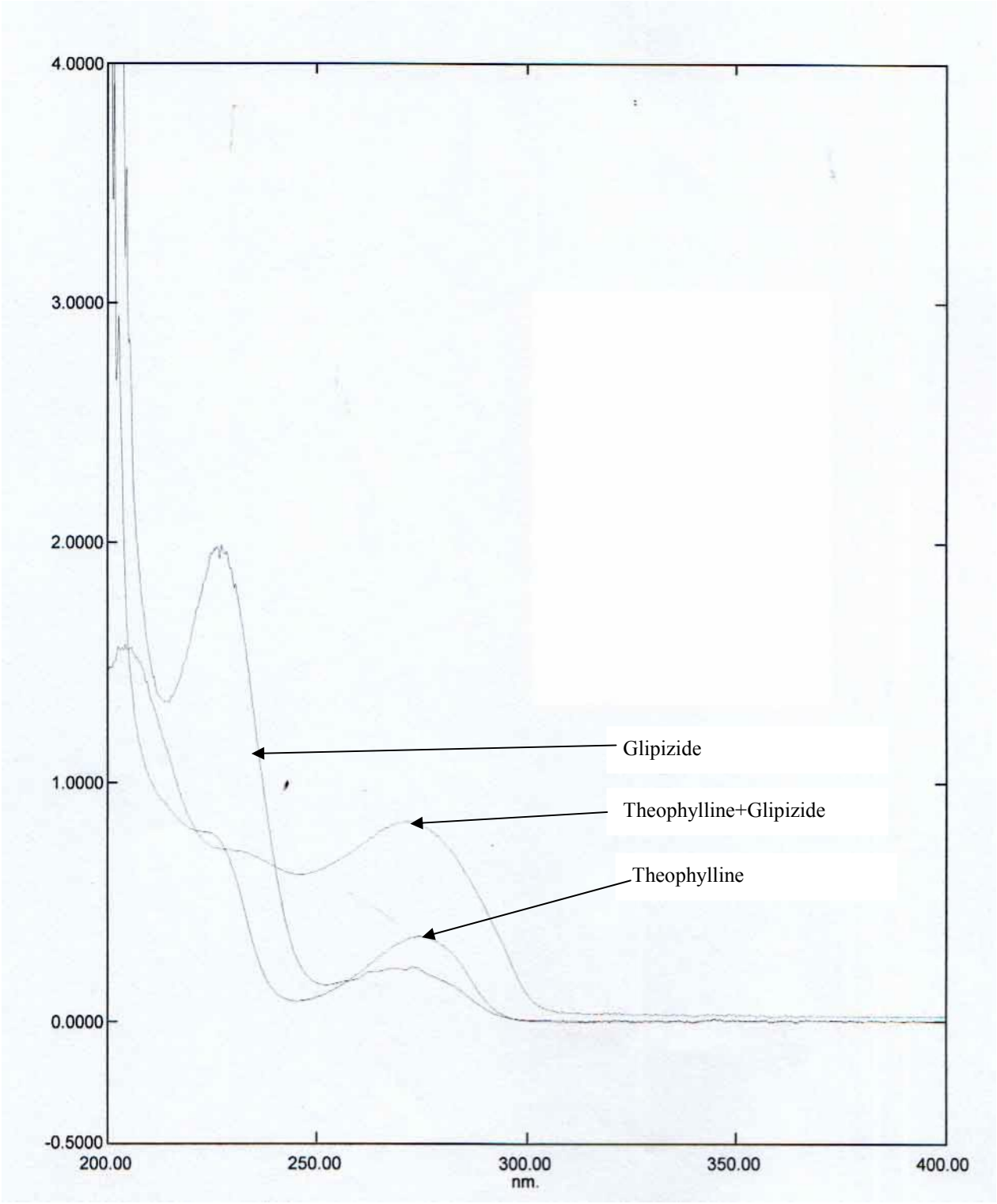


Fig.7.1.2.52: UV spectra of Theophylline – Glipizide systems at pH 6.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

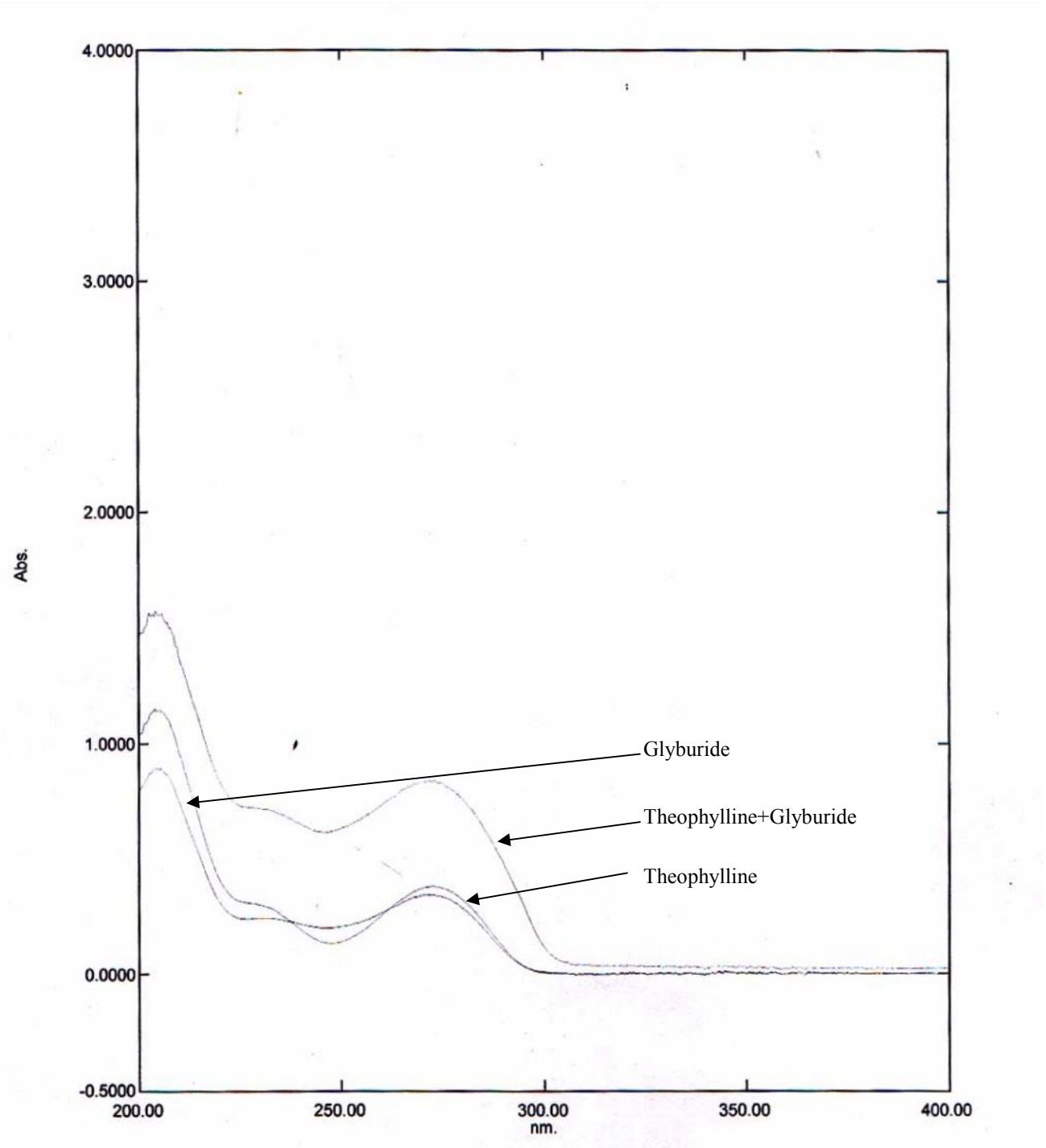


Fig.7.1.2.53: UV spectra of Theophylline – Glyburide systems at pH 6.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

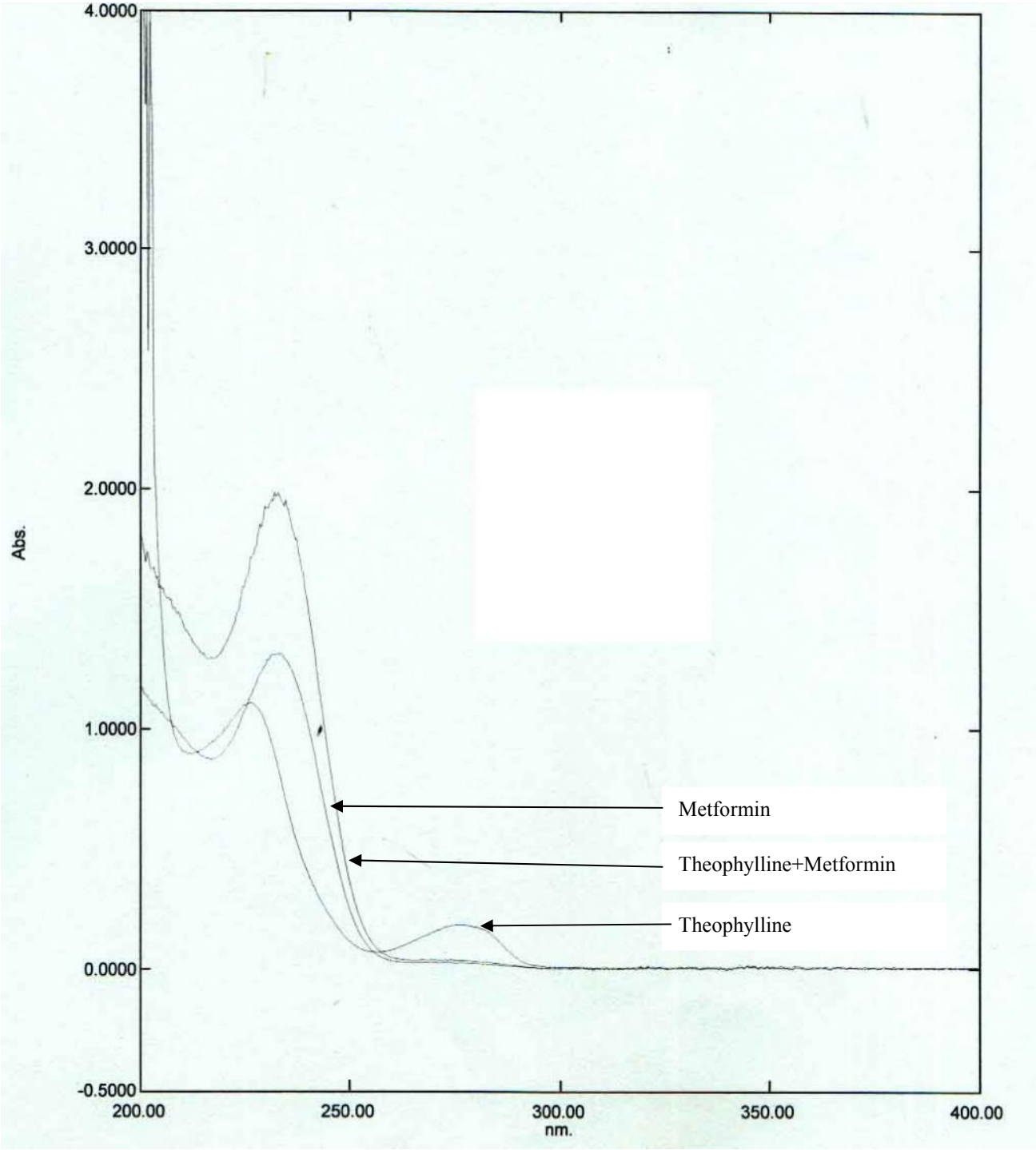


Fig.7.1.2.54: UV spectra of Theophylline – Metformin systems at pH 6.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

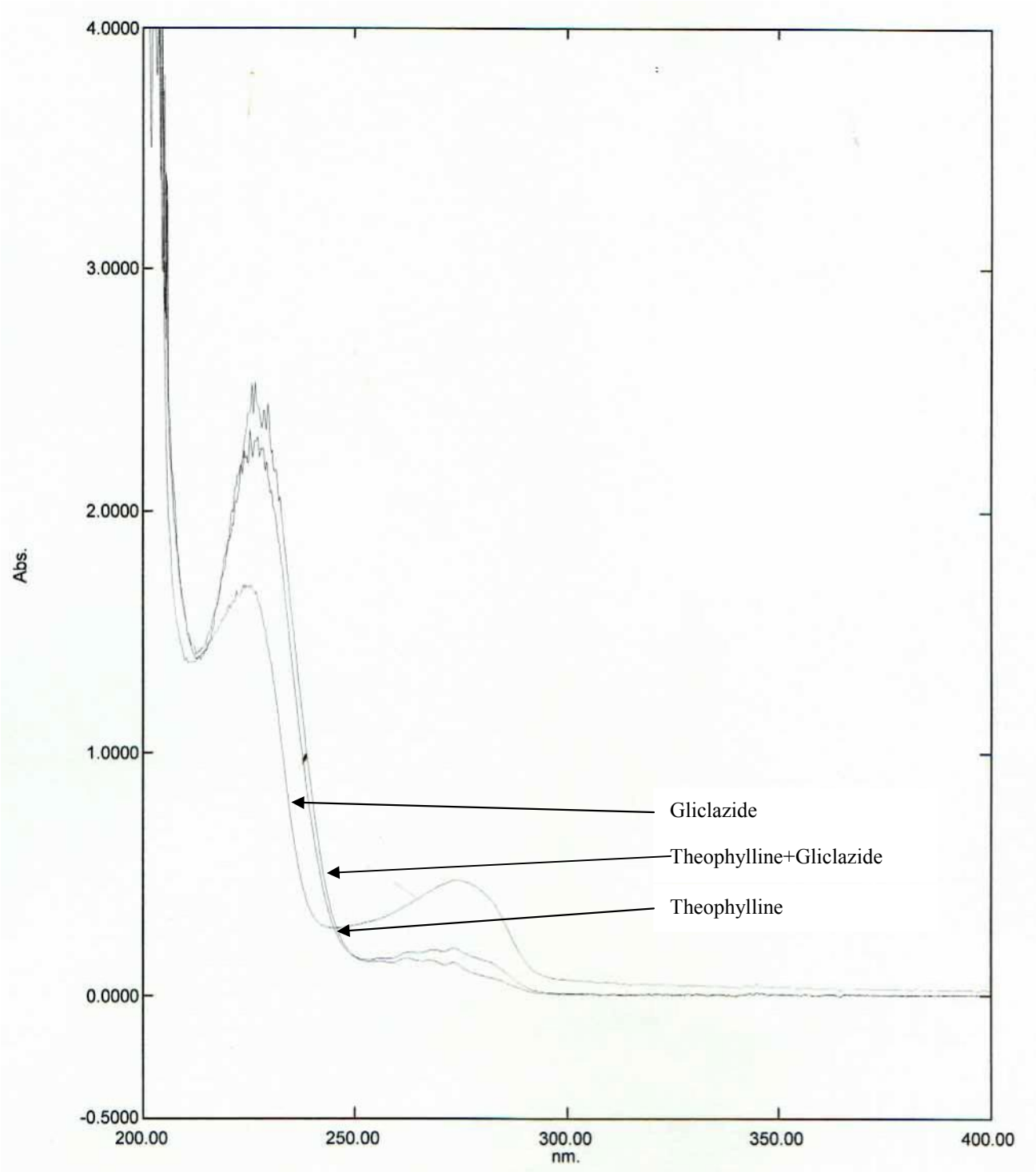


Fig.7.1.2.55: UV spectra of Theophylline – Gliclazide systems at pH 7.4 (Conc. of Theophylline = Conc. of Gliclazide = 0.0001 M)

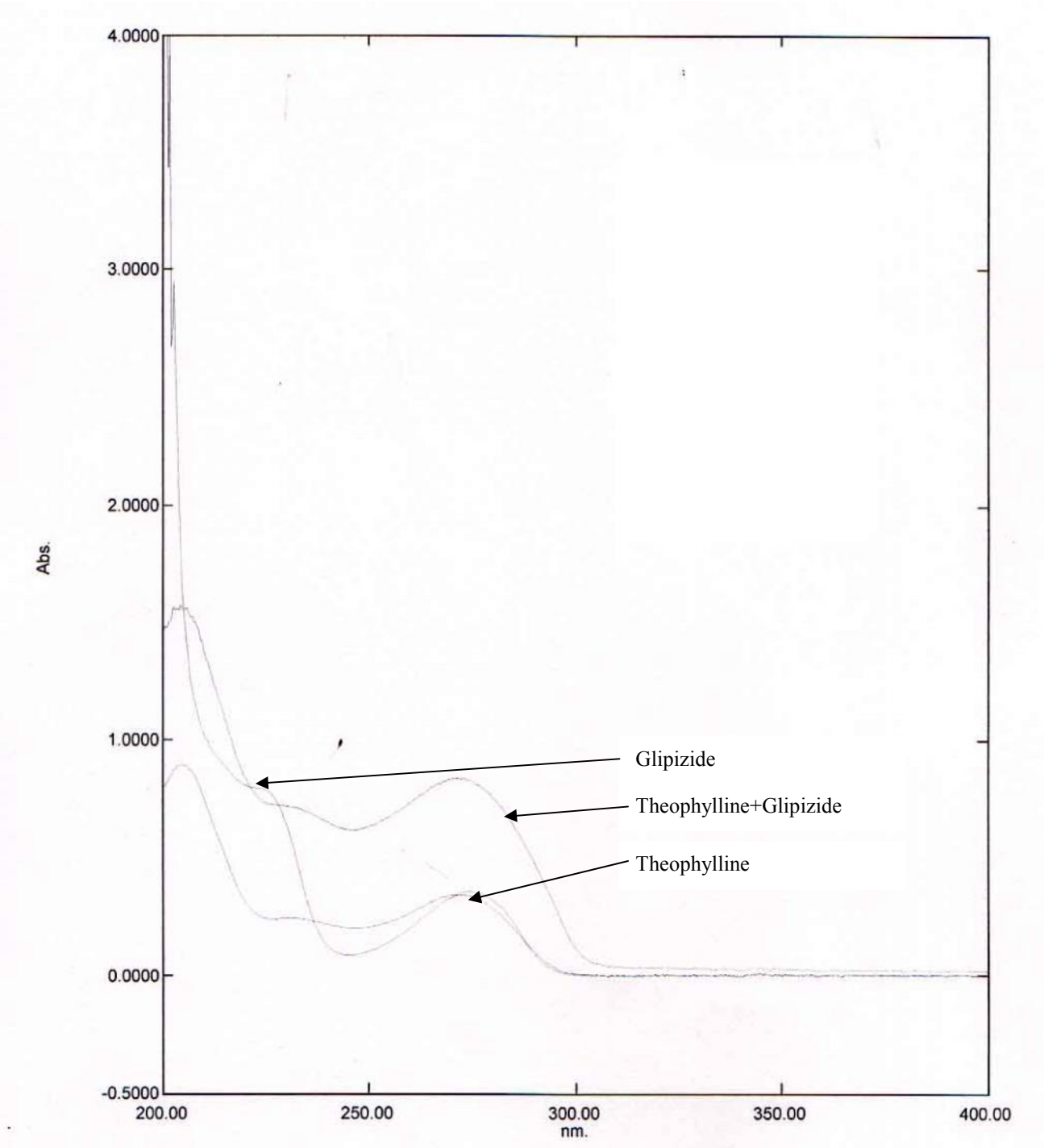


Fig.7.1.2.56: UV spectra of Theophylline – Glipizide systems at pH 7.4 (Conc. of Theophylline = Conc. of Glipizide = 0.0001 M)

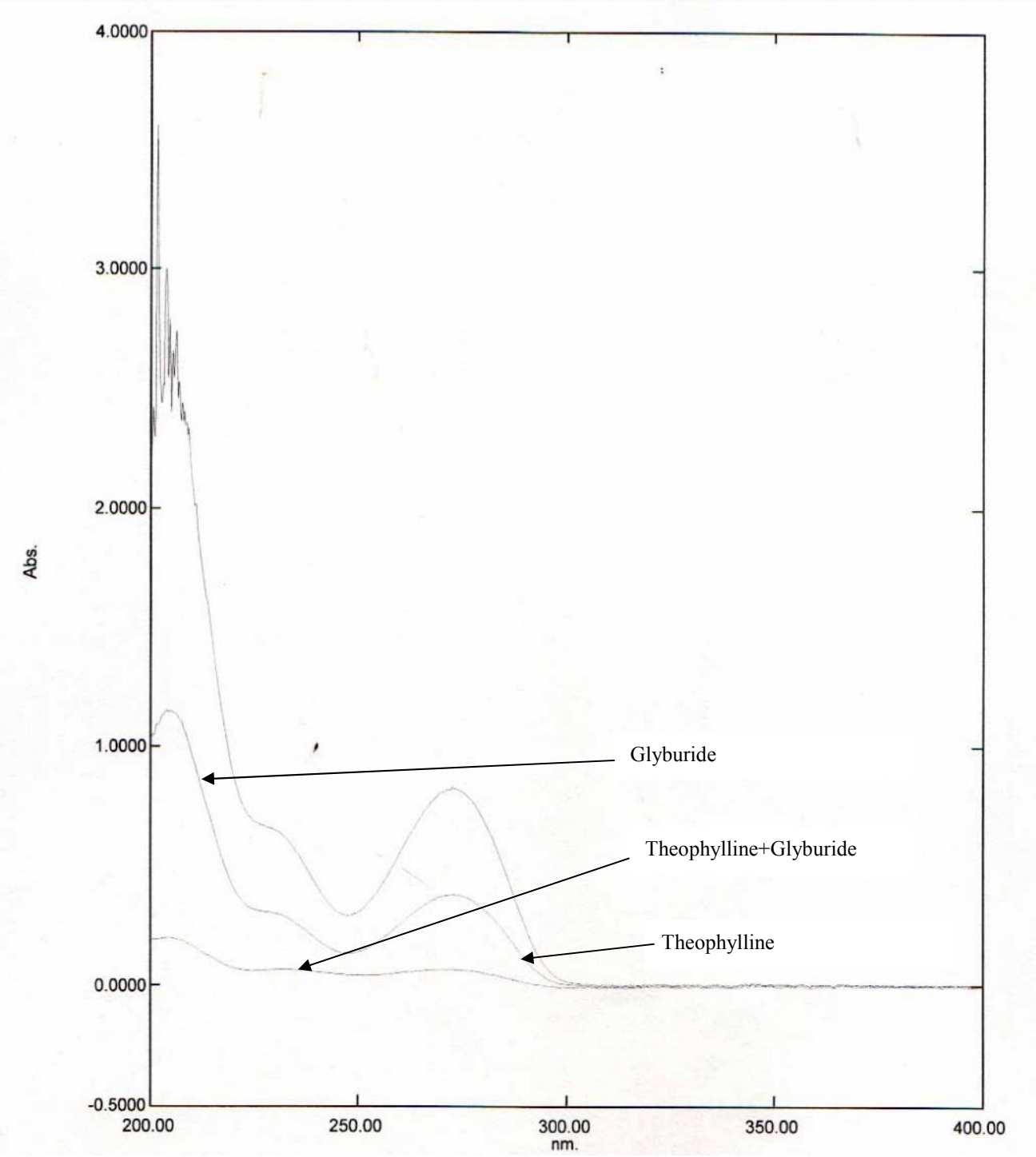


Fig.7.1.2.57: UV spectra of Theophylline – Glyburide systems at pH 7.4 (Conc. of Theophylline = Conc. of Glyburide = 0.0001 M)

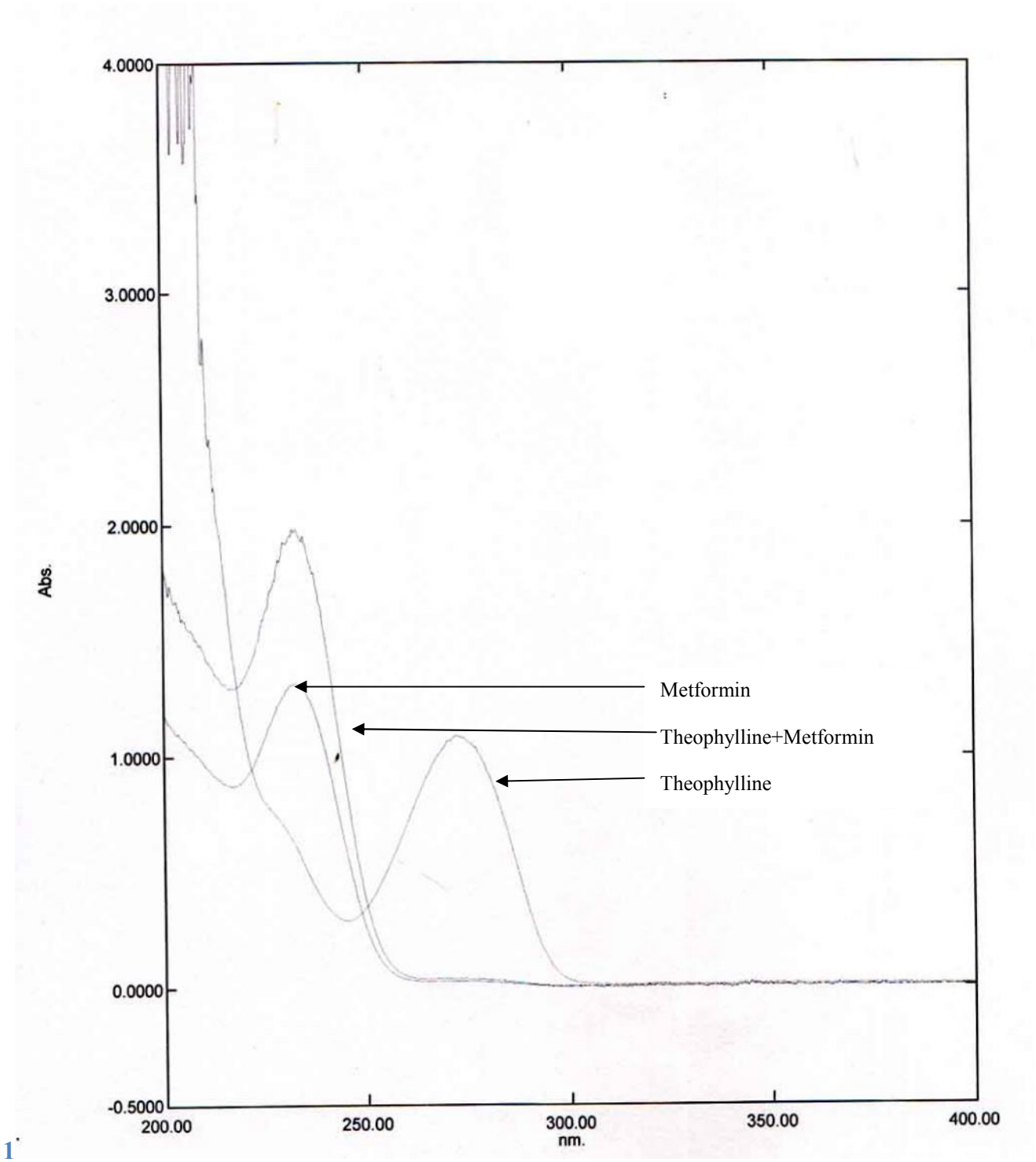


Fig.7.1.2.58: UV spectra of Theophylline – Metformin systems at pH 7.4 (Conc. of Theophylline = Conc. of Metformin = 0.0001 M)

From above spectrum, it can be inferred that the spectra of target molecules alone and the mixture (1:1) of theophylline with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of theophylline with the drugs that alter the absorption intensities as donor-acceptor complexation occurs.

7.1.3 INITIAL COMPLEXATION BY CONTINUOUS VARIATION METHOD

Job's method of continuous-variation plots have conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide.

Solution of different concentrations of caffeine, theophylline, gliclazide, glipizide, glyburide and metformin were prepared and a continuous-variation plots were prepared by plotting corrected absorbance (measured at 273 nm for caffeine and measured at 276 nm for theophylline) against the volume fraction of one reactant. [63]

In this method, the corrected absorbance was plotted against the volume fraction of one reactant, that was, $V_M/ (V_M+V_L)$, Where, V_M was the volume of the cation solution & V_L that of the ligand. [66]

Note that cation & ligand solutions with identical analytical concentrations are mixed in such a way that the total volume and the total moles of reactants in each mixture are constant but the mole ratio of reactants varies systematically.

The data for continuous-variation plots are given in tables 7.1.3.1 to 7.1.3.4. These plots (figs. 7.1.3.1 to 7.1.3.4) give straight lines with different intercepts, indicating the formation of 1:1 complexes for all systems of caffeine with gliclazide, glipizide, metformin and 1:2 complexes for caffeine with glyburide.

In Fig.7.1.3.1 $V_M/ (V_M+V_L)$ is 0.50 and $V_L/ (V_M+V_L)$ is 0.50; thus, V_M/ V_L is 0.50/0.50, which suggests that the complex has the formula ML i.e. 1:1.

In Fig.7.1.3.2 $V_M/ (V_M+V_L)$ is 0.50 and $V_L / (V_M+V_L)$ is 0.50; thus, V_M/ V_L is 0.50/0.50, which suggests that the complex has also the formula ML i.e. 1:1.

In Fig.7.1.3.3 $V_M/ (V_M+V_L)$ is 0.33 and $V_L/ (V_M+V_L)$ is 0.66; thus, V_M/ V_L is 0.33/0.66, which suggests that the complex has the formula ML i.e. 1:2.

Again, in Fig.7.1.3.4 $V_M/ (V_M+V_L)$ is 0.50 and $V_L / (V_M+V_L)$ is 0.50; thus, V_M/ V_L is 0.50/0.50, which suggests that the complex has also the formula ML i.e. 1:1.

So, Job's method of continuous-variation plots have conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide.

Table 7.1.3.1: Data for Continuous-variation plot for Caffeine-Gliclazide system

Volume of Caffeine = $V_{M,}$ Absorbance of Caffeine = 0.572 = A_C

Volume of Gliclazide = V_L, Absorbance of Gliclazide = 0.378 = A_G

Total moles of reactants = $V_M+V_{L,}$ Mixture of Absorbance = M_A

Volume fraction of Caffeine = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Gliclazide = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.378	0.000
02	18	20	0.1	0.9	0.760	0.190
04	16	20	0.2	0.8	0.575	0.375
06	14	20	0.3	0.7	0.400	0.550
08	12	20	0.4	0.6	0.325	0.625
10	10	20	0.5	0.5	0.300	0.650
12	08	20	0.6	0.4	0.324	0.626
14	06	20	0.7	0.3	0.398	0.552
16	04	20	0.8	0.2	0.574	0.376
18	02	20	0.9	0.1	0.759	0.191
20	00	20	1.0	0.0	0.572	0.000

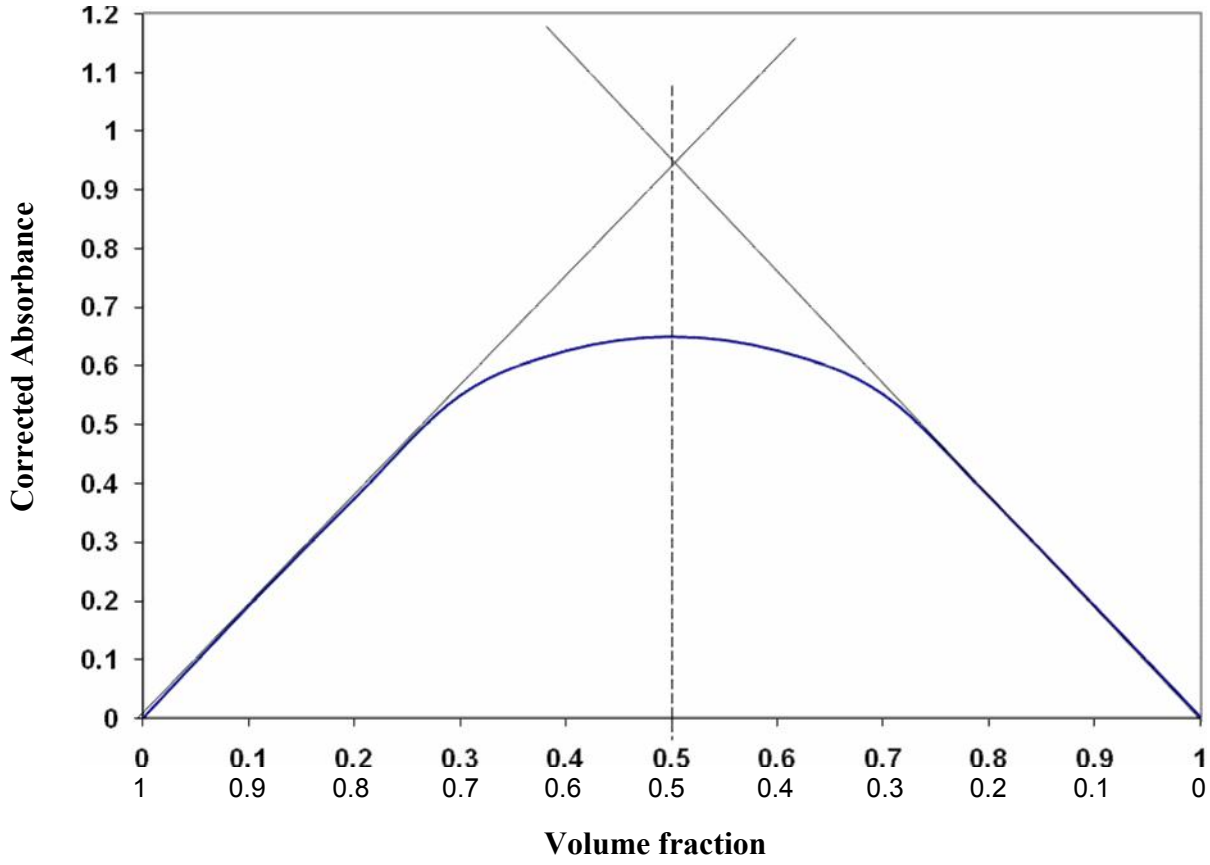


Fig. 7.1.3.1: Continuous-variation plot for Caffeine-Gliclazide system

Table 7.1.3.2: Data for Continuous-variation plot for Caffeine-Glipizide system

Volume of Caffeine = V_M , Absorbance of Caffeine = 0.572 = A_C

Volume of Glipizide = V_L, Absorbance of Glipizide = 0.372 = A_G

Total moles of reactants = V_M+V_L, Mixture of Absorbance = M_A

Volume fraction of Caffeine = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Glipizide = $V_L / (V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.372	0.000
02	18	20	0.1	0.9	0.760	0.183
04	16	20	0.2	0.8	0.575	0.367
06	14	20	0.3	0.7	0.400	0.544
08	12	20	0.4	0.6	0.325	0.617
10	10	20	0.5	0.5	0.300	0.641
12	08	20	0.6	0.4	0.324	0.618
14	06	20	0.7	0.3	0.398	0.543
16	04	20	0.8	0.2	0.574	0.366
18	02	20	0.9	0.1	0.759	0.181
20	00	20	1.0	0.0	0.572	0.000

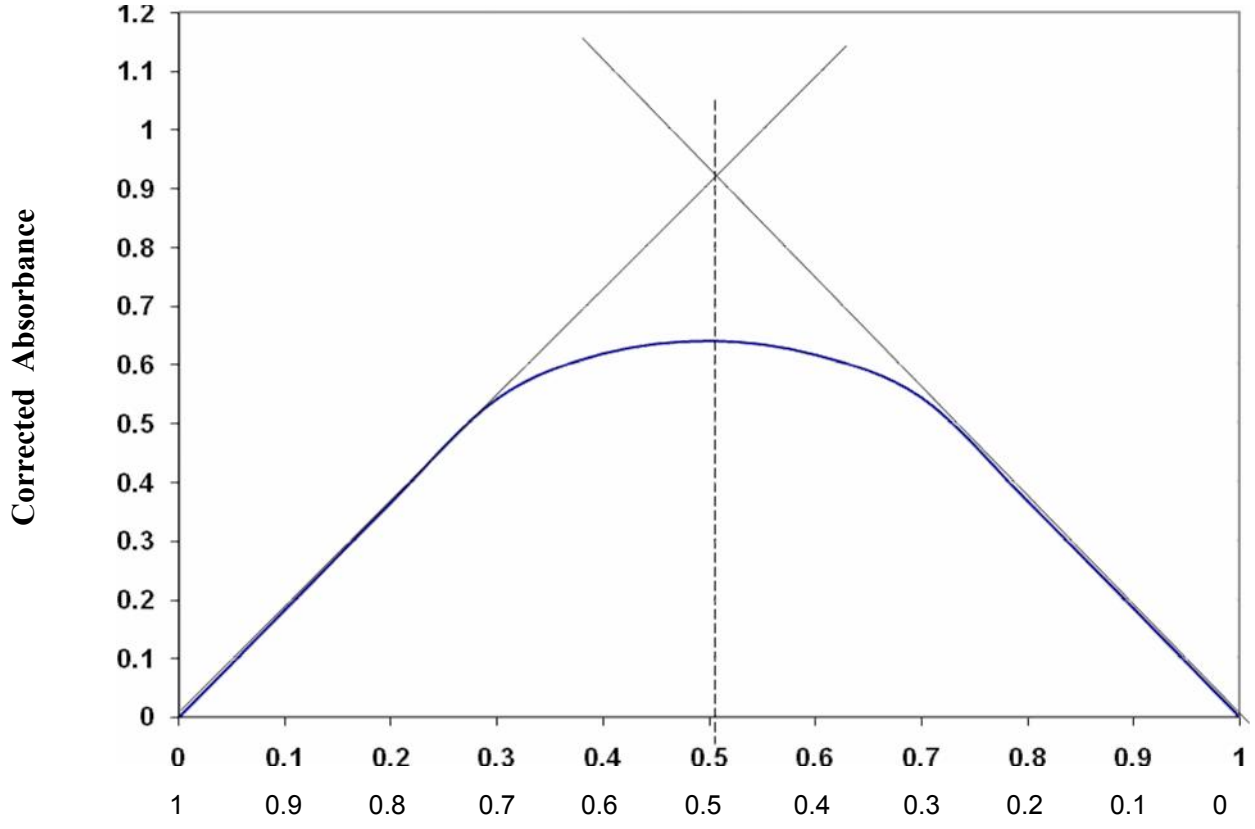


Fig. 7.1.3.2: Continuous-variation plot for Caffeine-Glipizide system

Table 7.1.3.3: Data for Continuous-variation plot for Caffeine-Glyburide system

Volume of Caffeine = $V_{M,}$ Absorbance of Caffeine = 0.572 = A_C

Volume of Glyburide = V_L, Absorbance of Glyburide = 0.353 = A_G

Total moles of reactants = $V_M+V_{L,}$ Mixture of Absorbance = M_A

Volume fraction of Caffeine = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Glyburide = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.353	0.000
02	18	20	0.1	0.9	0.712	0.231
04	16	20	0.2	0.8	0.502	0.423
06	14	20	0.3	0.7	0.347	0.578
08	12	20	0.4	0.6	0.324	0.601
10	10	20	0.5	0.5	0.397	0.528
12	08	20	0.6	0.4	0.496	0.429
14	06	20	0.7	0.3	0.600	0.325
16	04	20	0.8	0.2	0.702	0.223
18	02	20	0.9	0.1	0.822	0.103
20	00	20	1.0	0.0	0.572	0.000

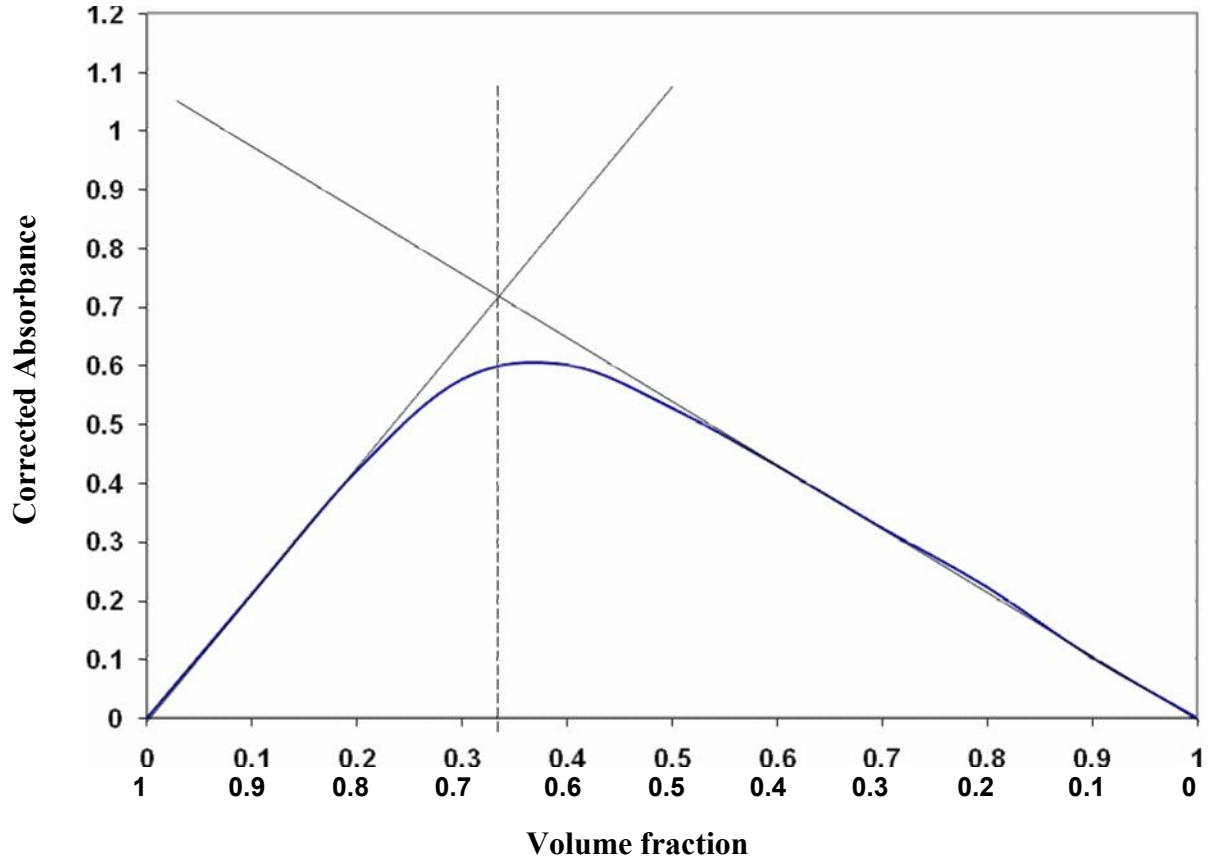


Fig. 7.1.3.3: Continuous-variation plot for Caffeine-Glyburide system

Table 7.1.3.4: Data for Continuous-variation plot for Caffeine- Metformin system

Volume of Caffeine = $V_{M,}$ Absorbance of Caffeine = 0.572 =A_C

Volume of Metformin = V_L, Absorbance of Metformin = 0.772= A_M

Total moles of reactants = V_M+$V_{L\,,}$ Mixture of Absorbance = M_A

Volume fraction of Caffeine = V_M/ (V_M+V_L) Corrected Absorbance = (A_C + $A_{M)}$ - M_A

and Volume fraction of Metformin = V_L /(V_M+V_L) = C_A

V_M	V_L	(V_M+V_L)	V_M/(V_M+V_L)	V_L/(V_M+V_L)	M_A	C_A
00	20	20	0.0	1.0	0.772	0.000
02	18	20	0.1	0.9	1.125	0.219
04	16	20	0.2	0.8	0.926	0.418
06	14	20	0.3	0.7	0.704	0.640
08	12	20	0.4	0.6	0.597	0.747
10	10	20	0.5	0.5	0.566	0.778
12	08	20	0.6	0.4	0.596	0.748
14	06	20	0.7	0.3	0.703	0.641
16	04	20	0.8	0.2	0.925	0.419
18	02	20	0.9	0.1	1.124	0.220
20	00	20	1.0	0.0	0.572	0.000

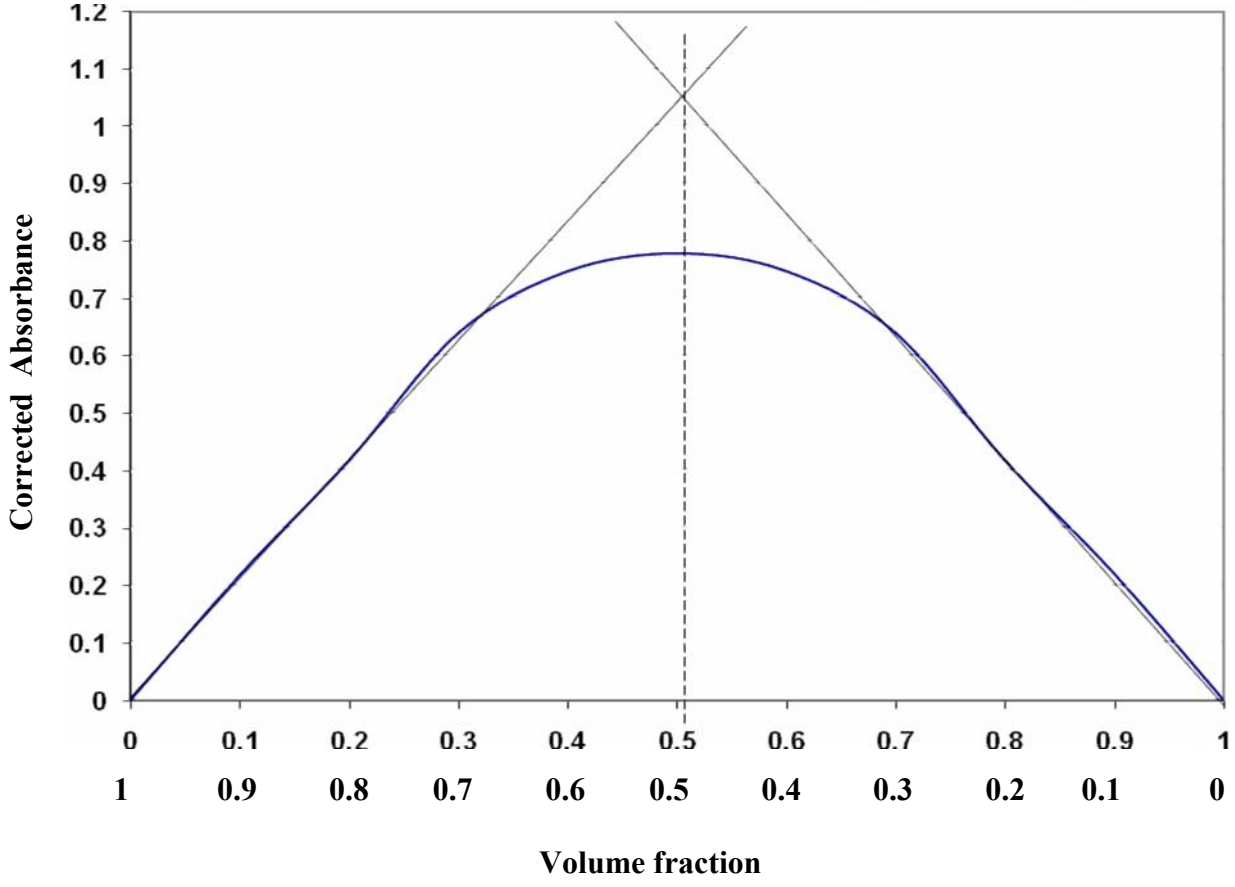


Fig. 7.1.3.4: Continuous-variation plot for Caffeine-Metformin system

On the other hand, continuous-variation plots have conformed the formation of 2:1 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide and 1:1 complexes of theophylline with metformin.

The data for continuous-variation plots are given in tables 7.3.5 to 7.3.8. These plots (figs. 7.3.5 to 7.3.8) give straight lines with different intercepts, indicating the formation of 2:1 complexes for all systems of theophylline with gliclazide, glipizide and 1:2 complexes for theophylline with glyburide and 1:1 complexes for theophylline with metformin. [63]

In Fig.7.3.5 $V_M/ (V_M+V_L)$ is 0.66 and $V_L/ (V_M+V_L)$ is 0.33; thus, V_M/ V_L is 0.66/0.33, which suggests that the complex has the formula M_L i.e. 2:1.

In Fig.7.3.6 $V_M/ (V_M+V_L)$ is 0.66 and $V_L/ (V_M+V_L)$ is 0.33; thus, V_M/ V_L is 0.66/0.33, which suggests that the complex has the formula M_L i.e. 2:1.

In Fig.7.3.7 $V_M/ (V_M+V_L)$ is 0.33 and $V_L/ (V_M+V_L)$ is 0.66; thus, V_M/ V_L is 0.33/0.66, which suggests that the complex has the formula ML i.e. 1:2.

Again, in Fig.7.2.8 $V_M/ (V_M+V_L)$ is 0.50 and $V_L / (V_M+V_L)$ is 0.50; thus, V_M/ V_L is 0.50/0.50, which suggests that the complex has also the formula M_L i.e. 1:1.

Thus, continuous-variation plots have conformed the formation of 2:1 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide and 1:1 complexes of theophylline with metformin.

Note that Job's method of continuous-variation plots conformed the formation of 1:1 complexes of both caffeine & theophylline with metformin.

Table 7.1.3.5: Data for Continuous-variation plot for Theophylline-Gliclazide system

Volume of Theophylline = $V_{M,}$ Absorbance of Theophylline = 0.553 = A_C

Volume of Gliclazide = V_L, Absorbance of Gliclazide = 0.378 = A_G

Total moles of reactants = $V_M+V_{L,}$ Mixture of Absorbance = M_A

Volume fraction of Theophylline = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Gliclazide = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.378	0.000
02	18	20	0.1	0.9	0.808	0.123
04	16	20	0.2	0.8	0.689	0.242
06	14	20	0.3	0.7	0.574	0.357
08	12	20	0.4	0.6	0.443	0.488
10	10	20	0.5	0.5	0..373	0.558
12	08	20	0.6	0.4	0.339	0.592
14	06	20	0.7	0.3	0.337	0.554
16	04	20	0.8	0.2	0.513	0.418
18	02	20	0.9	0.1	0.703	0.228
20	00	20	1.0	0.0	0.553	0.000

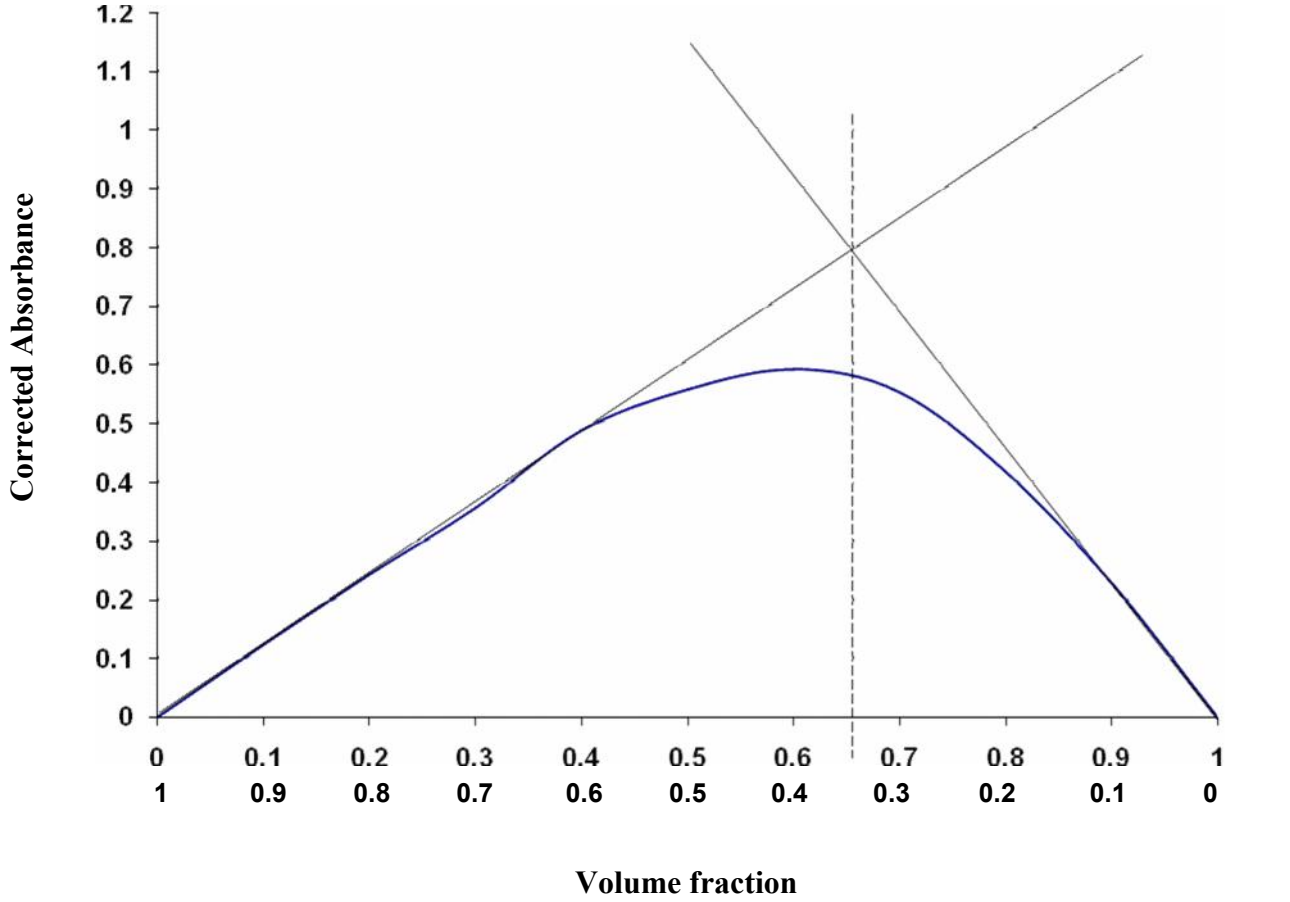


Fig. 7.1.3.5: Continuous-variation plot for Theophylline-Gliclazide system

Table 7.1.3.6: Data for Continuous-variation plot for Theophylline-Glipizide system

Volume of Theophylline = $V_{M,}$ Absorbance of Theophylline = 0.553 = A_C

Volume of Glipizide = V_L, Absorbance of Glipizide = 0.372 = A_G

Total moles of reactants = $V_M+V_{L,}$ Mixture of Absorbance = M_A

Volume fraction of Theophylline = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Glipizide = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.372	0.000
02	18	20	0.1	0.9	0.805	0.120
04	16	20	0.2	0.8	0.680	0.245
06	14	20	0.3	0.7	0.562	0.363
08	12	20	0.4	0.6	0.448	0.477
10	10	20	0.5	0.5	0.360	0.565
12	08	20	0.6	0.4	0.336	0.589
14	06	20	0.7	0.3	0.374	0.551
16	04	20	0.8	0.2	0.510	0.415
18	02	20	0.9	0.1	0.701	0.224
20	00	20	1.0	0.0	0.553	0.000

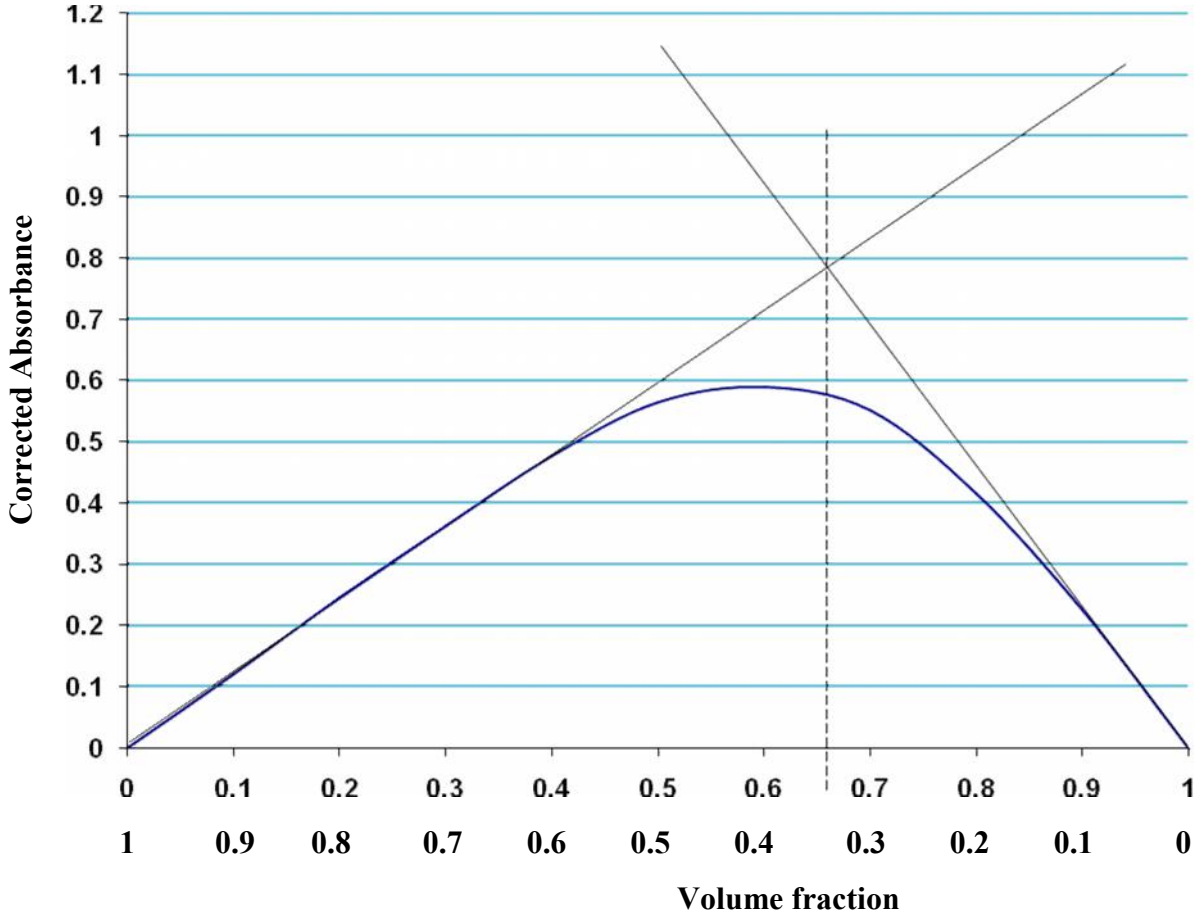


Fig. 7.1.3.6: Continuous-variation plot for Theophylline-Glipizide system

Table 7.1.3.7: Data for Continuous-variation plot for Theophylline-Glyburide system

Volume of Theophylline = V_M Absorbance of Theophylline = 0.572 = A_C

Volume of Glyburide = V_L Absorbance of Glyburide = 0.353 = A_G

Total moles of reactants = V_M+V_L, Mixture of Absorbance = M_A

Volume fraction of Theophylline = $V_M/ (V_M+V_L)$ Corrected Absorbance = $(A_C + A_G) - M_A$

and Volume fraction of Glyburide = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.353	0.000
02	18	20	0.1	0.9	0.693	0.213
04	16	20	0.2	0.8	0.494	0.412
06	14	20	0.3	0.7	0.338	0.568
08	12	20	0.4	0.6	0.311	0.595
10	10	20	0.5	0.5	0.386	0.520
12	08	20	0.6	0.4	0.487	0.419
14	06	20	0.7	0.3	0.588	0.318
16	04	20	0.8	0.2	0.690	0.216
18	02	20	0.9	0.1	0.836	0.097
20	00	20	1.0	0.0	0.553	0.000

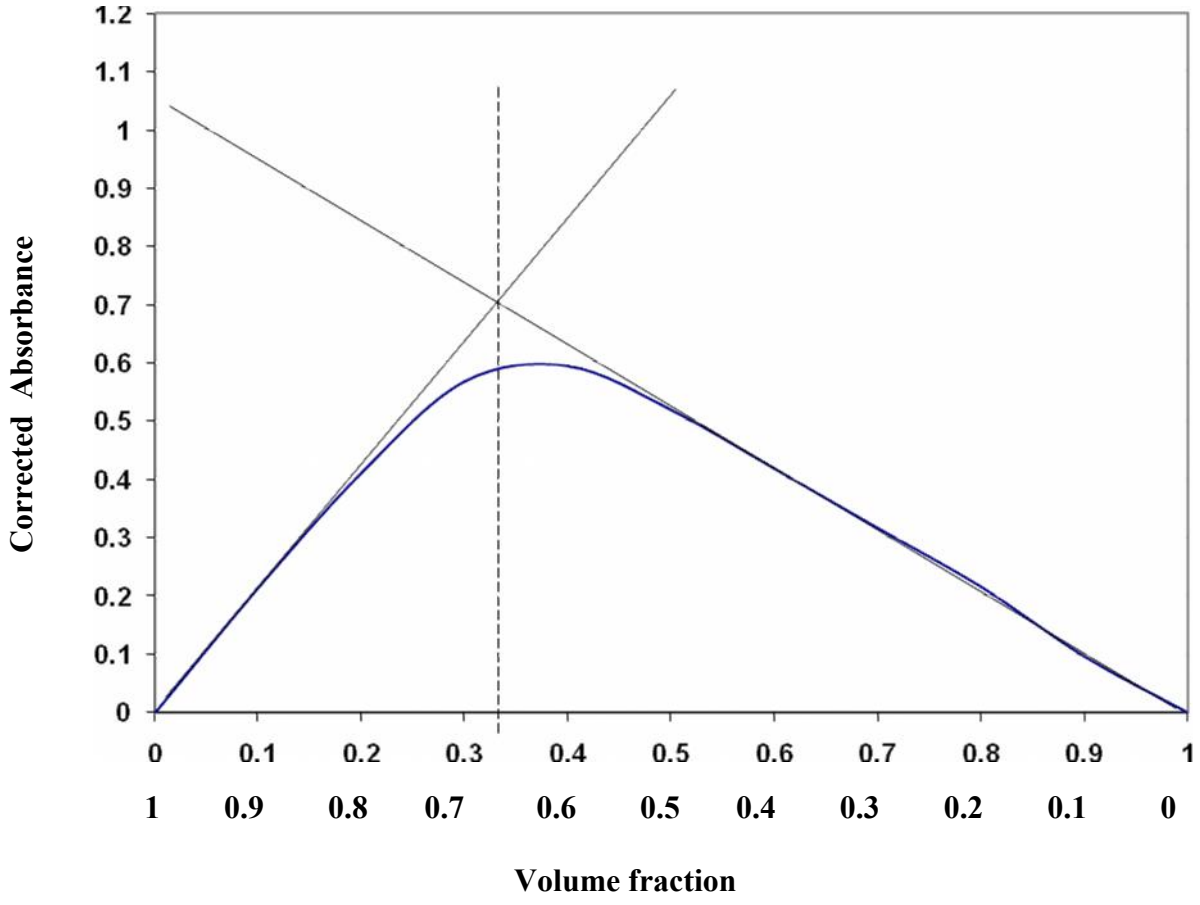


Fig. 7.1.3.7: Continuous-variation plot for Theophylline-Glyburide system

Table 7.1.3.8: Data for Continuous-variation plot for Theophylline-Metformin system

Volume of Theophylline = V_M Absorbance of Theophylline = 0.553 =A_C

Volume of Metformin = V_L Absorbance of Metformin = 0.772= A_M

Total moles of reactants = V_M+V_L Mixture of Absorbance = M_A

Volume fraction of Theophylline = $V_M/(V_M+V_L)$ Corrected Absorbance = $(A_C + A_M) - M_A$

and Volume fraction of Metformin = $V_L /(V_M+V_L)$ = C_A

V_M	V_L	(V_M+V_L)	$V_M/(V_M+V_L)$	$V_L/(V_M+V_L)$	M_A	C_A
00	20	20	0.0	1.0	0.772	0.000
02	18	20	0.1	0.9	0.925	0.200
04	16	20	0.2	0.8	0.739	0.386
06	14	20	0.3	0.7	0.570	0.555
08	12	20	0.4	0.6	0.468	0.657
10	10	20	0.5	0.5	0.435	0.690
12	08	20	0.6	0.4	0.457	0.668
14	06	20	0.7	0.3	0.559	0.566
16	04	20	0.8	0.2	0.738	0.387
18	02	20	0.9	0.1	0.933	0.192
20	00	20	1.0	0.0	0.553	0.000

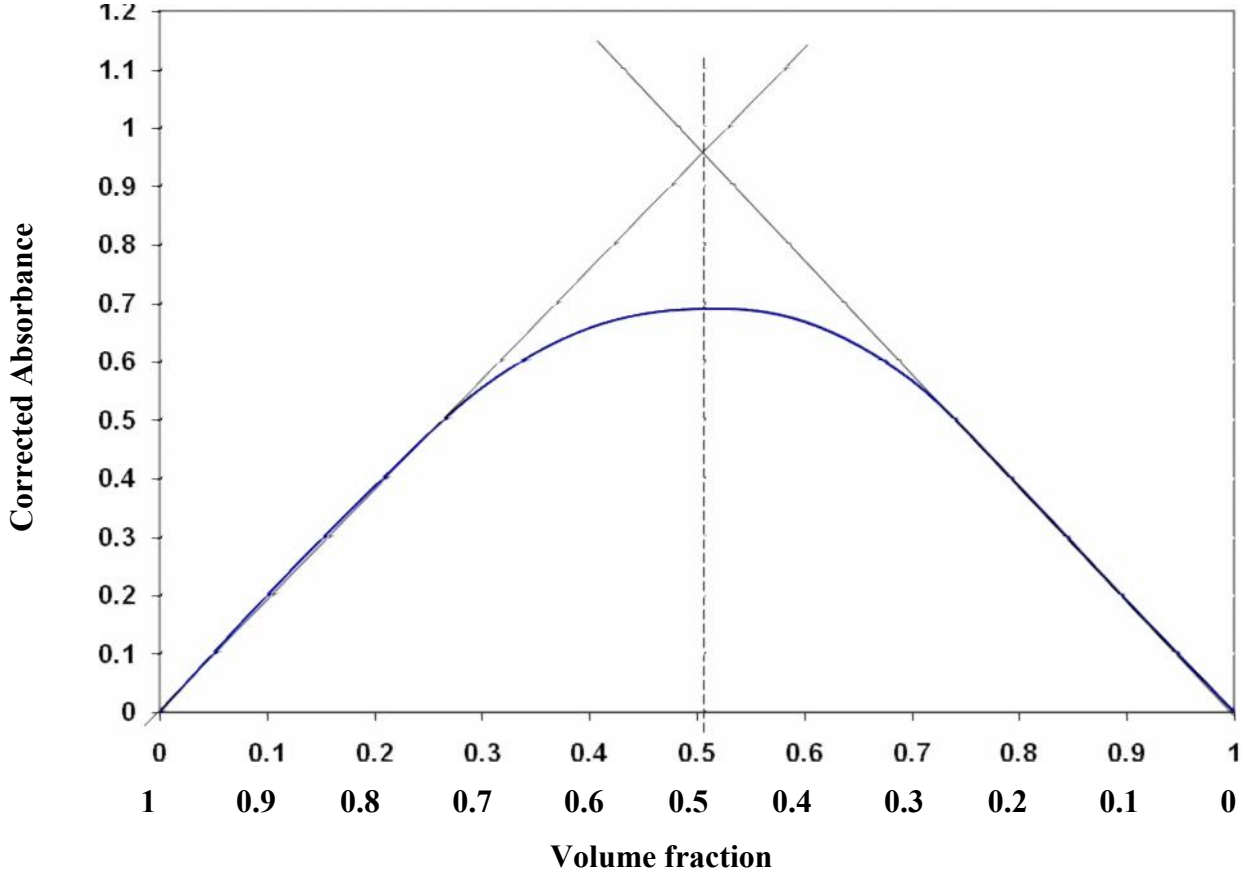


Fig. 7.1.3.8: Continuous-variation plot for Theophylline-Metformin system

7.1.4 INITIAL COMPLEXATION BY MOLE-RATIO METHOD

Mole-ratio plots conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide. On the other hand, Mole-ratio plots conformed the formation of 1:2 complexes of theophylline with gliclazide & glipizide and 1:2 complexes of caffeine with glyburide & metformin.

In this method, the absorbance was plotted against mole-ratio i.e. mole ligand per mole cation.In the mole-ratio method, a series of solutions were prepared in which the analytical concentration of one reactant was held constant while that of the other was varied. [63]

Solution of different concentrations of each caffeine & theophylline were prepared individually by using water and typical plots were prepared by plotting absorbance (measured at 273 nm for caffeine and at 276 nm for theophylline) against mole-ratio.

The data for mole-ratio plots are given in tables (7.1.4.1 to 7.1.4.8). These plots (figs. 7.1.4.1 to 7.1.4.4) give straight lines with different intercepts, indicating the formation of 1:1 complexes for all systems of caffeine with gliclazide, glipizide, metformin and 1: 2 complexes for caffeine with glyburide.

Note that the ligand of the 1:1 complex absorbs at the wavelength selected so that the slope beyond the equivalence point is greater than zero for all systems of caffeine with gliclazide, glipizide and metformin. In this case, we deduced that the uncomplexed cation involved in the 1:2 complexes absorbs, because the initial point has an absorbance greater than zero.But the ligand of the 1:2 complex absorbs at the wavelength selected so that the slope beyond the equivalence point is greater than zero for the system of caffeine with glyburide. In this case, we deduced that the uncomplexed cation involved in the 1:1 complexes absorbs, because the initial point has an absorbance greater than zero. [100]

In the figs. (7.1.4.1 to 7.1.4.4), mole-ratio plots for 1:1 and 1:2 complexes. The 1:1 complex is the more stable, as indicated by less curvature near the stoichiometric ratio for the system of caffeine with gliclazide, glipizide and metformin. But the 1:2 complexes is the more stable, as indicated by less curvature near the stoichiometric ratio for the system of caffeine with glyburide.

Table 7.1.4.1: Data for mole-ratio plot of caffeine-gliclazide system (1:1 mixture)

Conc. of caffeine	Conc. of gliclazide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.425
0.0002	0.0001	2	0.484
0.0003	0.0001	3	0.488
0.0004	0.0001	4	0.496

Table 7.1.4.2: Data for mole-ratio plot of caffeine-gliclazide system (1:2 mixtures)

Conc. of caffeine	Conc. of gliclazide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.301
0.0001	0.0001	1	0.712
0.0002	0.0001	2	0.965
0.0003	0.0001	3	1.102
0.0004	0.0001	4	1.112

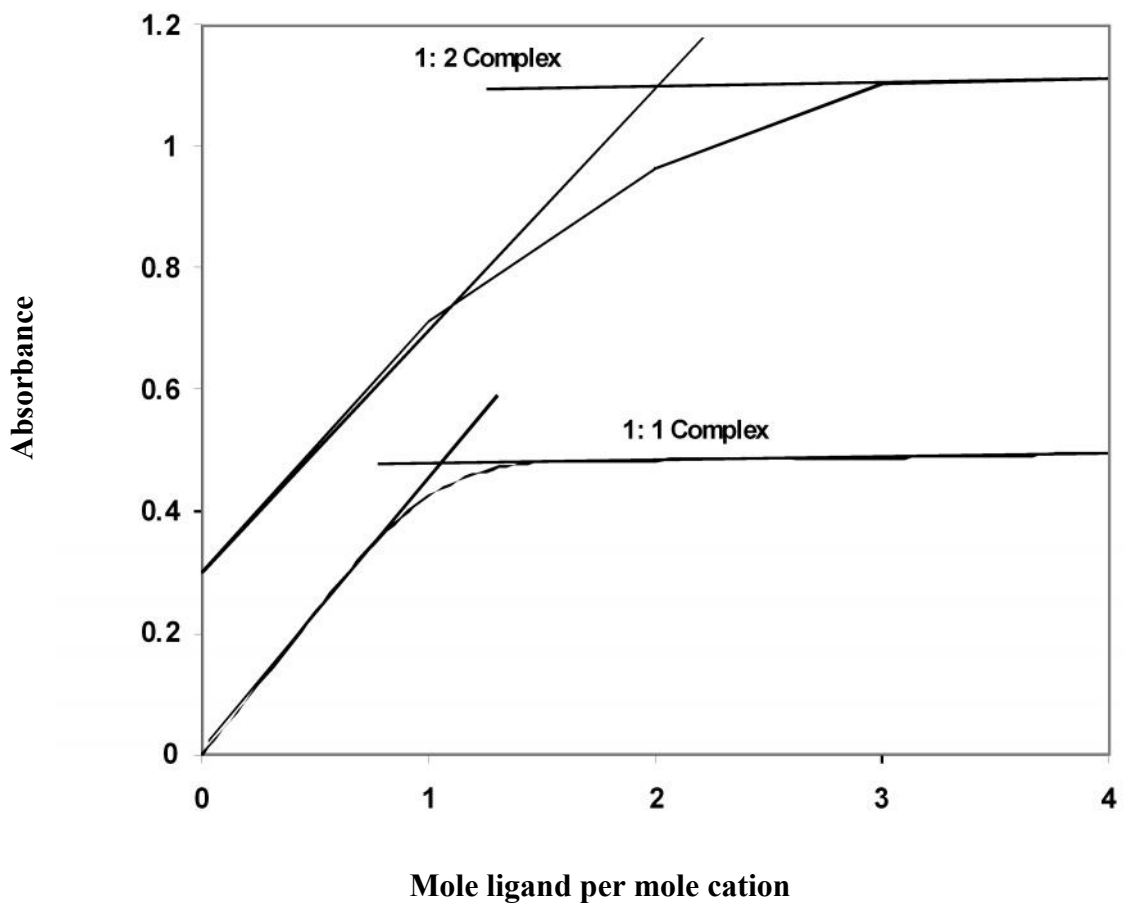


Fig. 7.1.4.1: Mole-ratio plot for caffeine-gliclazide system

Table 7.1.4.3: Data for mole-ratio plot of caffeine-glipizide system (1:1 mixture)

Conc. of caffeine	Conc. of glipizide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.421
0.0002	0.0001	2	0.478
0.0003	0.0001	3	0.483
0.0004	0.0001	4	0.489

Table 7.1.4.4: Data for mole-ratio plot of caffeine-glipizide system (1:2 mixtures)

Conc. of caffeine	Conc. of glipizide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.295
0.0001	0.0001	1	0.685
0.0002	0.0001	2	0.958
0.0003	0.0001	3	1.091
0.0004	0.0001	4	1.102

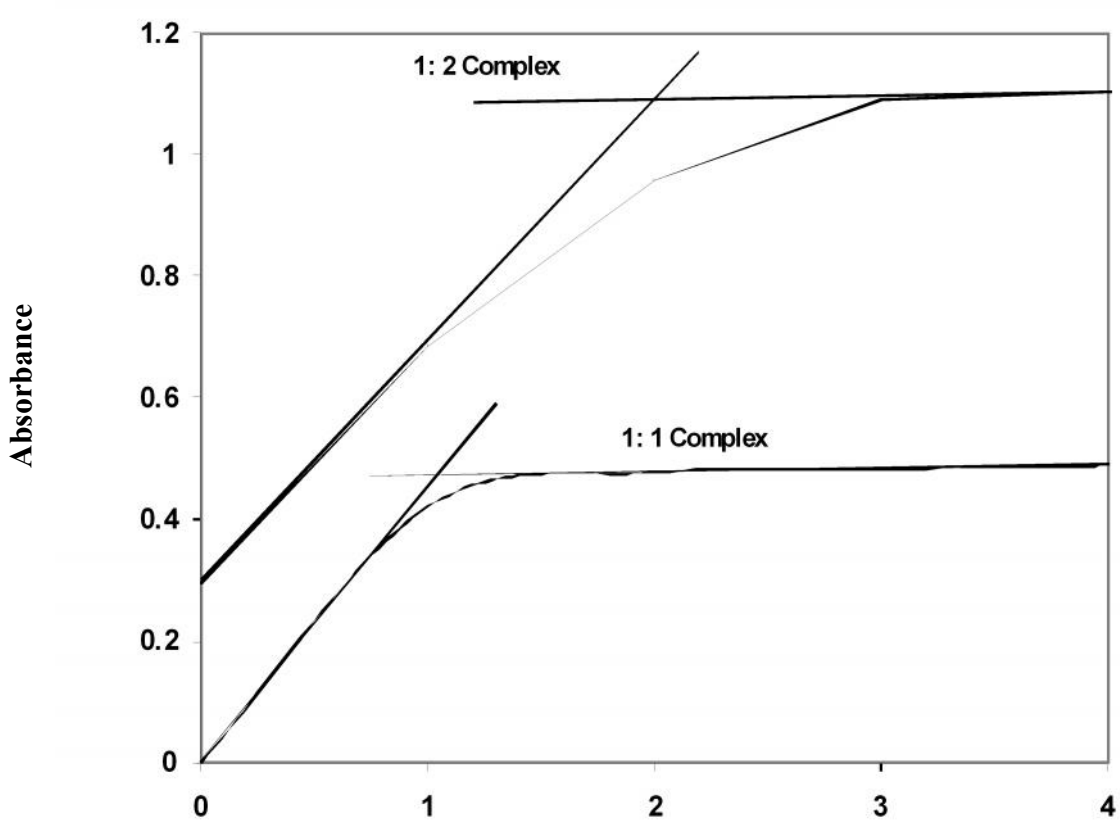


Fig. 7.1.4.2: Mole-ratio plot for caffeine-glipizide system

Table 7.1.4.5: Data for mole-ratio plot of caffeine-glyburide system (1:1 mixture)

Conc. of caffeine	Conc. of glyburide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.301
0.0001	0.0001	1	0.927
0.0002	0.0001	2	1.107
0.0003	0.0001	3	1.115
0.0004	0.0001	4	1.126

Table 7.1.4.6: Data for mole-ratio plot of caffeine-glyburide system (1:2 mixtures)

Conc. of caffeine	Conc. of glyburide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.225
0.0002	0.0001	2	0.435
0.0003	0.0001	3	0.488
0.0004	0.0001	4	0.499

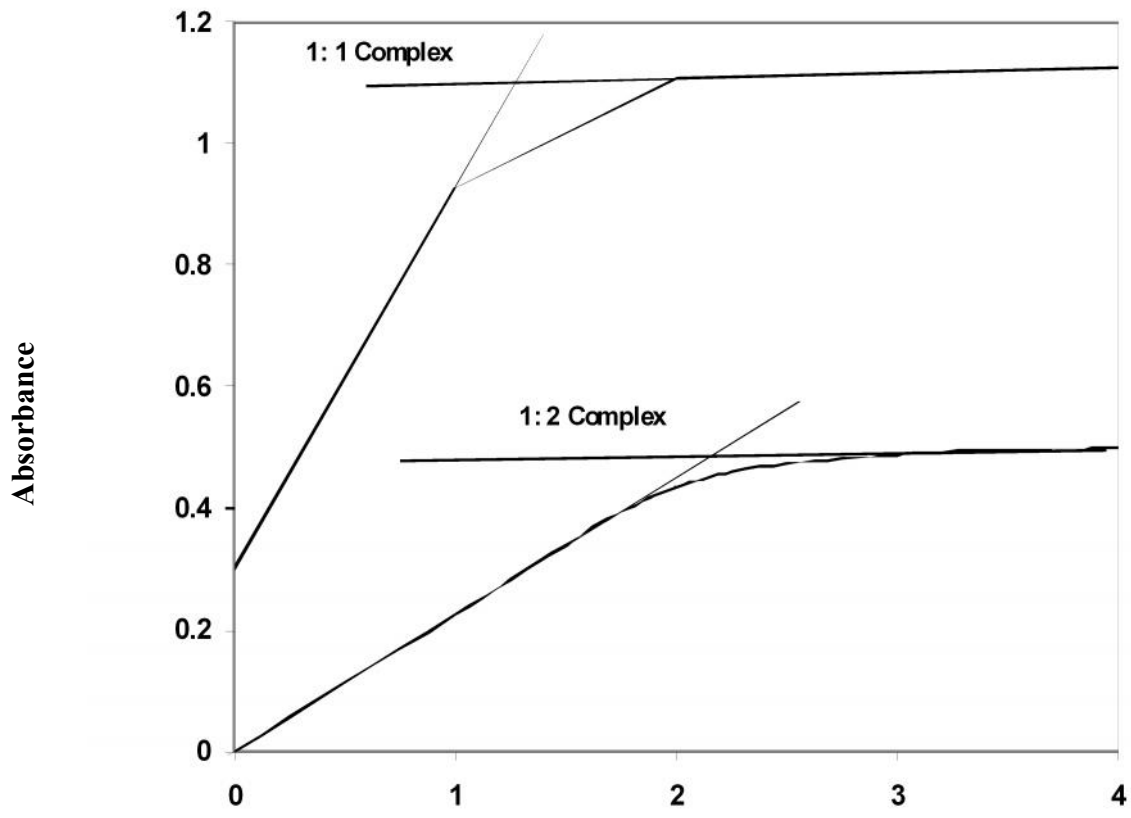


Fig. 7.1.4.3: Mole-ratio plot for caffeine-glyburide system

Table 7.1.4.7: Data for mole-ratio plot of caffeine-metformin system (1:1 mixture)

Conc. of caffeine	Conc. of metformin	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.523
0.0002	0.0001	2	0.578
0.0003	0.0001	3	0.586
0.0004	0.0001	4	0.595

Table 7.1.4.8: Data for mole-ratio plot of caffeine-metformin system (1:2 mixtures)

Conc. of caffeine	Conc. of metformin	Mole-ratio	Absorbance
0.0000	0.0001	0	0.403
0.0001	0.0001	1	0.815
0.0002	0.0001	2	1.035
0.0003	0.0001	3	1.203
0.0004	0.0001	4	1.223

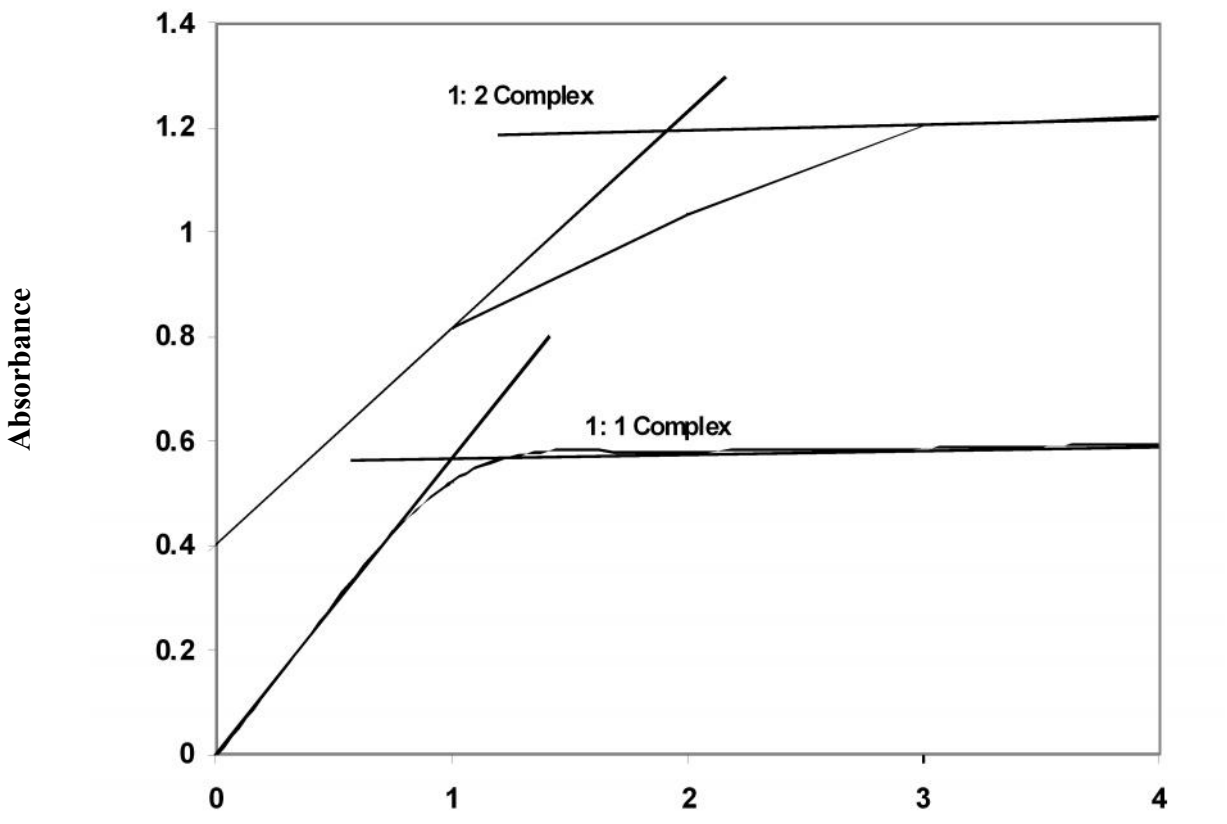


Fig. 7.1.4.4: Mole-ratio plot for caffeine-metformin system

On the other hand, the data for mole-ratio plots are given in tables (7.1.4.9 to 7.1.4.16). These plots (figs. 7.1.4.5 to 7.1.4.8) give straight lines with different intercepts, indicating the formation of 1:2 complexes for all systems of theophylline with gliclazide & glipizide and 1:1 complexes for all systems of theophylline with glyburide and metformin. [63]

Note that the ligand of the 1:2 complexes absorbs at the wavelength selected so that the slope beyond the equivalence point is greater than zero for all systems of theophylline with gliclazide & glipizide. In this case, we deduced that the uncomplexed cation involved in the 1:1 complexes absorbs, because the initial point has an absorbance greater than zero. But the ligand of the 1:1 complexes absorbs at the wavelength selected so that the slope beyond the equivalence point is greater than zero for all systems of theophylline with glyburide and metformin. In this case, we deduced that the uncomplexed cation involved in the 1:2 complexes absorbs, because the initial point has an absorbance greater than zero. [100]

In the figs. (7.1.4.5 to 7.1.4.8), mole-ratio plots for 1:1 and 1:2 complexes. The 1:2 complexes are the more stable, as indicated by less curvature near the stoichiometric ratio for the systems of theophylline with gliclazide & glipizide. But the 1:1 complexes are the more stable, as indicated by less curvature near the stoichiometric ratio for the system of theophylline with glyburide and metformin.

So, Mole-ratio plots conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide. On the other hand, Mole-ratio plots conformed the formation of 1:2 complexes of theophylline with gliclazide & glipizide and 1:2 complexes of theophylline with glyburide & metformin.

Note that Mole-ratio plot conformed the formation of 1:1 complexes of both caffeine & theophylline with metformin.

Table 7.1.4.9: Data for mole-ratio plot of theophylline-gliclazide system (1:1 mixture)

Conc. of theophylline	Conc. of gliclazide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.298
0.0001	0.0001	1	0.921
0.0002	0.0001	2	1.027
0.0003	0.0001	3	1.115
0.0004	0.0001	4	1.135

Table 7.1.4.10: Data for mole-ratio plot of theophylline-gliclazide system (1:2 mixtures)

Conc. of theophylline	Conc. of gliclazide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.223
0.0002	0.0001	2	0.427
0.0003	0.0001	3	0.481
0.0004	0.0001	4	0.495

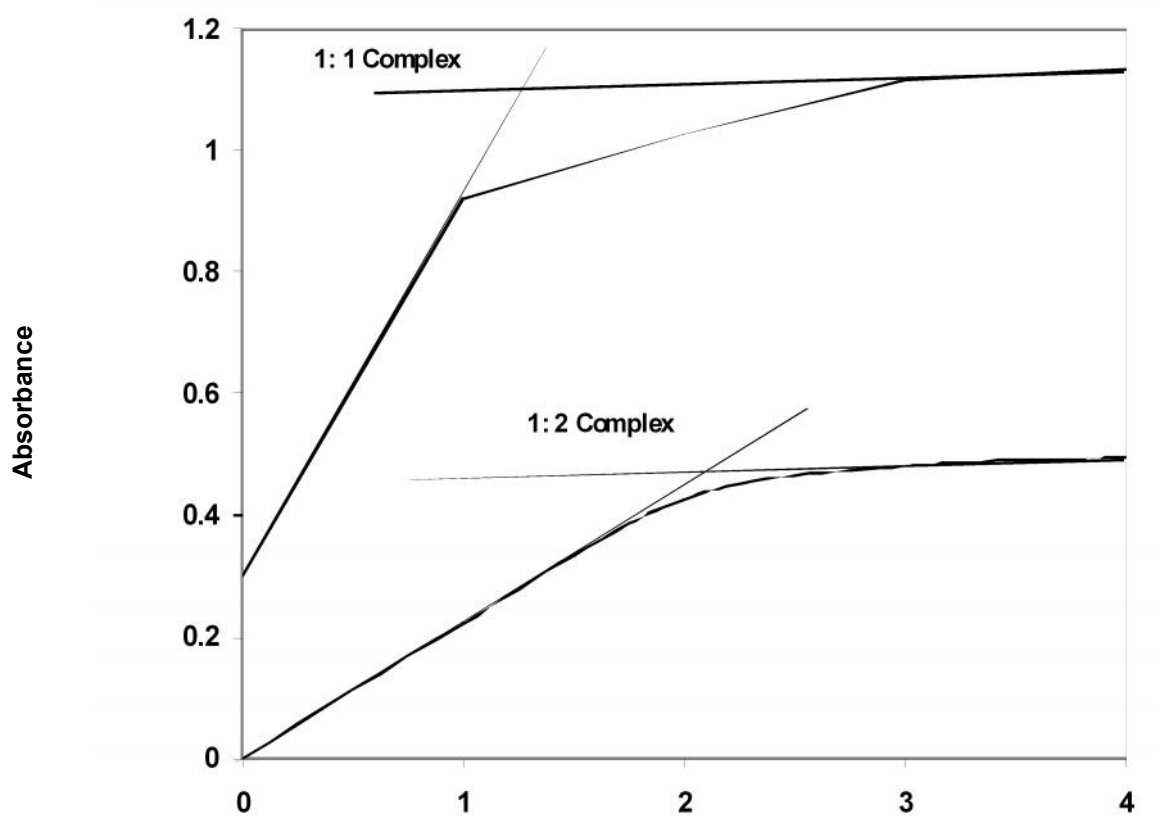


Fig. 7.1.4.5: Mole-ratio plot for theophylline-gliclazide system

Table 7.1.4.11: Data for mole-ratio plot of theophylline-glipizide system (1:1 mixture)

Conc. of theophylline	Conc. of glipizide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.278
0.0001	0.0001	1	0.881
0.0002	0.0001	2	1.033
0.0003	0.0001	3	1.123
0.0004	0.0001	4	1.132

Table 7.1.4.12: Data for mole-ratio plot of theophylline-glipizide system (1:2 mixtures)

Conc. of theophylline	Conc. of glipizide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.229
0.0002	0.0001	2	0.425
0.0003	0.0001	3	0.478
0.0004	0.0001	4	0.489

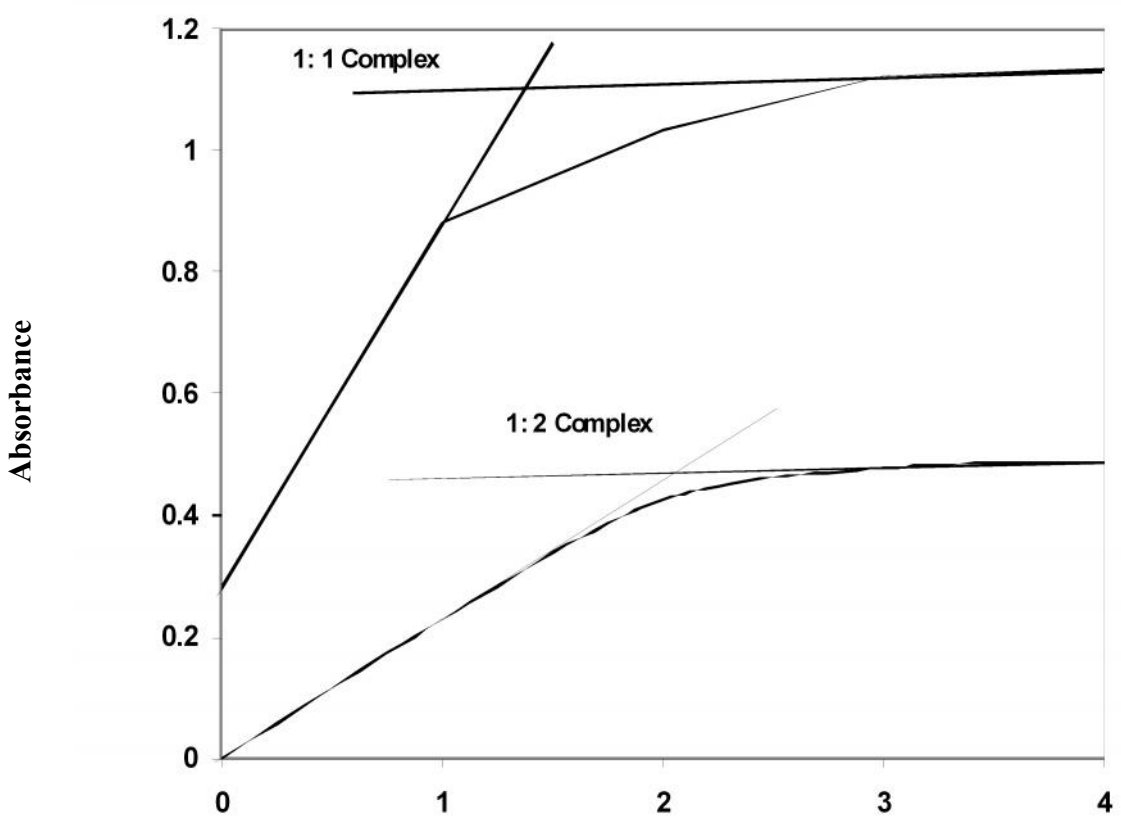


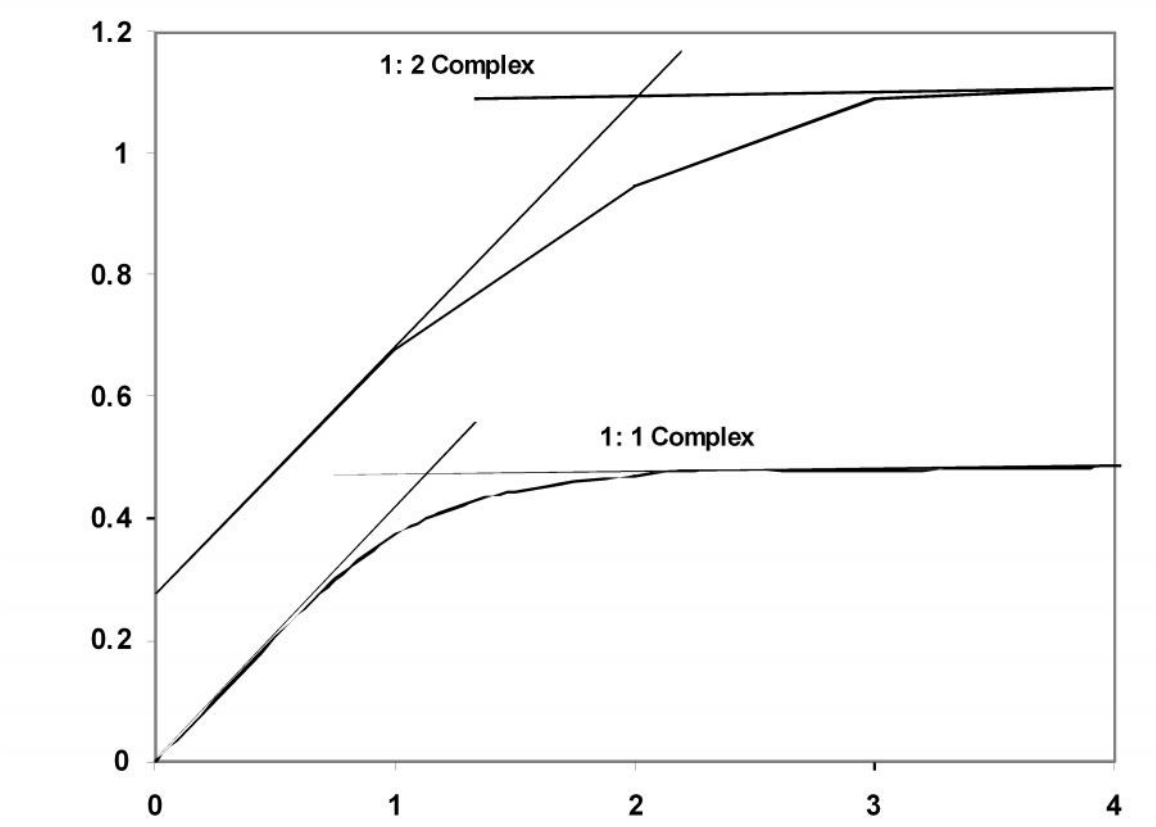
Fig. 7.1.4.6: Mole-ratio plot for theophylline-glipizide system

Table 7.1.4.13: Data for mole-ratio plot of theophylline-glyburide system (1:1 mixture)

Conc. of theophylline	Conc. of glyburide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.373
0.0002	0.0001	2	0.471
0.0003	0.0001	3	0.479
0.0004	0.0001	4	0.485

Table 7.1.4.14: Data for mole-ratio plot of theophylline-glyburide system (1:2 mixtures)

Conc. of theophylline	Conc. of glyburide	Mole-ratio	Absorbance
0.0000	0.0001	0	0.277
0.0001	0.0001	1	0.678
0.0002	0.0001	2	0.948
0.0003	0.0001	3	1.091
0.0004	0.0001	4	1.107



Mole ligand per mole cation

Fig. 7.1.4.7: Mole-ratio plot for theophylline-glyburide system

Table 7.1.4.15: Data for mole-ratio plot of theophylline-metformin system (1:1 mixture)

Conc. of theophylline	Conc. of metformin	Mole-ratio	Absorbance
0.0000	0.0001	0	0.000
0.0001	0.0001	1	0.479
0.0002	0.0001	2	0.565
0.0003	0.0001	3	0.576
0.0004	0.0001	4	0.575

Table 7.1.4.16: Data for mole-ratio plot of theophylline-metformin system (1:2 mixtures)

Conc. of theophylline	Conc. of metformin	Mole-ratio	Absorbance
0.0000	0.0001	0	0.413
0.0001	0.0001	1	0.821
0.0002	0.0001	2	1.042
0.0003	0.0001	3	1.198
0.0004	0.0001	4	1.218

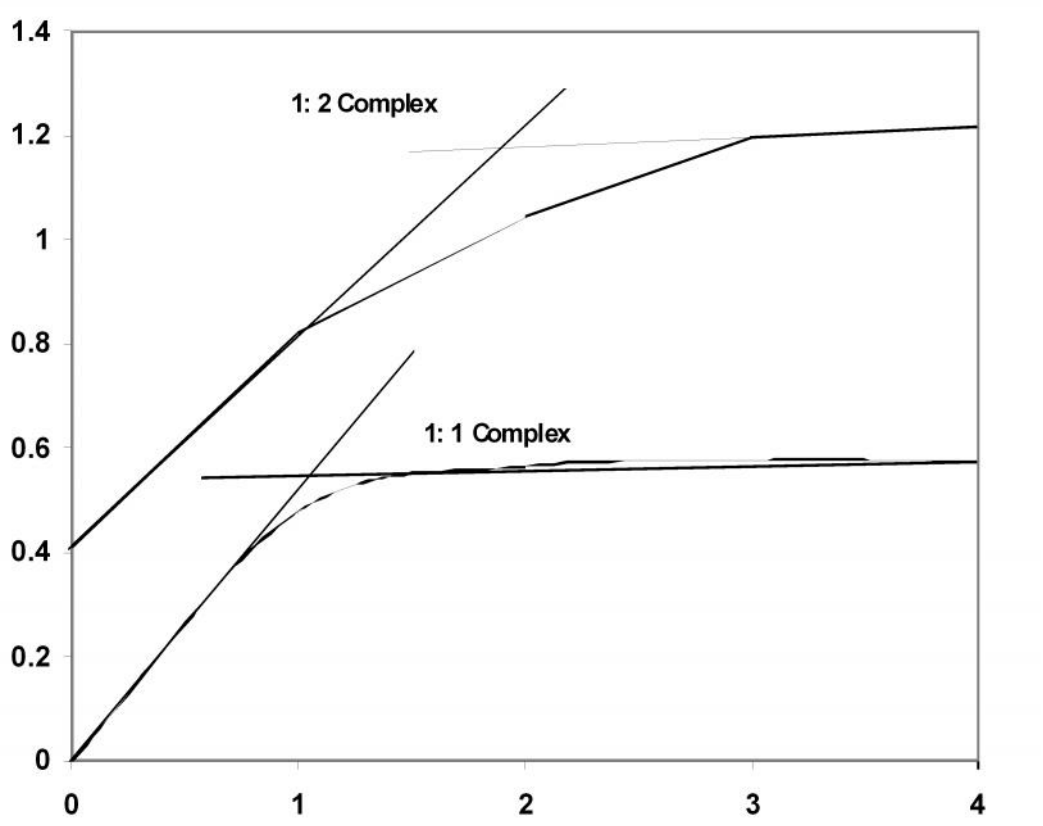


Fig. 7.1.4.8: Mole-ratio plot for theophylline-metformin system

7.1.5 COMPLEX FORMATION BY CONDUCTOMETRIC METHOD

It is well known that conductance is an inherent property of an ionic species. The conductance of one species in solution may be changed due to interaction with other species. Conductance changes as a function of varying molar ratios of the species in a mixture.

In this study, conductometric titrations in demineralized water system at different pHs were carried out to find out the molar ratios at which complexation occurred.[92][98] For each combination, two titrations were performed one was titrated against the other and vice-versa. [74]

For other interacting species, the same process was followed. The conductance value at each addition was recorded. Then conductance was plotted versus the molar ratios of the titrant for obtaining conductivity curves (7.1.5.1 to 7.1.5.56)

When caffeine was titrated with gliclazide at pH 1.4, three distinct breaks corresponding to caffeine-gliclazide molar ratios of 1:1, 2:1 and 3:1 were found in the conductivity curve (fig. 7.1.5.1). The reverse titration showed breaks at 1:1, 2:1 and 4:1 molar ratio (fig.7.1.5.1). These indicate that caffeine forms stable complex with gliclazide at 1:1 molar ratio through some unstable complex.

When caffeine was titrated with glipizide at pH 1.4, three distinct breaks also corresponding to caffeine-glipizide molar ratios of 1:1, 2:1 and 4:1 were found in the conductivity curve (fig.7.1.5.2). The reverse titration showed breaks at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.2). These indicate that caffeine forms stable complex with glipizide at 1:1 molar ratio through some unstable complex.

When caffeine was titrated with glyburide at pH 1.4, three distinct breaks also corresponding to caffeine-glyburide molar ratios of 1:1 and 3:1 were found in the conductivity curve (fig.7.5.3). The reverse titration showed breaks at 1:1 and 2:1 molar ratio (fig.7.5.3). These indicate that caffeine forms stable complex with glyburide at 1:1 molar ratio through some unstable complex.

When caffeine was titrated with metformin at pH 1.4, three distinct breaks also corresponding to caffeine-metformin molar ratios of 1:1 and 2:1 were found in the conductivity curve (fig.7.1.5.4). The reverse titration showed breaks at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.4). These indicate that caffeine forms stable complex with metformin at 1:1 molar ratio through some unstable complex.

The same titration of caffeine-gliclazide system at pH 2.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 molar ratio (fig.7.1.5.5). Here also the formation of a stable 1:1 complex of caffeine with gliclazide was indicated.

The same titration of caffeine-glipizide system at pH 2.4 showed distinct breaks at 1:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.6). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 2.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 2:1 molar ratio (fig.7.1.5.7). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 2.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.8). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated**.**

The same titration of caffeine-gliclazide system at pH 3.4 showed distinct breaks at 1:1, 2:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1, 2:1 and 4:1 molar ratio (fig.7.1.5.9). Here also the formation of a stable 1:1 complex of caffeine with gliclazide was indicated. **[57][59]**

The same titration of caffeine-glipizide system at pH 3.4 showed distinct breaks at 1:1, 2:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.10). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 3.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.11). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 3.4 showed distinct breaks at 1:1, 2:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.12). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated.

The same titration of caffeine-gliclazide system at pH 4.4 showed distinct breaks at 1:1 molar ratios, whereas the reverse titrations also showed a distinct break at 1:1 molar ratio (fig.7.1.5.13).Here, a stable 1:1 complex of caffeine with gliclazide was found.

The same titration of caffeine-glipizide system at pH 4.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.14). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 4.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 2:1 molar ratio (fig.7.1.5.15). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 4.4 showed distinct breaks at 1:1, 2:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1, 2:1 and 3:1 molar ratio (fig.7.1.5.16). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated.

The same titration of caffeine-gliclazide system at pH 5.4 gave distinct breaks at 1:1 and 4:1 molar ratios, whereas at the reverse titration showed breaks at 1:1 and 3:1 molar ratios (fig. 7.1.5.17). These also indicated the formation of a stable 1:1 complex of caffeine with gliclazide.

The same titration of caffeine-glipizide system at pH 5.4 showed distinct breaks at 1:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.18). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 5.4 showed distinct breaks at 1:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.19). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 5.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.20). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated.

The same titration of caffeine-gliclazide system at pH 6.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 molar ratio (fig. 7.1.5.21). Here also the formation of a stable 1:1 complex of caffeine with gliclazide was indicated.

The same titration of caffeine-glipizide system at pH 6.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.22). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 6.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 4:1 molar ratio (fig.7.1.5.23). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 6.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 4:1 molar ratio (fig.7.1.5.24). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated.

The same titration of caffeine-gliclazide system at pH 7.4 gave distinct breaks at 1:1 and 2:1 molar ratios, whereas at the reverse titration showed breaks at 1:1 and 3:1 molar ratios (fig.7.5.25). These also indicated the formation if a stable 1:1 complex of caffeine with gliclazide.

The same titration of caffeine-glipizide system at pH 7.4 showed distinct breaks at 1:1 and 3:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 2:1 molar ratio (fig.7.1.5.26). Here also the formation of a stable 1:1 complex of caffeine with glipizide was indicated.

The same titration of caffeine-glyburide system at pH 7.4 showed distinct breaks at 1:1 and 4:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.27). Here also the formation of a stable 1:1 complex of caffeine with glyburide was indicated.

The same titration of caffeine-metformin system at pH 7.4 showed distinct breaks at 1:1 and 2:1 molar ratios, whereas the reverse titration showed distinct break at 1:1 and 3:1 molar ratio (fig.7.1.5.28). Here also the formation of a stable 1:1 complex of caffeine with metformin was indicated.

By considering the above facts, it may be said that caffeine forms a stable 1:1 complexes with gliclazide and metformin at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4.

Table 7.1.5.1: Data for conductometric titration of caffeine-gliclazide System at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	2.50	3.10
0.5	40.50	0.125	2.60	3.15
1.0	41.00	0.250	2.70	3.25
1.5	41.50	0.375	2.80	3.30
2.0	42.00	0.500	2.90	3.40
2.5	42.50	0.625	3.00	3.50
3.0	43.00	0.750	2.98	3.53
3.5	43.50	0.875	2.95	3.55
4.0	44.00	1.000	2.95	3.57
4.5	44.50	1.125	2.94	3.60
5.0	45.00	1.250	2.93	3.60
5.5	45.50	1.375	3.00	3.63
6.0	46.00	1.500	3.10	3.68
6.5	46.50	1.625	3.17	3.80
7.0	47.00	1.750	3.28	3.95
7.5	47.50	1.875	3.30	4.05

8.0	48.00	2.000	3.35	4.20
8.5	48.50	2.125	3.40	4.00
9.0	49.00	2.250	3.40	3.97
9.5	49.50	2.375	3.41	3.92
10.0	50.00	2.500	3.40	3.90

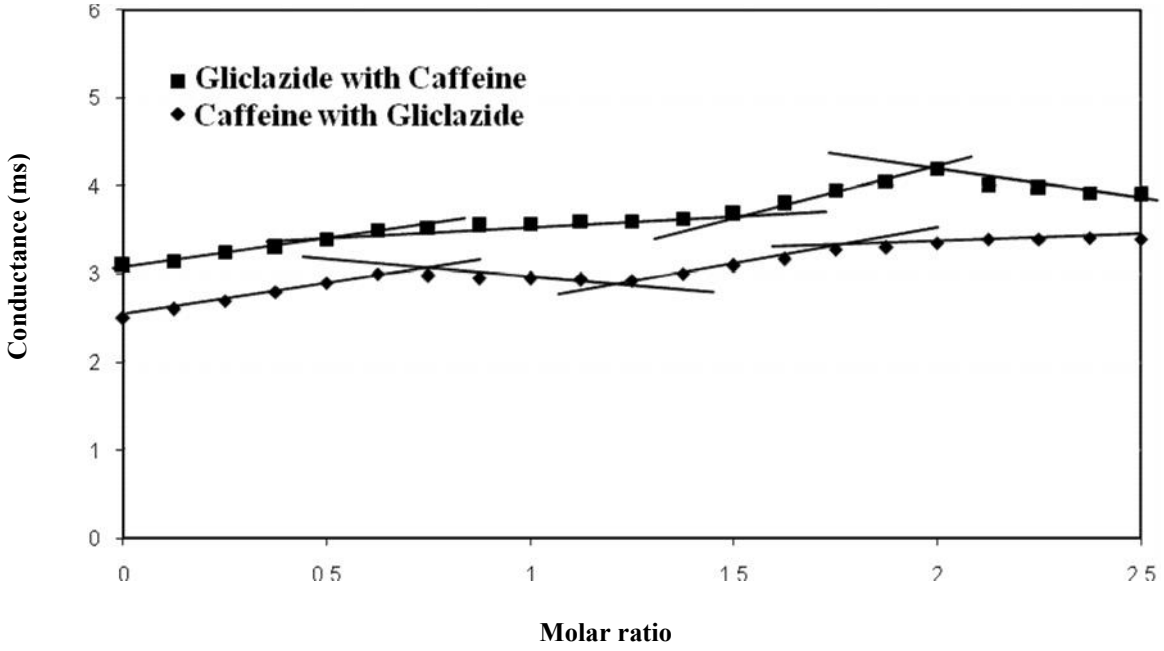


Fig. 7.1.5.1: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 1.4

Table 7.1.5.2: Data for conductometric titration of caffeine-glipizide System at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.52	3.07
0.5	40.50	0.125	2.62	3.12
1.0	41.00	0.250	2.75	3.23
1.5	41.50	0.375	2.85	3.28
2.0	42.00	0.500	2.88	3.35
2.5	42.50	0.625	2.98	3.48
3.0	43.00	0.750	2.95	3.51
3.5	43.50	0.875	2.91	3.52
4.0	44.00	1.000	2.92	3.55
4.5	44.50	1.125	2.90	3.57
5.0	45.00	1.250	2.91	3.58
5.5	45.50	1.375	3.02	3.60
6.0	46.00	1.500	3.07	3.65
6.5	46.50	1.625	3.15	3.75
7.0	47.00	1.750	3.25	3.91
7.5	47.50	1.875	3.28	4.00

8.0	48.00	2.000	3.31	4.05
8.5	48.50	2.125	3.32	3.98
9.0	49.00	2.250	3.34	3.87
9.5	49.50	2.375	3.35	3.83
10.0	50.00	2.500	3.33	3.79

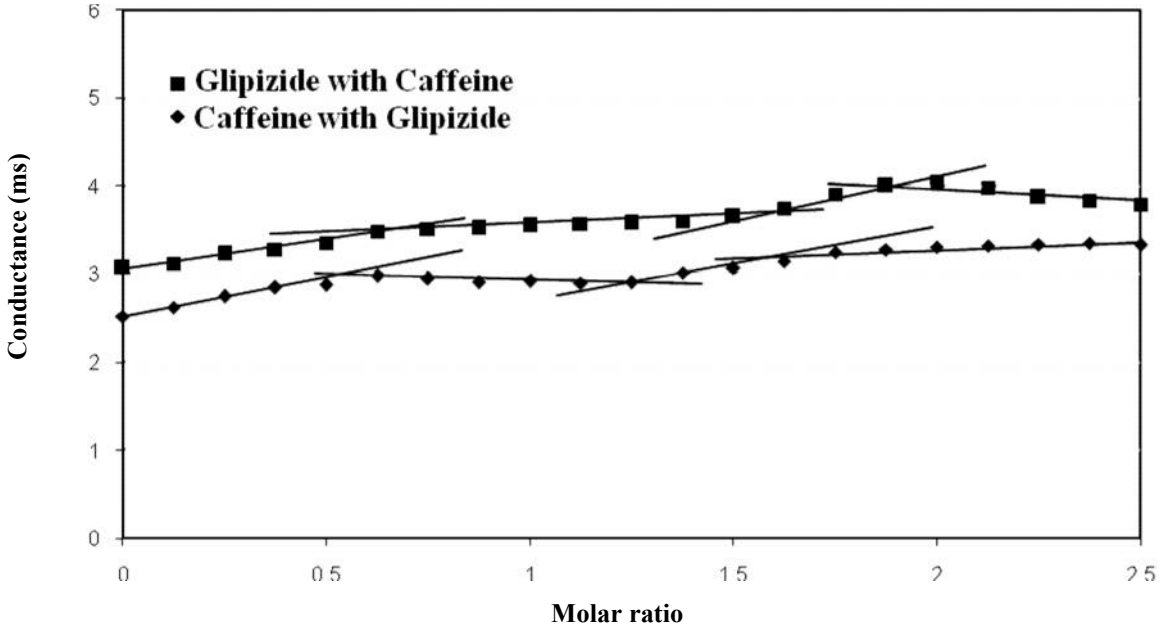


Fig. 7.1.5.2: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 1.4

Table 7.1.5.3: Data for conductometric titration of caffeine-glyburide system at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	2.25	3.17
0.5	40.50	0.125	2.28	3.25
1.0	41.00	0.250	2.38	3.27
1.5	41.50	0.375	2.35	3.32
2.0	42.00	0.500	2.40	3.31
2.5	42.50	0.625	2.63	3.37
3.0	43.00	0.750	2.82	3.65
3.5	43.50	0.875	2.98	3.88
4.0	44.00	1.000	3.13	4.13
4.5	44.50	1.125	3.38	4.36
5.0	45.00	1.250	3.45	4.42
5.5	45.50	1.375	3.56	4.38
6.0	46.00	1.500	3.57	4.28
6.5	46.50	1.625	3.58	4.3
7.0	47.00	1.750	3.59	4.25
7.5	47.50	1.875	3.62	4.22

8.0	48.00	2.000	3.61	4.25
8.5	48.50	2.125	3.63	4.17
9.0	49.00	2.250	3.62	4.15
9.5	49.50	2.375	3.63	4.12
10.0	50.00	2.500	3.60	4.10

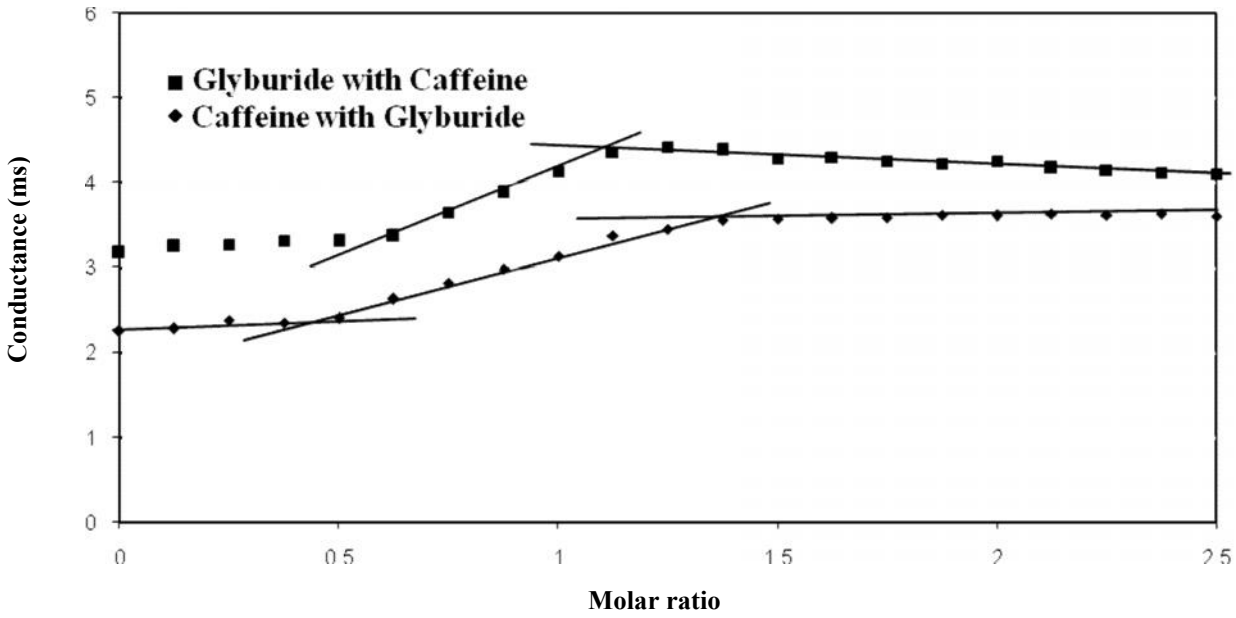


Fig. 7.1.5.3: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 1.4

Table 7.1.5.4: Data for conductometric titration of caffeine - metformin system at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	1.50	2.32
0.5	40.50	0.125	1.60	2.45
1.0	41.00	0.250	1.72	2.54
1.5	41.50	0.375	1.85	2.65
2.0	42.00	0.500	1.95	2.78
2.5	42.50	0.625	2.10	2.90
3.0	43.00	0.750	2.21	3.00
3.5	43.50	0.875	2.31	3.30
4.0	44.00	1.000	2.35	3.51
4.5	44.50	1.125	2.40	3.60
5.0	45.00	1.250	2.45	3.70
5.5	45.50	1.375	2.52	3.83
6.0	46.00	1.500	2.70	3.89
6.5	46.50	1.625	2.81	3.95
7.0	47.00	1.750	2.95	3.98
7.5	47.50	1.875	3.10	4.10

8.0	48.00	2.000	3.25	4.20
8.5	48.50	2.125	3.40	4.30
9.0	49.00	2.250	3.70	4.51
9.5	49.50	2.375	3.80	4.72
10.0	50.00	2.500	3.90	4.80

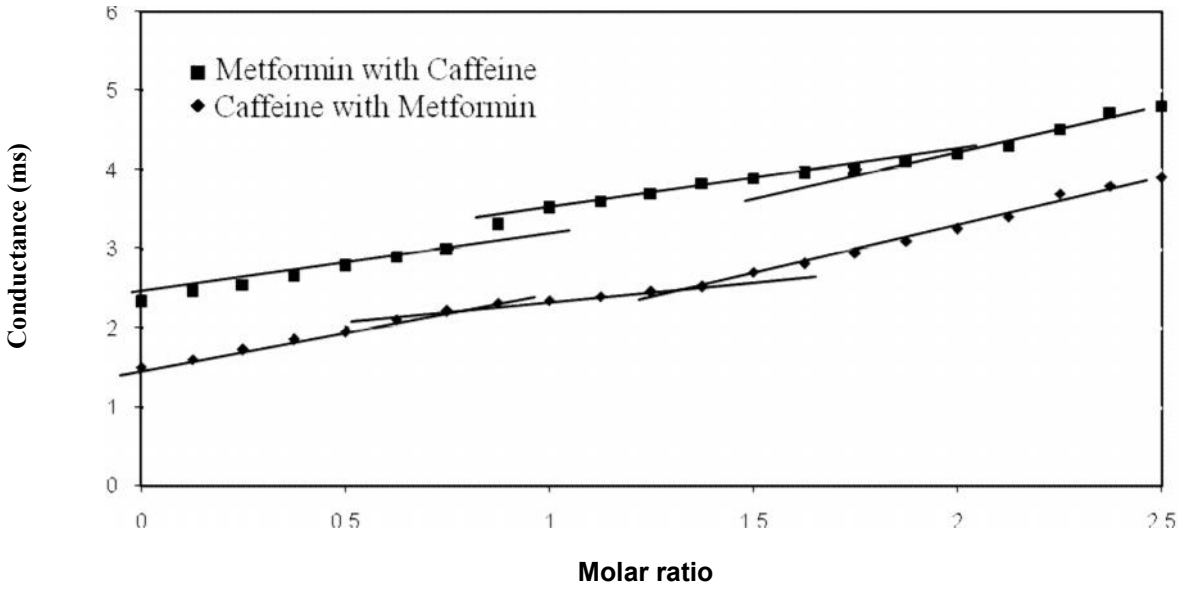


Fig. 7.1.5.4: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 1.4

Table 7.1.5.5: Data for conductometric titration of caffeine-gliclazide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	1.30	2.20
0.5	40.50	0.125	1.32	2.25
1.0	41.00	0.250	1.38	2.25
1.5	41.50	0.375	1.40	2.28
2.0	42.00	0.500	1.41	2.30
2.5	42.50	0.625	1.60	2.40
3.0	43.00	0.750	1.80	2.70
3.5	43.50	0.875	1.95	2.85
4.0	44.00	1.000	2.10	3.10
4.5	44.50	1.125	2.35	3.35
5.0	45.00	1.250	2.40	3.40
5.5	45.50	1.375	2.60	3.35
6.0	46.00	1.500	2.61	3.31
6.5	46.50	1.625	2.61	3.30
7.0	47.00	1.750	2.60	3.28
7.5	47.50	1.875	2.60	3.25

8.0	48.00	2.000	2.61	3.22
8.5	48.50	2.125	2.61	3.20
9.0	49.00	2.250	2.62	3.18
9.5	49.50	2.375	2.62	3.15
10.0	50.00	2.500	2.61	3.12

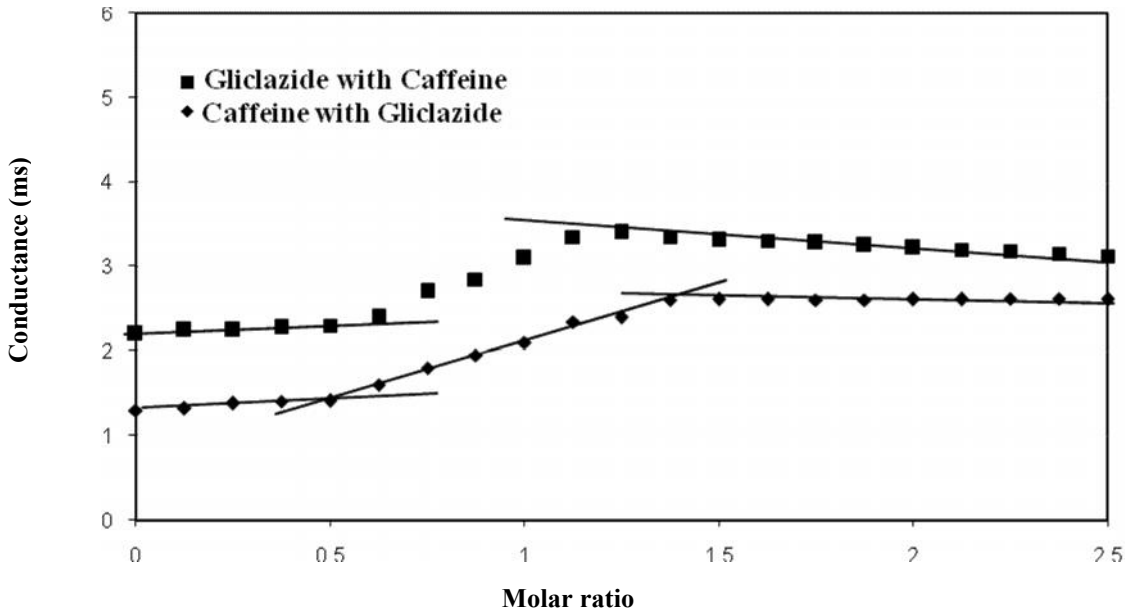


Fig. 7.1.5.5: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 2.4

Table 7.1.5.6: Data for conductometric titration of caffeine-glipizide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	1.63	2.53
0.5	40.50	0.125	1.67	2.55
1.0	41.00	0.250	1.69	2.58
1.5	41.50	0.375	1.71	2.59
2.0	42.00	0.500	1.73	2.61
2.5	42.50	0.625	1.83	2.43
3.0	43.00	0.750	1.98	2.65
3.5	43.50	0.875	2.08	2.83
4.0	44.00	1.000	2.21	3.02
4.5	44.50	1.125	2.38	3.32
5.0	45.00	1.250	2.45	3.51
5.5	45.50	1.375	2.58	3.55
6.0	46.00	1.500	2.62	3.53
6.5	46.50	1.625	2.63	3.55
7.0	47.00	1.750	2.61	3.56

7.5	47.50	1.875	2.59	3.54
8.0	48.00	2.000	2.60	3.53
8.5	48.50	2.125	2.62	3.52
9.0	49.00	2.250	2.64	3.58
9.5	49.50	2.375	2.65	3.55
10.0	50.00	2.500	2.63	3.52

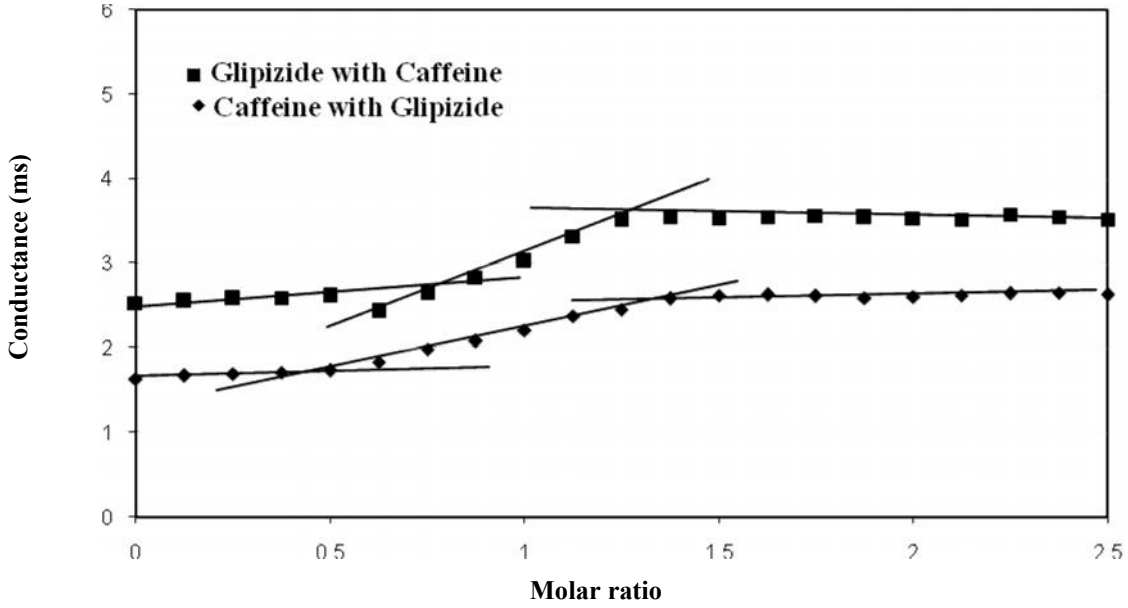


Fig. 7.1.5.6: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 2.4

Table 7.1.5.7: Data for conductometric titration of caffeine-glyburide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	1.05	1.95
0.5	40.50	0.125	1.23	2.08
1.0	41.00	0.250	1.35	2.23
1.5	41.50	0.375	1.45	2.29
2.0	42.00	0.500	1.65	2.48
2.5	42.50	0.625	1.75	2.51
3.0	43.00	0.750	1.01	2.70
3.5	43.50	0.875	1.05	2.85
4.0	44.00	1.000	2.11	2.88
4.5	44.50	1.125	2.25	2.93
5.0	45.00	1.250	2.29	2.98
5.5	45.50	1.375	2.43	3.05
6.0	46.00	1.500	2.49	3.12
6.5	46.50	1.625	2.51	3.25
7.0	47.00	1.750	2.61	3.29
7.5	47.50	1.875	2.52	3.38

8.0	48.00	2.000	2.51	3.36
8.5	48.50	2.125	2.47	3.32
9.0	49.00	2.250	2.45	3.28
9.5	49.50	2.375	2.44	3.25
10.0	50.00	2.500	2.42	3.23

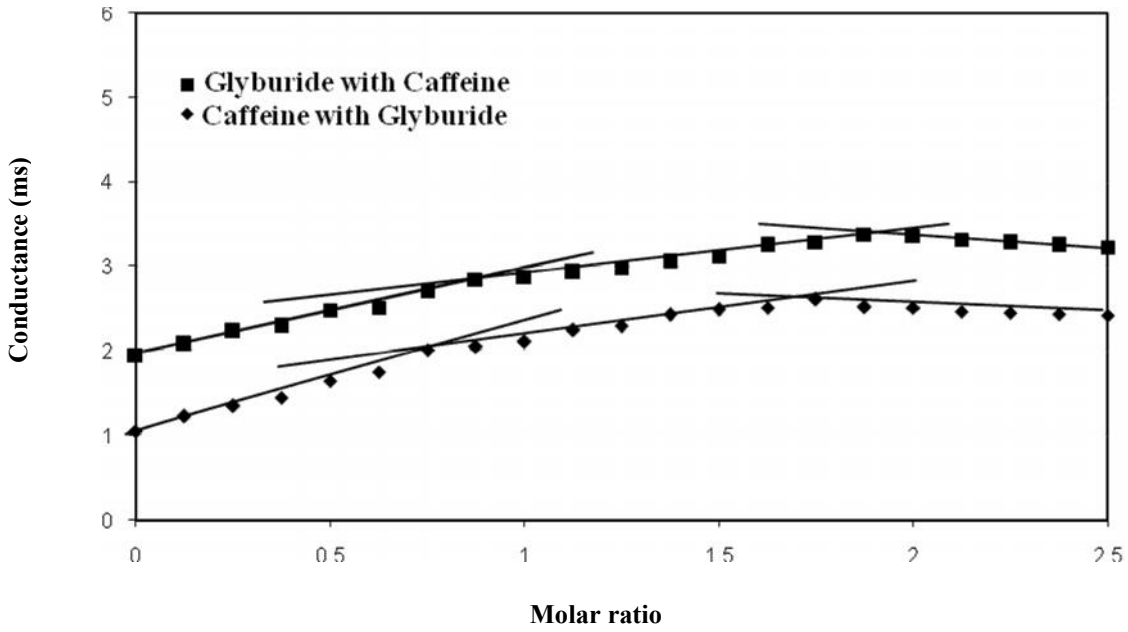


Fig. 7.1.5.7: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 2.4

Table 7.1.5.8: Data for conductometric titration of caffeine - metformin system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	1.51	2.78
0.5	40.50	0.125	1.65	2.88
1.0	41.00	0.250	1.69	3.01
1.5	41.50	0.375	1.91	3.19
2.0	42.00	0.500	2.09	3.31
2.5	42.50	0.625	2.32	3.52
3.0	43.00	0.750	2.46	3.82
3.5	43.50	0.875	2.82	4.21
4.0	44.00	1.000	3.12	4.50
4.5	44.50	1.125	3.42	4.90
5.0	45.00	1.250	3.71	5.30
5.5	45.50	1.375	4.01	5.62
6.0	46.00	1.500	4.31	5.85
6.5	46.50	1.625	4.51	5.56
7.0	47.00	1.750	4.62	5.55
7.5	47.50	1.875	4.72	5.52

8.0	48.00	2.000	4.82	5.51
8.5	48.50	2.125	4.91	5.52
9.0	49.00	2.250	4.97	5.50
9.5	49.50	2.375	5.06	5.51
10.0	50.00	2.500	5.16	5.50

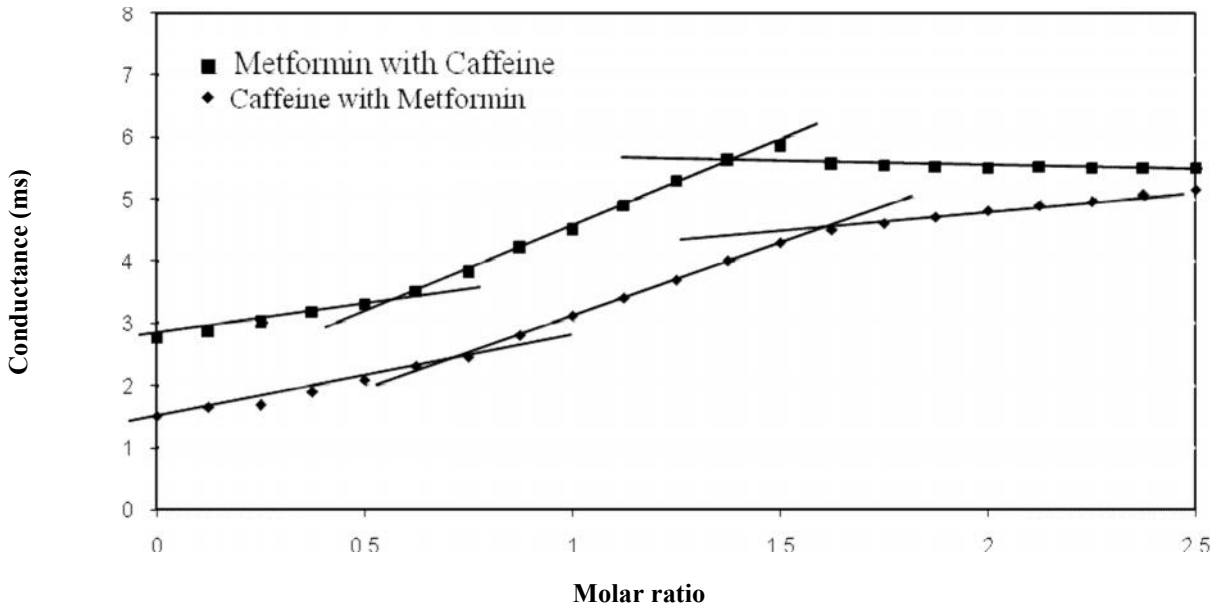


Fig. 7.1.5.8: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 2.4

Table 7.1.5.9: Data for conductometric titration of caffeine-gliclazide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	2.60	3.15
0.5	40.50	0.125	2.70	3.25
1.0	41.00	0.250	2.81	3.31
1.5	41.50	0.375	2.91	3.40
2.0	42.00	0.500	3.01	3.51
2.5	42.50	0.625	2.98	3.53
3.0	43.00	0.750	2.95	3.55
3.5	43.50	0.875	2.96	3.57
4.0	44.00	1.000	2.94	3.61
4.5	44.50	1.125	2.93	3.62
5.0	45.00	1.250	3.00	3.63
5.5	45.50	1.375	3.01	3.68
6.0	46.00	1.500	3.17	3.80
6.5	46.50	1.625	3.28	3.85
7.0	47.00	1.750	3.30	3.95
7.5	47.50	1.875	3.35	3.99

8.0	48.00	2.000	3.40	4.01
8.5	48.50	2.125	3.38	3.97
9.0	49.00	2.250	3.42	3.92
9.5	49.50	2.375	3.44	3.91
10.0	50.00	2.500	3.31	3.86

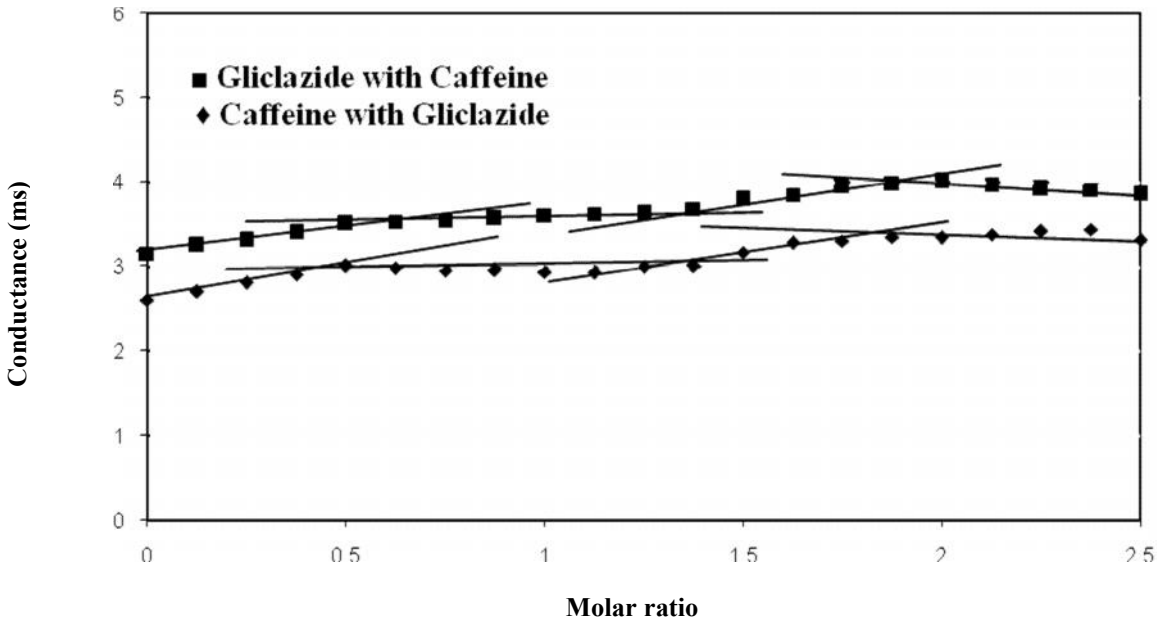


Fig. 7.1.5.9: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 3.4

Table 7.1.5.10: Data for conductometric titration of caffeine-glipizide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.57	3.16
0.5	40.50	0.125	2.68	3.22
1.0	41.00	0.250	2.75	3.25
1.5	41.50	0.375	2.87	3.35
2.0	42.00	0.500	2.95	3.42
2.5	42.50	0.625	2.91	3.51
3.0	43.00	0.750	2.88	3.45
3.5	43.50	0.875	2.90	3.53
4.0	44.00	1.000	2.89	3.59
4.5	44.50	1.125	2.91	3.61
5.0	45.00	1.250	3.97	3.62
5.5	45.50	1.375	3.21	3.67
6.0	46.00	1.500	3.24	3.76
6.5	46.50	1.625	3.18	3.83
7.0	47.00	1.750	3.30	3.85
7.5	47.50	1.875	3.25	3.88

8.0	48.00	2.000	3.24	3.89
8.5	48.50	2.125	3.28	3.87
9.0	49.00	2.250	3.22	3.88
9.5	49.50	2.375	3.23	3.89
10.0	50.00	2.500	3.25	3.90

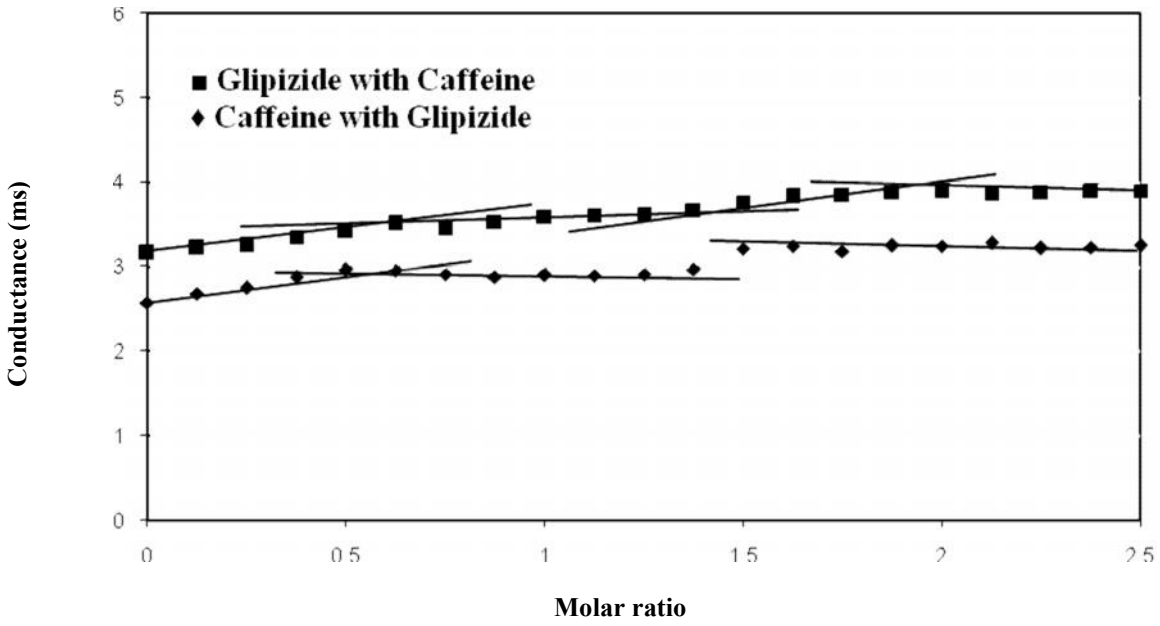


Fig. 7.1.5.10: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 3.4

Table 7.5.11: Data for conductometric titration of caffeine-glyburide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	2.28	3.07
0.5	40.50	0.125	2.42	3.23
1.0	41.00	0.250	2.53	3.29
1.5	41.50	0.375	2.72	3.37
2.0	42.00	0.500	2.78	3.41
2.5	42.50	0.625	2.83	3.43
3.0	43.00	0.750	2.97	3.48
3.5	43.50	0.875	2.97	3.56
4.0	44.00	1.000	2.95	3.58
4.5	44.50	1.125	2.94	3.59
5.0	45.00	1.250	2.91	3.58
5.5	45.50	1.375	2.93	3.66
6.0	46.00	1.500	2.91	3.75
6.5	46.50	1.625	2.98	3.81
7.0	47.00	1.750	3.05	3.85
7.5	47.50	1.875	3.13	3.89

8.0	48.00	2.000	3.15	3.95
8.5	48.50	2.125	3.25	4.02
9.0	49.00	2.250	3.22	4.01
9.5	49.50	2.375	3.23	4.07
10.0	50.00	2.500	3.31	4.05

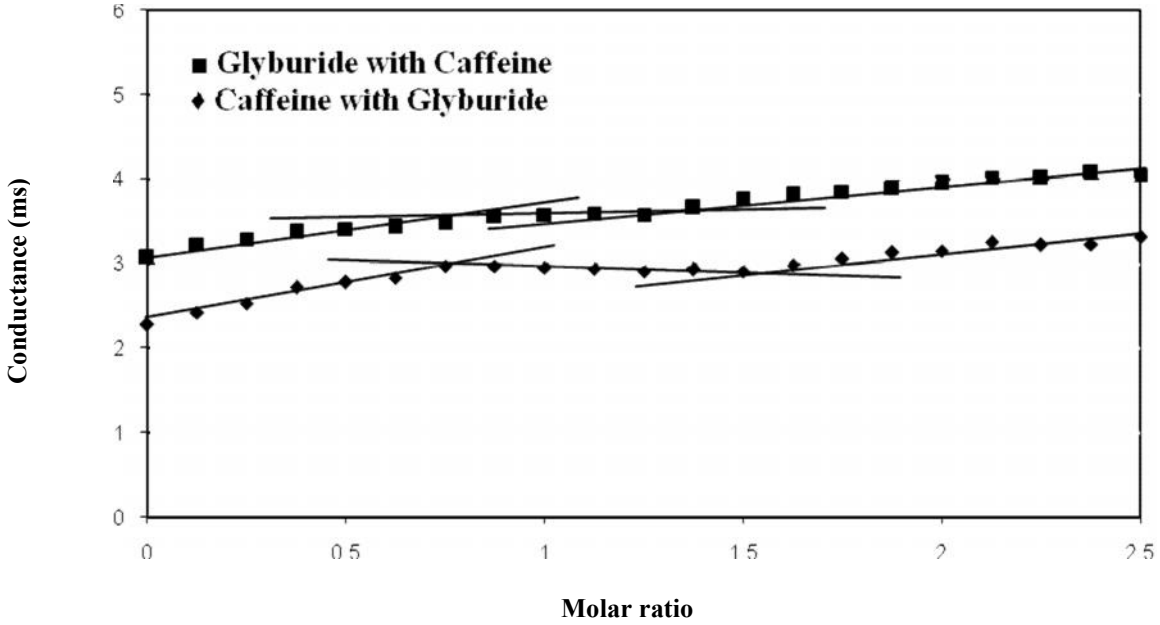


Fig. 7.1.5.11: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 3.4

Table 7.1.5.12: Data for conductometric titration of caffeine - metformin system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	1.62	2.36
0.5	40.50	0.125	1.72	2.42
1.0	41.00	0.250	1.86	2.52
1.5	41.50	0.375	1.96	2.62
2.0	42.00	0.500	2.12	2.67
2.5	42.50	0.625	2.22	2.82
3.0	43.00	0.750	2.32	3.00
3.5	43.50	0.875	2.36	3.31
4.0	44.00	1.000	2.40	3.51
4.5	44.50	1.125	2.42	3.61
5.0	45.00	1.250	2.44	3.71
5.5	45.50	1.375	2.47	3.70
6.0	46.00	1.500	2.49	3.72
6.5	46.50	1.625	2.70	3.73
7.0	47.00	1.750	3.10	3.75
7.5	47.50	1.875	3.50	3.85

8.0	48.00	2.000	3.70	3.95
8.5	48.50	2.125	3.80	4.05
9.0	49.00	2.250	3.90	4.10
9.5	49.50	2.375	4.00	4.15
10.0	50.00	2.500	4.10	4.25

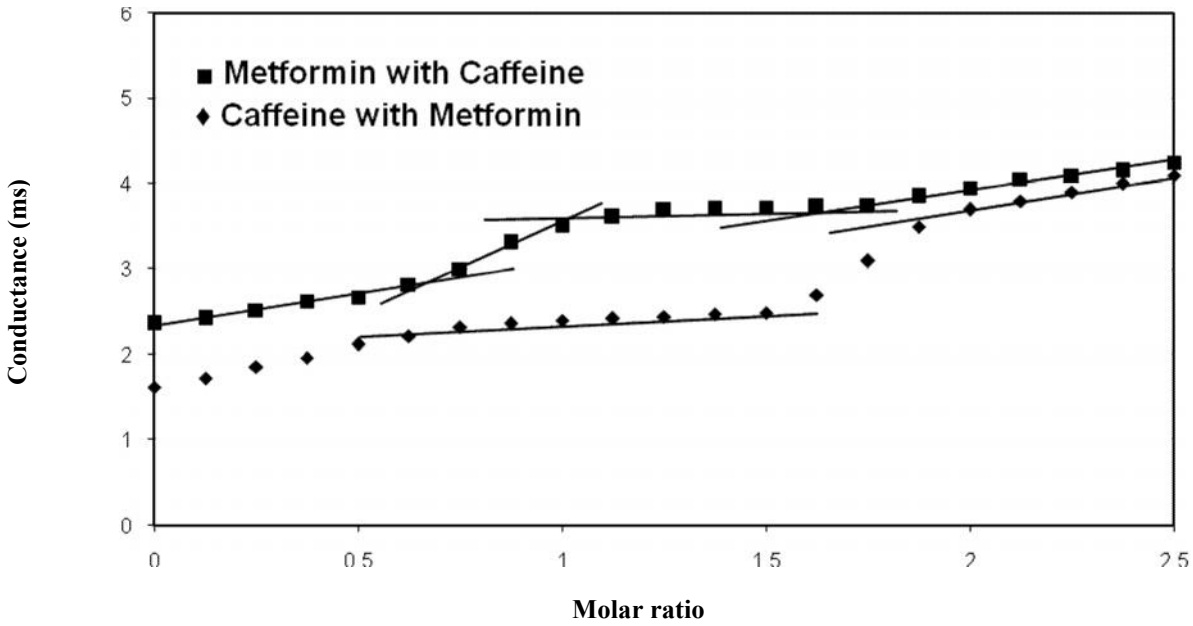


Fig. 7.1.5.12: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 3.4

Table 7.1.5.13: Data for conductometric titration of caffeine-gliclazide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	2.32	3.26
0.5	40.50	0.125	2.37	3.32
1.0	41.00	0.250	2.42	3.40
1.5	41.50	0.375	2.47	3.45
2.0	42.00	0.500	2.52	3.50
2.5	42.50	0.625	2.52	3.57
3.0	43.00	0.750	2.53	3.65
3.5	43.50	0.875	2.53	3.62
4.0	44.00	1.000	2.52	3.62
4.5	44.50	1.125	2.50	3.63
5.0	45.00	1.250	2.49	3.63
5.5	45.50	1.375	2.47	3.63
6.0	46.00	1.500	2.46	3.67
6.5	46.50	1.625	2.45	3.66
7.0	47.00	1.750	2.51	3.70
7.5	47.50	1.875	2.65	4.01

8.0	48.00	2.000	2.88	4.20
8.5	48.50	2.125	3.10	4.32
9.0	49.00	2.250	3.35	4.35
9.5	49.50	2.375	3.52	4.36
10.0	50.00	2.500	3.73	4.37

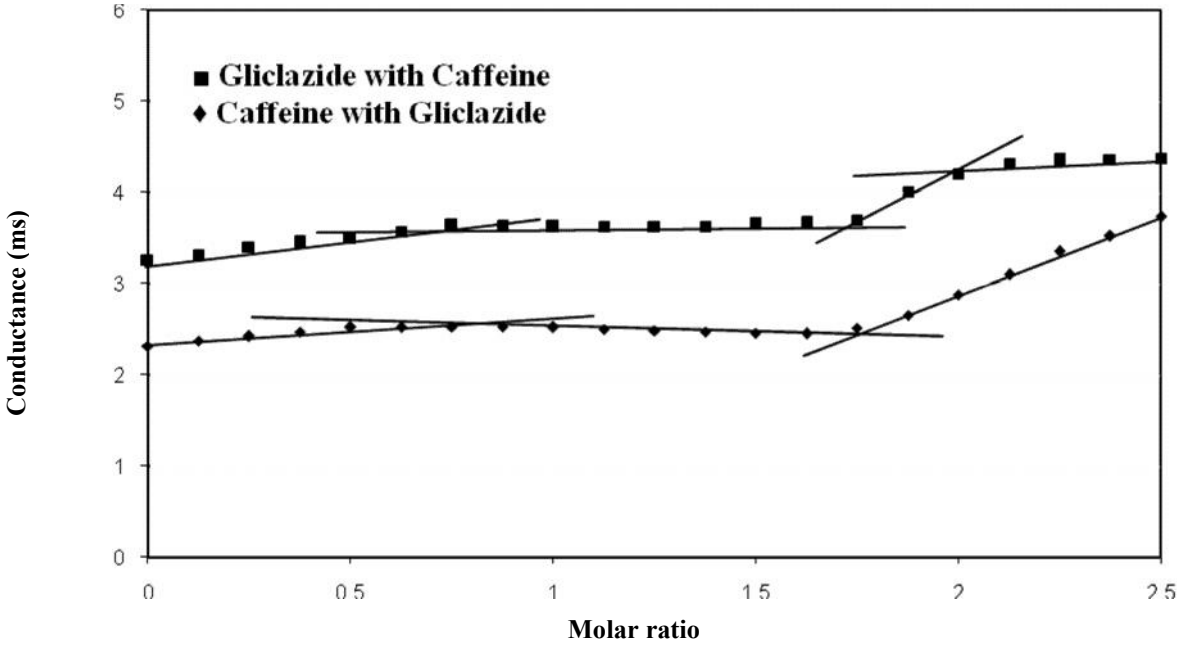


Fig. 7.1.5.13: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 4.4

Table 7.5.14: Data for conductometric titration of caffeine-glipizide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.23	3.23
0.5	40.50	0.125	2.35	3.25
1.0	41.00	0.250	2.46	3.36
1.5	41.50	0.375	2.49	3.43
2.0	42.00	0.500	2.62	3.48
2.5	42.50	0.625	2.71	3.57
3.0	43.00	0.750	2.75	3.62
3.5	43.50	0.875	2.74	3.56
4.0	44.00	1.000	2.72	3.55
4.5	44.50	1.125	2.75	3.57
5.0	45.00	1.250	2.79	3.62
5.5	45.50	1.375	2.78	3.64
6.0	46.00	1.500	2.69	3.61
6.5	46.50	1.625	2.85	3.63
7.0	47.00	1.750	2.98	3.68
7.5	47.50	1.875	3.13	3.91

8.0	48.00	2.000	2.25	4.13
8.5	48.50	2.125	3.39	4.29
9.0	49.00	2.250	3.45	4.33
9.5	49.50	2.375	3.58	4.36
10.0	50.00	2.500	3.69	4.53

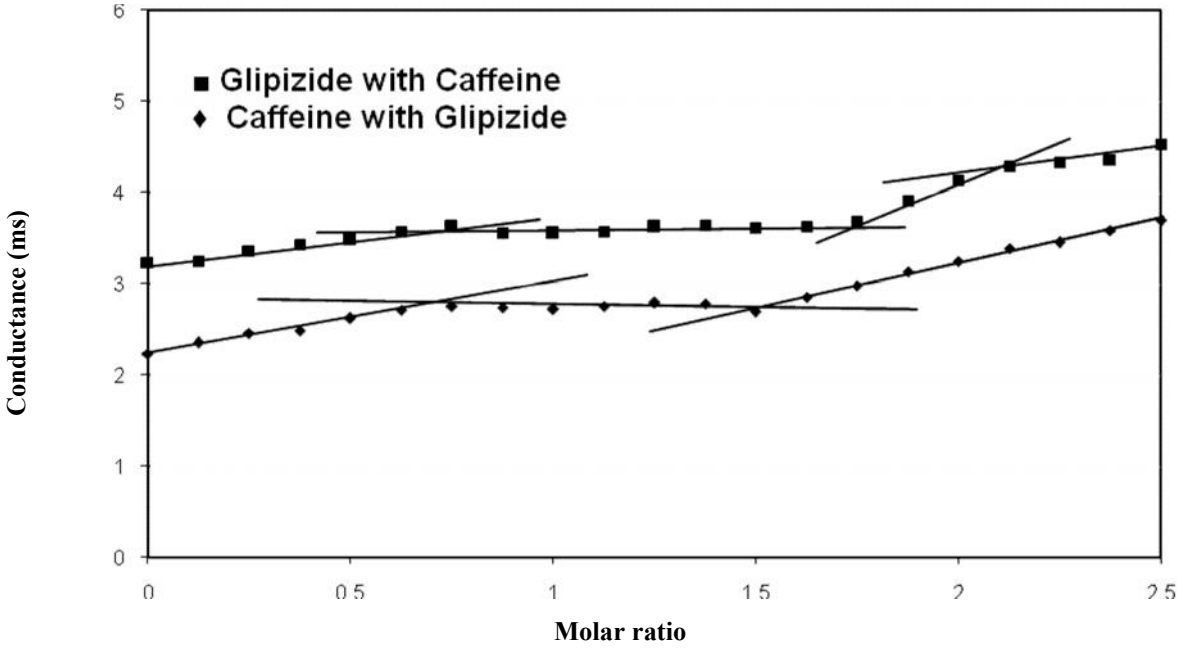


Fig. 7.1.5.14: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 4.4

Table 7.1.5.15: Data for conductometric titration of caffeine-glyburide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	2.21	3.28
0.5	40.50	0.125	2.27	3.35
1.0	41.00	0.250	2.32	3.44
1.5	41.50	0.375	2.38	3.45
2.0	42.00	0.500	2.39	3.48
2.5	42.50	0.625	2.49	3.56
3.0	43.00	0.750	2.59	3.61
3.5	43.50	0.875	2.55	3.62
4.0	44.00	1.000	2.54	3.58
4.5	44.50	1.125	2.53	3.59
5.0	45.00	1.250	2.55	3.61
5.5	45.50	1.375	2.57	3.62
6.0	46.00	1.500	2.55	3.63
6.5	46.50	1.625	2.54	3.65
7.0	47.00	1.750	2.53	3.88
7.5	47.50	1.875	2.63	4.02

8.0	48.00	2.000	2.86	4.21
8.5	48.50	2.125	3.05	4.36
9.0	49.00	2.250	3.23	4.53
9.5	49.50	2.375	3.43	4.73
10.0	50.00	2.500	3.71	4.85

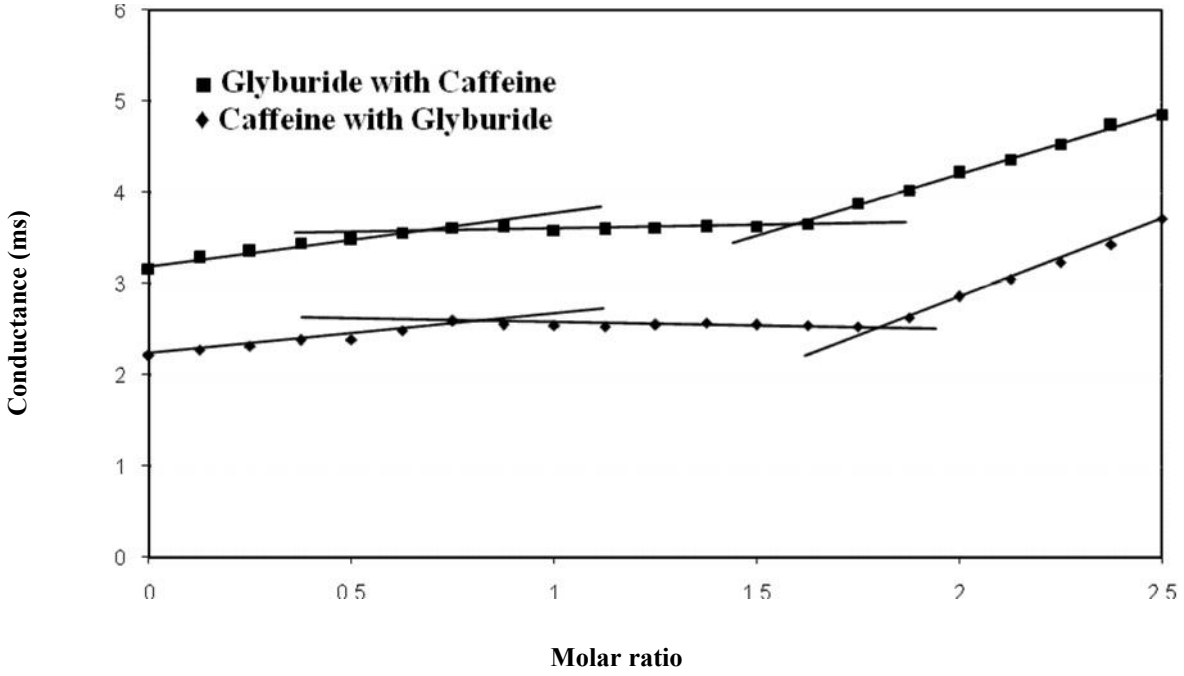


Fig. 7.1.5.15: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 4.4

Table 7.1.5.16: Data for conductometric titration of caffeine-metformin system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	1.26	2.26
0.5	40.50	0.125	1.30	2.32
1.0	41.00	0.250	1.34	2.40
1.5	41.50	0.375	1.38	2.50
2.0	42.00	0.500	1.40	2.60
2.5	42.50	0.625	1.45	2.70
3.0	43.00	0.750	1.60	2.78
3.5	43.50	0.875	1.85	2.80
4.0	44.00	1.000	2.00	2.81
4.5	44.50	1.125	2.25	2.82
5.0	45.00	1.250	2.15	2.83
5.5	45.50	1.375	2.10	2.85
6.0	46.00	1.500	2.05	2.86
6.5	46.50	1.625	2.00	2.87
7.0	47.00	1.750	1.95	2.60
7.5	47.50	1.875	2.01	2.45

8.0	48.00	2.000	2.10	2.70
8.5	48.50	2.125	2.25	3.00
9.0	49.00	2.250	2.40	3.40
9.5	49.50	2.375	2.50	3.70
10.0	50.00	2.500	2.60	4.01

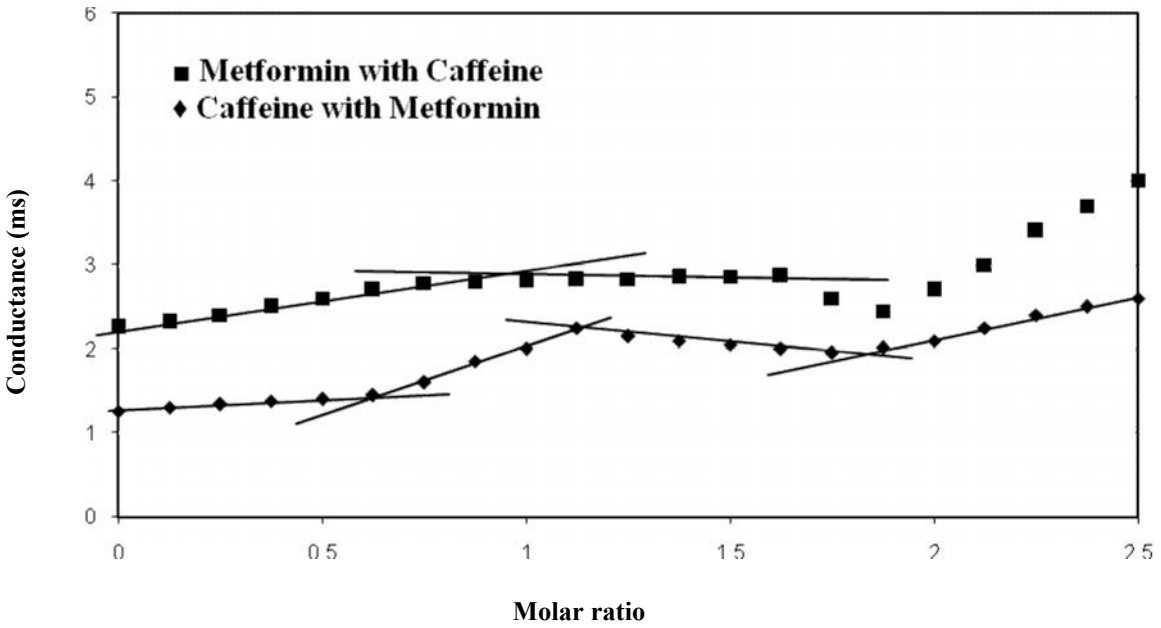


Fig. 7.5.16: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 4.4

Table 7.1.5.17: Data for conductometric titration of caffeine-gliclazide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	1.42	2.42
0.5	40.50	0.125	1.72	2.62
1.0	41.00	0.250	2.02	2.82
1.5	41.50	0.375	2.27	2.95
2.0	42.00	0.500	2.52	3.15
2.5	42.50	0.625	2.67	3.35
3.0	43.00	0.750	2.82	3.40
3.5	43.50	0.875	2.92	3.50
4.0	44.00	1.000	3.02	3.60
4.5	44.50	1.125	3.12	3.70
5.0	45.00	1.250	3.22	3.75
5.5	45.50	1.375	3.30	3.85
6.0	46.00	1.500	3.41	3.91
6.5	46.50	1.625	3.56	3.99
7.0	47.00	1.750	3.70	4.02
7.5	47.50	1.875	3.70	4.07

8.0	48.00	2.000	3.72	4.12
8.5	48.50	2.125	3.72	4.17
9.0	49.00	2.250	3.68	4.22
9.5	49.50	2.375	3.70	4.25
10.0	50.00	2.500	3.70	4.30

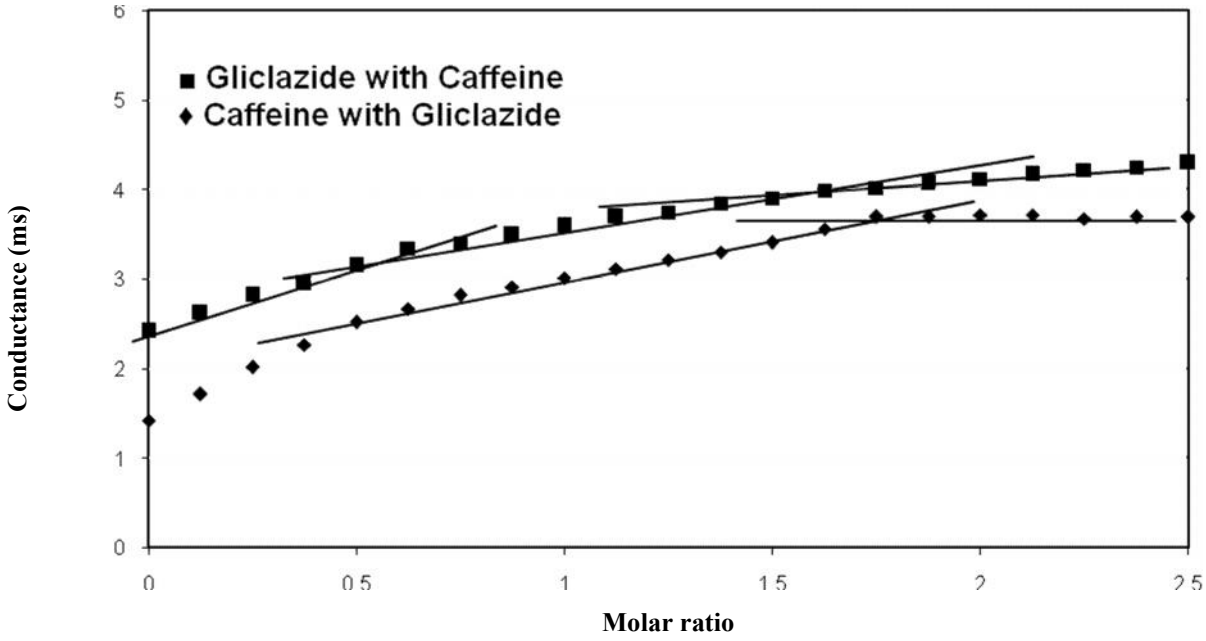


Fig. 7.5.17: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 5.4

Table 7.1.5.18: Data for conductometric titration of caffeine-glipizide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	1.53	2.32
0.5	40.50	0.125	1.79	2.55
1.0	41.00	0.250	2.00	2.75
1.5	41.50	0.375	2.23	2.91
2.0	42.00	0.500	2.46	3.07
2.5	42.50	0.625	2.62	3.25
3.0	43.00	0.750	2.73	3.96
3.5	43.50	0.875	2.75	3.43
4.0	44.00	1.000	2.93	3.53
4.5	44.50	1.125	2.95	3.65
5.0	45.00	1.250	2.99	3.71
5.5	45.50	1.375	3.13	3.81
6.0	46.00	1.500	3.19	3.89
6.5	46.50	1.625	3.28	3.91
7.0	47.00	1.750	3.41	3.98
7.5	47.50	1.875	3.44	4.00

8.0	48.00	2.000	3.43	4.07
8.5	48.50	2.125	3.38	4.12
9.0	49.00	2.250	3.35	4.19
9.5	49.50	2.375	3.38	4.23
10.0	50.00	2.500	3.35	4.21

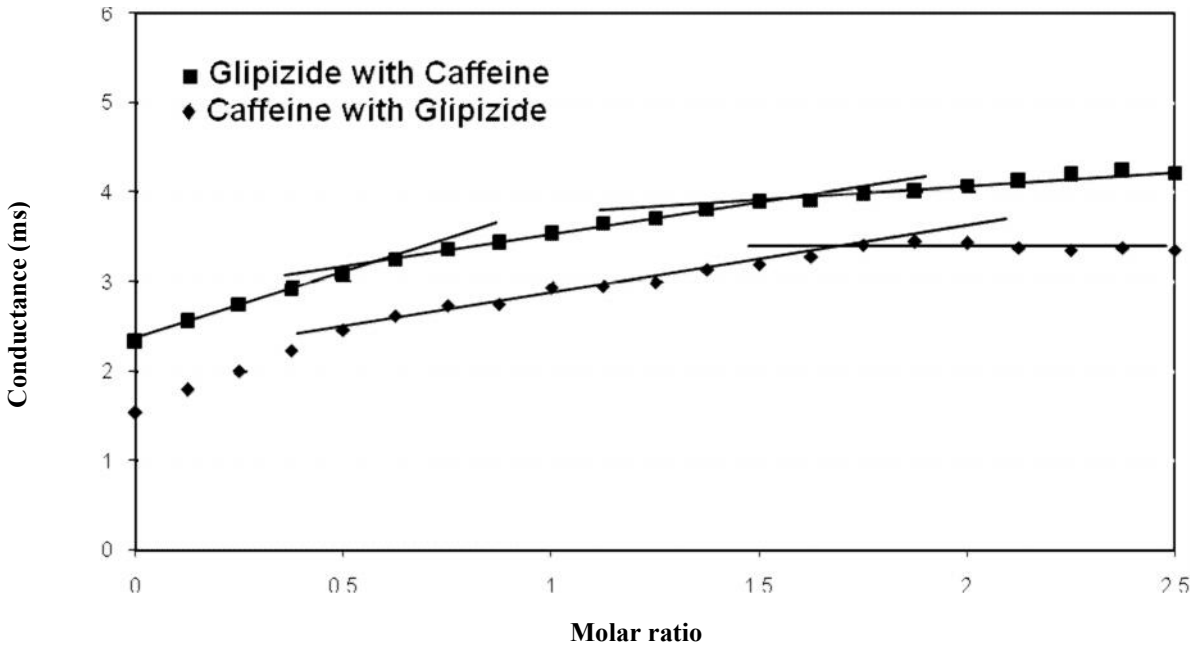


Fig. 7.5.18: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 5.4

Table 7.1.5.19: Data for conductometric titration of caffeine-glyburide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Gliyburide with caffeine
0.0	40.00	0.000	1.75	2.35
0.5	40.50	0.125	1.89	2.53
1.0	41.00	0.250	2.11	2.75
1.5	41.50	0.375	2.28	2.91
2.0	42.00	0.500	2.55	3.05
2.5	42.50	0.625	2.65	3.23
3.0	43.00	0.750	2.75	3.33
3.5	43.50	0.875	2.91	3.42
4.0	44.00	1.000	2.95	3.53
4.5	44.50	1.125	3.07	3.61
5.0	45.00	1.250	3.15	3.63
5.5	45.50	1.375	3.26	3.75
6.0	46.00	1.500	3.39	3.88
6.5	46.50	1.625	3.45	3.89
7.0	47.00	1.750	3.48	3.92
7.5	47.50	1.875	3.43	3.95

8.0	48.00	2.000	3.45	3.98
8.5	48.50	2.125	3.47	3.98
9.0	49.00	2.250	3.48	4.02
9.5	49.50	2.375	3.49	4.08
10.0	50.00	2.500	3.48	4.09

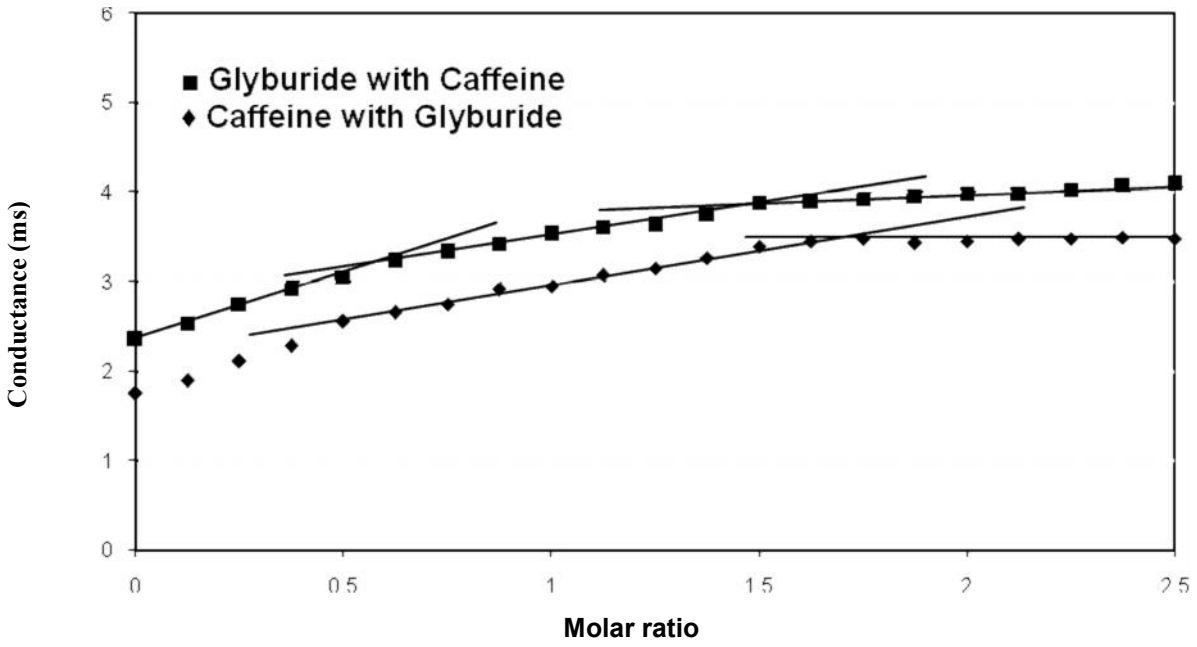


Fig. 7.5.19: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 5.4

Table 7.1.5.20: Data for conductometric titration of caffeine-metformin system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	2.20	2.72
0.5	40.50	0.125	2.20	2.82
1.0	41.00	0.250	2.22	2.88
1.5	41.50	0.375	2.23	2.95
2.0	42.00	0.500	2.25	3.00
2.5	42.50	0.625	2.28	3.10
3.0	43.00	0.750	2.30	3.12
3.5	43.50	0.875	2.32	3.15
4.0	44.00	1.000	2.35	3.17
4.5	44.50	1.125	2.60	3.19
5.0	45.00	1.250	2.75	3.20
5.5	45.50	1.375	3.00	3.20
6.0	46.00	1.500	3.20	3.22
6.5	46.50	1.625	3.40	3.22
7.0	47.00	1.750	3.60	3.23
7.5	47.50	1.875	3.50	3.23

8.0	48.00	2.000	3.45	3.30
8.5	48.50	2.125	3.40	3.40
9.0	49.00	2.250	3.35	3.50
9.5	49.50	2.375	3.30	3.60
10.0	50.00	2.500	3.30	3.70

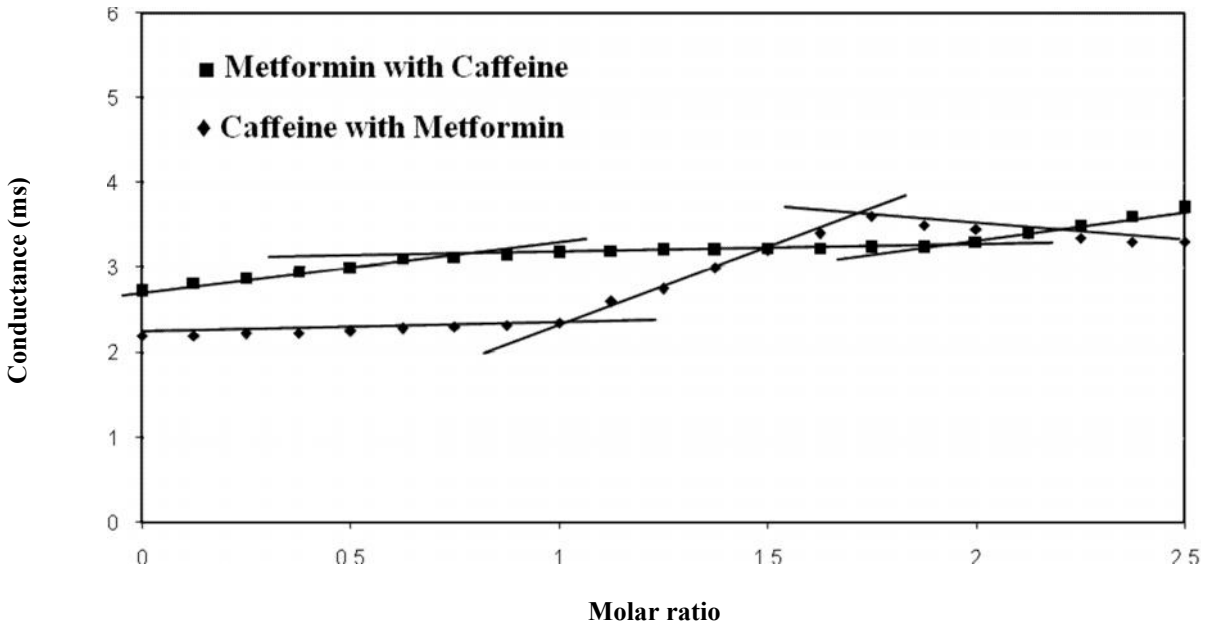


Fig. 7.1.5.20: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 5.4

Table 7.1.5.21: Data for conductometric titration of caffeine-gliclazide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	2.28	3.22
0.5	40.50	0.125	2.32	3.28
1.0	41.00	0.250	2.38	3.30
1.5	41.50	0.375	2.40	3.32
2.0	42.00	0.500	2.42	3.34
2.5	42.50	0.625	2.60	3.40
3.0	43.00	0.750	2.80	3.70
3.5	43.50	0.875	2.95	3.85
4.0	44.00	1.000	3.11	4.10
4.5	44.50	1.125	3.35	4.35
5.0	45.00	1.250	3.40	4.40
5.5	45.50	1.375	3.60	4.35
6.0	46.00	1.500	3.60	4.32
6.5	46.50	1.625	3.62	4.35
7.0	47.00	1.750	3.62	4.28
7.5	47.50	1.875	3.64	4.25

8.0	48.00	2.000	3.64	4.28
8.5	48.50	2.125	3.66	4.20
9.0	49.00	2.250	3.66	4.18
9.5	49.50	2.375	3.68	4.15
10.0	50.00	2.500	3.68	4.12

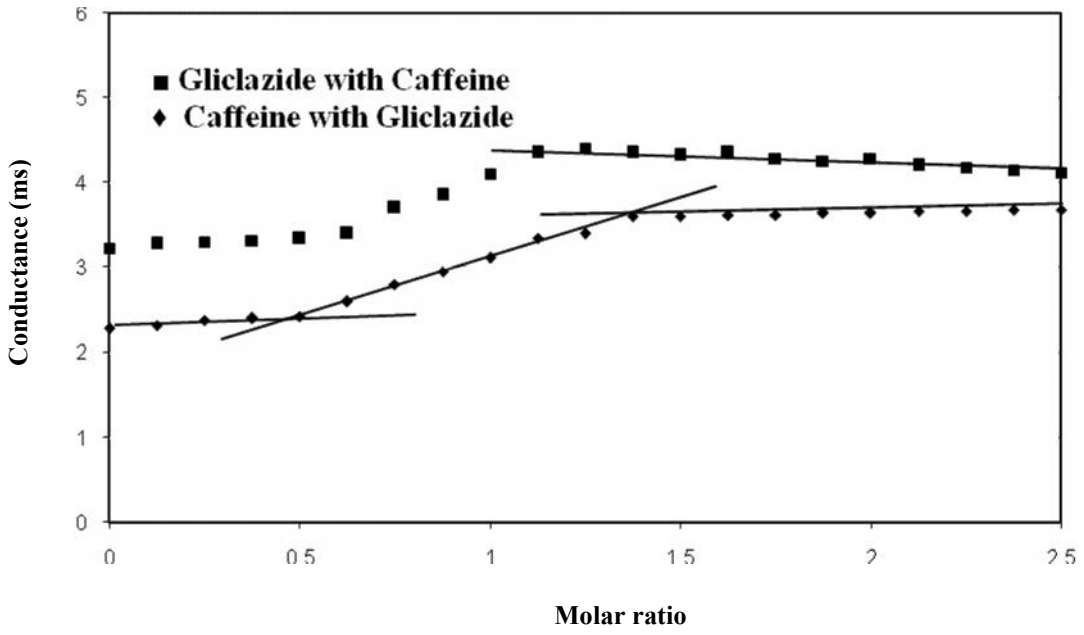


Fig. 7.5.21: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 6.4

Table 7.1.5.22: Data for conductometric titration of caffeine-glipizide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.31	3.17
0.5	40.50	0.125	2.35	3.19
1.0	41.00	0.250	2.39	3.23
1.5	41.50	0.375	2.42	3.25
2.0	42.00	0.500	2.52	3.24
2.5	42.50	0.625	2.60	3.28
3.0	43.00	0.750	2.68	3.35
3.5	43.50	0.875	2.85	3.55
4.0	44.00	1.000	3.05	3.65
4.5	44.50	1.125	3.20	3.85
5.0	45.00	1.250	3.39	3.96
5.5	45.50	1.375	3.51	4.12
6.0	46.00	1.500	3.55	4.22
6.5	46.50	1.625	3.51	4.23
7.0	47.00	1.750	3.48	4.19
7.5	47.50	1.875	3.38	4.13

8.0	48.00	2.000	3.32	4.19
8.5	48.50	2.125	3.29	4.12
9.0	49.00	2.250	3.28	4.16
9.5	49.50	2.375	3.23	4.13
10.0	50.00	2.500	3.19	4.02

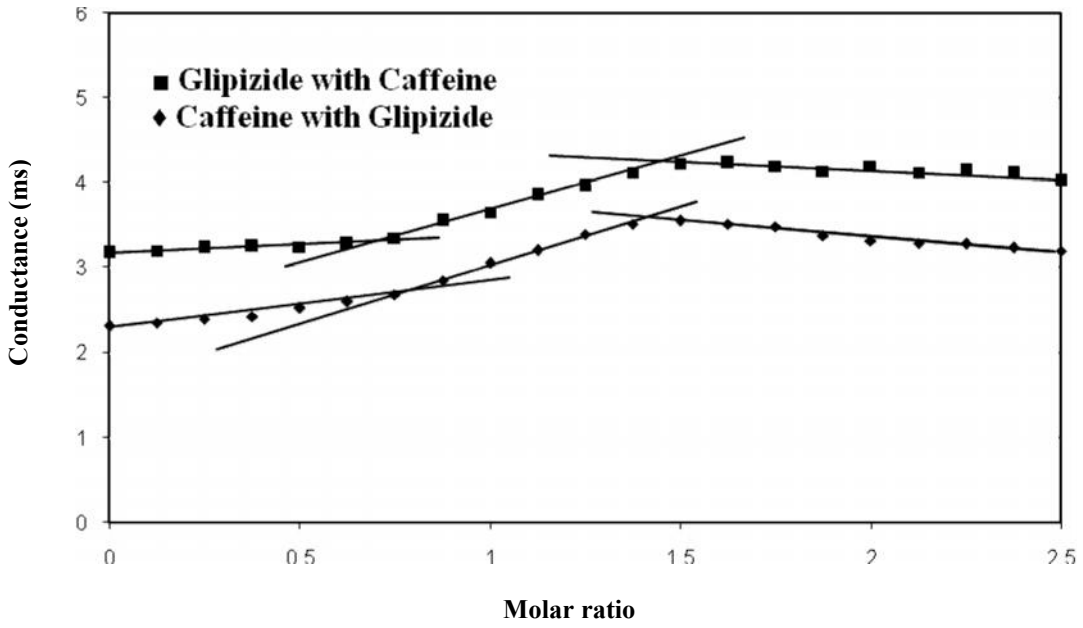


Fig. 7.1.5.22: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 6.4

Table 7.1.5.23: Data for conductometric titration of caffeine-glyburide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	2.15	3.18
0.5	40.50	0.125	2.23	3.21
1.0	41.00	0.250	2.35	3.25
1.5	41.50	0.375	2.42	3.28
2.0	42.00	0.500	2.45	3.30
2.5	42.50	0.625	2.58	3.40
3.0	43.00	0.750	2.70	3.59
3.5	43.50	0.875	2.85	3.68
4.0	44.00	1.000	3.01	3.85
4.5	44.50	1.125	3.23	4.00
5.0	45.00	1.250	3.36	4.15
5.5	45.50	1.375	3.50	4.23
6.0	46.00	1.500	3.55	4.32
6.5	46.50	1.625	3.57	4.40
7.0	47.00	1.750	3.59	4.39
7.5	47.50	1.875	3.58	4.35

8.0	48.00	2.000	3.54	4.32
8.5	48.50	2.125	3.60	4.33
9.0	49.00	2.250	3.58	4.37
9.5	49.50	2.375	3.57	4.39
10.0	50.00	2.500	3.58	4.42

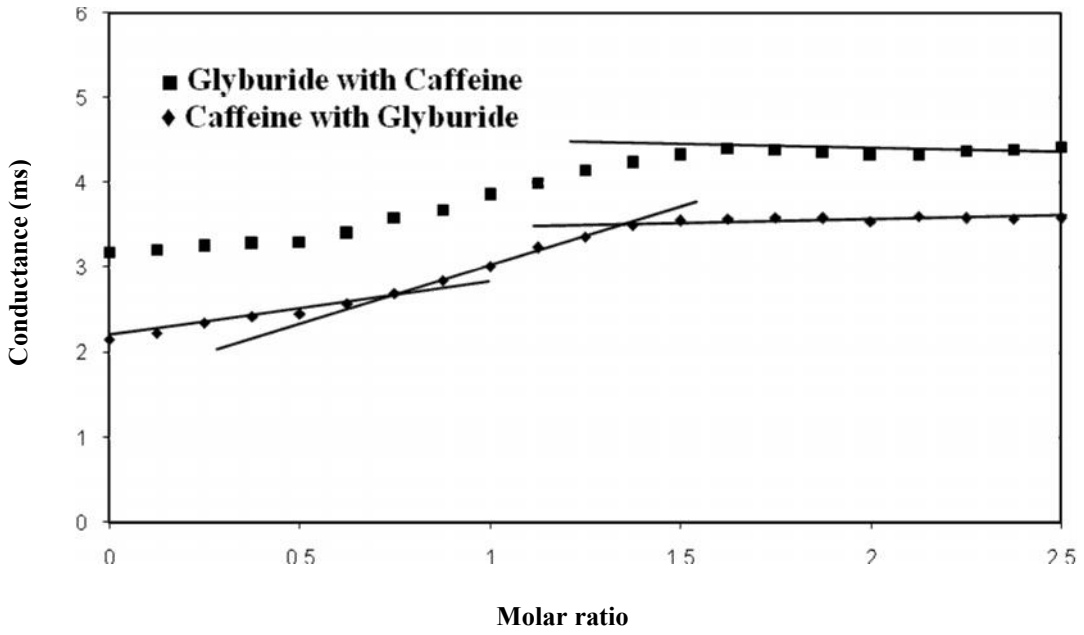


Fig. 7.1.5.23: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 6.4

Table 7.1.5.24: Data for conductometric titration of caffeine–metformin system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	1.48	2.78
0.5	40.50	0.125	1.68	2.88
1.0	41.00	0.250	1.88	3.00
1.5	41.50	0.375	2.08	3.18
2.0	42.00	0.500	2.32	3.32
2.5	42.50	0.625	2.48	3.52
3.0	43.00	0.750	2.78	3.82
3.5	43.50	0.875	3.12	4.22
4.0	44.00	1.000	3.42	4.52
4.5	44.50	1.125	3.72	4.92
5.0	45.00	1.250	4.02	5.32
5.5	45.50	1.375	4.32	5.62
6.0	46.00	1.500	4.51	5.88
6.5	46.50	1.625	4.61	5.58
7.0	47.00	1.750	4.71	5.58
7.5	47.50	1.875	4.81	5.54

8.0	48.00	2.000	4.91	5.52
8.5	48.50	2.125	4.98	5.52
9.0	49.00	2.250	5.08	5.55
9.5	49.50	2.375	5.18	5.55
10.0	50.00	2.500	5.22	5.55

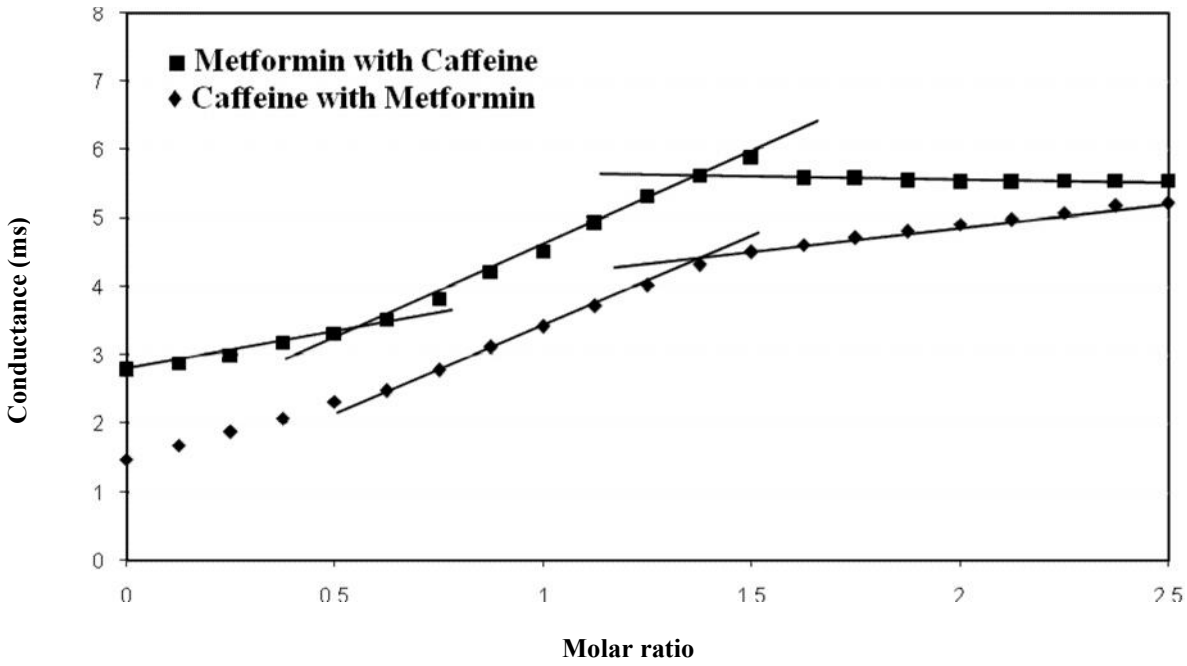


Fig. 7.1.5.24: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 6.4

Table 7.1.5.25: Data for conductometric titration of caffeine-gliclazide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with gliclazide	Gliclazide with caffeine
0.0	40.00	0.000	1.42	2.38
0.5	40.50	0.125	1.72	2.58
1.0	41.00	0.250	2.02	2.78
1.5	41.50	0.375	2.25	2.98
2.0	42.00	0.500	2.42	3.15
2.5	42.50	0.625	2.60	3.40
3.0	43.00	0.750	2.80	3.70
3.5	43.50	0.875	2.95	3.85
4.0	44.00	1.000	3.11	4.10
4.5	44.50	1.125	3.35	4.35
5.0	45.00	1.250	3.40	4.40
5.5	45.50	1.375	3.60	4.35
6.0	46.00	1.500	3.60	4.32
6.5	46.50	1.625	3.62	4.35
7.0	47.00	1.750	3.62	4.28
7.5	47.50	1.875	3.64	4.25

8.0	48.00	2.000	3.64	4.28
8.5	48.50	2.125	3.66	4.20
9.0	49.00	2.250	3.66	4.18
9.5	49.50	2.375	3.70	4.23
10.0	50.00	2.500	3.70	4.28

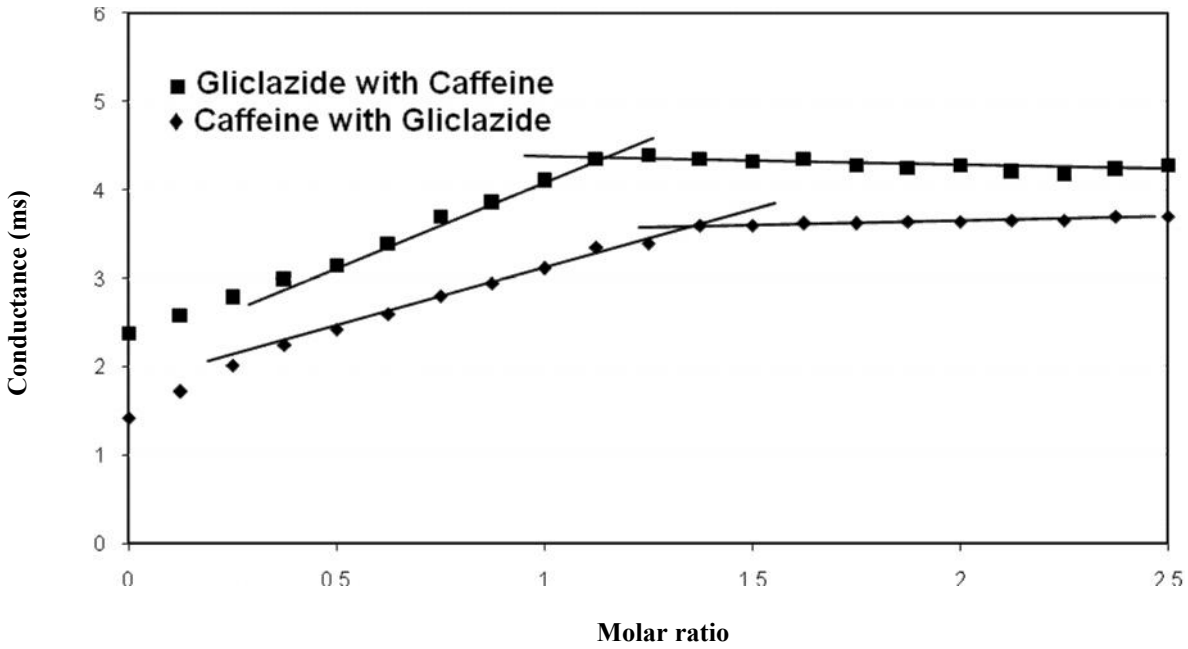


Fig. 7.1.5.25: Conductometric titration of caffeine with gliclazide and gliclazide with caffeine at pH 7.4

Table 7.1.5.26: Data for conductometric titration of caffeine-glipizide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glipizide	Glipizidewith caffeine
0.0	40.00	0.000	1.48	2.36
0.5	40.50	0.125	1.71	2.55
1.0	41.00	0.250	1.89	2.68
1.5	41.50	0.375	2.15	2.98
2.0	42.00	0.500	2.42	3.15
2.5	42.50	0.625	2.60	3.40
3.0	43.00	0.750	2.70	3.50
3.5	43.50	0.875	2.95	3.55
4.0	44.00	1.000	3.03	3.73
4.5	44.50	1.125	3.25	3.95
5.0	45.00	1.250	3.40	4.03
5.5	45.50	1.375	3.40	4.12
6.0	46.00	1.500	3.42	4.22
6.5	46.50	1.625	3.43	4.15
7.0	47.00	1.750	3.42	4.28
7.5	47.50	1.875	3.41	4.25

8.0	48.00	2.000	3.44	4.28
8.5	48.50	2.125	3.43	4.25
9.0	49.00	2.250	3.46	4.28
9.5	49.50	2.375	3.45	4.25
10.0	50.00	2.500	3.46	4.24

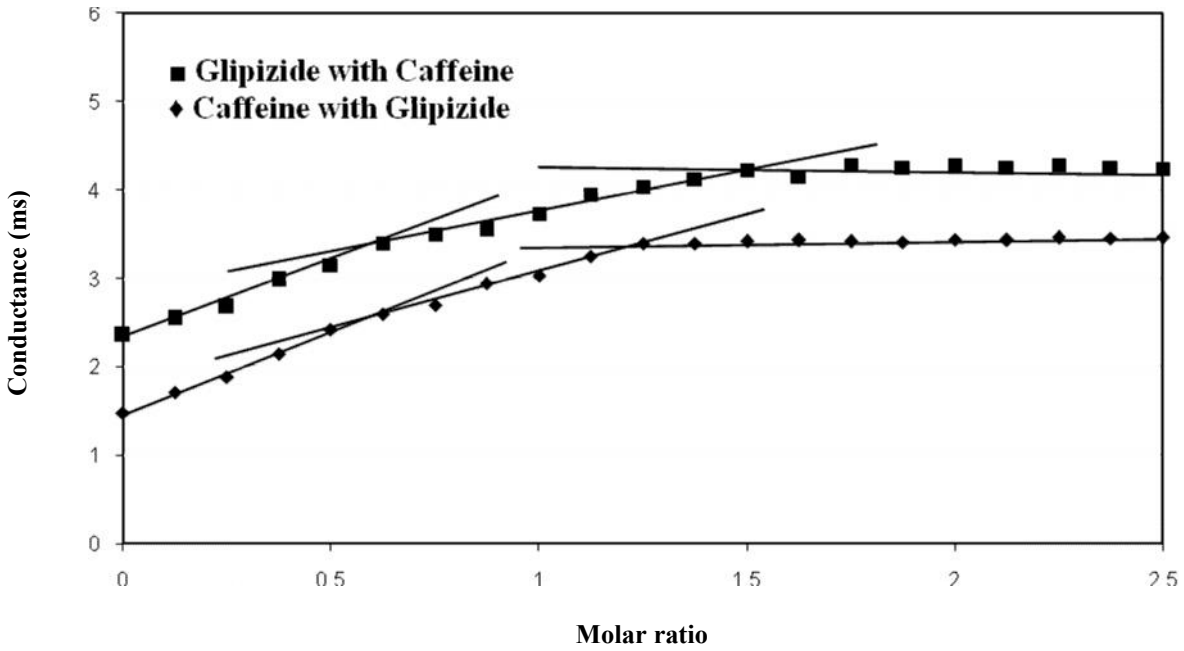


Fig. 7.1.5.26: Conductometric titration of caffeine with glipizide and glipizide with caffeine at pH 7.4

Table 7.1.5.27: Data for conductometric titration of caffeine-glyburide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with glyburide	Glyburide with caffeine
0.0	40.00	0.000	1.28	2.26
0.5	40.50	0.125	1.52	2.47
1.0	41.00	0.250	1.88	2.61
1.5	41.50	0.375	2.08	2.88
2.0	42.00	0.500	2.22	3.10
2.5	42.50	0.625	2.40	3.28
3.0	43.00	0.750	2.60	3.46
3.5	43.50	0.875	2.75	3.72
4.0	44.00	1.000	3.95	3.91
4.5	44.50	1.125	3.15	3.98
5.0	45.00	1.250	3.20	4.04
5.5	45.50	1.375	3.30	4.05
6.0	46.00	1.500	3.27	4.02
6.5	46.50	1.625	3.28	4.01
7.0	47.00	1.750	3.29	4.08
7.5	47.50	1.875	3.33	4.18

8.0	48.00	2.000	3.34	4.21
8.5	48.50	2.125	3.37	4.22
9.0	49.00	2.250	3.41	4.21
9.5	49.50	2.375	3.39	4.22
10.0	50.00	2.500	3.40	4.25

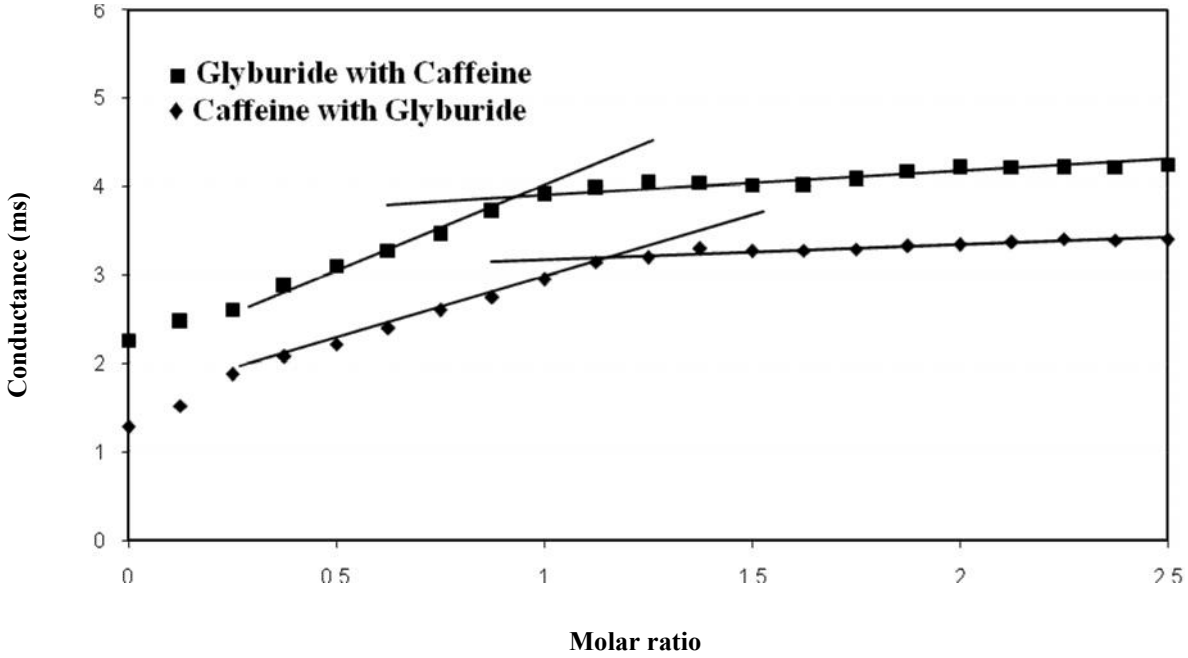


Fig. 7.1.5.27: Conductometric titration of caffeine with glyburide and glyburide with caffeine at pH 7.4

Table 7.1.5.28: Data for conductometric titration of caffeine - metformin system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Caffeine with metformin	Metformin with caffeine
0.0	40.00	0.000	2.20	2.68
0.5	40.50	0.125	2.22	2.78
1.0	41.00	0.250	2.23	2.85
1.5	41.50	0.375	2.25	2.95
2.0	42.00	0.500	2.28	3.00
2.5	42.50	0.625	2.30	3.10
3.0	43.00	0.750	2.32	3.12
3.5	43.50	0.875	2.36	3.13
4.0	44.00	1.000	2.58	3.15
4.5	44.50	1.125	2.73	3.20
5.0	45.00	1.250	3.02	3.20
5.5	45.50	1.375	3.20	3.22
6.0	46.00	1.500	3.40	3.22
6.5	46.50	1.625	3.60	3.23
7.0	47.00	1.750	3.50	3.23
7.5	47.50	1.875	3.45	3.30

8.0	48.00	2.000	3.40	3.40
8.5	48.50	2.125	3.35	3.50
9.0	49.00	2.250	3.30	3.60
9.5	49.50	2.375	3.30	3.70
10.0	50.00	2.500	3.20	3.80

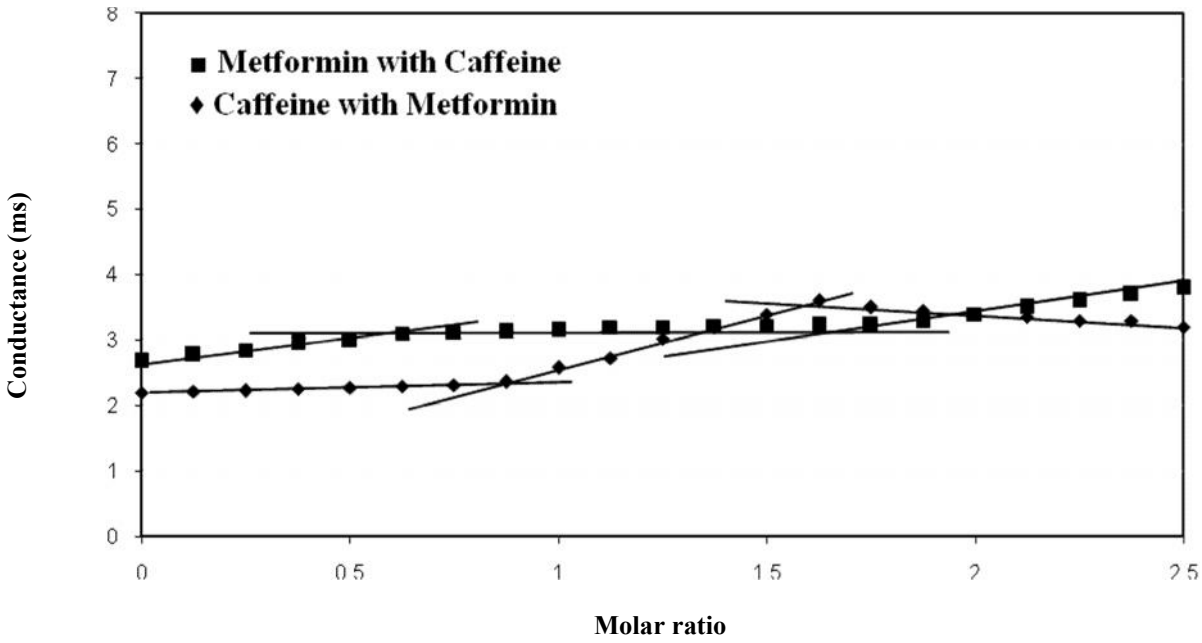


Fig. 7.1.5.28: Conductometric titration of caffeine with metformin and metformin with caffeine at pH 7.4

Thus again, when theophylline was titrated with gliclazide (fig.7.1.5.29, 7.1.5.33, 7.1.5.37, 7.1.5.41, 7.1.5.45, 7.1.5.49 & 7.1.5.53), it has been found that theophylline forms a 1:1 complex with gliclazide at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 and 4:1 complexes at pH 3.4, 4.4 & 5.4 through some other unstable intermediates.

Again, when theophylline was titrated with glipizide (fig.7.1.5.30, 7.1.5.34, 7.1.5.38, 7.1.5.42, 7.1.5.46, 7.1.5.50 & 7.1.5.54), it has been found that theophylline forms a 1:1 complex with glipizide at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 and 3:1 complexes at pH 5.4 & 6.4 through some other unstable intermediates.

Again, when theophylline was titrated with glyburide (fig.7.1.5.31, 7.1.5.35, 7.1.5.39, 7.1.5.43, 7.1.5.47, 7.1.5.51 & 7.1.5.55), it has been found that theophylline forms a 1:1 complex with glyburide at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 and 4:1 complexes at pH 2.4, 3.4 & 7.4 through some other unstable intermediates.

Again, when theophylline was titrated with metformin (fig.7.1.5.32, 7.1.5.36, 7.1.5.40, 7.1.5.44, 7.1.5.48, 7.1.5.52 & 7.1.5.56), it has been found that theophylline forms a 1:1 complex with metformin at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 and 4:1 complexes at pH 2.4 & 5.4 through some other unstable intermediates.

By considering the above facts, it may be said that theophylline forms a stable 1:1 complexes with gliclazide and metformin at pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4. [92][98]

Table 7.1.5.29: Data for conductometric titration of theophylline-gliclazide System at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with Theophylline
0.0	40.00	0.000	2.41	3.02
0.5	40.50	0.125	2.51	3.12
1.0	41.00	0.250	2.62	3.21
1.5	41.50	0.375	2.73	3.33
2.0	42.00	0.500	2.81	3.48
2.5	42.50	0.625	2.93	3.69
3.0	43.00	0.750	2.95	3.81
3.5	43.50	0.875	2.88	3.98
4.0	44.00	1.000	2.89	4.05
4.5	44.50	1.125	2.87	4.12
5.0	45.00	1.250	2.83	4.15
5.5	45.50	1.375	2.98	4.20
6.0	46.00	1.500	3.01	4.23
6.5	46.50	1.625	3.13	4.25
7.0	47.00	1.750	3.21	4.28
7.5	47.50	1.875	3.24	4.31
8.0	48.00	2.000	3.25	4.28

8.5	48.50	2.125	3.28	4.24
9.0	49.00	2.250	3.33	4.21
9.5	49.50	2.375	3.36	4.23
10.0	50.00	2.500	3.38	4.25

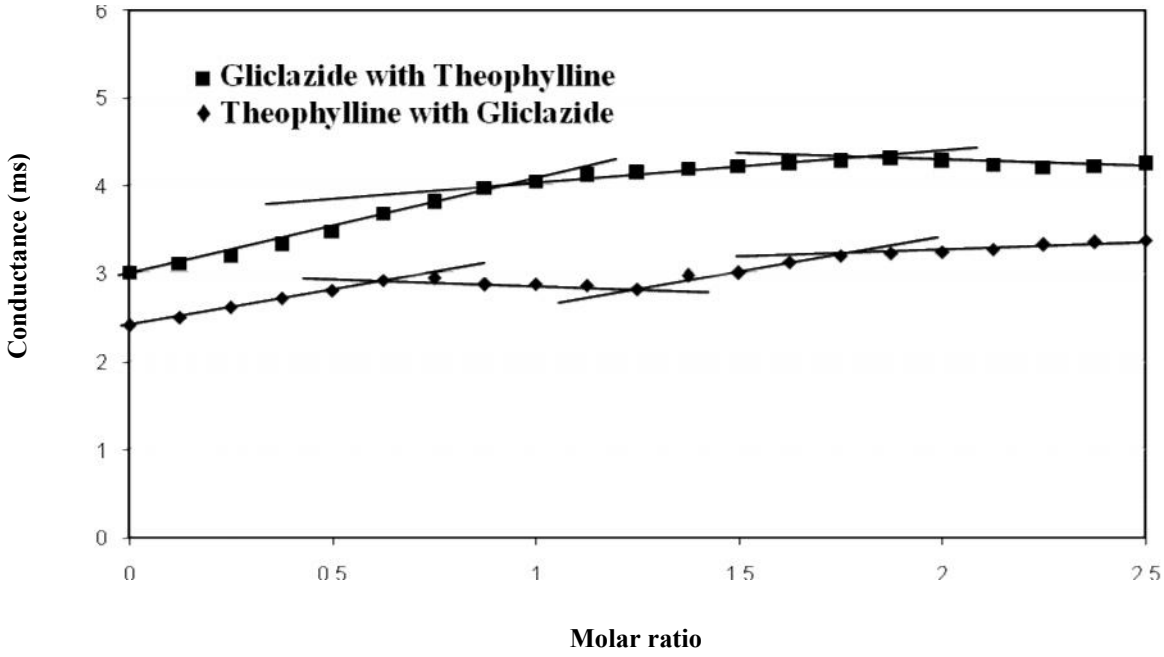


Fig. 7.1.5.29: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 1.4

Table 7.1.5.30: Data for conductometric titration of theophylline-glipizide system at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with theophylline
0.0	40.00	0.000	2.22	2.93
0.5	40.50	0.125	2.33	3.08
1.0	41.00	0.250	2.39	3.18
1.5	41.50	0.375	2.55	3.39
2.0	42.00	0.500	2.68	3.42
2.5	42.50	0.625	2.76	3.63
3.0	43.00	0.750	2.85	3.69
3.5	43.50	0.875	2.93	3.75
4.0	44.00	1.000	2.98	3.78
4.5	44.50	1.125	3.07	3.79
5.0	45.00	1.250	3.20	3.89
5.5	45.50	1.375	3.22	3.93
6.0	46.00	1.500	3.21	3.97
6.5	46.50	1.625	3.17	4.08
7.0	47.00	1.750	3.12	4.03
7.5	47.50	1.875	3.08	4.05
8.0	48.00	2.000	3.05	4.09

8.5	48.50	2.125	3.01	4.05
9.0	49.00	2.250	2.96	3.97
9.5	49.50	2.375	2.97	3.91
10.0	50.00	2.500	2.98	3.89

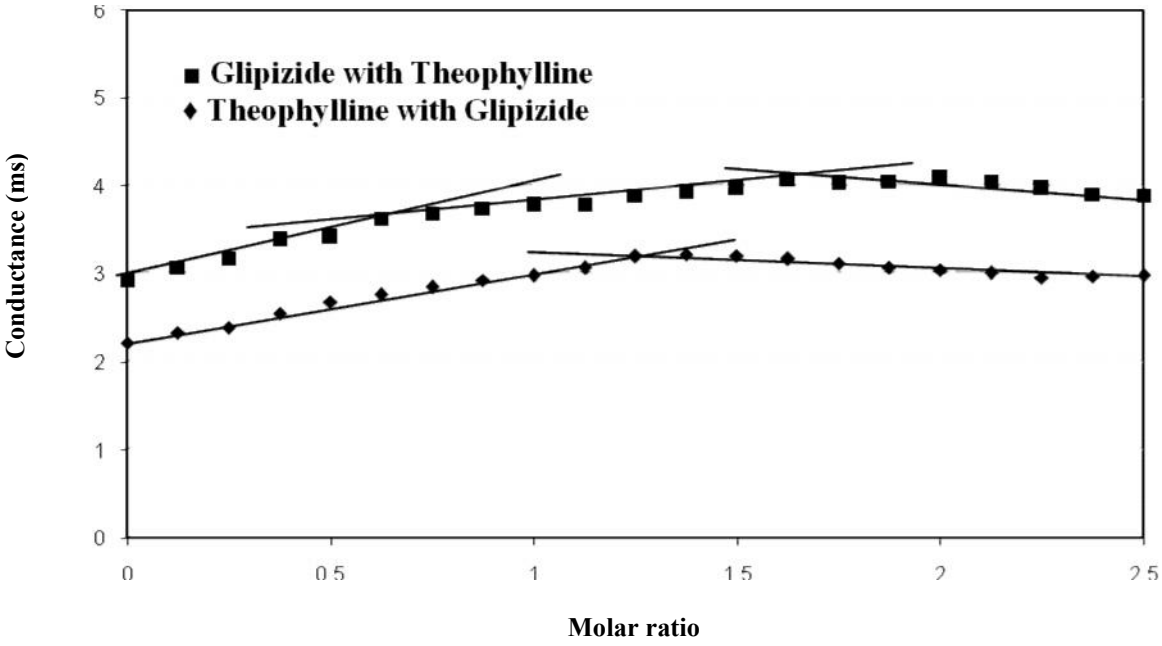


Fig. 7.1.5.30: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 1.4

Table 7.1.5.31: Data for conductometric titration of theophylline-glyburide system at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with theophylline
0.0	40.00	0.000	2.20	2.88
0.5	40.50	0.125	2.38	2.98
1.0	41.00	0.250	2.52	3.15
1.5	41.50	0.375	2.61	3.31
2.0	42.00	0.500	2.66	3.41
2.5	42.50	0.625	2.78	3.52
3.0	43.00	0.750	2.88	3.53
3.5	43.50	0.875	2.95	3.57
4.0	44.00	1.000	2.99	3.59
4.5	44.50	1.125	3.11	3.63
5.0	45.00	1.250	3.05	3.65
5.5	45.50	1.375	3.08	3.69
6.0	46.00	1.500	3.05	3.78
6.5	46.50	1.625	2.95	3.81
7.0	47.00	1.750	3.00	3.93
7.5	47.50	1.875	3.03	4.03
8.0	48.00	2.000	3.15	4.02

8.5	48.50	2.125	3.21	4.02
9.0	49.00	2.250	3.25	3.93
9.5	49.50	2.375	3.23	3.95
10.0	50.00	2.500	3.26	3.92

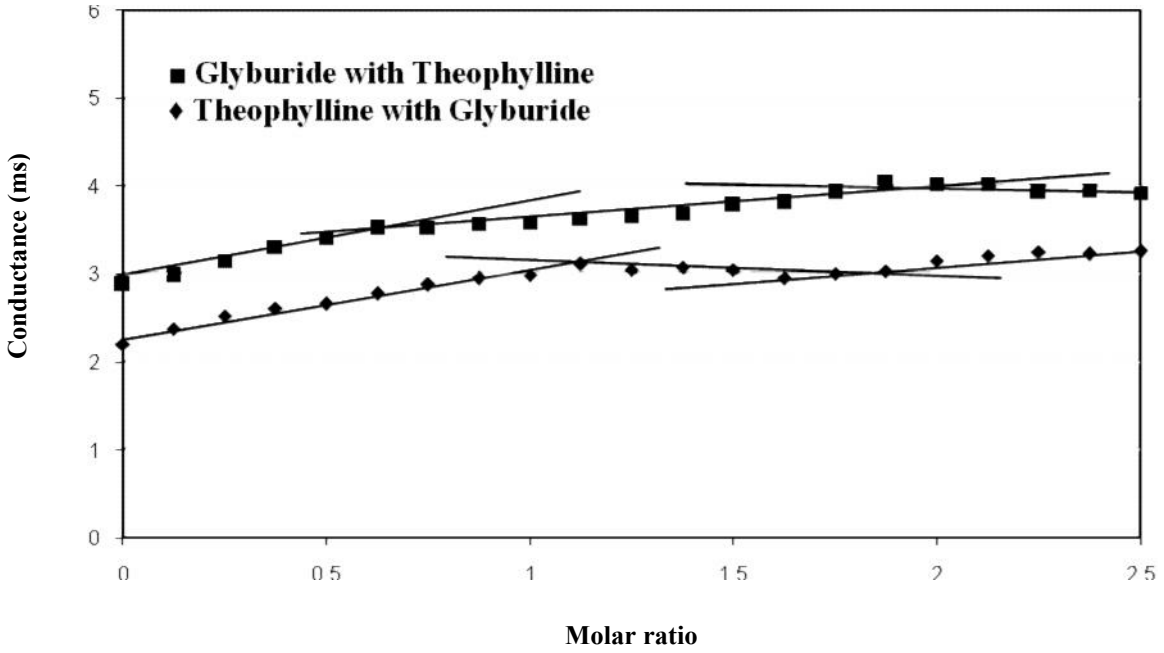


Fig. 7.1.5.31: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 1.4

Table 7.1.5.32: Data for conductometric titration of theophylline-metformin system at pH 1.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with Theophylline
0.0	40.00	0.000	1.48	2.28
0.5	40.50	0.125	1.59	2.44
1.0	41.00	0.250	1.70	2.56
1.5	41.50	0.375	1.82	2.68
2.0	42.00	0.500	1.92	2.85
2.5	42.50	0.625	2.05	2.96
3.0	43.00	0.750	2.17	3.23
3.5	43.50	0.875	2.19	3.32
4.0	44.00	1.000	2.25	3.48
4.5	44.50	1.125	2.36	3.58
5.0	45.00	1.250	2.38	3.68
5.5	45.50	1.375	2.45	3.82
6.0	46.00	1.500	2.63	3.85
6.5	46.50	1.625	2.75	3.93
7.0	47.00	1.750	2.88	3.96
7.5	47.50	1.875	3.08	4.05
8.0	48.00	2.000	3.28	4.21

8.5	48.50	2.125	3.42	4.32
9.0	49.00	2.250	3.61	4.46
9.5	49.50	2.375	3.73	4.63
10.0	50.00	2.500	3.88	4.78

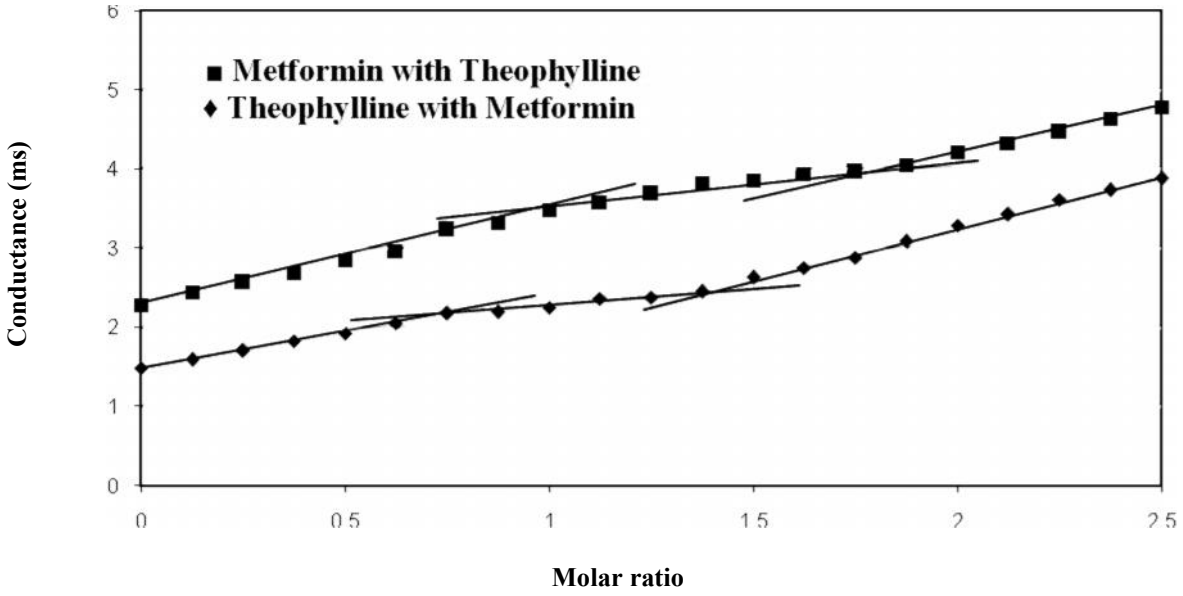


Fig. 7.1.5.32: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 1.4

Table 7.1.5.33: Data for conductometric titration of theophylline-gliclazide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with theophylline
0.0	40.00	0.000	1.25	2.14
0.5	40.50	0.125	1.29	2.15
1.0	41.00	0.250	1.35	2.24
1.5	41.50	0.375	1.37	2.26
2.0	42.00	0.500	1.37	2.29
2.5	42.50	0.625	1.48	2.32
3.0	43.00	0.750	1.76	2.53
3.5	43.50	0.875	1.89	2.65
4.0	44.00	1.000	2.08	3.78
4.5	44.50	1.125	2.23	3.05
5.0	45.00	1.250	2.38	3.24
5.5	45.50	1.375	2.52	3.32
6.0	46.00	1.500	2.53	3.33
6.5	46.50	1.625	2.54	3.34
7.0	47.00	1.750	2.55	3.32
7.5	47.50	1.875	2.56	3.26
8.0	48.00	2.000	2.53	3.21

8.5	48.50	2.125	2.49	3.19
9.0	49.00	2.250	2.47	3.17
9.5	49.50	2.375	2.43	3.16
10.0	50.00	2.500	2.35	3.11

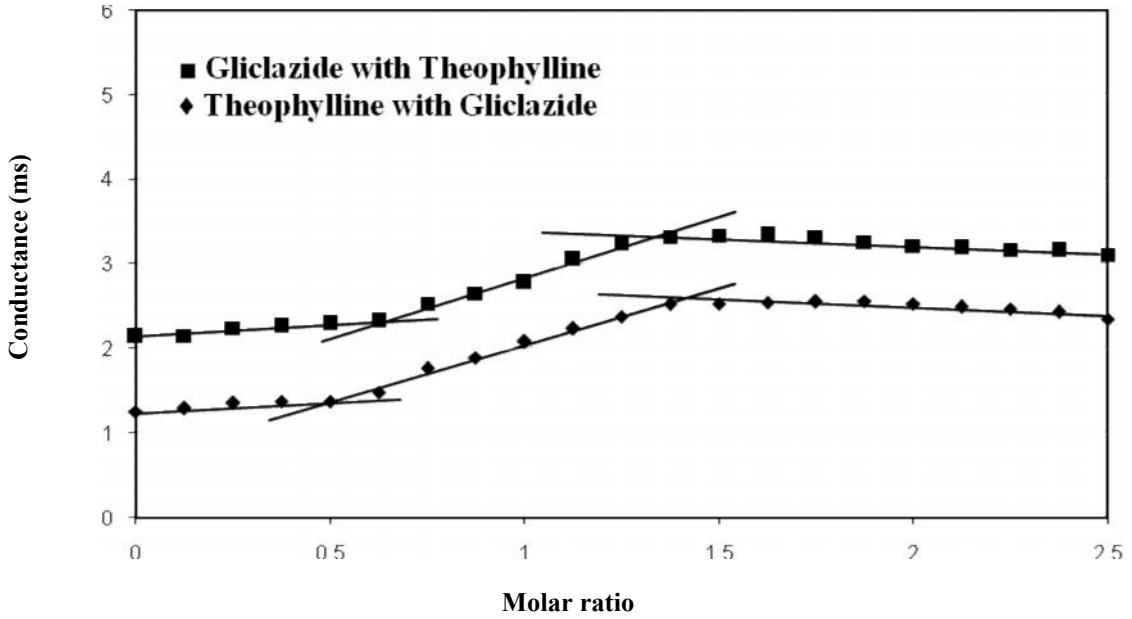


Fig. 7.5.33: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 2.4

Table 7.1.5.34: Data for conductometric titration of theophylline-glipizide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with theophylline
0.0	40.00	0.000	1.31	2.19
0.5	40.50	0.125	1.33	2.24
1.0	41.00	0.250	1.37	2.26
1.5	41.50	0.375	1.43	2.35
2.0	42.00	0.500	1.46	2.38
2.5	42.50	0.625	1.61	2.46
3.0	43.00	0.750	1.71	2.63
3.5	43.50	0.875	1.88	2.68
4.0	44.00	1.000	2.05	3.82
4.5	44.50	1.125	2.24	3.95
5.0	45.00	1.250	2.39	3.12
5.5	45.50	1.375	2.35	3.33
6.0	46.00	1.500	2.32	3.32
6.5	46.50	1.625	2.29	3.33
7.0	47.00	1.750	2.28	3.25
7.5	47.50	1.875	2.26	3.25
8.0	48.00	2.000	2.24	3.24

8.5	48.50	2.125	2.23	3.22
9.0	49.00	2.250	2.22	3.21
9.5	49.50	2.375	2.22	3.16
10.0	50.00	2.500	2.21	3.13

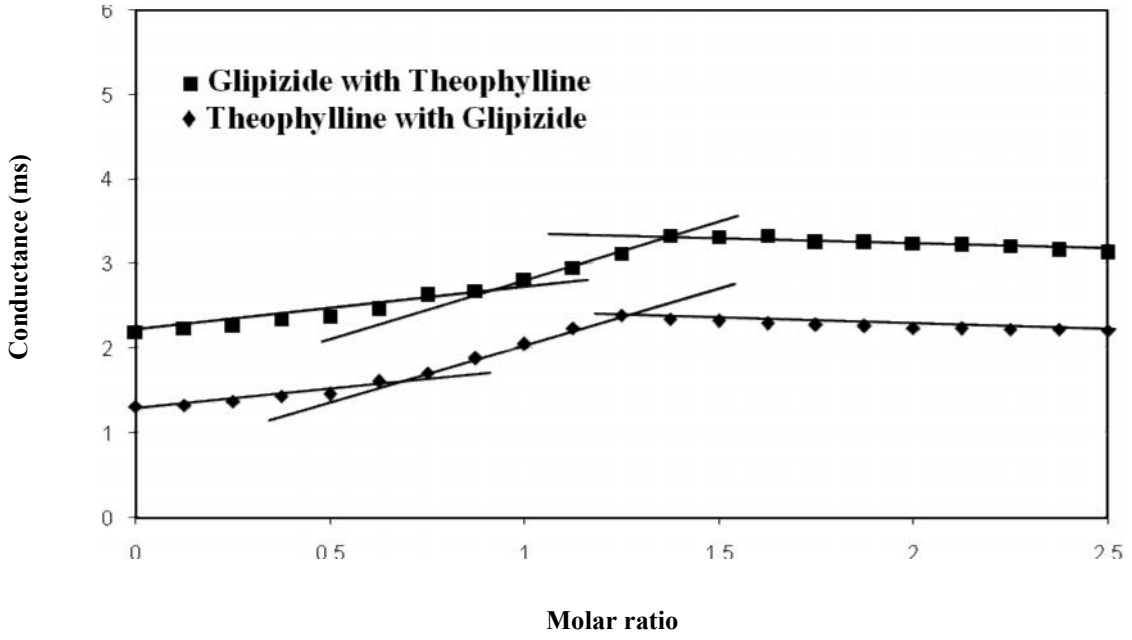


Fig. 7.1.5.34: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 2.4

Table 7.1.5.35: Data for conductometric titration of theophylline-glyburide system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with theophylline
0.0	40.00	0.000	1.22	2.12
0.5	40.50	0.125	1.29	2.19
1.0	41.00	0.250	1.31	2.21
1.5	41.50	0.375	1.33	2.25
2.0	42.00	0.500	1.35	2.23
2.5	42.50	0.625	1.48	2.41
3.0	43.00	0.750	1.68	2.51
3.5	43.50	0.875	1.78	2.65
4.0	44.00	1.000	1.88	2.75
4.5	44.50	1.125	2.04	2.85
5.0	45.00	1.250	2.16	2.95
5.5	45.50	1.375	2.19	3.15
6.0	46.00	1.500	2.41	3.28
6.5	46.50	1.625	2.48	3.27
7.0	47.00	1.750	2.55	3.26
7.5	47.50	1.875	2.45	3.23
8.0	48.00	2.000	2.44	3.21

8.5	48.50	2.125	2.41	3.22
9.0	49.00	2.250	2.42	3.11
9.5	49.50	2.375	2.43	3.13
10.0	50.00	2.500	2.34	3.10

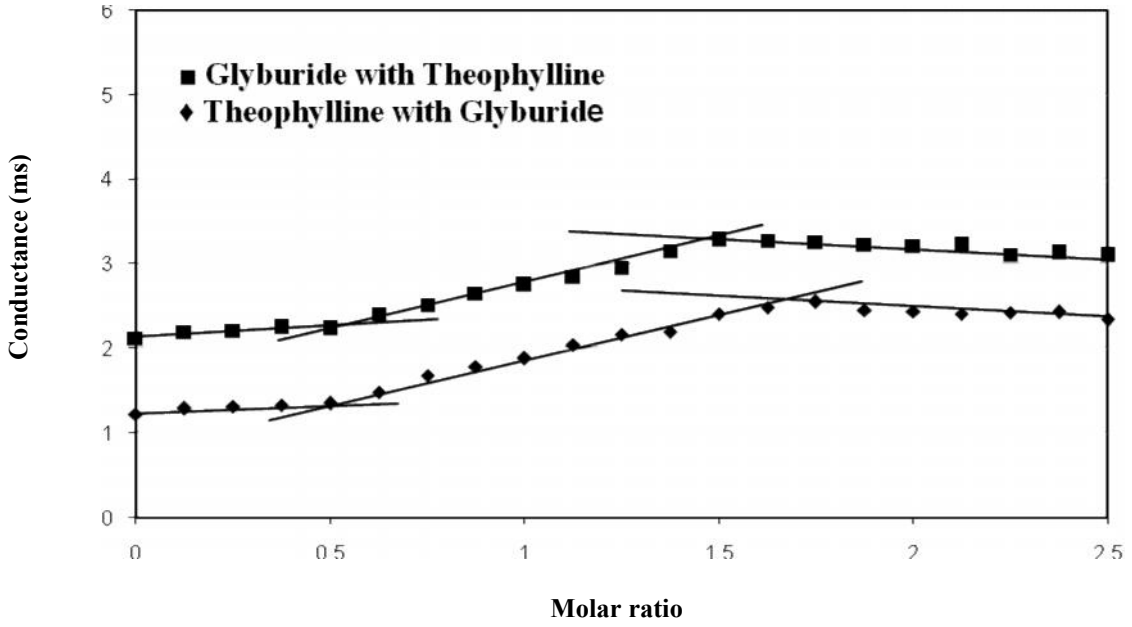


Fig. 7.5.35: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 2.4

Table 7.1.5.36: Data for conductometric titration of theophylline-metformin system at pH 2.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with theophylline
0.0	40.00	0.000	1.50	2.82
0.5	40.50	0.125	1.68	2.93
1.0	41.00	0.250	1.73	3.05
1.5	41.50	0.375	1.93	3.23
2.0	42.00	0.500	2.07	3.38
2.5	42.50	0.625	2.31	3.55
3.0	43.00	0.750	2.75	3.85
3.5	43.50	0.875	3.03	4.12
4.0	44.00	1.000	3.37	4.42
4.5	44.50	1.125	3.75	4.75
5.0	45.00	1.250	4.17	4.83
5.5	45.50	1.375	4.21	4.95
6.0	46.00	1.500	4.24	5.03
6.5	46.50	1.625	4.26	5.07
7.0	47.00	1.750	4.30	5.15
7.5	47.50	1.875	4.31	5.12
8.0	48.00	2.000	4.32	5.13

8.5	48.50	2.125	4.35	5.15
9.0	49.00	2.250	4.34	5.19
9.5	49.50	2.375	4.33	5.21
10.0	50.00	2.500	4.36	5.23

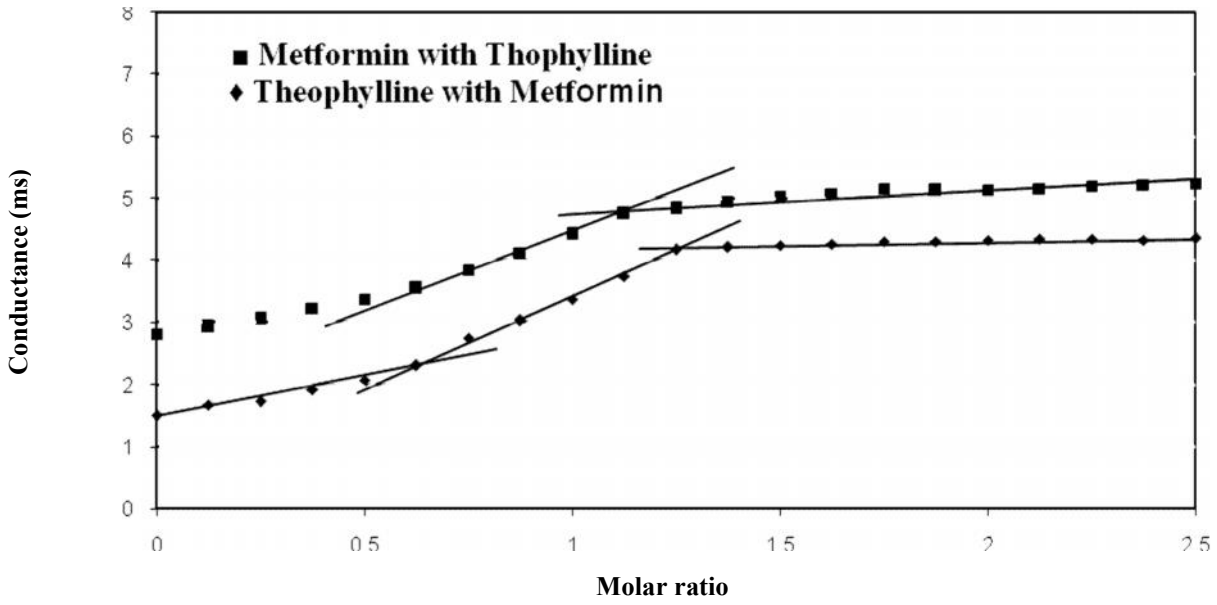


Fig. 7.5.36: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 2.4

Table 7.1.5.37: Data for conductometric titration of theophylline-gliclazide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with theophylline
0.0	40.00	0.000	2.36	3.05
0.5	40.50	0.125	2.48	3.08
1.0	41.00	0.250	2.56	3.19
1.5	41.50	0.375	2.63	3.29
2.0	42.00	0.500	2.75	3.39
2.5	42.50	0.625	2.89	3.51
3.0	43.00	0.750	2.88	3.53
3.5	43.50	0.875	2.86	3.55
4.0	44.00	1.000	2.84	3.57
4.5	44.50	1.125	2.79	3.59
5.0	45.00	1.250	2.78	3.61
5.5	45.50	1.375	3.00	3.62
6.0	46.00	1.500	3.12	3.78
6.5	46.50	1.625	3.25	3.83
7.0	47.00	1.750	3.27	3.92
7.5	47.50	1.875	3.23	4.0
8.0	48.00	2.000	3.16	4.01

8.5	48.50	2.125	3.15	3.97
9.0	49.00	2.250	3.14	3.95
9.5	49.50	2.375	3.13	3.93
10.0	50.00	2.500	3.11	3.92

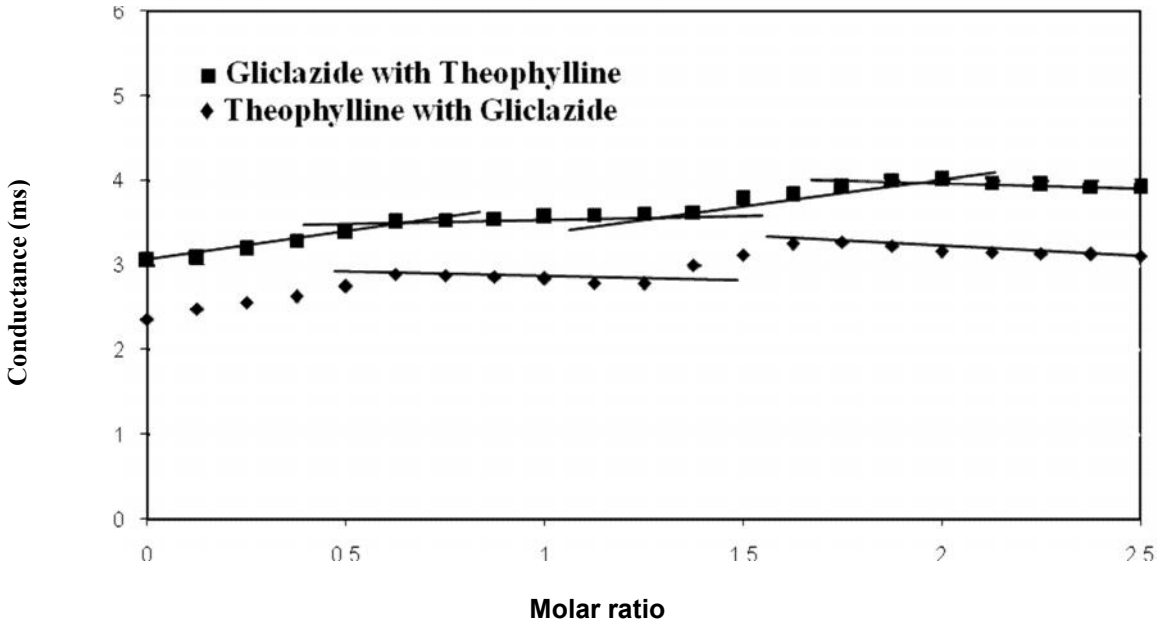


Fig. 7.1.5.37: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 3.4

Table 7.1.5.38: Data for conductometric titration of theophylline-glipizide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with Theophylline
0.0	40.00	0.000	2.33	3.09
0.5	40.50	0.125	2.43	3.19
1.0	41.00	0.250	2.54	3.32
1.5	41.50	0.375	2.63	3.47
2.0	42.00	0.500	2.74	3.57
2.5	42.50	0.625	2.82	3.68
3.0	43.00	0.750	2.95	3.88
3.5	43.50	0.875	2.96	3.94
4.0	44.00	1.000	2.94	3.96
4.5	44.50	1.125	2.93	3.99
5.0	45.00	1.250	2.95	3.96
5.5	45.50	1.375	2.98	4.01
6.0	46.00	1.500	2.92	3.95
6.5	46.50	1.625	2.74	3.85
7.0	47.00	1.750	2.65	3.74
7.5	47.50	1.875	2.55	3.65
8.0	48.00	2.000	2.45	3.52

8.5	48.50	2.125	2.53	3.68
9.0	49.00	2.250	2.61	3.72
9.5	49.50	2.375	2.63	3.75
10.0	50.00	2.500	2.65	3.78

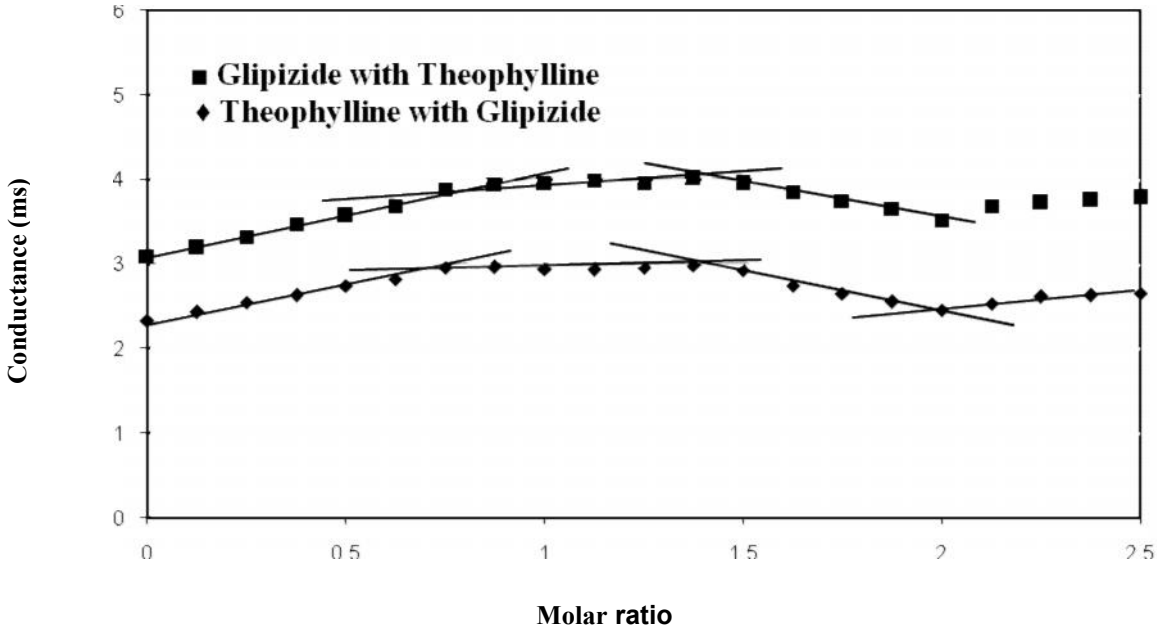


Fig. 7.5.38: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 3.4

Table 7.1.5.39: Data for conductometric titration of theophylline-glyburide system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with Theophylline
0.0	40.00	0.000	2.57	3.15
0.5	40.50	0.125	2.68	3.25
1.0	41.00	0.250	2.85	3.41
1.5	41.50	0.375	2.89	3.52
2.0	42.00	0.500	3.01	3.65
2.5	42.50	0.625	3.08	3.76
3.0	43.00	0.750	3.12	3.78
3.5	43.50	0.875	3.13	3.76
4.0	44.00	1.000	3.05	3.68
4.5	44.50	1.125	3.02	3.65
5.0	45.00	1.250	3.00	3.61
5.5	45.50	1.375	3.23	3.75
6.0	46.00	1.500	3.35	3.88
6.5	46.50	1.625	3.55	3.95
7.0	47.00	1.750	3.78	4.21
7.5	47.50	1.875	3.59	4.35
8.0	48.00	2.000	3.45	4.45

8.5	48.50	2.125	3.38	4.35
9.0	49.00	2.250	3.32	4.21
9.5	49.50	2.375	3.20	4.02
10.0	50.00	2.500	3.03	3.85

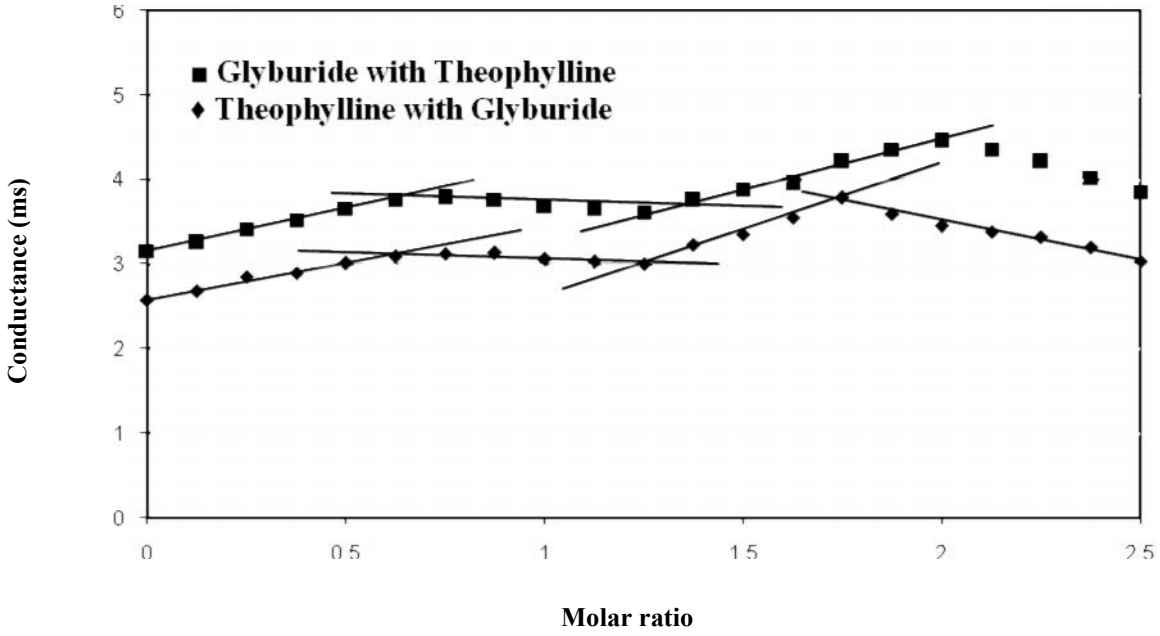


Fig. 7.1.5.39: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 3.4

Table 7.1.5.40: Data for conductometric titration of theophylline-metformin system at pH 3.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with theophylline
0.0	40.00	0.000	1.55	2.73
0.5	40.50	0.125	1.70	2.78
1.0	41.00	0.250	1.85	2.85
1.5	41.50	0.375	1.96	2.96
2.0	42.00	0.500	2.12	3.02
2.5	42.50	0.625	2.22	3.12
3.0	43.00	0.750	2.32	3.05
3.5	43.50	0.875	2.26	3.12
4.0	44.00	1.000	2.33	3.13
4.5	44.50	1.125	2.36	3.36
5.0	45.00	1.250	2.45	3.63
5.5	45.50	1.375	2.37	3.75
6.0	46.00	1.500	2.42	3.98
6.5	46.50	1.625	2.75	4.25
7.0	47.00	1.750	3.15	4.45
7.5	47.50	1.875	3.45	4.43
8.0	48.00	2.000	3.35	4.38

8.5	48.50	2.125	3.27	4.21
9.0	49.00	2.250	3.23	4.12
9.5	49.50	2.375	3.17	4.08
10.0	50.00	2.500	3.13	3.98

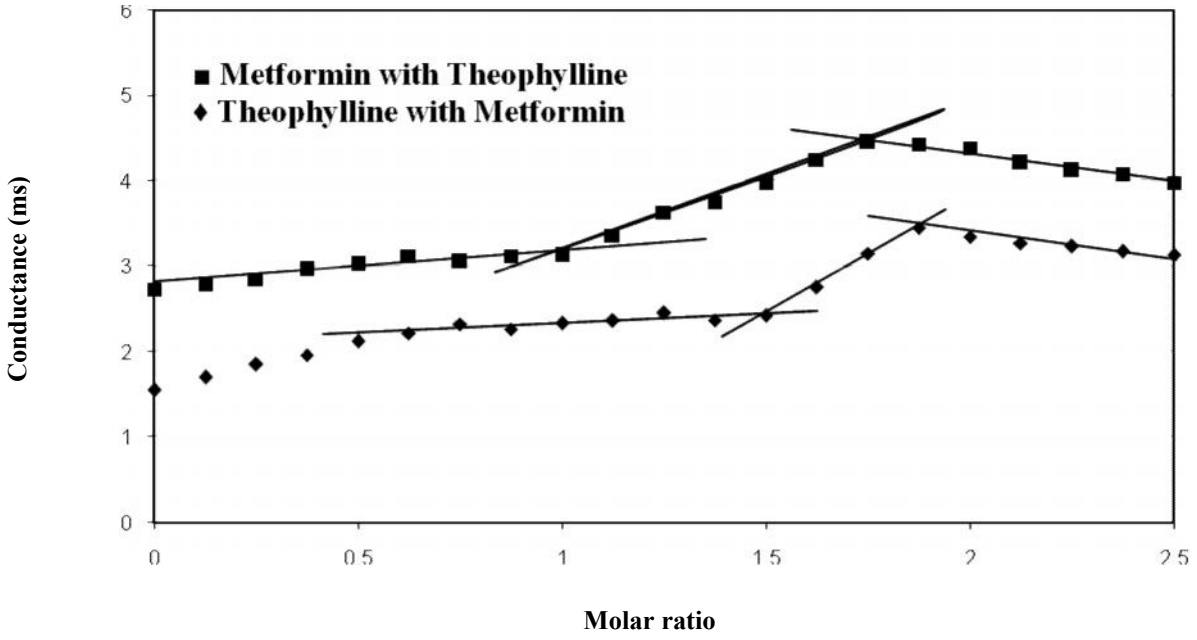


Fig. 7.1.5.40: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 3.4

Table 7.1.5.41: Data for conductometric titration of theophylline-gliclazide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with Theophylline
0.0	40.00	0.000	2.25	3.15
0.5	40.50	0.125	2.31	3.32
1.0	41.00	0.250	2.35	3.42
1.5	41.50	0.375	2.49	3.49
2.0	42.00	0.500	2.55	3.62
2.5	42.50	0.625	2.65	3.68
3.0	43.00	0.750	2.73	3.69
3.5	43.50	0.875	2.73	3.75
4.0	44.00	1.000	2.71	3.75
4.5	44.50	1.125	2.68	3.68
5.0	45.00	1.250	2.65	3.68
5.5	45.50	1.375	2.68	3.63
6.0	46.00	1.500	2.63	3.79
6.5	46.50	1.625	2.73	3.96
7.0	47.00	1.750	2.86	4.05
7.5	47.50	1.875	3.05	4.01
8.0	48.00	2.000	3.23	4.23

8.5	48.50	2.125	3.36	4.25
9.0	49.00	2.250	3.47	4.26
9.5	49.50	2.375	3.57	4.28
10.0	50.00	2.500	3.67	4.29

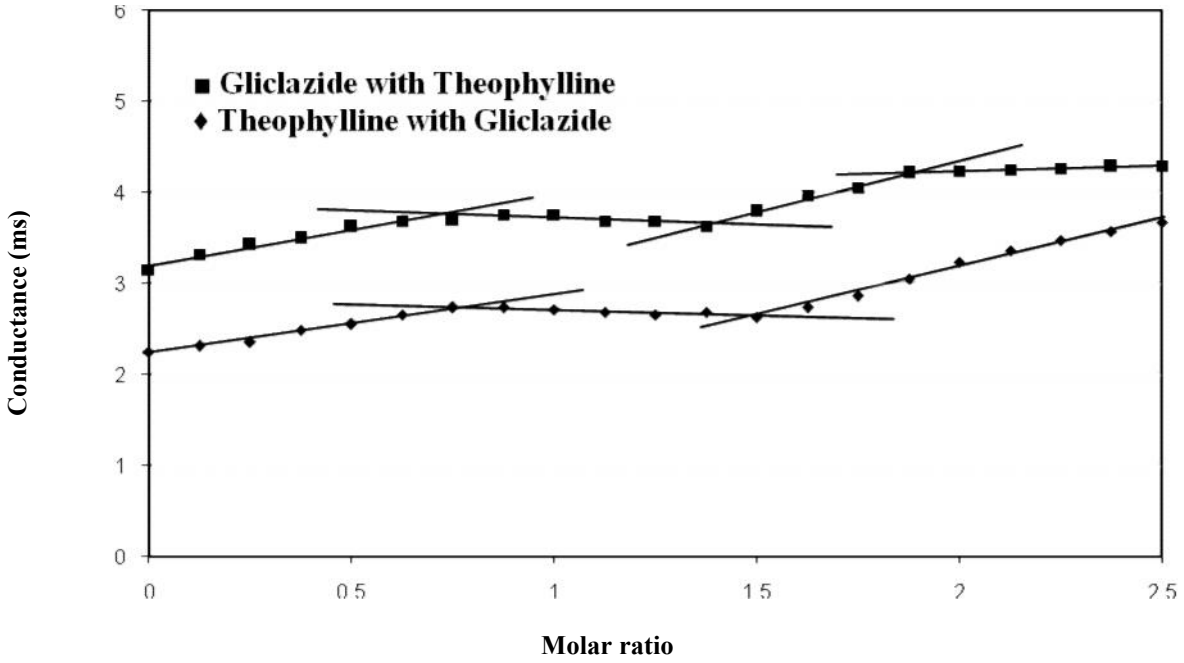


Fig. 7.5.41: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 4.4

Table 7.1.5.42: Data for conductometric titration of theophylline-glipizide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.25	3.21
0.5	40.50	0.125	2.37	3.32
1.0	41.00	0.250	2.46	3.44
1.5	41.50	0.375	2.56	3.55
2.0	42.00	0.500	2.63	3.59
2.5	42.50	0.625	2.75	3.67
3.0	43.00	0.750	2.85	3.75
3.5	43.50	0.875	2.98	3.88
4.0	44.00	1.000	3.05	3.98
4.5	44.50	1.125	2.93	4.2
5.0	45.00	1.250	2.82	4.08
5.5	45.50	1.375	2.67	3.99
6.0	46.00	1.500	2.52	3.87
6.5	46.50	1.625	2.65	3.88
7.0	47.00	1.750	2.75	3.71
7.5	47.50	1.875	2.92	4.01
8.0	48.00	2.000	3.01	4.23

8.5	48.50	2.125	3.11	4.48
9.0	49.00	2.250	3.22	4.36
9.5	49.50	2.375	3.28	4.36
10.0	50.00	2.500	3.37	4.32

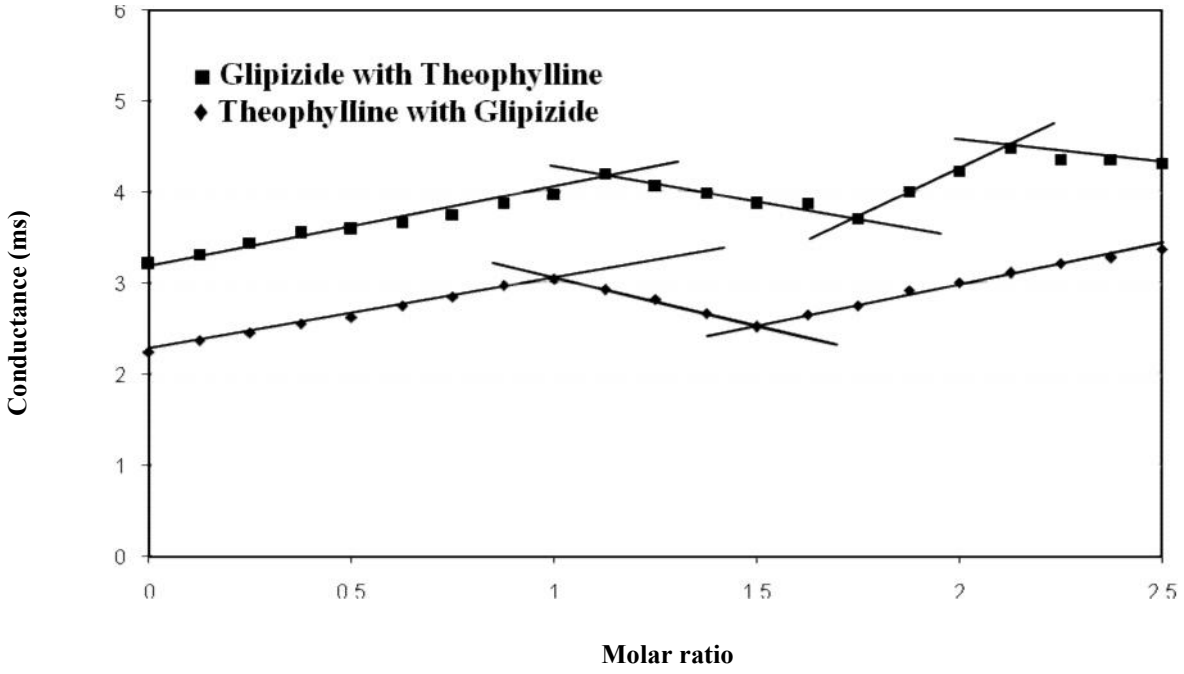


Fig. 7.1.5.42: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 4.4

Table 7.1.5.43: Data for conductometric titration of theophylline-glyburide system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with Theophylline
0.0	40.00	0.000	2.22	3.26
0.5	40.50	0.125	2.28	3.32
1.0	41.00	0.250	2.43	3.51
1.5	41.50	0.375	2.57	3.55
2.0	42.00	0.500	2.62	3.59
2.5	42.50	0.625	2.72	3.68
3.0	43.00	0.750	2.78	3.75
3.5	43.50	0.875	2.93	3.92
4.0	44.00	1.000	2.96	3.92
4.5	44.50	1.125	2.95	4.12
5.0	45.00	1.250	2.93	4.21
5.5	45.50	1.375	2.97	4.18
6.0	46.00	1.500	2.96	4.03
6.5	46.50	1.625	2.95	3.79
7.0	47.00	1.750	2.91	3.72
7.5	47.50	1.875	2.79	4.01

8.0	48.00	2.000	3.02	4.21
8.5	48.50	2.125	3.21	4.31
9.0	49.00	2.250	3.35	4.35
9.5	49.50	2.375	3.51	4.36
10.0	50.00	2.500	3.73	4.37

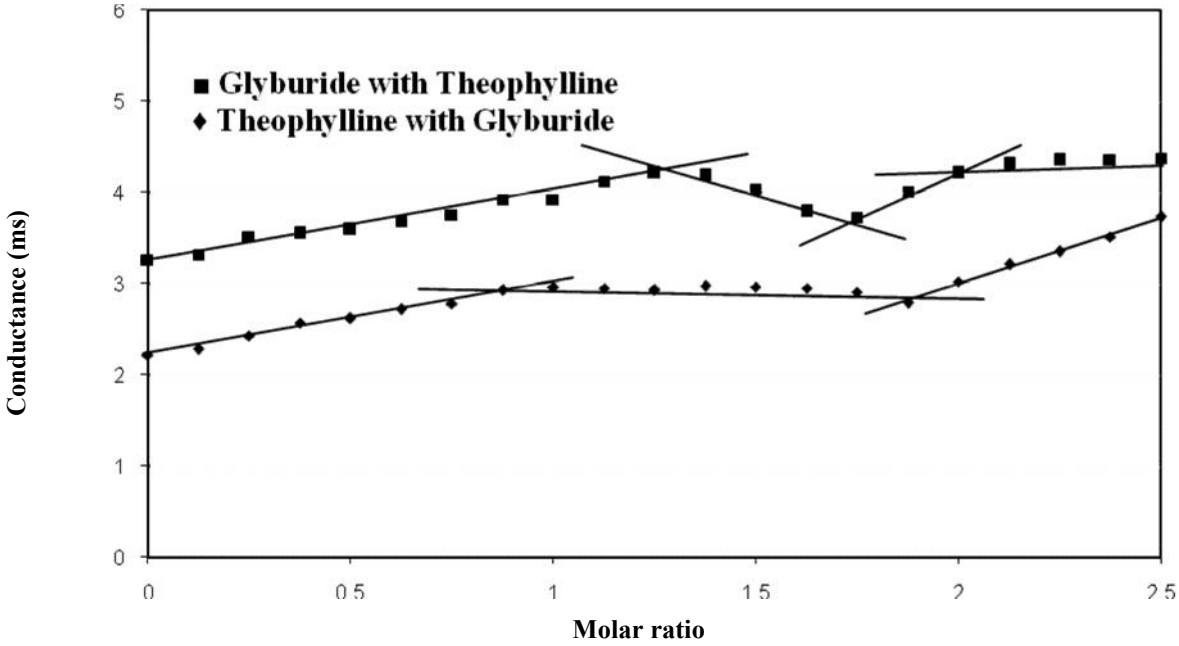


Fig. 7.1.5.43: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 4.4

Table 7.1.5.44: Data for conductometric titration of theophylline-metformin system at pH 4.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with Theophylline
0.0	40.00	0.000	1.28	2.26
0.5	40.50	0.125	1.32	2.42
1.0	41.00	0.250	1.37	2.59
1.5	41.50	0.375	1.43	2.73
2.0	42.00	0.500	1.47	2.91
2.5	42.50	0.625	1.49	3.22
3.0	43.00	0.750	1.61	3.35
3.5	43.50	0.875	1.85	3.38
4.0	44.00	1.000	2.09	3.41
4.5	44.50	1.125	2.25	3.43
5.0	45.00	1.250	2.15	3.44
5.5	45.50	1.375	2.11	3.36
6.0	46.00	1.500	2.04	3.41
6.5	46.50	1.625	1.98	3.27
7.0	47.00	1.750	1.84	3.15
7.5	47.50	1.875	1.75	2.85

8.0	48.00	2.000	1.95	2.72
8.5	48.50	2.125	2.05	2.96
9.0	49.00	2.250	2.32	3.41
9.5	49.50	2.375	2.49	3.72
10.0	50.00	2.500	2.61	4.05

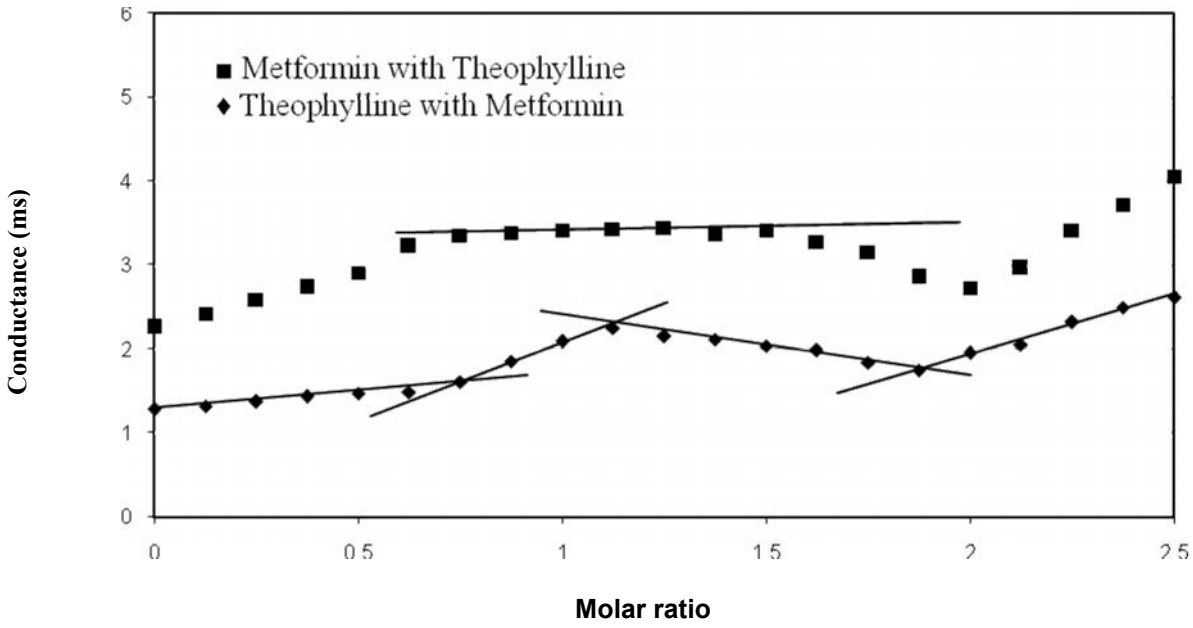


Fig. 7.1.5.44: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 4.4

Table 7.1.5.45: Data for conductometric titration of theophylline-gliclazide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with theophylline
0.0	40.00	0.000	1.43	2.39
0.5	40.50	0.125	1.69	2.60
1.0	41.00	0.250	1.95	2.81
1.5	41.50	0.375	2.12	3.08
2.0	42.00	0.500	2.33	3.29
2.5	42.50	0.625	2.52	3.48
3.0	43.00	0.750	2.82	3.58
3.5	43.50	0.875	2.88	3.77
4.0	44.00	1.000	3.05	3.85
4.5	44.50	1.125	3.13	4.03
5.0	45.00	1.250	3.24	4.15
5.5	45.50	1.375	3.32	4.25
6.0	46.00	1.500	3.42	4.33
6.5	46.50	1.625	3.56	4.35
7.0	47.00	1.750	3.59	4.34
7.5	47.50	1.875	3.61	4.33
8.0	48.00	2.000	3.58	4.28

8.5	48.50	2.125	3.56	4.26
9.0	49.00	2.250	3.55	4.25
9.5	49.50	2.375	3.53	4.24
10.0	50.00	2.500	3.52	4.22

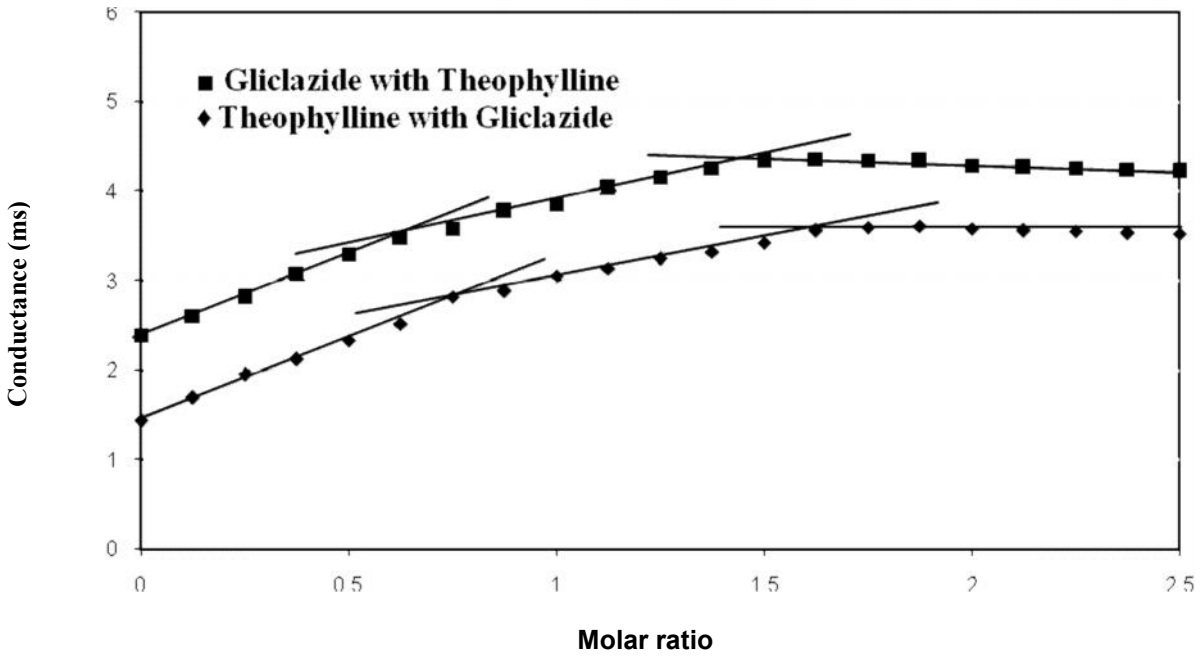


Fig. 7.1.5.45: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 5.4

Table 7.1.5.46: Data for conductometric titration of theophylline-glipizide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.26	3.22
0.5	40.50	0.125	2.38	3.33
1.0	41.00	0.250	2.47	3.44
1.5	41.50	0.375	2.57	3.55
2.0	42.00	0.500	2.67	3.63
2.5	42.50	0.625	2.77	3.73
3.0	43.00	0.750	2.86	3.82
3.5	43.50	0.875	2.96	3.93
4.0	44.00	1.000	3.06	4.03
4.5	44.50	1.125	3.02	3.95
5.0	45.00	1.250	3.01	3.94
5.5	45.50	1.375	2.95	3.89
6.0	46.00	1.500	2.88	3.81
6.5	46.50	1.625	2.78	3.82
7.0	47.00	1.750	2.85	3.72
7.5	47.50	1.875	2.95	4.03
8.0	48.00	2.000	3.05	4.23

8.5	48.50	2.125	3.16	4.48
9.0	49.00	2.250	3.19	4.32
9.5	49.50	2.375	3.31	4.34
10.0	50.00	2.500	3.39	4.33

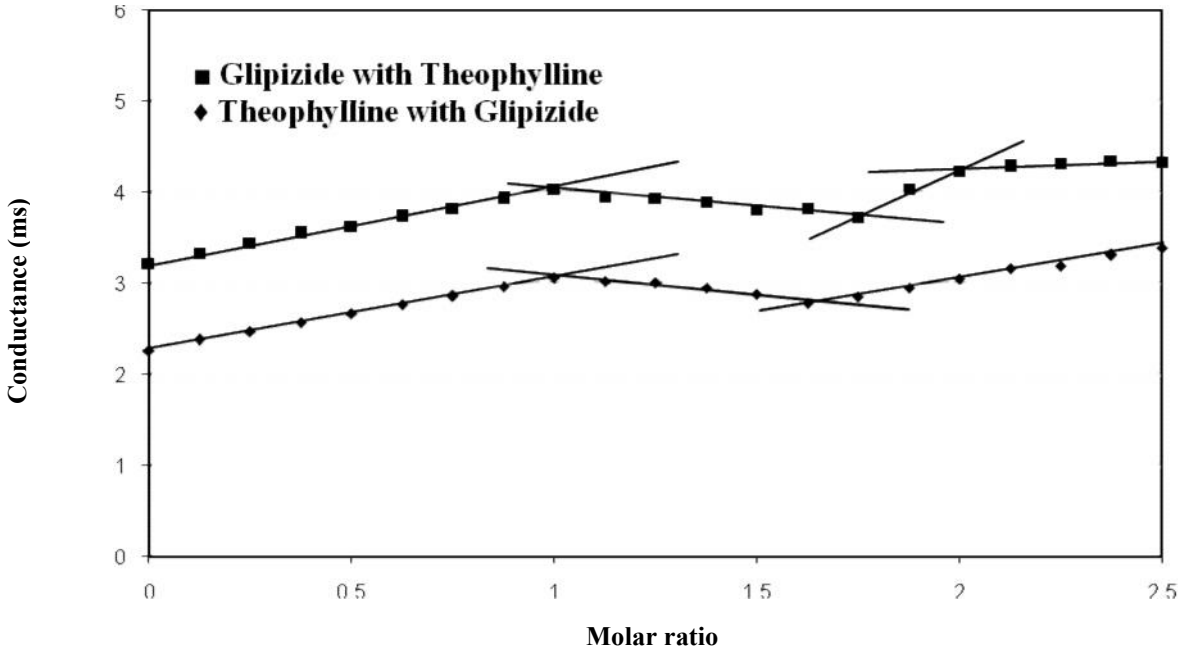


Fig. 7.1.5.46: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 5.4

Table 7.1.5.47: Data for conductometric titration of theophylline-glyburide system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with Theophylline
0.0	40.00	0.000	2.21	3.25
0.5	40.50	0.125	2.32	3.36
1.0	41.00	0.250	2.45	3.48
1.5	41.50	0.375	2.55	3.57
2.0	42.00	0.500	2.66	3.65
2.5	42.50	0.625	2.78	3.78
3.0	43.00	0.750	2.86	3.84
3.5	43.50	0.875	3.05	3.92
4.0	44.00	1.000	3.13	3.98
4.5	44.50	1.125	3.12	3.99
5.0	45.00	1.250	3.11	3.96
5.5	45.50	1.375	3.09	3.87
6.0	46.00	1.500	3.08	3.87
6.5	46.50	1.625	3.06	3.80
7.0	47.00	1.750	3.01	3.95
7.5	47.50	1.875	3.02	4.15
8.0	48.00	2.000	3.08	4.25

8.5	48.50	2.125	3.23	4.31
9.0	49.00	2.250	3.37	4.33
9.5	49.50	2.375	3.52	4.35
10.0	50.00	2.500	3.63	4.39

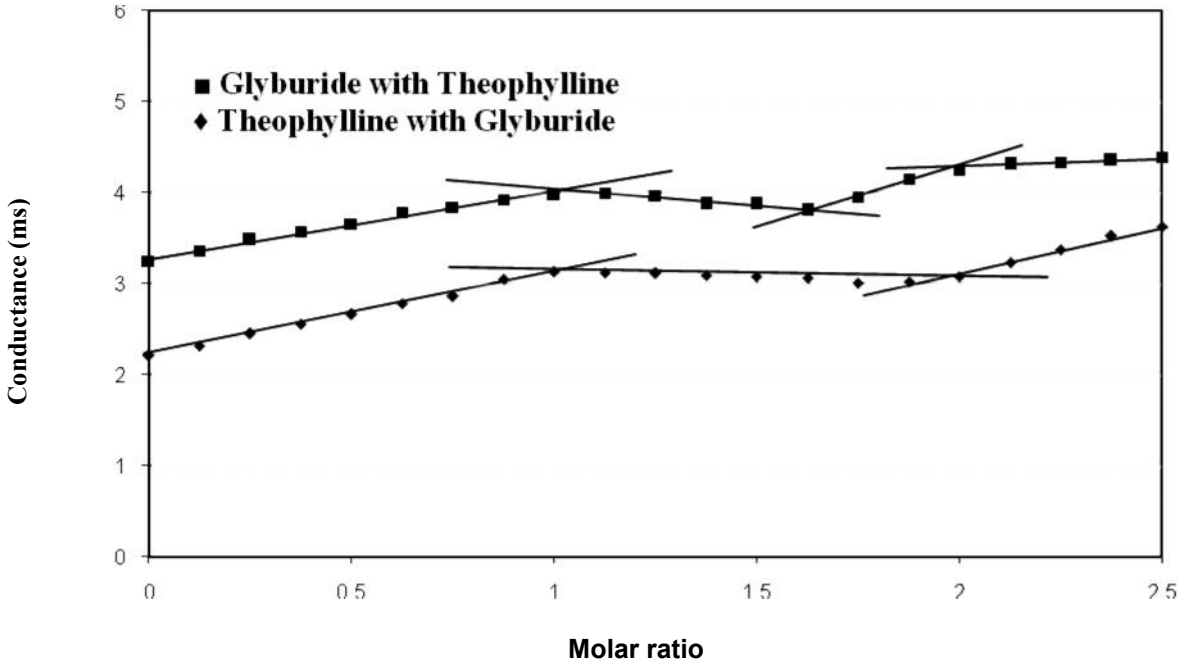


Fig. 7.1.5.47: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 5.4

Table 7.1.5.48: Data for conductometric titration of theophylline-metformin system at pH 5.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with Theophylline
0.0	40.00	0.000	1.43	2.59
0.5	40.50	0.125	1.46	2.63
1.0	41.00	0.250	1.48	2.73
1.5	41.50	0.375	1.52	2.88
2.0	42.00	0.500	1.65	2.99
2.5	42.50	0.625	1.63	3.21
3.0	43.00	0.750	1.61	3.29
3.5	43.50	0.875	1.85	3.28
4.0	44.00	1.000	1.98	3.31
4.5	44.50	1.125	2.03	3.33
5.0	45.00	1.250	2.15	3.34
5.5	45.50	1.375	2.08	3.36
6.0	46.00	1.500	2.01	3.37
6.5	46.50	1.625	1.96	3.38
7.0	47.00	1.750	1.85	3.4
7.5	47.50	1.875	2.01	3.42
8.0	48.00	2.000	2.11	3.58

8.5	48.50	2.125	2.15	3.66
9.0	49.00	2.250	2.31	3.73
9.5	49.50	2.375	2.48	3.82
10.0	50.00	2.500	2.58	3.98

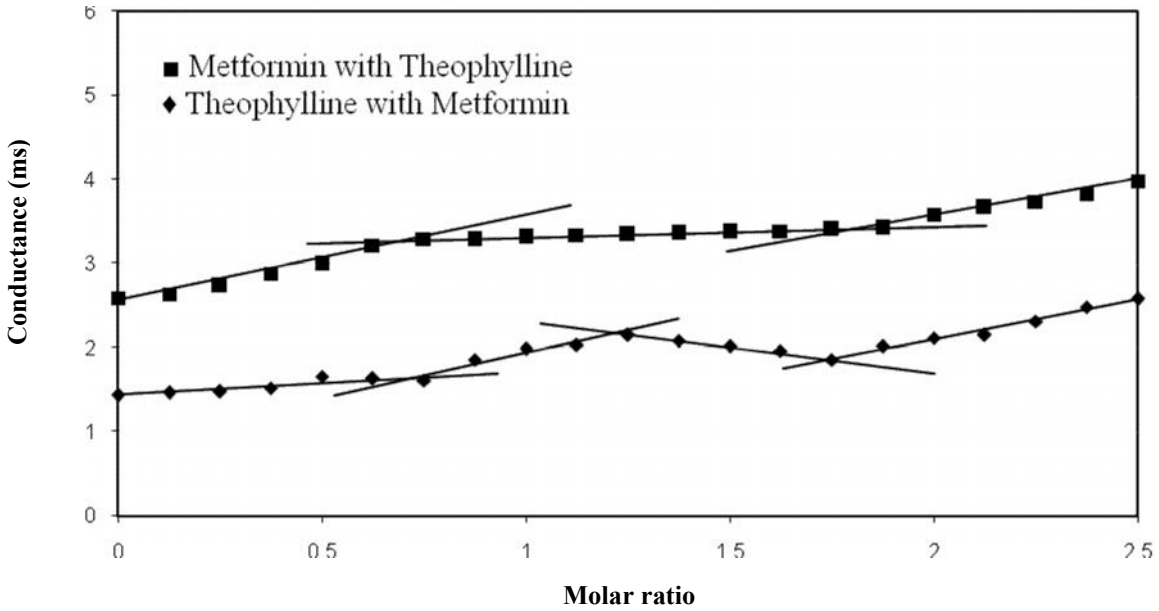


Fig. 7.5.48: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 5.4

Table 7.1.5.49: Data for conductometric titration of theophylline-gliclazide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazidewith theophylline
0.0	40.00	0.000	2.25	3.19
0.5	40.50	0.125	2.28	3.26
1.0	41.00	0.250	2.33	3.29
1.5	41.50	0.375	2.35	3.31
2.0	42.00	0.500	2.39	3.33
2.5	42.50	0.625	2.45	3.41
3.0	43.00	0.750	2.58	3.43
3.5	43.50	0.875	2.71	3.63
4.0	44.00	1.000	2.83	3.78
4.5	44.50	1.125	2.98	3.92
5.0	45.00	1.250	3.08	4.02
5.5	45.50	1.375	3.25	4.23
6.0	46.00	1.500	3.35	4.18
6.5	46.50	1.625	3.46	4.15
7.0	47.00	1.750	3.44	4.12
7.5	47.50	1.875	3.42	4.10
8.0	48.00	2.000	3.41	4.08

8.5	48.50	2.125	3.38	4.06
9.0	49.00	2.250	3.36	4.04
9.5	49.50	2.375	3.34	4.02
10.0	50.00	2.500	3.33	3.99

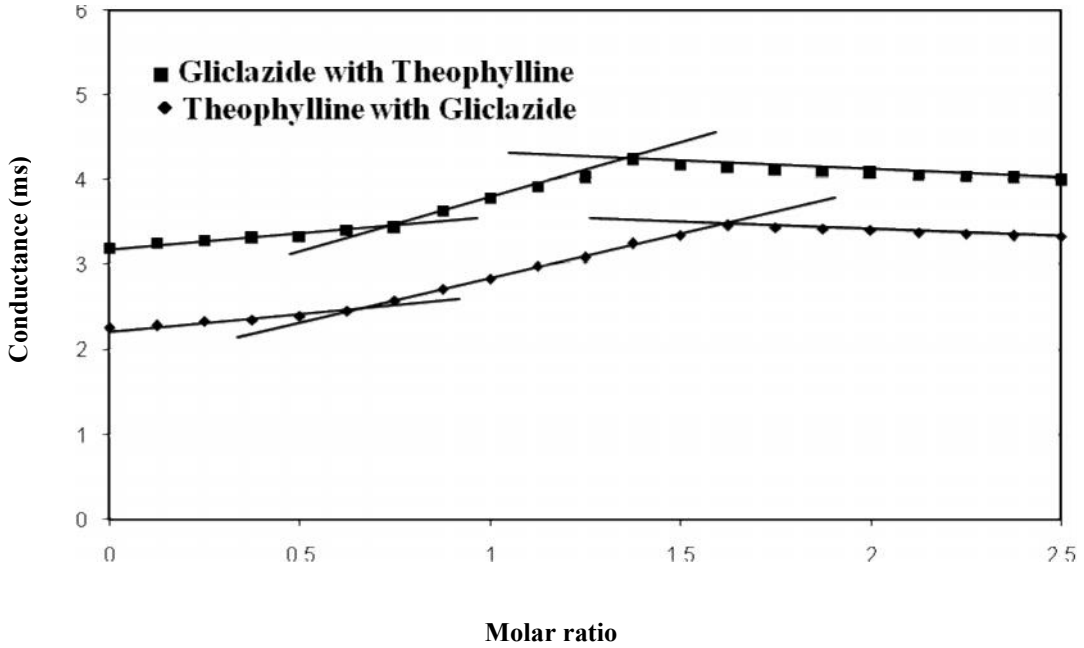


Fig. 7.1.5.49: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 6.4

Table 7.1.5.50: Data for conductometric titration of theophylline-glipizide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with caffeine
0.0	40.00	0.000	2.12	2.98
0.5	40.50	0.125	2.24	3.08
1.0	41.00	0.250	2.35	3.23
1.5	41.50	0.375	2.49	3.31
2.0	42.00	0.500	2.61	3.43
2.5	42.50	0.625	2.72	3.63
3.0	43.00	0.750	2.82	3.76
3.5	43.50	0.875	2.92	3.91
4.0	44.00	1.000	3.06	3.99
4.5	44.50	1.125	3.05	3.94
5.0	45.00	1.250	3.05	3.96
5.5	45.50	1.375	3.03	3.88
6.0	46.00	1.500	3.02	3.82
6.5	46.50	1.625	3.05	3.98
7.0	47.00	1.750	3.13	4.02
7.5	47.50	1.875	3.18	4.08
8.0	48.00	2.000	3.21	4.19

8.5	48.50	2.125	3.23	4.25
9.0	49.00	2.250	3.25	4.29
9.5	49.50	2.375	3.33	4.32
10.0	50.00	2.500	3.38	4.34

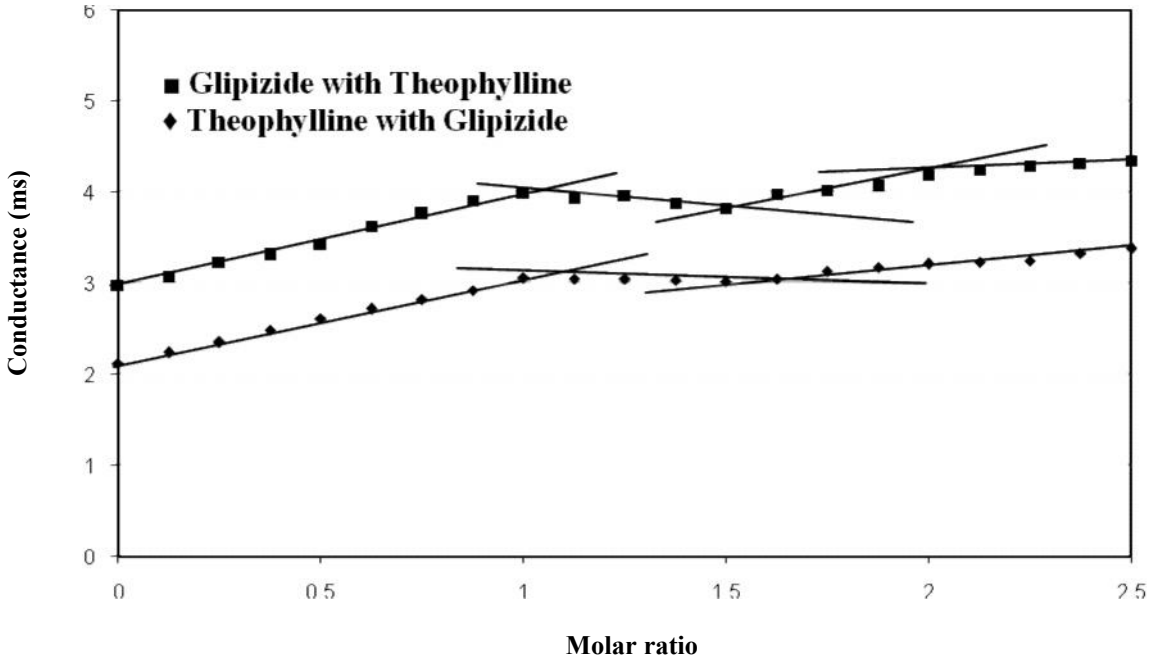


Fig. 7.1.5.50: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 6.4

Table 7.1.5.51: Data for conductometric titration of theophylline-glyburide system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with Theophylline
0.0	40.00	0.000	2.45	3.39
0.5	40.50	0.125	2.53	3.42
1.0	41.00	0.250	2.59	3.52
1.5	41.50	0.375	2.73	3.59
2.0	42.00	0.500	2.76	3.65
2.5	42.50	0.625	2.92	3.73
3.0	43.00	0.750	2.98	3.81
3.5	43.50	0.875	3.04	3.85
4.0	44.00	1.000	3.15	3.82
4.5	44.50	1.125	3.12	3.89
5.0	45.00	1.250	3.12	3.88
5.5	45.50	1.375	3.10	3.86
6.0	46.00	1.500	3.08	3.88
6.5	46.50	1.625	3.07	3.94
7.0	47.00	1.750	3.09	4.02
7.5	47.50	1.875	3.13	4.14
8.0	48.00	2.000	3.21	4.24

8.5	48.50	2.125	3.28	4.29
9.0	49.00	2.250	3.39	4.31
9.5	49.50	2.375	3.45	4.28
10.0	50.00	2.500	3.53	4.27

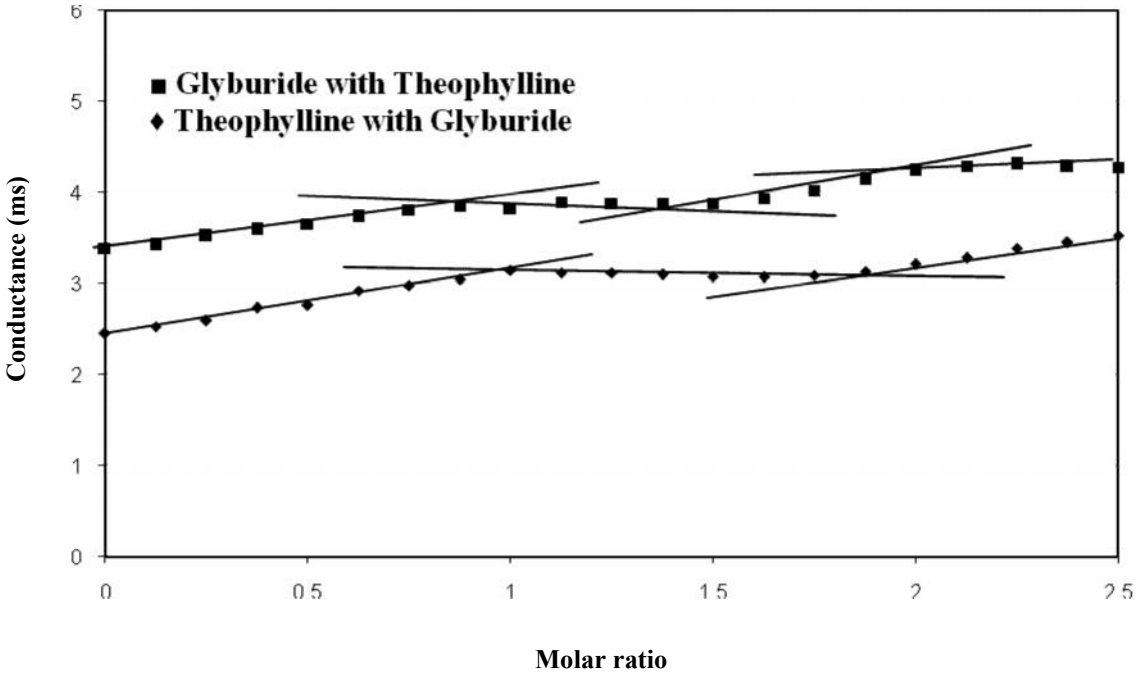


Fig. 7.1.5.51: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 6.4

Table 7.5.52: Data for conductometric titration of theophylline-metformin system at pH 6.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with Theophylline
0.0	40.00	0.000	1.45	2.55
0.5	40.50	0.125	1.65	2.64
1.0	41.00	0.250	1.82	2.75
1.5	41.50	0.375	1.93	2.87
2.0	42.00	0.500	2.02	2.96
2.5	42.50	0.625	2.12	3.05
3.0	43.00	0.750	2.25	3.21
3.5	43.50	0.875	2.36	3.25
4.0	44.00	1.000	2.37	3.28
4.5	44.50	1.125	2.38	3.26
5.0	45.00	1.250	2.41	3.33
5.5	45.50	1.375	2.43	3.34
6.0	46.00	1.500	2.44	3.41
6.5	46.50	1.625	2.45	3.39
7.0	47.00	1.750	2.49	3.49
7.5	47.50	1.875	3.56	3.56
8.0	48.00	2.000	3.61	3.61

8.5	48.50	2.125	3.66	3.66
9.0	49.00	2.250	3.77	3.77
9.5	49.50	2.375	3.85	3.85
10.0	50.00	2.500	3.99	3.99

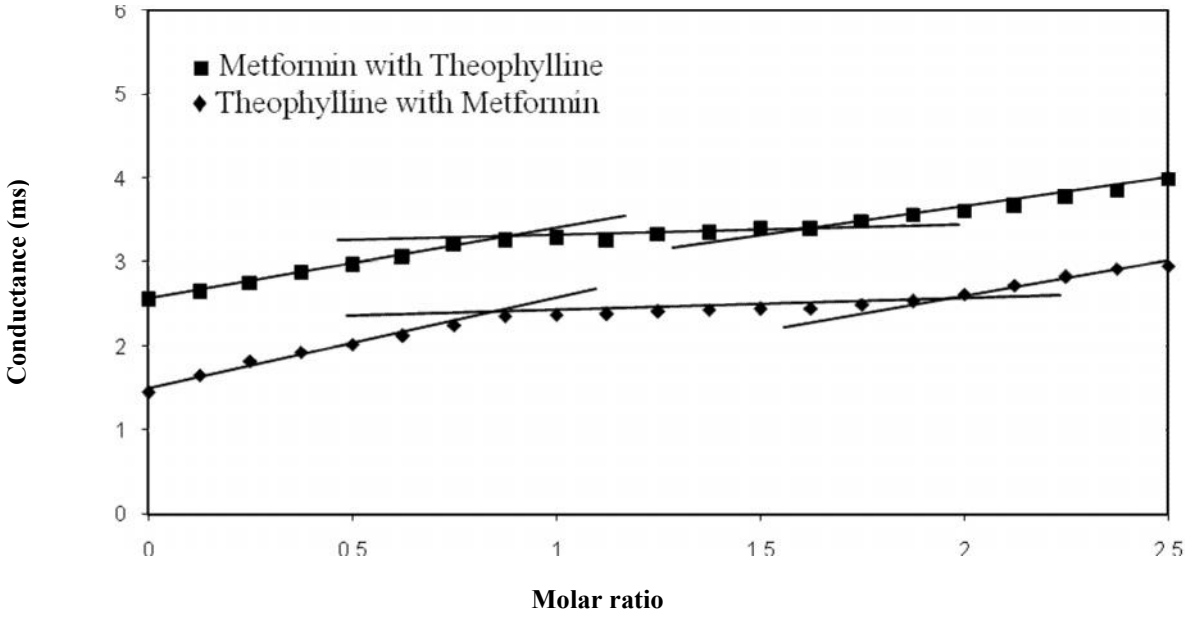


Fig. 7.1.5.52: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 6.4

Table 7.1.5.53: Data for conductometric titration of theophylline-gliclazide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with gliclazide	Gliclazide with theophylline
0.0	40.00	0.000	2.12	2.75
0.5	40.50	0.125	2.22	2.98
1.0	41.00	0.250	2.33	3.13
1.5	41.50	0.375	2.43	3.21
2.0	42.00	0.500	2.53	3.35
2.5	42.50	0.625	2.63	3.42
3.0	43.00	0.750	2.69	3.51
3.5	43.50	0.875	2.68	3.56
4.0	44.00	1.000	2.78	3.58
4.5	44.50	1.125	2.79	3.63
5.0	45.00	1.250	2.78	3.62
5.5	45.50	1.375	2.92	3.61
6.0	46.00	1.500	2.98	3.78
6.5	46.50	1.625	3.13	3.81
7.0	47.00	1.750	3.21	3.91
7.5	47.50	1.875	3.28	4.01
8.0	48.00	2.000	3.26	3.90

8.5	48.50	2.125	3.23	3.88
9.0	49.00	2.250	3.19	3.82
9.5	49.50	2.375	3.15	3.80
10.0	50.00	2.500	3.12	3.78

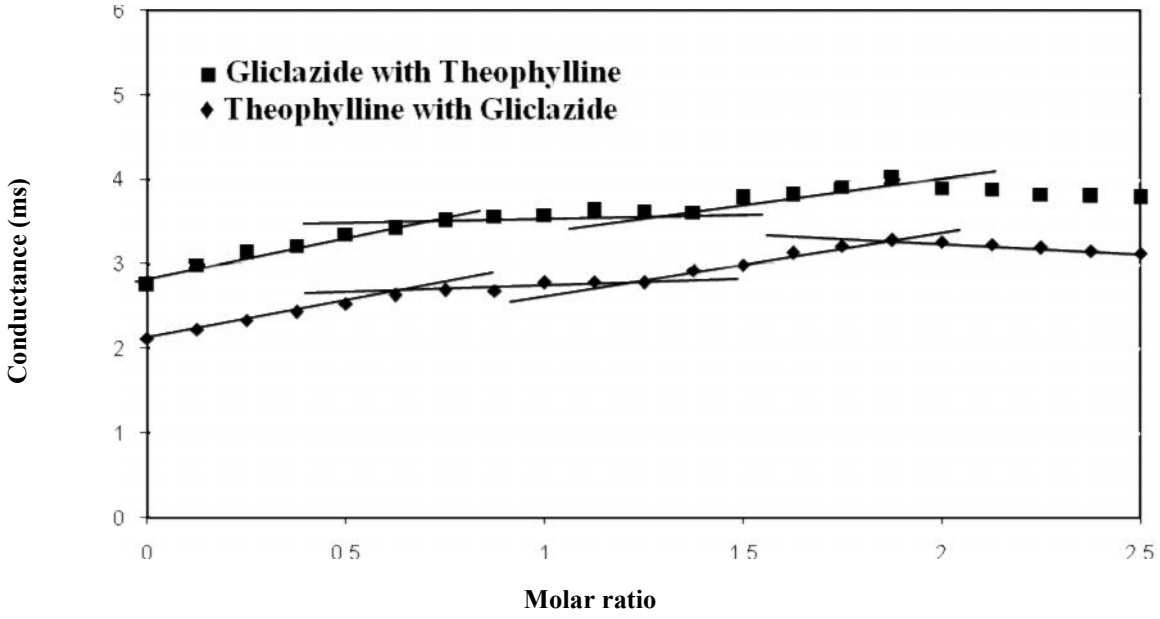


Fig. 7.1.5.53: Conductometric titration of theophylline with gliclazide and gliclazide with theophylline at pH 7.4

Table 7.1.5.54: Data for conductometric titration of theophylline-glipizide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glipizide	Glipizide with Theophylline
0.0	40.00	0.000	2.13	3.05
0.5	40.50	0.125	2.22	3.21
1.0	41.00	0.250	2.38	3.33
1.5	41.50	0.375	2.53	3.46
2.0	42.00	0.500	2.65	3.56
2.5	42.50	0.625	2.82	3.72
3.0	43.00	0.750	2.95	3.91
3.5	43.50	0.875	2.90	3.94
4.0	44.00	1.000	2.85	3.92
4.5	44.50	1.125	2.75	3.95
5.0	45.00	1.250	2.66	3.96
5.5	45.50	1.375	2.63	3.95
6.0	46.00	1.500	2.59	3.85
6.5	46.50	1.625	2.58	3.72
7.0	47.00	1.750	2.57	3.56
7.5	47.50	1.875	2.48	3.51
8.0	48.00	2.000	2.45	3.53

8.5	48.50	2.125	2.54	3.56
9.0	49.00	2.250	2.62	3.55
9.5	49.50	2.375	2.64	3.56
10.0	50.00	2.500	2.66	3.55

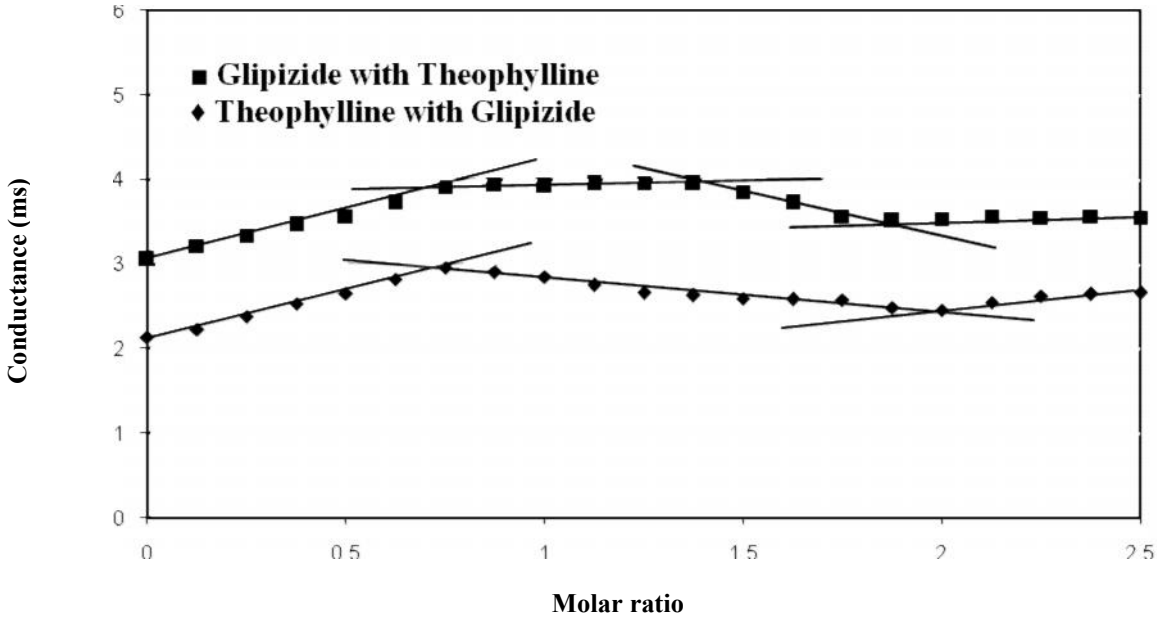


Fig. 7.1.5.54: Conductometric titration of theophylline with glipizide and glipizide with theophylline at pH 7.4

Table 7.1.5.55: Data for conductometric titration of theophylline-glyburide system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with glyburide	Glyburide with Theophylline
0.0	40.00	0.000	2.37	3.16
0.5	40.50	0.125	2.48	3.25
1.0	41.00	0.250	2.63	3.42
1.5	41.50	0.375	2.79	3.52
2.0	42.00	0.500	2.95	3.66
2.5	42.50	0.625	3.08	3.78
3.0	43.00	0.750	3.05	3.77
3.5	43.50	0.875	3.01	3.75
4.0	44.00	1.000	3.02	3.66
4.5	44.50	1.125	3.00	3.65
5.0	45.00	1.250	2.90	3.65
5.5	45.50	1.375	3.01	3.75
6.0	46.00	1.500	3.15	3.88
6.5	46.50	1.625	3.25	4.02
7.0	47.00	1.750	3.38	4.15
7.5	47.50	1.875	3.58	4.31
8.0	48.00	2.000	3.46	4.13

8.5	48.50	2.125	3.39	4.03
9.0	49.00	2.250	3.33	3.93
9.5	49.50	2.375	3.21	3.83
10.0	50.00	2.500	3.05	3.73

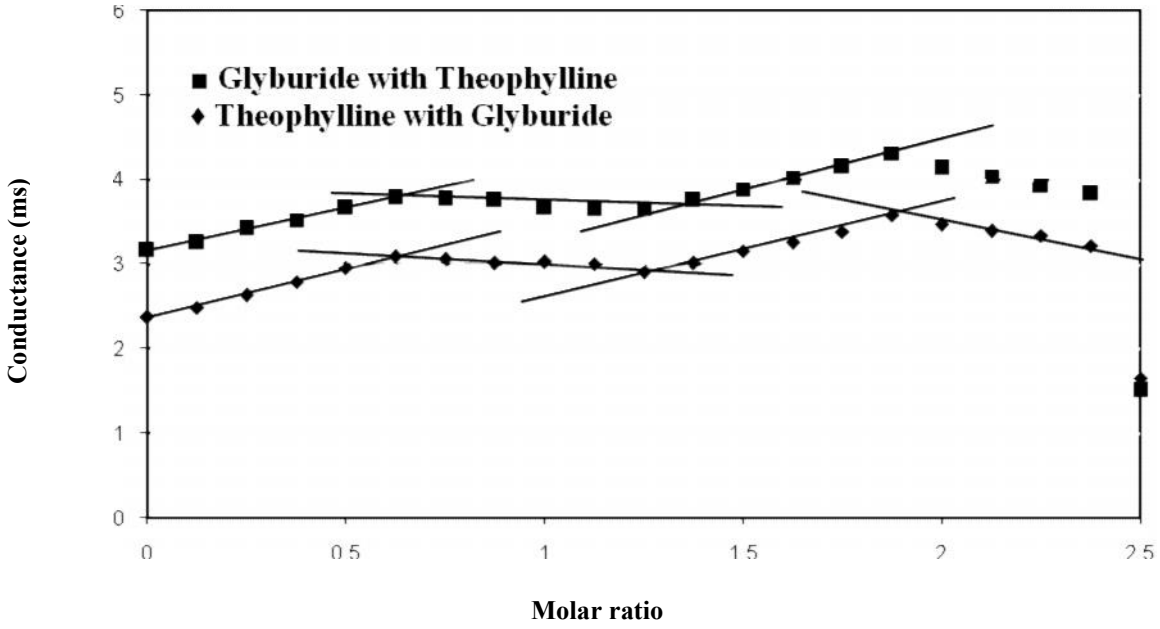


Fig. 7.1.5.55: Conductometric titration of theophylline with glyburide and glyburide with theophylline at pH 7.4

Table 7.1.5.56: Data for conductometric titration of theophylline-metformin system at pH 7.4

Initial volume of solution = 40 ml
Concentration of added species = 0.01 M
Concentration of initial species = 0.001 M

Volume of Added species (ml)	Volume of Mixture (ml)	Fraction of added species	Conductance (ms) for titration	
			Theophylline with metformin	Metformin with theophylline
0.0	40.00	0.000	1.28	2.25
0.5	40.50	0.125	1.44	2.43
1.0	41.00	0.250	1.45	2.58
1.5	41.50	0.375	1.59	2.72
2.0	42.00	0.500	1.69	2.92
2.5	42.50	0.625	1.79	3.12
3.0	43.00	0.750	1.89	3.25
3.5	43.50	0.875	2.02	3.31
4.0	44.00	1.000	2.11	3.23
4.5	44.50	1.125	2.28	3.22
5.0	45.00	1.250	2.25	3.18
5.5	45.50	1.375	2.19	3.16
6.0	46.00	1.500	2.16	3.12
6.5	46.50	1.625	2.12	3.03
7.0	47.00	1.750	2.06	2.95
7.5	47.50	1.875	2.04	2.93
8.0	48.00	2.000	2.0	3.22

8.5	48.50	2.125	2.23	3.33
9.0	49.00	2.250	2.36	3.56
9.5	49.50	2.375	2.55	3.72
10.0	50.00	2.500	2.65	4.03

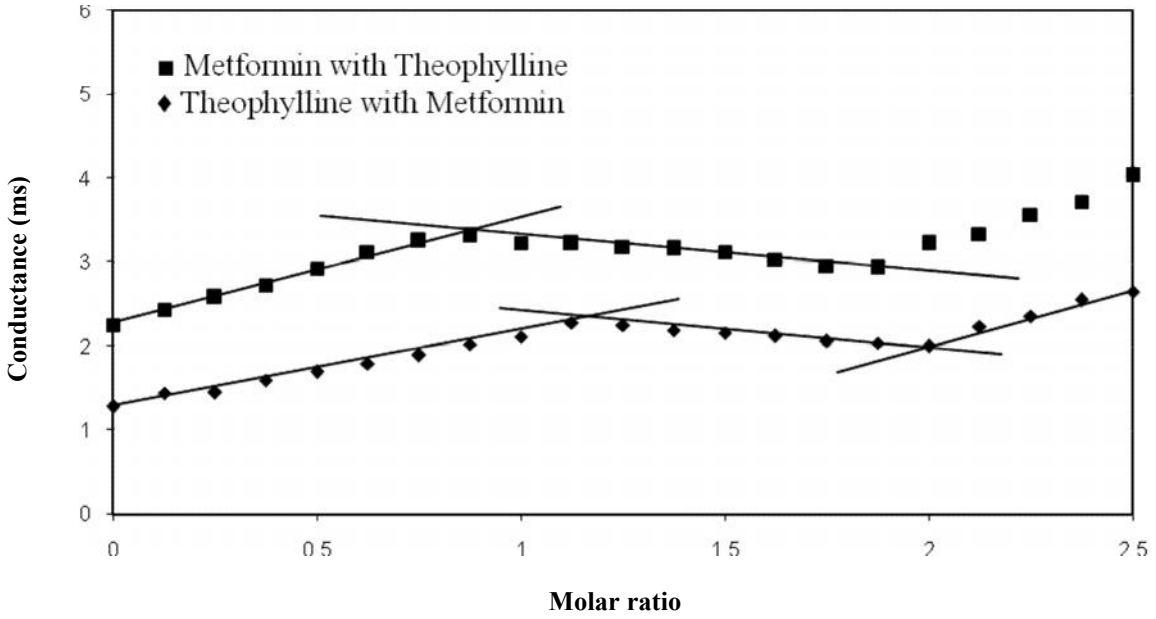


Fig. 7.1.5.56: Conductometric titration of theophylline with metformin and metformin with theophylline at pH 7.4

7.1.6 STUDY OF STRENGTH OF COMPLEX FORMATION BY ARDON'S SPECTROPHOTOMETRIC METHOD

Ardon's plots conformed the formation of 1:1 complexes of caffeine and theophylline with gliclazide, glipizide, glyburide & metformin. [65] These plots were obtained by plotting 1/ (D-E_AC) versus 1/ [B] using the Ardon's equation.

$$1/ (D - E_A C) = 1/ KC (E_{com} - E_{A)} [B]^n + 1/ C (E_{com} - E_{A)}$$

Where, D = absorbance of mixture
C = molar concentration of the drug
B = molar concentration of the ligand (the drug, which is the target)
E_{com} = molar extinction co-efficient of the complex.
E_A = molar extinction co-efficient of the drug.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for 1/ (D - E_AC) was plotted versus 1/ [B] to get the straight lines. The concentration of each caffeine and theophylline were kept constant at 5 x 10^{-5}**M** (denoted by C in the equation). The 1:1 complex gave a straight line in the plots with an intercept and slope.

All the experiments were performed at different pH values. The absorbance of solutions having p^H 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 & 7.4 were measured at 273 nm for caffeine and at 276 nm for theophylline using UV – Visible spectrophotometer. The data for Ardon's plots are given in tables 7.1.6.1 to 7.1.6.28. These plots (figs. 7.1.6.1 to 7.1.6.28) give straight lines with different intercepts, indicating the formation of 1:1 complexes for all systems.

Determination of stability constant

The stability constants are the strength of complexes. Stability constants for caffeine–gliclazide, caffeine–glipizide, caffeine–glyburide and caffeine-metformin systems were obtained from the Ardon's plots, which are straight lines (fig. 7.1.6.1 to 7.1.6.14) and theophylline–gliclazide, theophylline–glipizide, theophylline–glyburide and theophylline–metformin systems were obtained from the Ardon's plots, which are straight lines (fig. 7.1.6.15 to 7.1.6.28).The stability constants were calculated from the slopes and intercepts of these plots. The above equation shows that the stability constant (K) is the ratio between intercepts and slope e.g.

$$K = \text{intercept} / \text{slope}$$

Table 7.1.6.1: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 1.4
Concentration of caffeine = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M caffeine = 0.535
Molar extinction co-efficient of caffeine, E_A = 10700

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.020	2.06	1.00
1.5	1.317	1.28	0.67
2.0	1.415	1.14	0.50
2.5	1.517	1.02	0.40
3.0	1.618	0.92	0.33
3.5	1.715	0.85	0.29
4.0	1.813	0.78	0.25
4.5	1.919	0.72	0.22
5.0	2.015	0.68	0.20

Caffeine-glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.825	3.45	1.00
1.5	0.976	2.27	0.67
2.0	1.135	1.67	0.50
2.5	1.355	1.22	0.40
3.0	1.466	1.07	0.33
3.5	1.518	1.02	0.29
4.0	1.655	0.89	0.25
4.5	1.695	0.86	0.22
5.0	1.915	0.72	0.20

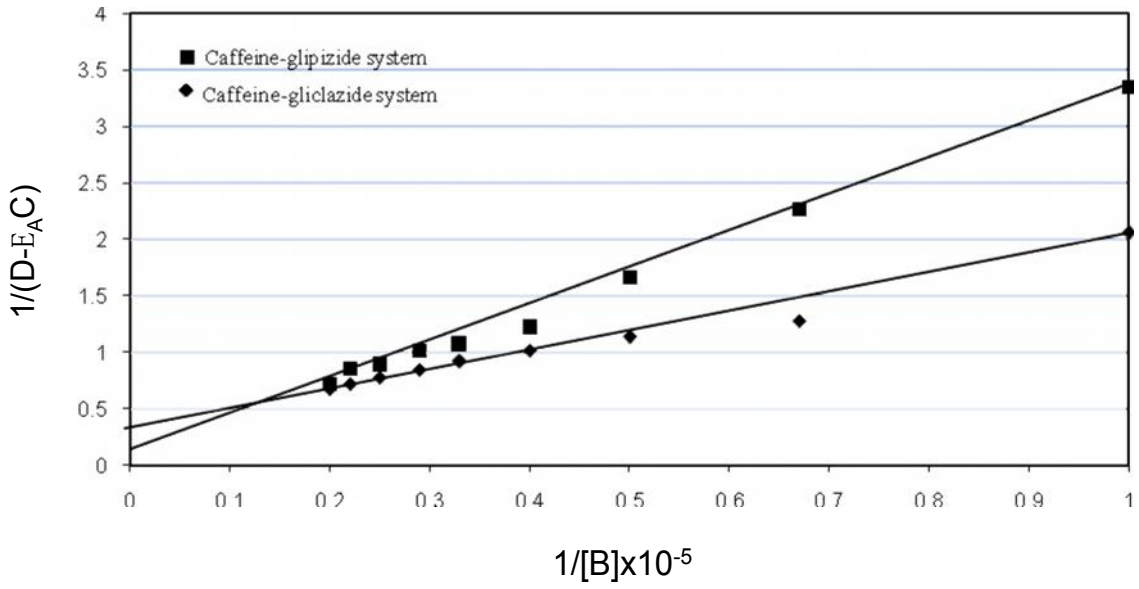


Fig.7.1.6.1: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 1.4

Table 7.1.6.2: Values for Ardon's plot for caffeine with glyburide and metformin at pH 1.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.535

Molar extinction co-efficient of caffeine, E_A = 10700

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.095	1.79	1.00
1.5	1.362	1.21	0.67
2.0	1.490	1.05	0.50
2.5	1.592	0.95	0.40
3.0	1.693	0.86	0.33
3.5	1.791	0.80	0.29
4.0	1.888	0.74	0.25
4.5	1.995	0.68	0.22
5.0	2.092	0.64	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.902	2.72	1.00
1.5	0.963	2.34	0.67
2.0	1.162	1.59	0.50
2.5	1.384	1.18	0.40
3.0	1.494	1.04	0.33
3.5	1.548	0.99	0.29
4.0	1.685	0.87	0.25
4.5	1.721	0.84	0.22
5.0	1.971	0.70	0.20

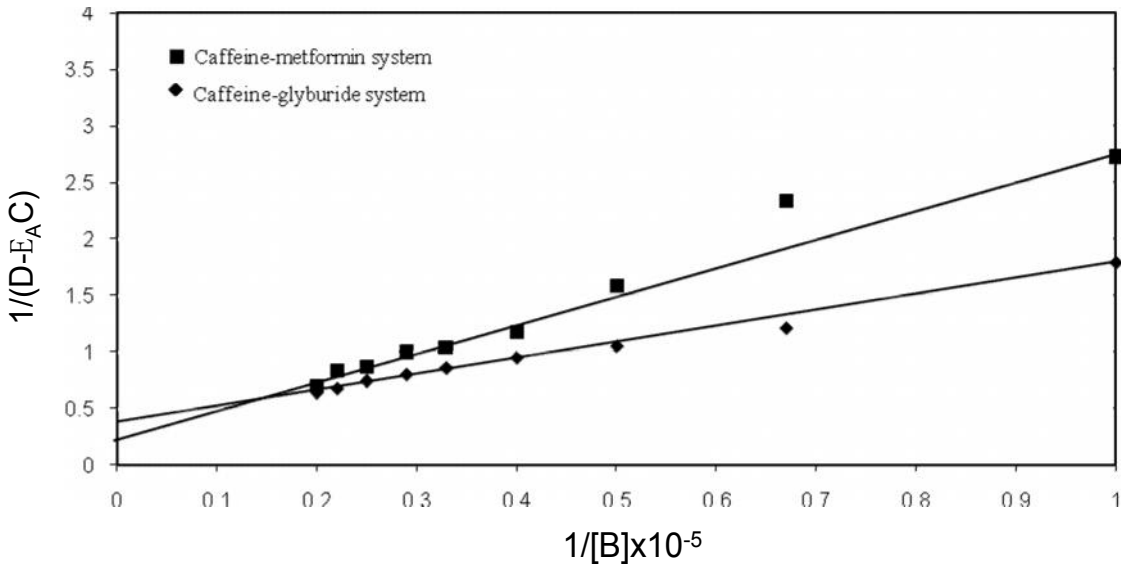


Fig.7.1.6.2: Ardon's plot for complexation of caffeine with glyburide and Metformin at pH 1.4

Table 7.1.6.3: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 2.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.527

Molar extinction co-efficient of caffeine, E_A = 10540

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.102	1.74	1.00
1.5	1.325	1.25	0.67
2.0	1.524	1.00	0.50
2.5	1.615	0.92	0.40
3.0	1.715	0.84	0.33
3.5	1.815	0.78	0.29
4.0	1.921	0.72	0.25
4.5	2.024	0.67	0.22
5.0	2.128	0.62	0.20

1/(D-E_AC)

Caffeine - glipizide System

1/[B]x10^{-5}

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.009	2.07	1.00
1.5	1.197	1.49	0.67
2.0	1.272	1.34	0.50
2.5	1.397	1.15	0.40
3.0	1.489	1.04	0.33
3.5	1.595	0.94	0.29
4.0	1.697	0.85	0.25
4.5	1.795	0.79	0.22
5.0	1.897	0.73	0.20

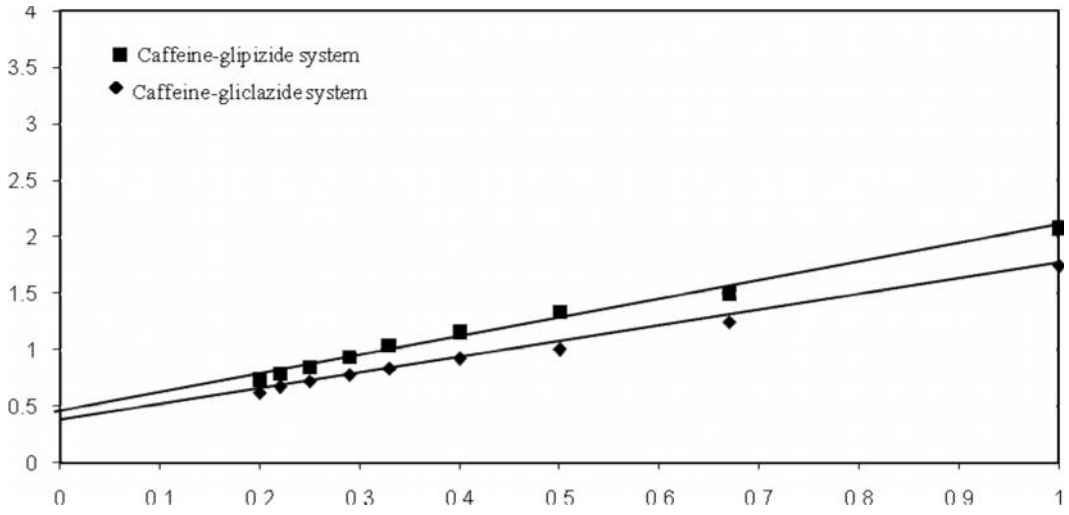


Fig.7.1.6.3: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 2.4

Table 7.1.6.4: Values for Ardon's plot for caffeine with glyburide and metformin at pH 2.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.527

Molar extinction co-efficient of caffeine, E_A = 10540

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.028	2.00	1.00
1.5	1.328	1.25	0.67
2.0	1.427	1.11	0.50
2.5	1.529	1.00	0.40
3.0	1.628	0.91	0.33
3.5	1.727	0.83	0.29
4.0	1.828	0.77	0.25
4.5	1.929	0.71	0.22
5.0	2.038	0.66	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.975	2.23	1.00
1.5	1.012	2.06	0.67
2.0	1.165	1.57	0.50
2.5	1.235	1.41	0.40
3.0	1.338	1.23	0.33
3.5	1.537	0.99	0.29
4.0	1.672	0.87	0.25
4.5	1.724	0.84	0.22
5.0	1.926	0.71	0.20

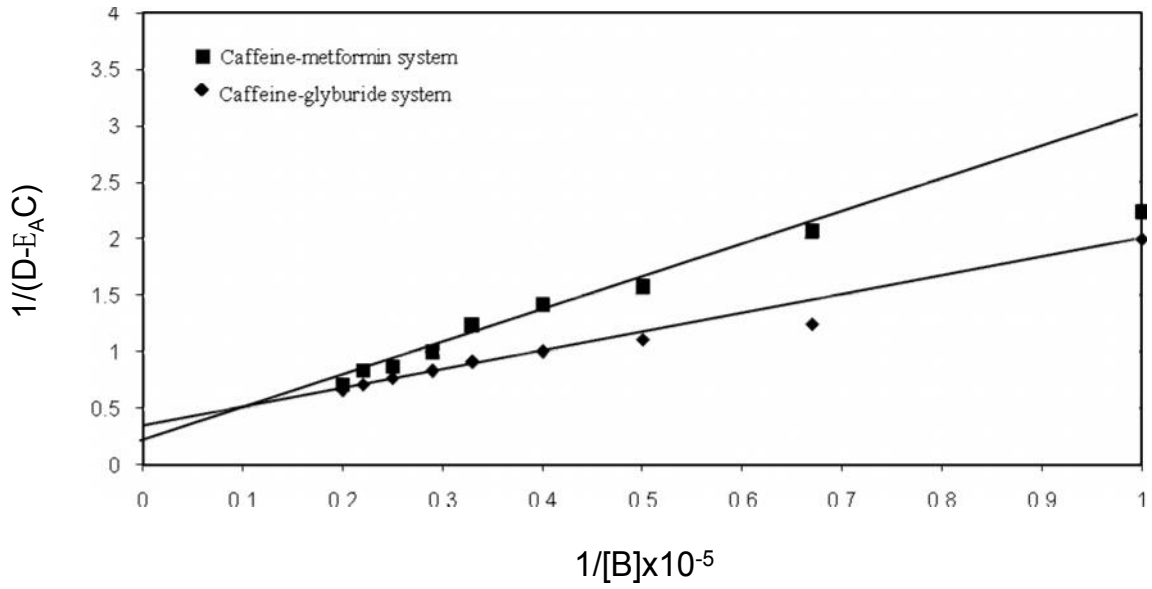


Fig.7.1.6.4: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 2.4

Table 7.1.6.5: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 3.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.521

Molar extinction co-efficient of caffeine, E_A = 10420

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.922	2.49	1.00
1.5	0.988	2.14	0.67
2.0	1.119	1.67	0.50
2.5	1.239	1.39	0.40
3.0	1.395	1.14	0.33
3.5	1.501	1.02	0.29
4.0	1.619	0.91	0.25
4.5	1.775	0.80	0.22
5.0	1.928	0.71	0.20

Caffeine - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.978	2.19	1.00
1.5	1.079	1.79	0.67
2.0	1.145	1.60	0.50
2.5	1.226	1.42	0.40
3.0	1.255	1.36	0.33
3.5	1.263	1.35	0.29
4.0	1.269	1.34	0.25
4.5	1.278	1.32	0.22
5.0	1.323	1.25	0.20

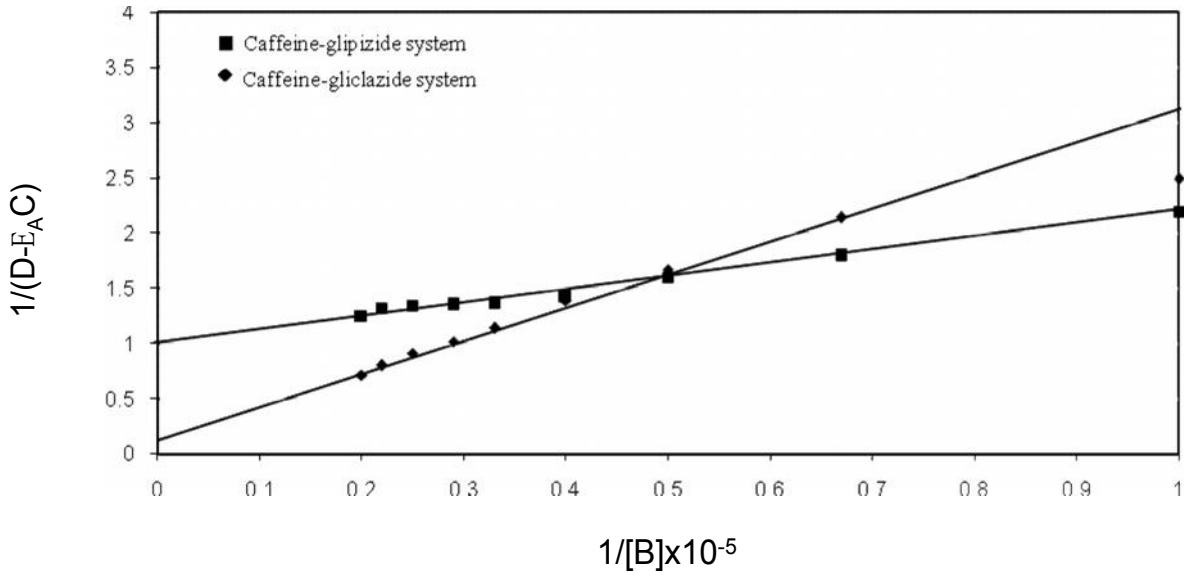


Fig.7.1.6.5: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 3.4

Table 7.1.6.6: Values for Ardon's plot for caffeine with glyburide and metformin at pH 3.4
Concentration of caffeine = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M caffeine = 0.521
Molar extinction co-efficient of caffeine, E_A = 10420

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.146	1.60	1.00
1.5	1.329	1.24	0.67
2.0	1.428	1.10	0.50
2.5	1.535	0.99	0.40
3.0	1.627	0.90	0.33
3.5	1.724	0.83	0.29
4.0	1.828	0.77	0.25
4.5	1.927	0.71	0.22
5.0	2.023	0.67	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.961	2.27	1.00
1.5	1.038	1.93	0.67
2.0	1.109	1.70	0.50
2.5	1.141	1.61	0.40
3.0	1.159	1.57	0.33
3.5	1.187	1.50	0.29
4.0	1.232	1.41	0.25
4.5	1.284	1.31	0.22
5.0	1.328	1.24	0.20

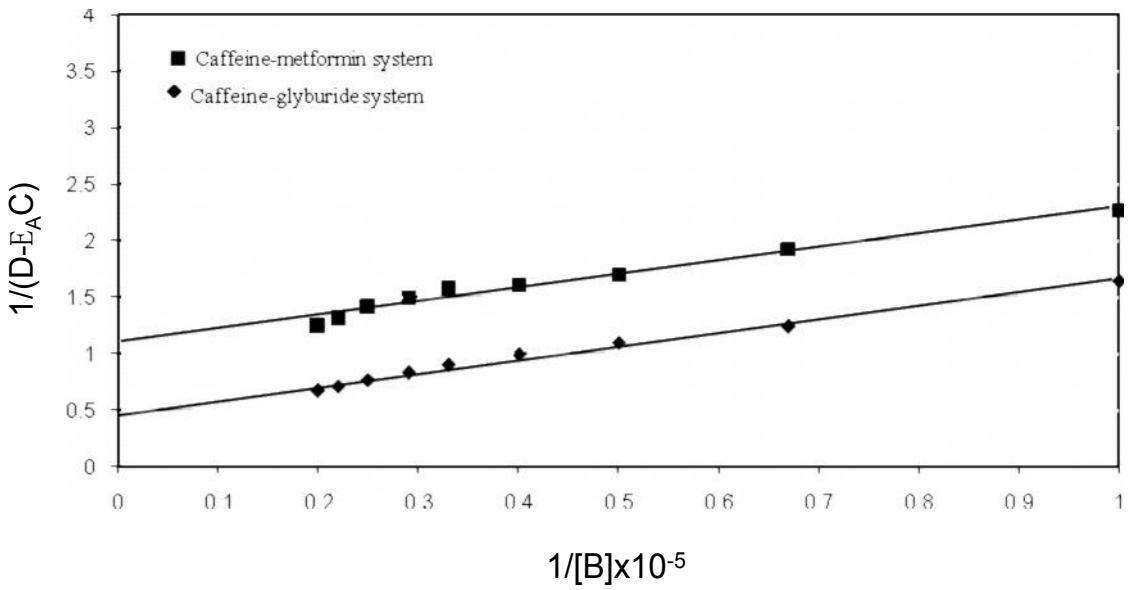


Fig.7.1.6.6: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 3.4

Table 7.1.6.7: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 4.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.502

Molar extinction co-efficient of caffeine, E_A = 10040

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.996	2.02	1.00
1.5	1.286	1.28	0.67
2.0	1.422	1.09	0.50
2.5	1.815	0.76	0.40
3.0	2.415	0.52	0.33
3.5	2.978	0.40	0.29
4.0	2.682	0.46	0.25
4.5	3.452	0.34	0.22
5.0	4.351	0.26	0.20

Caffeine - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	1.125	1.61	1.00
1.5	1.378	1.14	0.67
2.0	1.661	0.86	0.50
2.5	1.936	0.70	0.40
3.0	2.245	0.57	0.33
3.5	2.525	0.49	0.29
4.0	2.765	0.44	0.25
4.5	3.225	0.37	0.22
5.0	3.323	0.35	0.20

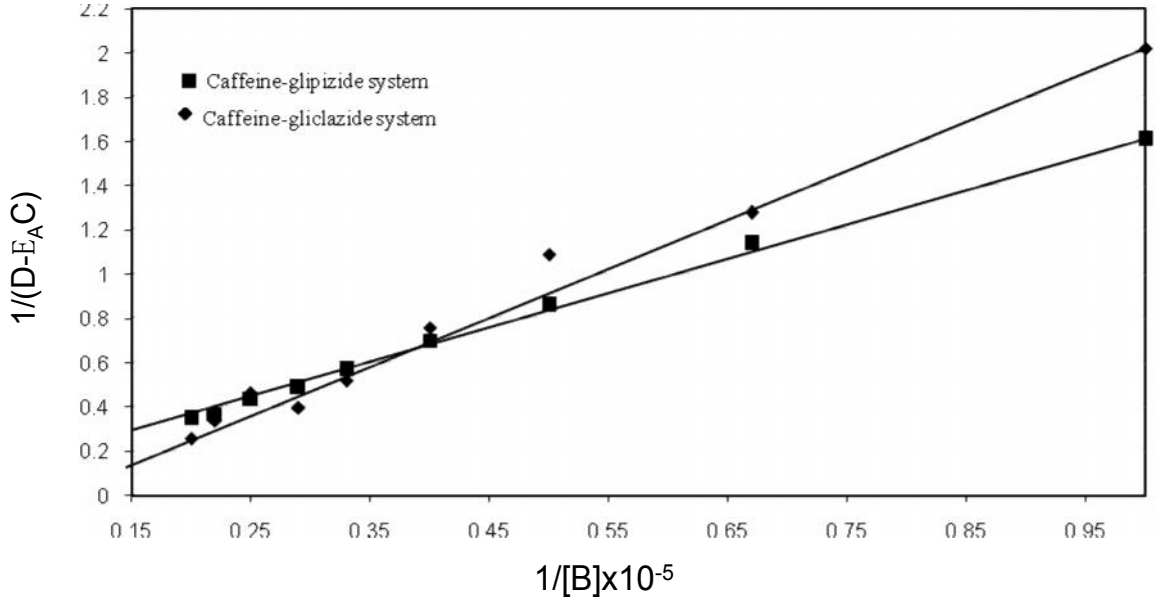


Fig.7.1.6.7: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 4.4

Table 7.1.6.8: Values for Ardon's plot for caffeine with glyburide and metformin at pH 4.4

Concentration of caffeine = $5x10^{-5}$ M

Absorbance of $5x10^{-5}$ M caffeine = 0.502

Molar extinction co-efficient of caffeine, E_A = 10040

Caffeine - glyburide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.989	2.05	1.00
1.5	1.227	1.38	0.67
2.0	1.392	1.12	0.50
2.5	1.505	1.00	0.40
3.0	1.668	0.86	0.33
3.5	1.698	0.84	0.29
4.0	1.789	0.78	0.25
4.5	1.908	0.71	0.22
5.0	2.023	0.66	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.188	1.46	1.00
1.5	1.443	1.06	0.67
2.0	1.762	0.79	0.50
2.5	2.036	0.65	0.40
3.0	2.344	0.54	0.33
3.5	2.618	0.47	0.29
4.0	2.868	0.42	0.25
4.5	3.128	0.38	0.22
5.0	3.328	0.35	0.20

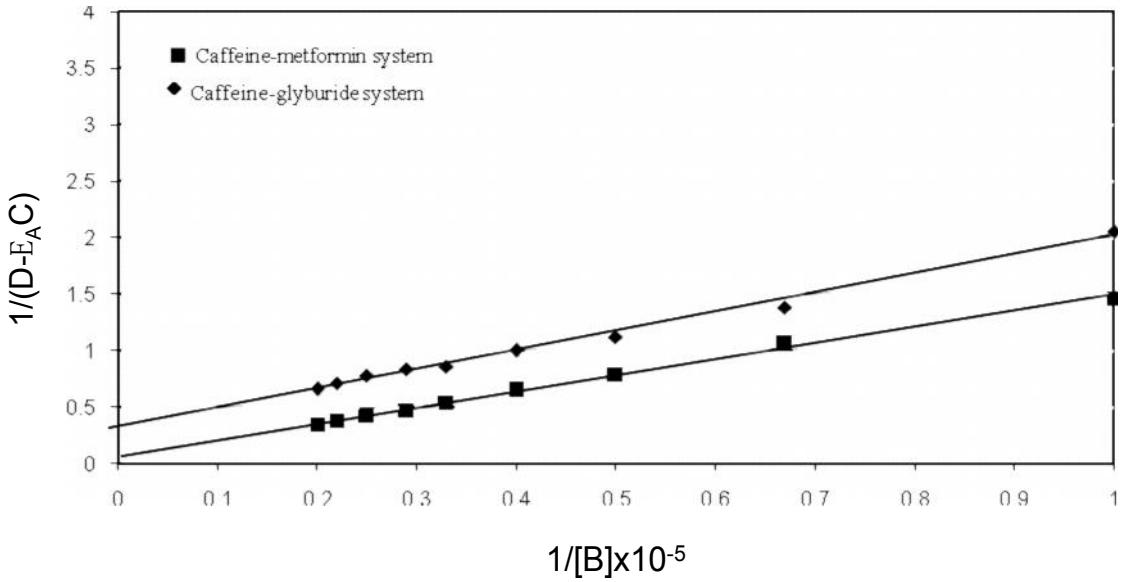


Fig.7.1.6.8: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 4.4

Table 7.1.6.9: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 5.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.488

Molar extinction co-efficient of caffeine, E_A = 9760

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.967	2.09	1.00
1.5	0.998	1.96	0.67
2.0	1.123	1.57	0.50
2.5	1.157	1.49	0.40
3.0	1.215	1.38	0.33
3.5	1.288	1.25	0.29
4.0	1.335	1.18	0.25
4.5	1.399	1.10	0.22
5.0	1.425	1.07	0.20

Caffeine - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.887	2.51	1.00
1.5	1.045	1.80	0.67
2.0	1.139	1.54	0.50
2.5	1.303	1.23	0.40
3.0	1.419	1.07	0.33
3.5	1.535	0.96	0.29
4.0	1.668	0.85	0.25
4.5	1.701	0.82	0.22
5.0	1.902	0.71	0.20

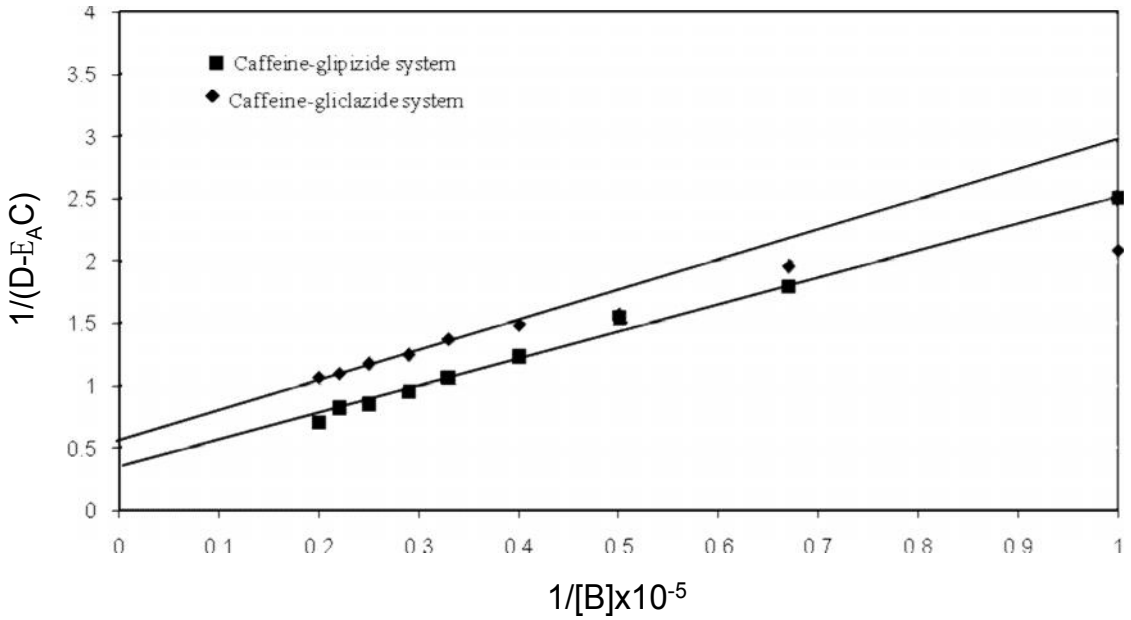


Fig. 7.1.6.9: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 5.4

Table 7.1.6.10: Values for Ardon's plot for caffeine with glyburide and metformin at pH 5.4
Concentration of caffeine = 5x10^{-5} M
Absorbance of 5x10^{-5} M caffeine = 0.488
Molar extinction co-efficient of caffeine, E_A = 9760

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.136	1.54	1.00
1.5	1.333	1.18	0.67
2.0	1.435	1.06	0.50
2.5	1.536	0.95	0.40
3.0	1.635	0.87	0.33
3.5	1.738	0.80	0.29
4.0	1.834	0.74	0.25
4.5	1.937	0.69	0.22
5.0	2.036	0.65	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.953	2.15	1.00
1.5	1.012	1.91	0.67
2.0	1.169	1.47	0.50
2.5	1.325	1.19	0.40
3.0	1.428	1.06	0.33
3.5	1.543	0.95	0.29
4.0	1.678	0.84	0.25
4.5	1.723	0.81	0.22
5.0	1.932	0.69	0.20

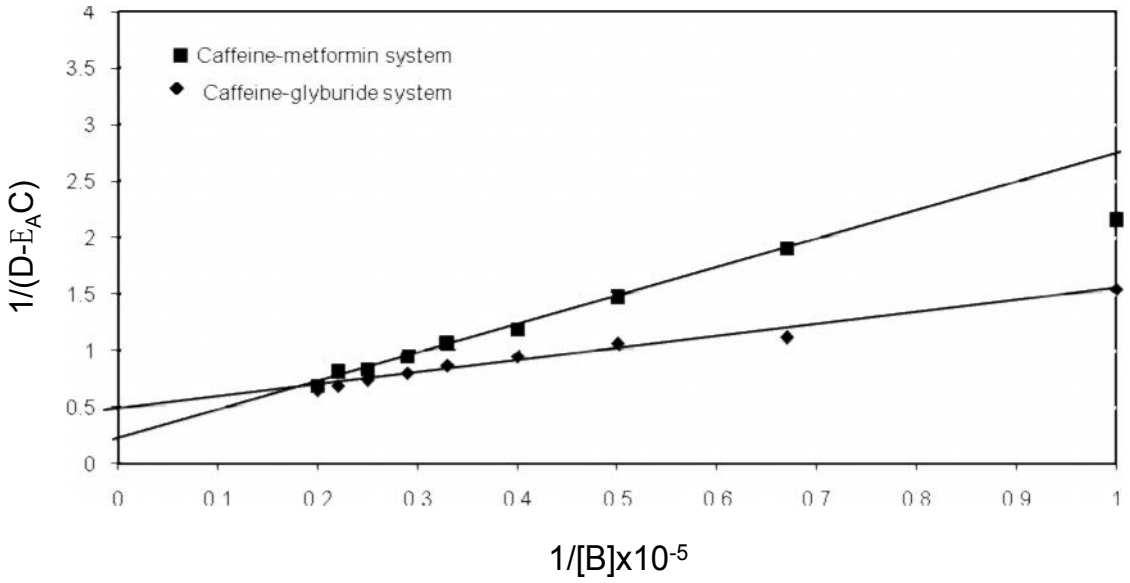


Fig. 7.1.6.10: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 5.4

Table 7.1.6.11: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 6.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.469

Molar extinction co-efficient of caffeine, E_A = 9380

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.921	2.21	1.00
1.5	1.072	1.66	0.67
2.0	1.225	1.32	0.50
2.5	1.367	1.11	0.40
3.0	1.549	0.93	0.33
3.5	1.691	0.82	0.29
4.0	1.821	0.74	0.25
4.5	1.996	0.65	0.22
5.0	2.102	0.61	0.20

Caffeine - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.221	1.33	1.00
1.5	1.495	0.97	0.67
2.0	1.789	0.76	0.50
2.5	1.923	0.69	0.40
3.0	2.275	0.55	0.33
3.5	2.585	0.47	0.29
4.0	2.875	0.42	0.25
4.5	3.168	0.37	0.22
5.0	3.378	0.34	0.20

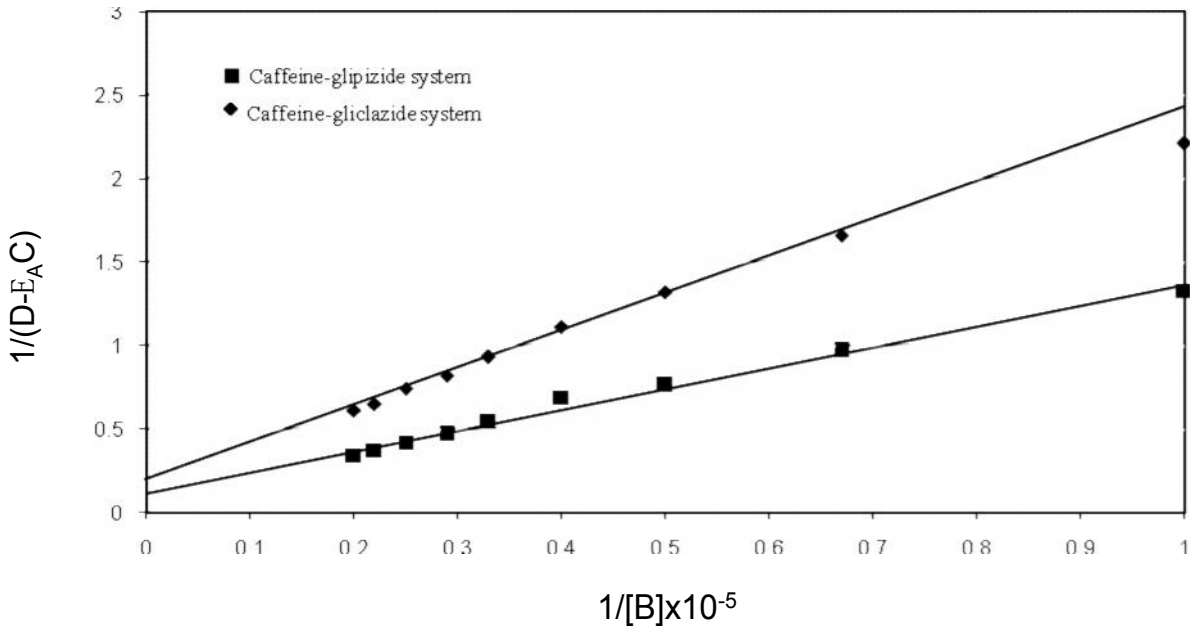


Fig. 7.1.6.11: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 6.4

Table 7.1.6.12: Values for Ardon's plot for caffeine with glyburide and metformin at pH 6.4
Concentration of caffeine = 5x10^{-5} M
Absorbance of 5x10^{-5} M caffeine = 0.469
Molar extinction co-efficient of caffeine, E_A = 9380

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.078	1.64	1.00
1.5	1.277	1.24	0.67
2.0	1.422	1.05	0.50
2.5	1.528	0.94	0.40
3.0	1.633	0.86	0.33
3.5	1.723	0.80	0.29
4.0	1.825	0.74	0.25
4.5	1.928	0.69	0.22
5.0	2.042	0.64	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.171	1.42	1.00
1.5	1.444	1.03	0.67
2.0	1.689	0.82	0.50
2.5	1.991	0.66	0.40
3.0	2.248	0.56	0.33
3.5	2.528	0.49	0.29
4.0	2.822	0.42	0.25
4.5	3.068	0.38	0.22
5.0	3.328	0.35	0.20

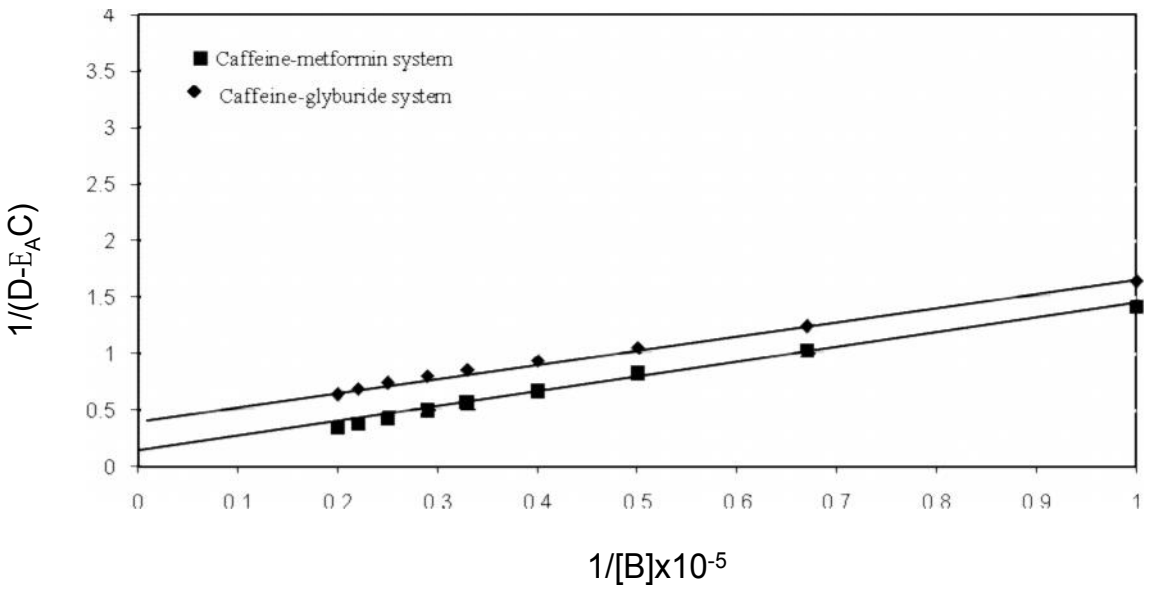


Fig. 7.1.6.12: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 6.4

Table 7.1.6.13: Values for Ardon's plot for caffeine with gliclazide and glipizide at pH 7.4

Concentration of caffeine = 5x10^{-5} M

Absorbance of 5x10^{-5} M caffeine = 0.457

Molar extinction co-efficient of caffeine, E_A = 9140

Caffeine - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.832	2.67	1.00
1.5	0.955	2.01	0.67
2.0	1.150	1.44	0.50
2.5	1.43	1.03	0.40
3.0	1.492	0.97	0.33
3.5	1.576	0.89	0.29
4.0	1.745	0.78	0.25
4.5	1.915	0.69	0.22
5.0	2.125	0.60	0.20

Caffeine - glipizide System

Caffeine-glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	1.079	1.61	1.00
1.5	1.375	1.09	0.67
2.0	1.678	0.82	0.50
2.5	1.978	0.66	0.40
3.0	2.365	0.52	0.33
3.5	2.466	0.50	0.29
4.0	2.769	0.43	0.25
4.5	3.078	0.38	0.22
5.0	3.375	0.34	0.20

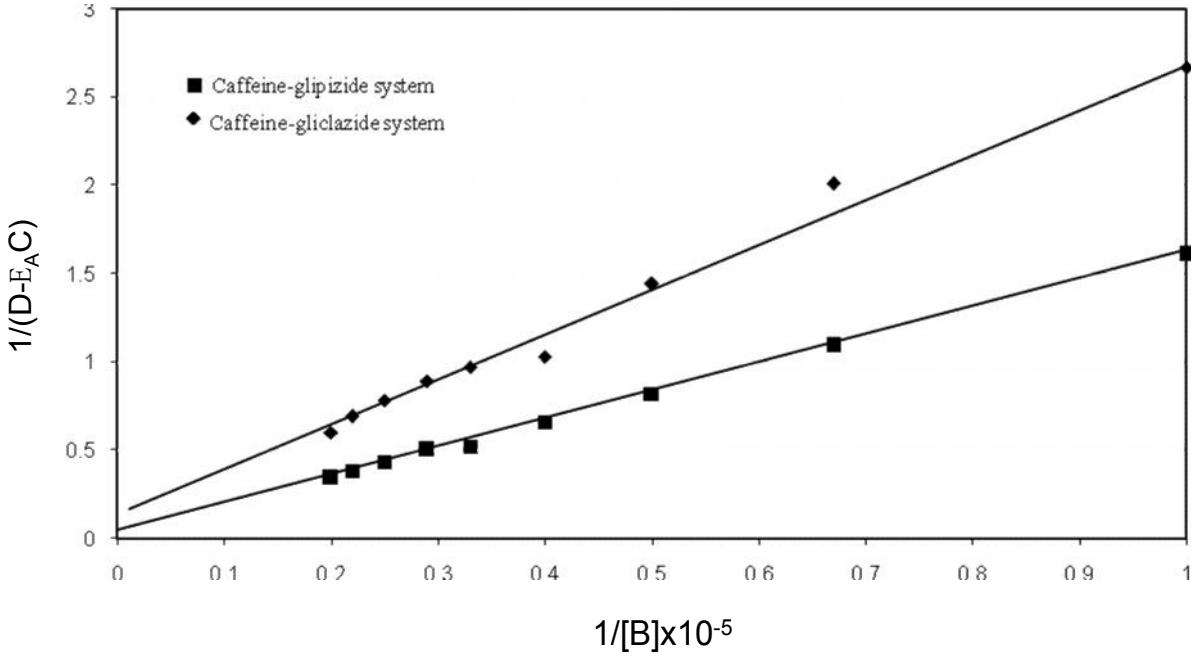


Fig. 7.1.6.13: Ardon's plot for complexation of caffeine with gliclazide and glipizide at pH 7.4

Table 7.1.6.14: Values for Ardon's plot for caffeine with glyburide and metformin at pH 7.4
Concentration of caffeine = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M caffeine = 0.457
Molar extinction co-efficient of caffeine, E_A = 9140

Caffeine - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-EAC)	1/[B]x10^{-5}
1.0	0.916	2.18	1.00
1.5	1.225	1.30	0.67
2.0	1.442	1.02	0.50
2.5	1.547	0.92	0.40
3.0	1.649	0.84	0.33
3.5	1.748	0.77	0.29
4.0	1.855	0.72	0.25
4.5	1.978	0.66	0.22
5.0	2.055	0.63	0.20

Caffeine - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-EAC)	1/[B]x10^{-5}
1.0	1.028	1.75	1.00
1.5	1.302	1.18	0.67
2.0	1.623	0.86	0.50
2.5	1.888	0.70	0.40
3.0	1.923	0.68	0.33
3.5	2.459	0.50	0.29
4.0	2.703	0.45	0.25
4.5	3.005	0.39	0.22
5.0	3.323	0.35	0.20

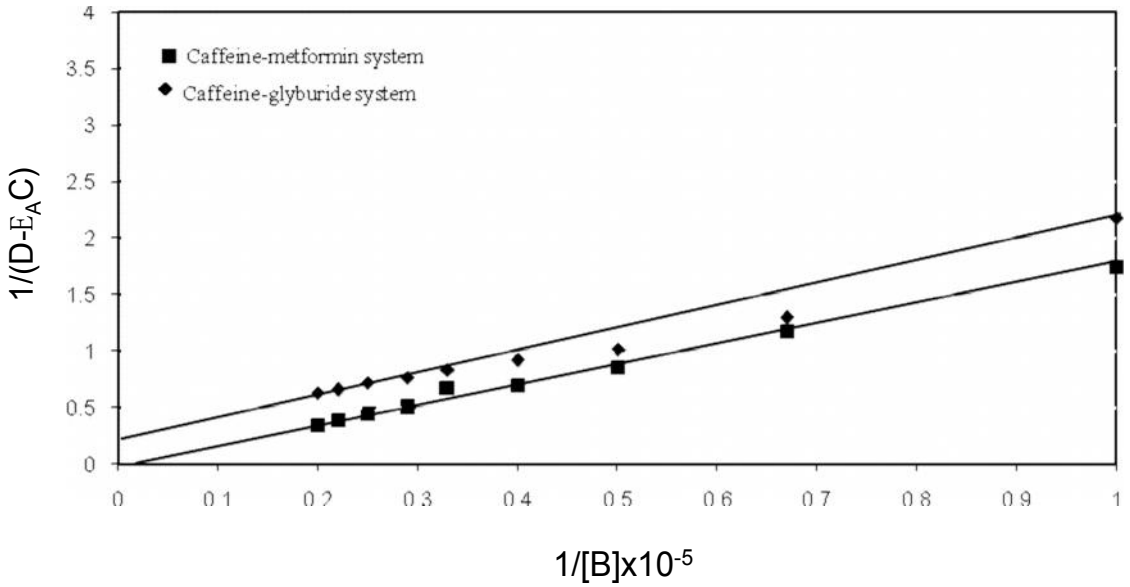


Fig. 7.1.6.14: Ardon's plot for complexation of caffeine with glyburide and metformin at pH 7.4

The stability constants for different systems are given in the following table.

Systems	Stability constants K x 10^{-3}/mole

	p^H 1.4	p^H 2.4	p^H 3.4	p^H 4.4	p^H 5.4	p^H 6.4	p^H 7.4
Caffeine-gliclazide system	2.57	2.48	35.67	3.78	3.75	3.45	2.35
Caffeine-glipizide system	4.53	4.45	4.76	4.65	3.22	2.73	2.15
Caffeine-glyburide system	3.51	3.44	3.36	3.28	3.25	2.65	2.32
Caffeine-metformin system	5.48	5.42	3.65	2.72	2.45	2.33	2.23

From the above table, it is found that the values of stability constant for each of the systems (caffeine-gliclazide, caffeine-glipizide, caffeine-glyburide and caffeine-metformin system) remain quite close to each other at all pH systems except pH 3.4. At pH 3.4 the stability constant for caffeine-gliclazide system is higher than all other systems. The values of stability constants are moderately large in these cases also. So, we can conclude that at pH 3.4 quite a stable complex is formed for the systems while at other pH conditions relatively weak complexes are formed. It is uncertain if these complex substances (adducts) can be separated as solid materials.

From the stability constants, we observe that the stability constant values for caffeine-metformin system at lower pH values are higher and the stability constants of caffeine-gliclazide system at pH 3.4 is very high indicating a stronger complex formation between these two drugs at this pH.

Table 7.1.6.15: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 1.4
Concentration of theophylline = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M theophylline = 0.463
Molar extinction co-efficient of theophylline, E_A = 9260

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.079	1.62	1.00
1.5	1.388	1.08	0.67
2.0	1.485	0.98	0.50
2.5	1.479	0.98	0.40
3.0	1.588	0.89	0.33
3.5	1.692	0.81	0.29
4.0	1.801	0.75	0.25
4.5	1.893	0.70	0.22
5.0	1.985	0.66	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.887	2.36	1.00
1.5	0.976	1.95	0.67
2.0	1.119	1.52	0.50
2.5	1.189	1.38	0.40
3.0	1.396	1.07	0.33
3.5	1.458	1.01	0.29
4.0	1.588	0.89	0.25
4.5	1.623	0.86	0.22
5.0	1.875	0.71	0.20

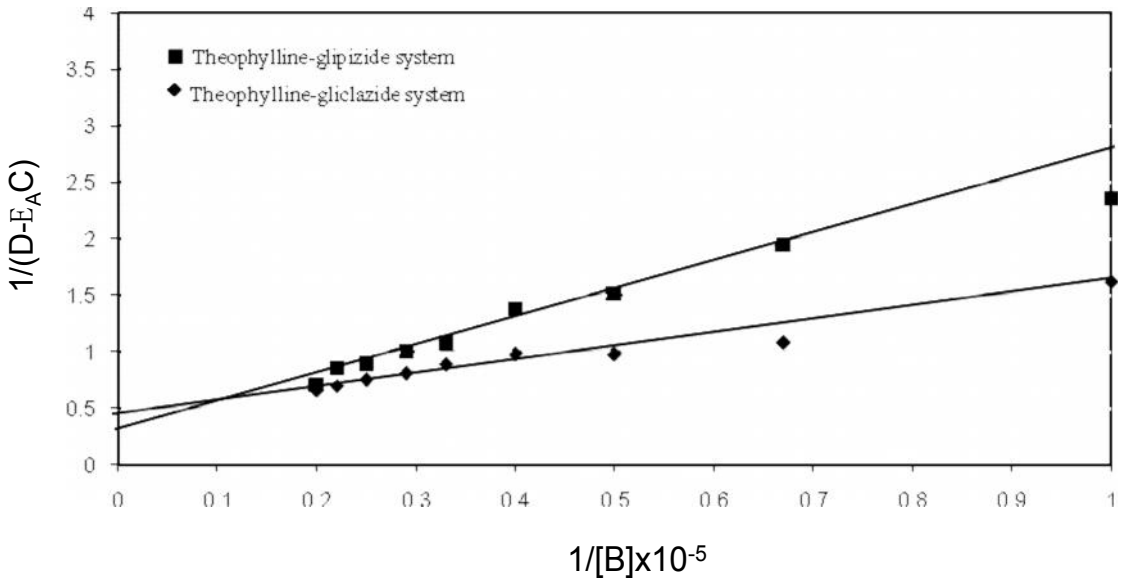


Fig. 7.1.6.15: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 1.4

Table 7.1.6.16: Values for Ardon's plot for theophylline with glyburide and metformin at pH 1.4

Concentration of theophylline = 5x10^{-5} M

Absorbance of 5x10^{-5} M theophylline = 0.463

Molar extinction co-efficient of theophylline, E_A = 9260

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.019	1.80	1.00
1.5	1.225	1.31	0.67
2.0	1.452	1.01	0.50
2.5	1.535	0.93	0.40
3.0	1.648	0.84	0.33
3.5	1.735	0.79	0.29
4.0	1.823	0.74	0.25
4.5	1.929	0.68	0.22
5.0	2.005	0.65	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.925	2.16	1.00
1.5	0.969	1.98	0.67
2.0	1.168	1.42	0.50
2.5	1.389	1.08	0.40
3.0	1.498	0.97	0.33
3.5	1.544	0.93	0.29
4.0	1.683	0.82	0.25
4.5	1.728	0.79	0.22
5.0	1.978	0.66	0.20

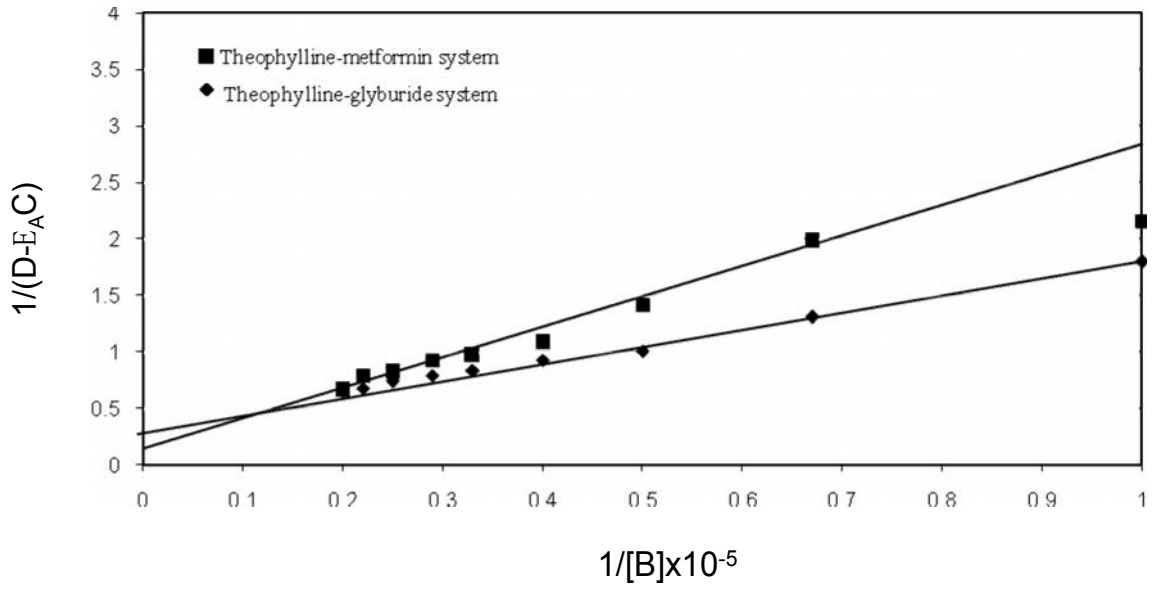


Fig. 7.1.6.16: Ardon's plot for complexation of theophylline with glyburide and Metformin at pH 1.4

Table 7.1.6.17: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 2.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.467
Molar extinction co-efficient of theophylline, E_A = 9340

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.049	1.72	1.00
1.5	1.328	1.16	0.67
2.0	1.521	0.95	0.50
2.5	1.628	0.86	0.40
3.0	1.778	0.76	0.33
3.5	1.978	0.66	0.29
4.0	2.035	0.64	0.25
4.5	2.125	0.60	0.22
5.0	2.223	0.57	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.778	3.22	1.00
1.5	0.897	2.33	0.67
2.0	1.028	1.78	0.50
2.5	1.123	1.52	0.40
3.0	1.222	1.32	0.33
3.5	1.288	1.22	0.29
4.0	1.355	1.13	0.25
4.5	1.588	0.89	0.22
5.0	1.625	0.86	0.20

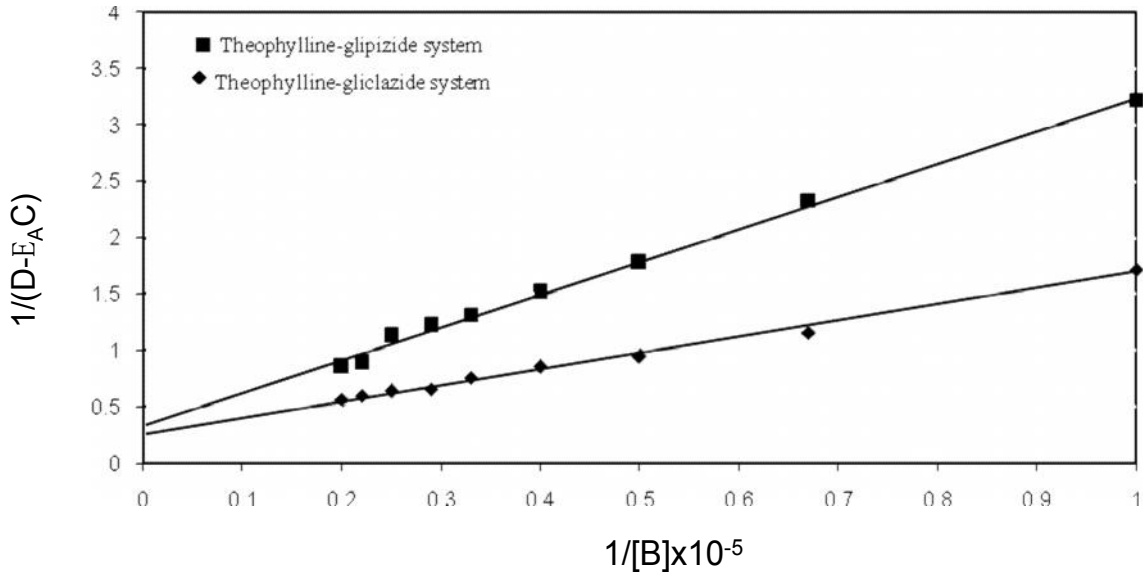


Fig. 7.1.6.17: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 2.4

Table 7.1.6.18: Values for Ardon's plot for theophylline with glyburide and metformin at pH 2.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.467
Molar extinction co-efficient of theophylline, E_A = 9340

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.019	1.80	1.00
1.5	1.225	1.31	0.67
2.0	1.352	1.12	0.50
2.5	1.435	1.03	0.40
3.0	1.735	0.79	0.33
3.5	1.972	0.66	0.29
4.0	2.323	0.54	0.25
4.5	2.421	0.51	0.22
5.0	2.525	0.48	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.877	2.42	1.00
1.5	1.023	1.79	0.67
2.0	1.155	1.45	0.50
2.5	1.308	1.18	0.40
3.0	1.408	1.06	0.33
3.5	1.512	0.95	0.29
4.0	1.621	0.86	0.25
4.5	1.723	0.79	0.22
5.0	1.875	0.71	0.20

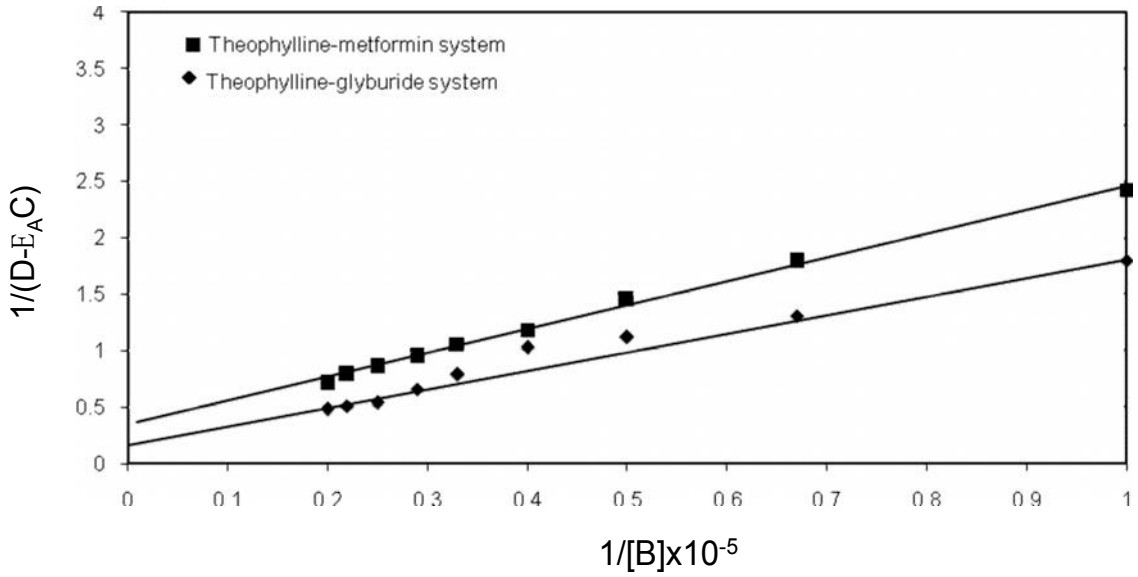


Fig. 7.1.6.18: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 2.4

Table 7.1.6.19: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 3.4

Concentration of theophylline = 5x10^{-5} M

Absorbance of 5x10^{-5} M theophylline = 0.471

Molar extinction co-efficient of theophylline, E_A = 9420

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.922	2.22	1.00
1.5	1.128	1.52	0.67
2.0	1.225	1.33	0.50
2.5	1.373	1.11	0.40
3.0	1.485	0.99	0.33
3.5	1.575	0.91	0.29
4.0	1.725	0.80	0.25
4.5	1.888	0.71	0.22
5.0	1.935	0.68	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.882	2.43	1.00
1.5	0.935	2.16	0.67
2.0	1.007	1.87	0.50
2.5	1.101	1.59	0.40
3.0	1.159	1.45	0.33
3.5	1.221	1.33	0.29
4.0	1.273	1.25	0.25
4.5	1.373	1.11	0.22
5.0	1.438	1.03	0.20

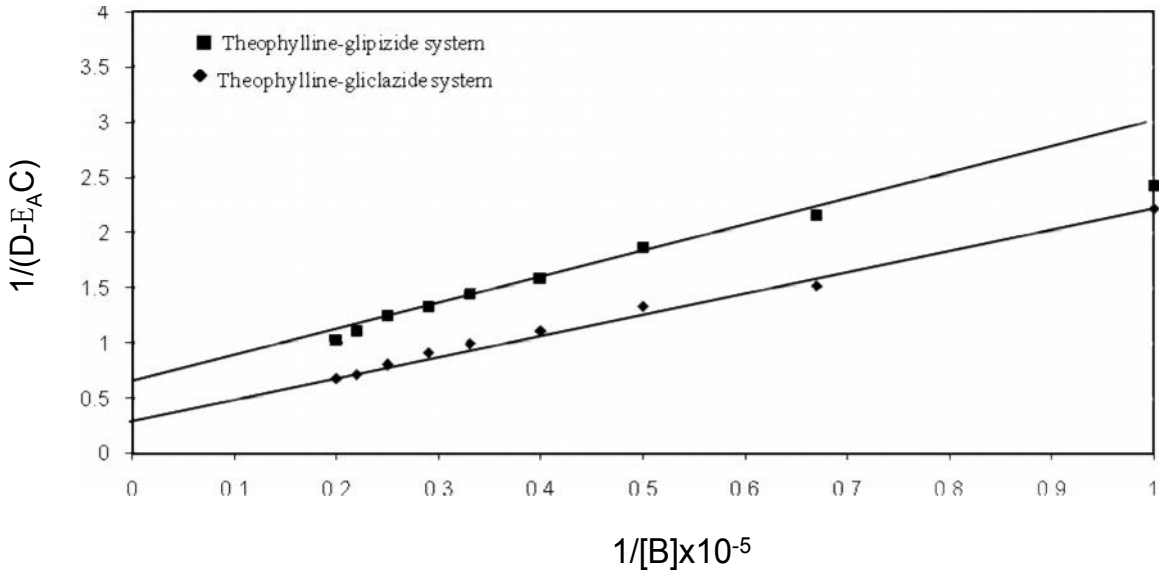


Fig. 7.1.6.19: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 3.4

Table 7.1.6.20: Values for Ardon's plot for theophylline with glyburide and metformin at pH 3.4
Concentration of theophylline = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M theophylline = 0.471
Molar extinction co-efficient of theophylline, E_A = 9420

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.223	1.33	1.00
1.5	1.418	1.06	0.67
2.0	1.519	0.95	0.50
2.5	1.619	0.87	0.40
3.0	1.738	0.79	0.33
3.5	1.828	0.74	0.29
4.0	1.923	0.69	0.25
4.5	2.012	0.65	0.22
5.0	2.115	0.61	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.872	2.49	1.00
1.5	1.021	1.82	0.67
2.0	1.138	1.50	0.50
2.5	1.284	1.23	0.40
3.0	1.434	1.04	0.33
3.5	1.539	0.94	0.29
4.0	1.635	0.86	0.25
4.5	1.701	0.81	0.22
5.0	1.773	0.77	0.20

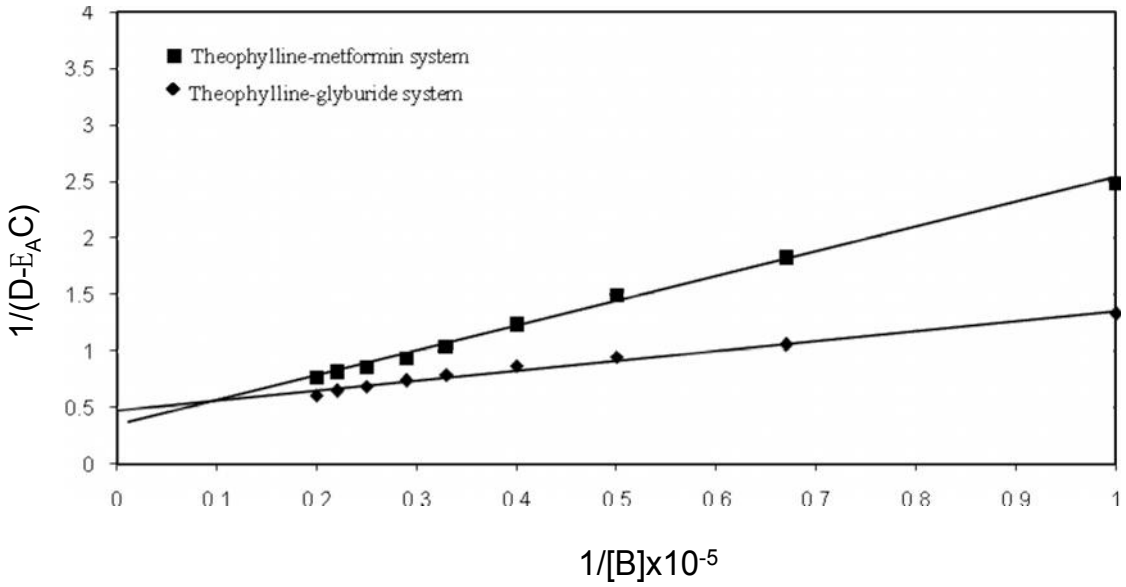


Fig. 7.1.6.20: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 3.4

Table 7.1.6.21: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 4.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.475
Molar extinction co-efficient of theophylline, E_A = 9500

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	1.229	1.33	1.00
1.5	1.445	1.03	0.67
2.0	1.964	0.67	0.50
2.5	2.136	0.60	0.40
3.0	2.375	0.53	0.33
3.5	2.728	0.44	0.29
4.0	2.965	0.40	0.25
4.5	3.228	0.36	0.22
5.0	3.375	0.34	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.922	2.24	1.00
1.5	1.245	1.30	0.67
2.0	1.464	1.01	0.50
2.5	1.636	0.86	0.40
3.0	1.875	0.71	0.33
3.5	2.028	0.64	0.29
4.0	2.225	0.57	0.25
4.5	2.525	0.49	0.22
5.0	2.776	0.43	0.20

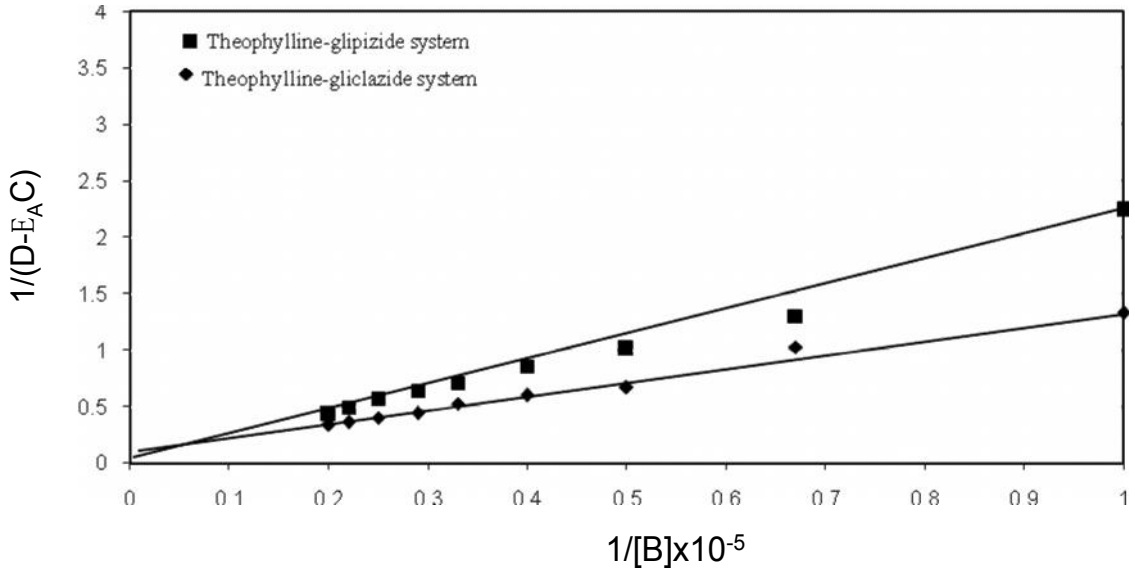


Fig. 7.1.6.21: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 4.4

Table 7.1.6.22: Values for Ardon's plot for theophylline with glyburide and metformin at pH 4.4
Concentration of theophylline = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M theophylline = 0.475
Molar extinction co-efficient of theophylline, E_A = 9500

Theophylline - glyburide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.001	1.90	1.00
1.5	1.259	1.28	0.67
2.0	1.338	1.16	0.50
2.5	1.448	1.03	0.40
3.0	1.588	0.90	0.33
3.5	1.665	0.84	0.29
4.0	1.778	0.77	0.25
4.5	1.875	0.71	0.22
5.0	1.973	0.67	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.875	2.50	1.00
1.5	0.972	2.01	0.67
2.0	1.102	1.59	0.50
2.5	1.138	1.51	0.40
3.0	1.296	1.22	0.33
3.5	1.321	1.18	0.29
4.0	1.385	1.10	0.25
4.5	1.428	1.05	0.22
5.0	1.472	1.00	0.20

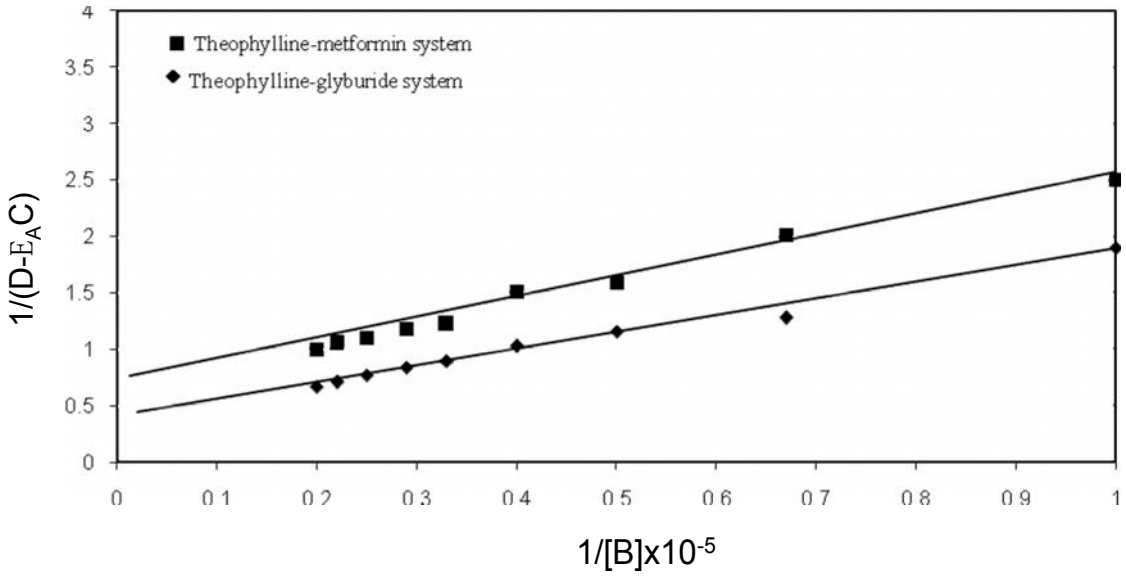


Fig. 7.1.6.22: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 4.4

Table 7.1.6.23: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 5.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.482
Molar extinction co-efficient of theophylline, E_A = 9640

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.878	2.53	1.00
1.5	0.958	2.10	0.67
2.0	1.088	1.65	0.50
2.5	1.128	1.55	0.40
3.0	1.239	1.32	0.33
3.5	1.365	1.13	0.29
4.0	1.435	1.05	0.25
4.5	1.478	1.00	0.22
5.0	1.525	0.96	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	0.978	2.02	1.00
1.5	1.132	1.54	0.67
2.0	1.235	1.33	0.50
2.5	1.308	1.21	0.40
3.0	1.325	1.19	0.33
3.5	1.363	1.14	0.29
4.0	1.405	1.08	0.25
4.5	1.415	1.07	0.22
5.0	1.433	1.05	0.20

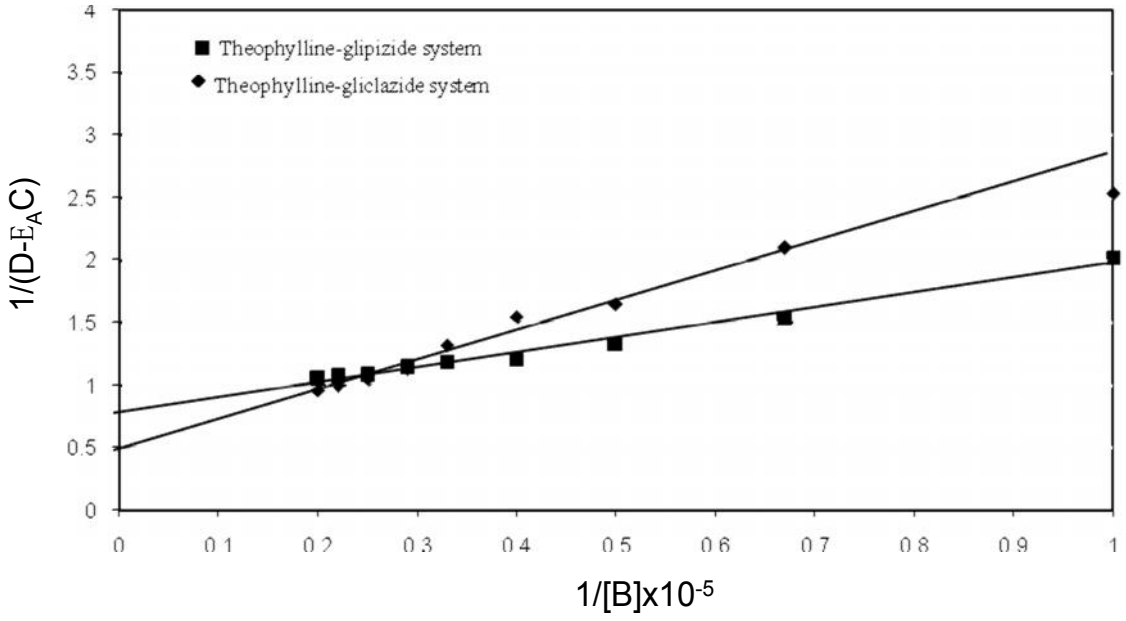


Fig. 7.1.6.23: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 5.4

Table 7.1.6.24: Values for Ardon's plot for theophylline with glyburide and metformin at pH 5.4
Concentration of theophylline = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M theophylline = 0.482
Molar extinction co-efficient of theophylline, E_A = 9640

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.028	1.83	1.00
1.5	1.316	1.20	0.67
2.0	1.419	1.07	0.50
2.5	1.518	0.97	0.40
3.0	1.779	0.77	0.33
3.5	1.978	0.67	0.29
4.0	2.175	0.59	0.25
4.5	2.228	0.57	0.22
5.0	2.338	0.54	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.913	2.32	1.00
1.5	0.968	2.06	0.67
2.0	1.166	1.46	0.50
2.5	1.293	1.23	0.40
3.0	1.378	1.12	0.33
3.5	1.475	1.01	0.29
4.0	1.635	0.87	0.25
4.5	1.722	0.81	0.22
5.0	1.92	0.70	0.20

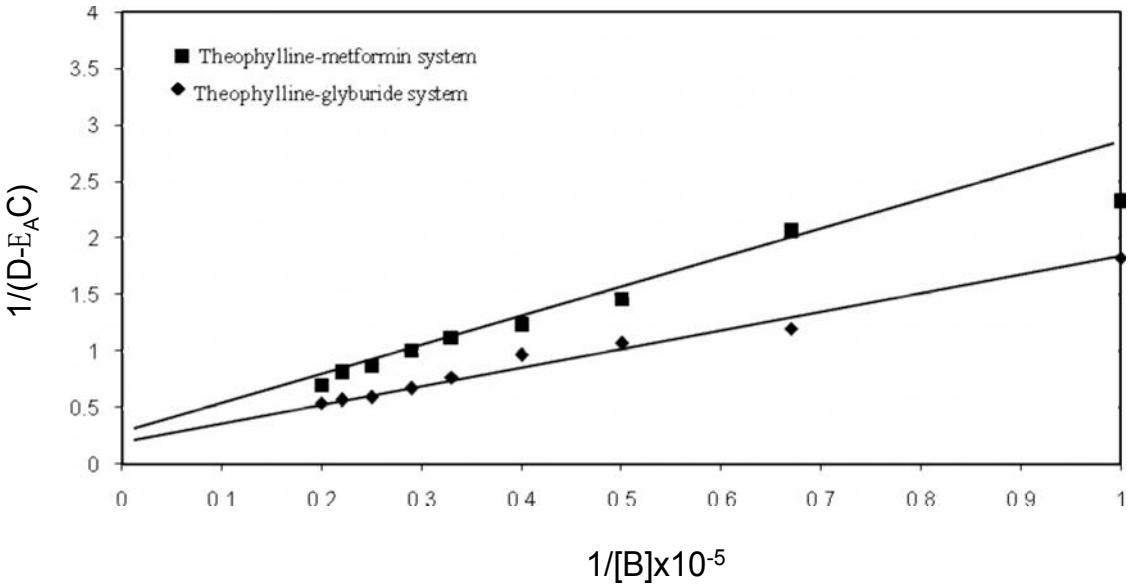


Fig. 7.1.6.24: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 5.4

Table 7.1.6.25: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 6.4

Concentration of theophylline = 5x10^{-5} M

Absorbance of 5x10^{-5} M theophylline = 0.489

Molar extinction co-efficient of theophylline, E_A = 9780

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	1.173	1.46	1.00
1.5	1.455	1.04	0.67
2.0	1.688	0.83	0.50
2.5	1.993	0.66	0.40
3.0	2.245	0.57	0.33
3.5	2.523	0.49	0.29
4.0	2.821	0.43	0.25
4.5	3.065	0.39	0.22
5.0	3.324	0.35	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	$1/(D-E_AC)$	$1/[B]x10^{-5}$
1.0	1.075	1.71	1.00
1.5	1.245	1.32	0.67
2.0	1.495	0.99	0.50
2.5	1.592	0.91	0.40
3.0	1.725	0.81	0.33
3.5	1.895	0.71	0.29
4.0	1.962	0.68	0.25
4.5	2.163	0.60	0.22
5.0	2.322	0.55	0.20

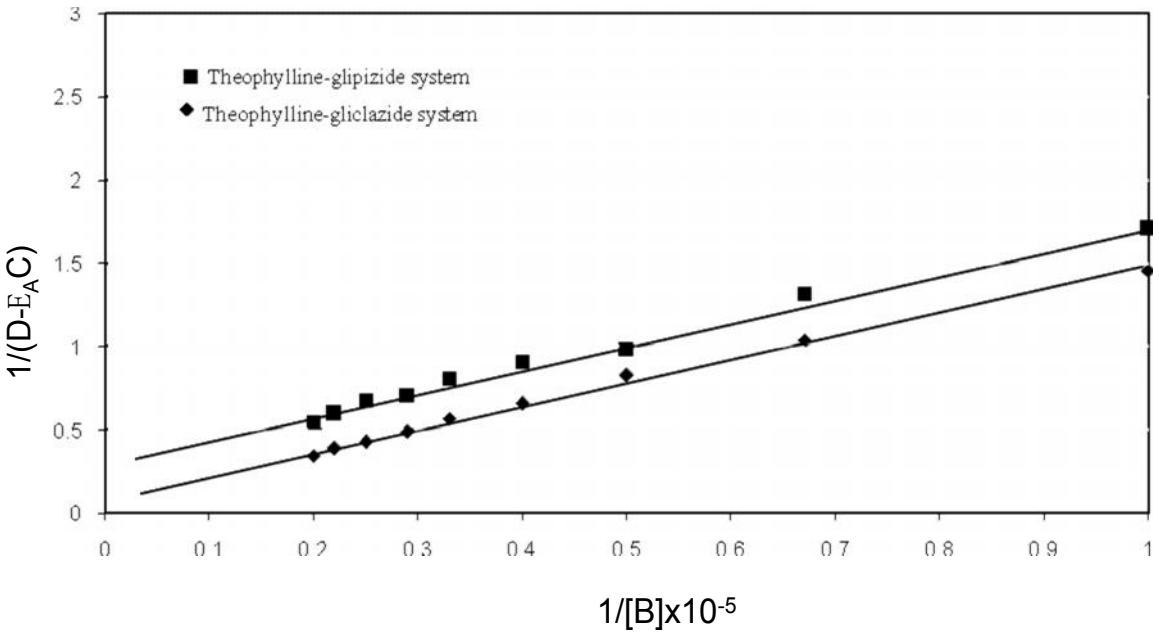


Fig. 7.1.6.25: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 6.4

Table 7.1.6.26: Values for Ardon's plot for theophylline with glyburide and metformin at pH 6.4
Concentration of theophylline = $5x10^{-5}$ M
Absorbance of $5x10^{-5}$ M theophylline = 0.489
Molar extinction co-efficient of theophylline, E_A = 9780

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.017	1.89	1.00
1.5	1.287	1.25	0.67
2.0	1.385	1.12	0.50
2.5	1.477	1.01	0.40
3.0	1.619	0.88	0.33
3.5	1.717	0.81	0.29
4.0	1.818	0.75	0.25
4.5	1.921	0.70	0.22
5.0	2.115	0.62	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.869	2.63	1.00
1.5	0.983	2.02	0.67
2.0	1.062	1.75	0.50
2.5	1.184	1.44	0.40
3.0	1.298	1.24	0.33
3.5	1.348	1.16	0.29
4.0	1.453	1.04	0.25
4.5	1.472	1.02	0.22
5.0	1.575	0.92	0.20

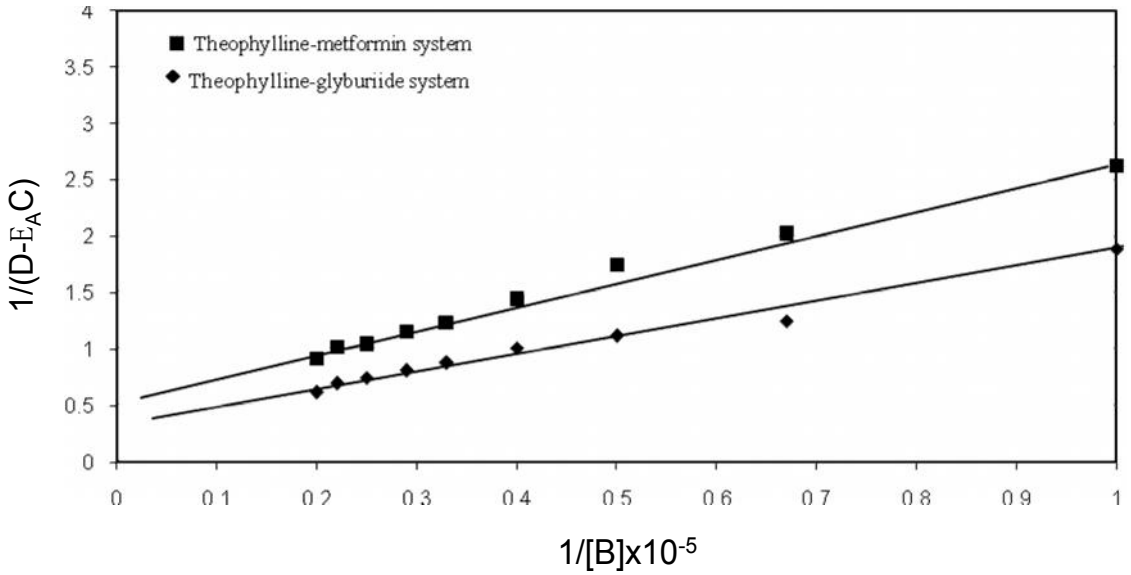


Fig. 7.1.6.26: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 6.4

Table 7.1.6.27: Values for Ardon's plot for theophylline with gliclazide and glipizide at pH 7.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.501
Molar extinction co-efficient of theophylline, E_A = 10020

Theophylline - gliclazide System

Conc.of gliclazide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.025	1.91	1.00
1.5	1.308	1.24	0.67
2.0	1.625	0.89	0.50
2.5	1.885	0.72	0.40
3.0	2.325	0.55	0.33
3.5	2.461	0.51	0.29
4.0	2.709	0.45	0.25
4.5	3.001	0.40	0.22
5.0	3.326	0.35	0.20

Theophylline - glipizide System

Conc.of glipizide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.928	2.34	1.00
1.5	1.102	1.66	0.67
2.0	1.227	1.38	0.50
2.5	1.481	1.02	0.40
3.0	1.512	0.99	0.33
3.5	1.659	0.86	0.29
4.0	1.783	0.78	0.25
4.5	1.988	0.67	0.22
5.0	2.128	0.61	0.20

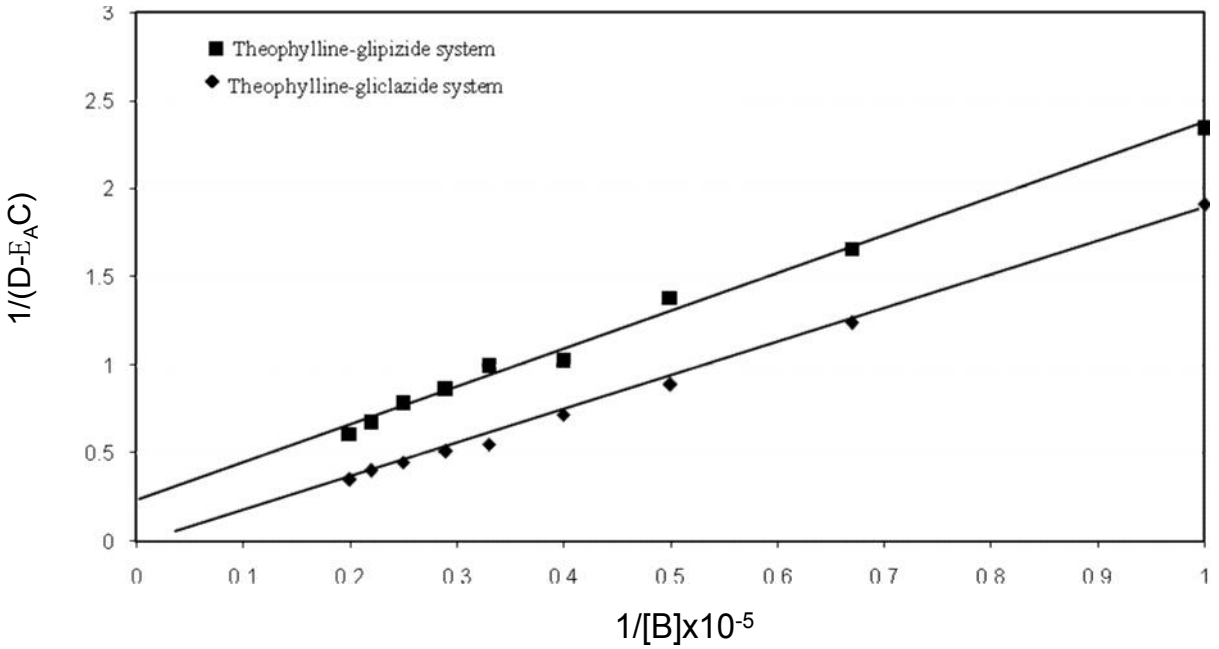


Fig. 7.1.6.27: Ardon's plot for complexation of theophylline with gliclazide and glipizide at pH 7.4

Table 7.1.6.28: Values for Ardon's plot for theophylline with glyburide and metformin at pH 7.4
Concentration of theophylline = 5x10^{-5} M
Absorbance of 5x10^{-5} M theophylline = 0.501
Molar extinction co-efficient of theophylline, E_A = 10020

Theophylline - glyburide System

Conc.of glyburide [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	1.000	2.00	1.00
1.5	1.207	1.42	0.67
2.0	1.403	1.11	0.50
2.5	1.497	1.00	0.40
3.0	1.603	0.91	0.33
3.5	1.696	0.84	0.29
4.0	1.808	0.77	0.25
4.5	1.921	0.70	0.22
5.0	1.988	0.67	0.20

Theophylline - metformin System

Conc.of metformin [B], Mx10^5	Abs. of mixture (D)	1/(D-E_AC)	1/[B]x10^{-5}
1.0	0.912	2.43	1.00
1.5	1.063	1.78	0.67
2.0	1.163	1.51	0.50
2.5	1.286	1.27	0.40
3.0	1.364	1.16	0.33
3.5	1.423	1.08	0.29
4.0	1.485	1.02	0.25
4.5	1.545	0.96	0.22
5.0	1.573	0.93	0.20

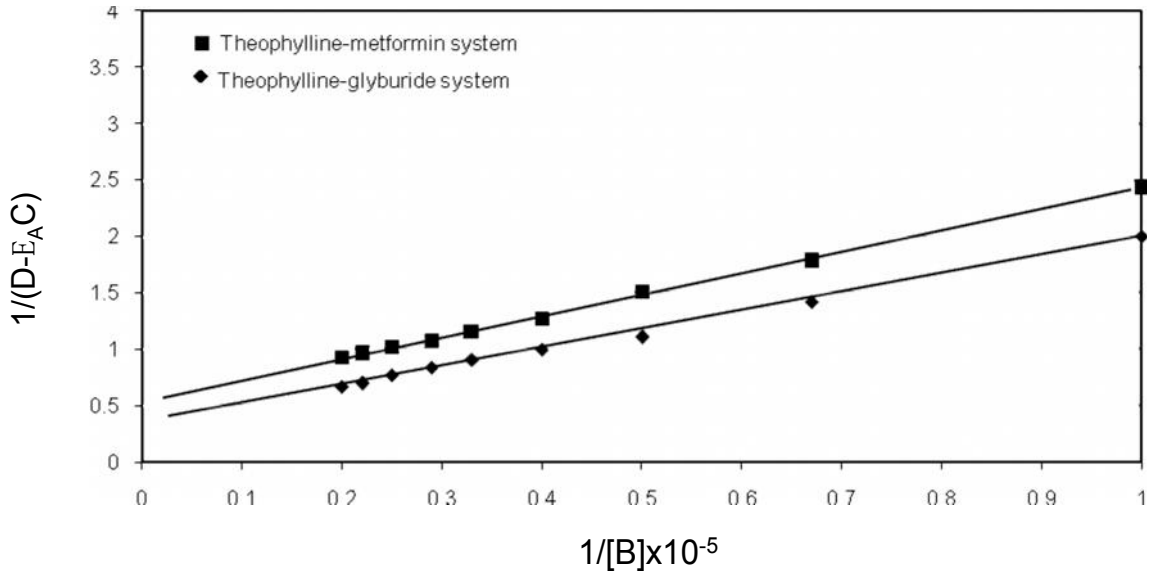


Fig. 7.1.6.28: Ardon's plot for complexation of theophylline with glyburide and metformin at pH 7.4

The stability constants for different systems are given in the following table.

Systems	Stability constants K x 10^{-3}/mole						
	p^H 1.4	p^H 2.4	p^H 3.4	p^H 4.4	p^H 5.4	p^H 6.4	p^H 7.4
Theophylline-gliclazide system	2.38	2.32	3.85	3.76	15.78	2.62	2.28
Theophylline -glipizide system	2.27	2.22	3.66	3.48	2.76	2.54	2.43
Theophylline -glyburide system	3.35	3.38	3.95	3.77	2.75	2.65	2.55
Theophylline -metformin system	2.36	2.31	3.72	2.62	2.35	2.26	2.21

From the above table, it is found that the values of stability constant for each of the systems (theophylline-gliclazide, theophylline-glipizide, theophylline-glyburide and theophylline-metformin systems) remain quite close to each other at all pH systems except pH 5.4. At pH 5.4 the stability constant for theophylline-gliclazide system is higher than all other systems. The values of stability constants are moderately large in these cases also. So, we can conclude that at pH 5.4 quite a stable complex is formed for the systems while at other pH conditions relatively weak complexes are formed. It is uncertain if these complex substances (adducts) can be separated as solid materials.

From the stability constants, we observe that the stability constant values for theophylline-glyburide system at lower pH 1.4 values are higher and the stability constants of theophylline-gliclazide system at pH 5.4 is very high indicating a stronger complex formation between these two drugs at this pH.

The studies of interaction between oral anti-diabetic drugs and other agents have been carried out recently.[75] [76] [77] The results of the present study indicated that caffeine and theophylline form 1:1

complexes with gliclazide, glipizide, glyburide and metformin.Observations of IR and UV spectral data have revealed the possibility of interaction of each caffeine and theophylline with gliclazide, glipizide, glyburide and metformin. Spectral observation studies, Job's method of continuous-variation and spectrophotometric mole-ratio indicated initial complexation. Continuous-variation plots have conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide. But continuous-variation plots have conformed the formation of 2:1 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide and 1:1 complexes of theophylline with metformin. It can be inferred that Job's method of continuous-variation plots conformed the formation of 1:1 complexes of both caffeine & theophylline with metformin. Mole-ratio plots conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide. But mole-ratio plots conformed the formation of 1:2 complexes of theophylline with gliclazide & glipizide and 1:2 complexes of Theophylline with glyburide & metformin.The conductometric method was used to further ascertain about the nature of interaction and stoichiometries. Conductrometric titrations have showed that 1:1 complexes are formed between caffeine and each of the interacting species. It has been also showed that 1:1 complexes are formed between theophylline and each of the interacting species. It has been found that along with stable complexes some unstable intermediates are formed between caffeine & theophylline with each of their four interacting molecules.

The Ardon's Spectrophotometric method confirmed the formation of 1:1 molecular complexes and led to calculate the stability constants.The stability constants of the complexes are estimated from these straight lines using the Ardon equation. It has been observed that the stability constans for caffeine-gliclazide system were higher than that of all other systems in all pH conditions. It has indicated a comparatively weak interaction in the all other systems. From the stability constants, we observe that the stability constant values for caffeine-metformin system at lower pH values are higher and the stability constants of caffeine-gliclazide system at pH 3.4 is very high indicating a stronger complex formation between these two drugs at this pH. But we observe that the stability constant values for theophylline-glyburide system at lower pH 1.4 values are higher and the stability constants of theophylline-gliclazide system at pH 5.4 is very high indicating a stronger complex formation between these two drugs at this pH.

In-vitro studies sometimes contradict with *in-vivo* studies by the same method and in the same experimental models.In a recent study, it was found that in the *in-vitro* study ibuprofen decrease the protein binding of glipizide and gliclazide but when studied *in-vivo* in rat model the plasma concentration of glipizide and gliclazide did not changed significantly.[77] If we compare the results of the present study with our previous studies, we infer that the results of those studies do not differ significantly in most the methods studied, except the Ardon's method at pH 3.4. Probably caffeine forms strong complex with gliclazide in acidic environment. This might be due to the presence of amino, carbonyl and sulfonyl groups in caffeine and gliclazide which might from hydrogen bonding network between these two compounds.

7.2 IN-VITRO EFFECT OF CAFFEINE AND THEOPHYLLINE ON PROTEIN BINDING OF GLICLAZIDE, GLIPIZIDE, GLYBURIDE AND METFORMIN

In-vitro estimation of percentage of protein binding of gliclazide, glipizide, glyburide & metformin and the effects of caffeine & theophylline on protein (bovine serum albumin) binding have been studied by equilibrium dialysis method at physiological temperature (37 ± 0.5^{o}C) and pH 7.4. All absorbance have been measured at 227 nm, 228 nm, 248 nm & 233 nm which are the absorption maximum for gliclazide, glipizide, glyburide and metformin respectively and where caffeine & theophylline do also absorb highly. Scatchard plots were prepared to reveal the number of binding sites and the affinity for protein binding. [78] [79] [80]

7.2.1 Percentage of protein binding

Calculation of percentage of protein binding helps to determine the binding affinity of a compound /drug to the protein. An increase or decrease in percentage of protein binding with the change in concentration of a material can be observed by plotting percentage of protein binding versus concentration of the species in protein solution after equilibrium.

Here, percentage of protein binding of gliclazide, glipizide, glyburide and metformin and that of the 1:1 mixtures of gliclazide, glipizide, glyburide and metformin with caffeine & theophylline were determined by the equilibrium dialysis method. Data for protein binding are given in tables 7.2.1.1-7.2.1.12. Plots of percentage of binding versus concentration are shown in the figures 7.2.1.1 to 7.2.1.12.

Protein binding of gliclazide

Protein binding versus concentration of gliclazide (figure 7.2.1.1) show that at low concentration (1 x 10^{-5}M to 5 x 10^{-5}M), the percentage of protein binding decreases with the increase in concentration of the drug. But at higher concentrations, the percentage attains a steady plateau indicating the saturation zone for the binding of gliclazide to bovine serum albumin (B.S.A.).

The usual dose of gliclazide is 80 mg three times daily. Literature shows that gliclazide is bound about 95% to human plasma protein. But in the present study, the percentage of binding to B.S.A at saturation level is about 92.46. This is perhaps due to inherent difference between human plasma protein and B.S.A.

Effect of caffeine on protein binding of gliclazide

From the table 7.2.1.2 and figure 7.2.1.2, it is found that the highest percentage of protein binding of gliclazide at saturation level was about 83.99 in presence of caffeine. By comparing this with that of gliclazide alone, it can be inferred that caffeine has significant effect on the protein binding of gliclazide. This is obviously due to a good affinity of the complex and also caffeine for the protein. The significant lowering of protein binding of gliclazide due to caffeine interference indicates that binding of caffeine is also site specific. On the whole, it is clear that a combination of caffeine with gliclazide is not always desirable.

Effect of theophylline on protein binding of gliclazide

From the table 7.2.1.3 and figure 7.2.1.3, it is found that the highest percentage of protein binding of gliclazide at saturation level was about 85.09 in presence of theophylline. By comparing this with that of gliclazide alone, it can be inferred that theophylline has significant effect on the protein binding of

gliclazide. This is obviously due to a good affinity of the complex and also theophylline for the protein. The significant lowering of protein binding of gliclazide due to theophylline interference indicates that binding of theophylline is also site specific. On the whole, it is clear that a combination of theophylline with gliclazide is not always desirable.

Protein binding of glipizide

Protein binding versus concentration of glipizide (figure 7.2.1.4) show that at low concentration (1 x 10^{-5}M to 5 x 10^{-5}M), the percentage of protein binding decreases with the increase in concentration of the drug. But at higher concentrations, the percentage attains a steady plateau indicating the saturation zone for the binding of glipizide to bovine serum albumin (B.S.A.).

The usual dose of glipizide is 10 mg three times daily. Literature shows that glipizide is bound about 99% to human plasma protein. But in the present study, the percentage of binding to B.S.A at saturation level is about 93.30. This is perhaps due to inherent difference between human plasma protein and B.S.A.

Effect of caffeinee on protein binding of glipizide

From the table 7.2.1.5 and figure 7.2.1.5, it is found that the highest percentage of protein binding of glipizide at saturation level was about 84.10 in presence of caffeine. By comparing this with that of glipizide alone, it can be inferred that caffeine has significant effect on the protein binding of glipizide. This is obviously due to a good affinity of the complex and also caffeine for the protein. The significant lowering of protein binding of glipizide due to caffeine interference indicates that binding of caffeine is also site specific. On the whole, it is clear that a combination of caffeine with glipizide is not always desirable.

Effect of theophylline on protein binding of glipizide

From the table 7.2.1.6 and figure 7.2.1.6, it is found that the highest percentage of protein binding of glipizide at saturation level was about 84.88 in presence of theophylline. By comparing this with that of theophylline alone, it can be inferred that theophylline has significant effect on the protein binding of glipizide. This is obviously due to a good affinity of the complex and also theophylline for the protein. The significant lowering of protein binding of glipizide due to theophylline interference indicates that binding of theophylline is also site specific. On the whole, it is clear that a combination of theophylline with glipizide is not always desirable.

Protein binding of glyburide

Protein binding versus concentration of glyburide (figure 7.2.1.7) show that at low concentration (1 x 10^{-5}M to 5 x 10^{-5}M), the percentage of protein binding decreases with the increase in concentration of the drug. But at higher concentrations, the percentage attains a steady plateau indicating the saturation zone for the binding of glyburide to bovine serum albumin (B.S.A.).

The usual dose of glyburide is 5 mg three times daily. Literature shows that glipizide is bound about 98% to human plasma protein. But in the present study, the percentage of binding to B.S.A at saturation level is about 92.44. This is perhaps due to inherent difference between human plasma protein and B.S.A.

Effect of caffeine on protein binding of glyburide

From the table 7.2.1.8 and figure 7.2.1.8, it is found that the highest percentage of protein binding of glyburide at saturation level was about 86.31 in presence of caffeine. By comparing this with that of glyburide alone, it can be inferred that caffeine has significant effect on the protein binding of

glyburide. This is obviously due to a good affinity of the complex and also caffeine for the protein. The significant lowering of protein binding of glyburide due to caffeine interference indicates that binding of caffeine is also site specific. On the whole, it is clear that a combination of caffeine with glyburide is not always desirable.

Effect of theophylline on protein binding of glyburide

From the table 7.2.1.9 and figure 7.2.1.9, it is found that the highest percentage of protein binding of glyburide at saturation level was about 85.94 in presence of theophylline. By comparing this with that of glyburide alone, it can be inferred that theophylline has significant effect on the protein binding of glyburide. This is obviously due to a good affinity of the complex and also theophylline for the protein. The significant lowering of protein binding of glyburide due to theophylline interference indicates that binding of theophylline is also site specific. On the whole, it is clear that a combination of theophylline with glyburide is not always desirable.

Protein binding of metformin

Protein binding versus concentration of metformin (figure 7.2.1.10) show that at low concentration (1 x 10^{-5}M to 5 x 10^{-5}M), the percentage of protein binding decreases with the increase in concentration of the drug. But at higher concentrations, the percentage attains a steady plateau indicating the saturation zone for the binding of metformin to bovine serum albumin (B.S.A.).

The usual dose of metformin is 500 mg three times daily. Literature shows that glipizide is bound about 93% to human plasma protein. But in the present study, the percentage of binding to B.S.A at saturation level is about 91.52. This is perhaps due to inherent difference between human plasma protein and B.S.A.

Effect of caffeine on protein binding of metformin

From the table 7.2.1.11 and figure 7.2.1.11, it is found that the highest percentage of protein binding of metformin at saturation level was about 81.84 in presence of caffeine. So, by comparing this with that of metformin alone, it can be inferred that caffeine has significant effect on the protein binding of metformin. This is obviously due to a good affinity of the complex and also caffeine for the protein. The significant lowering of protein binding of metformin due to caffeine interference indicates that binding of caffeine is also site specific. On the whole, it is clear that a combination of caffeine with metformin is not always desirable.

Effect of theophylline on protein binding of metformin

From the table 7.2.1.12 and figure 7.2.1.12, it is found that the highest percentage of protein binding of metformin at saturation level was about 83.07 in presence of theophylline. So, by comparing this with that of metformin alone, it can be inferred that theophylline has significant effect on the protein binding of metformin. This is obviously due to a good affinity of the complex and also theophylline for the protein. The significant lowering of protein binding of metformin due to theophylline interference indicates that binding of theophylline is also site specific. On the whole, it is clear that a combination of theophylline with metformin is not always desirable.

Table 7.2.1.1: Data for percentage of protein binding of gliclazide alone

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.123

Conc. of gliclazide in protein sol. M x 10^5	After equilibrium			
	Abs. of free gliclazide in buffer, $A = A^o - a$	Conc. of free gliclazide in buffer [A] x 10^5 M	Conc. of gliclazide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.005	0.03	0.98	96.94
2	0.014	0.08	1.91	95.81
3	0.028	0.15	2.18	93.12
4	0.053	0.27	3.77	92.84
5	0.077	0.39	4.66	91.63
6	0.107	0.53	5.52	90.40
7	0.128	0.64	6.59	90.29
8	0.139	0.67	7.41	90.96
9	0.144	0.75	8.48	91.16
10	0.160	0.79	9.28	91.49

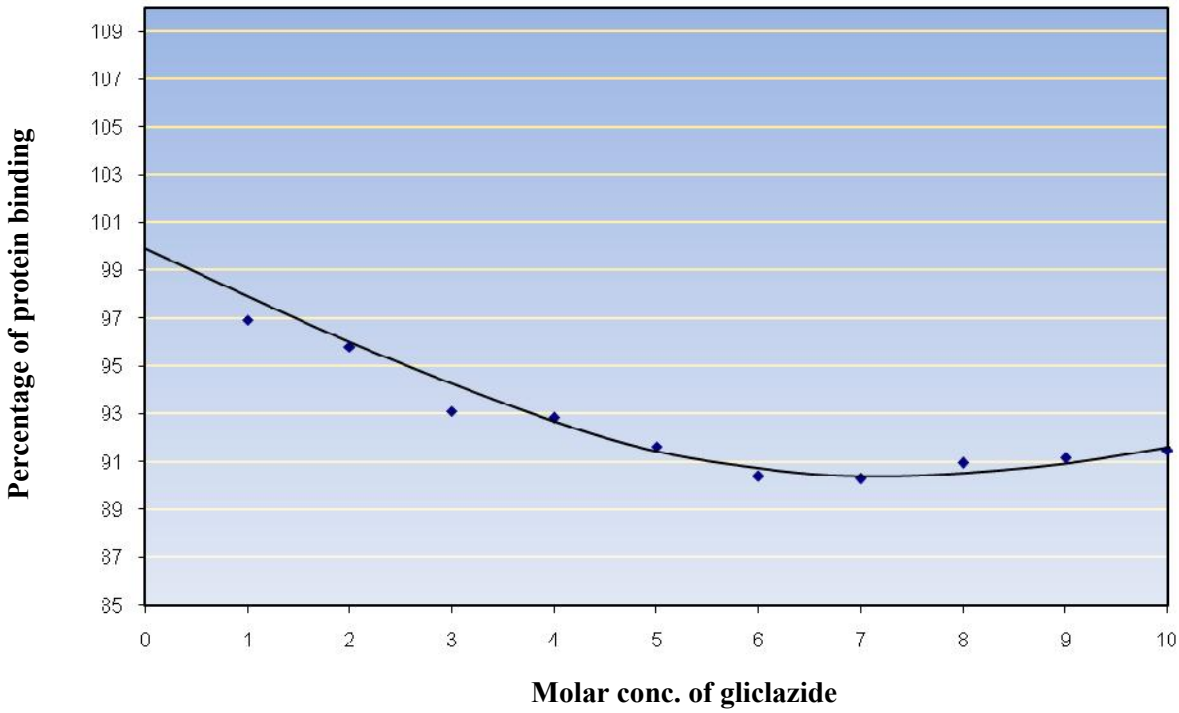


Fig. 7.2.1.1: Protein binding of gliclazide alone

Table 7.2.1.2: Data for percentage of protein binding of 1:1 mixture of gliclazide with caffeine

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.123

Conc. of gliclazide in protein sol. M x 10^5	After equilibrium			
	Abs. of free gliclazide in buffer, $A = A^o - a$	Conc. of free gliclazide in buffer [A] x 10^5 M	Conc. of gliclazide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.022	0.13	1.21	89.26
2	0.045	0.29	1.95	85.13
3	0.082	0.39	2.85	86.32
4	0.127	0.64	3.62	82.32
5	0.158	0.78	4.85	83.92
6	0.193	0.95	5.45	82.57
7	0.213	1.03	6.22	83.44
8	0.247	1.22	7.21	83.08
9	0.284	1.43	7.95	82.01
10	0.318	1.64	8.88	81.53

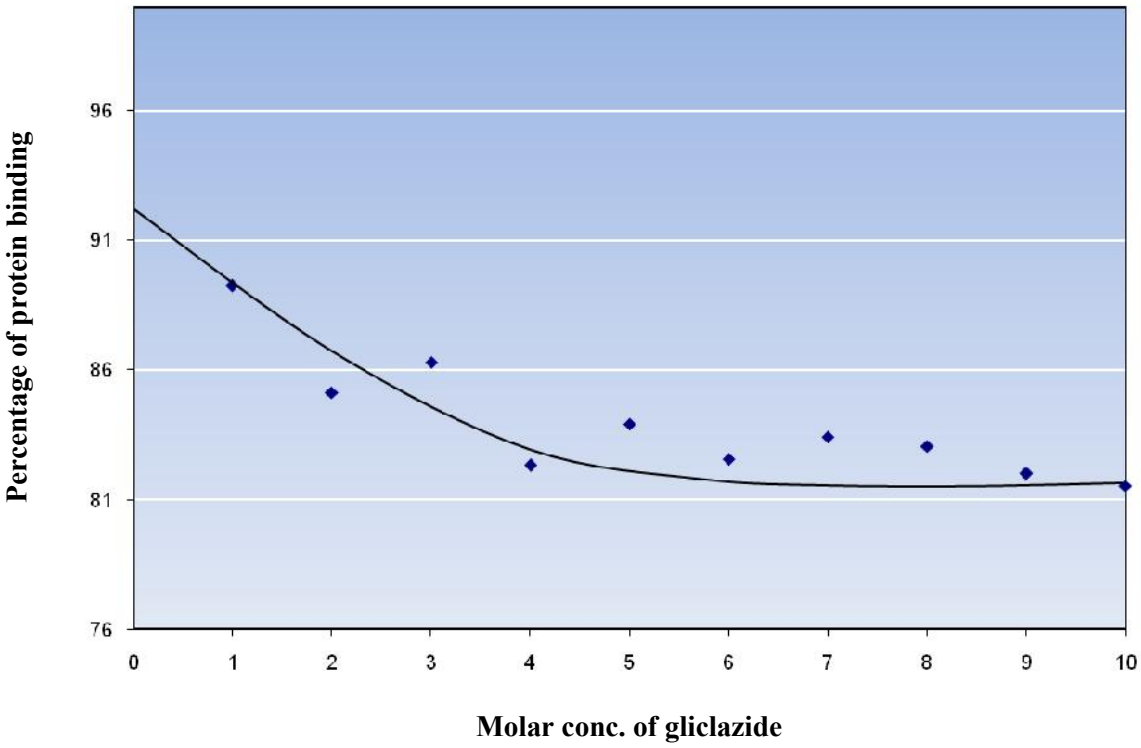


Fig. 7.2.1.2: Protein binding of gliclazide in presence of caffeine (1:1 mixture)

Table 7.2.1.3: Data for percentage of protein binding of 1:1 mixture of gliclazide with theophylline

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.123

Conc. of gliclazide in protein sol. M x 10^5	After equilibrium			
	Abs. of free gliclazide in buffer, $A = A^o - a$	Conc. of free gliclazide in buffer [A] x 10^5 M	Conc. of gliclazide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.014	0.09	0.97	90.72
2	0.035	0.19	1.75	89.14
3	0.074	0.35	2.65	86.79
4	0.115	0.52	3.38	84.62
5	0.147	0.68	4.21	83.85
6	0.182	0.85	5.07	83.23
7	0.203	0.93	5.98	84.45
8	0.238	1.13	6.78	83.33
9	0.272	1.32	7.72	82.90
10	0.313	1.58	8.69	81.91

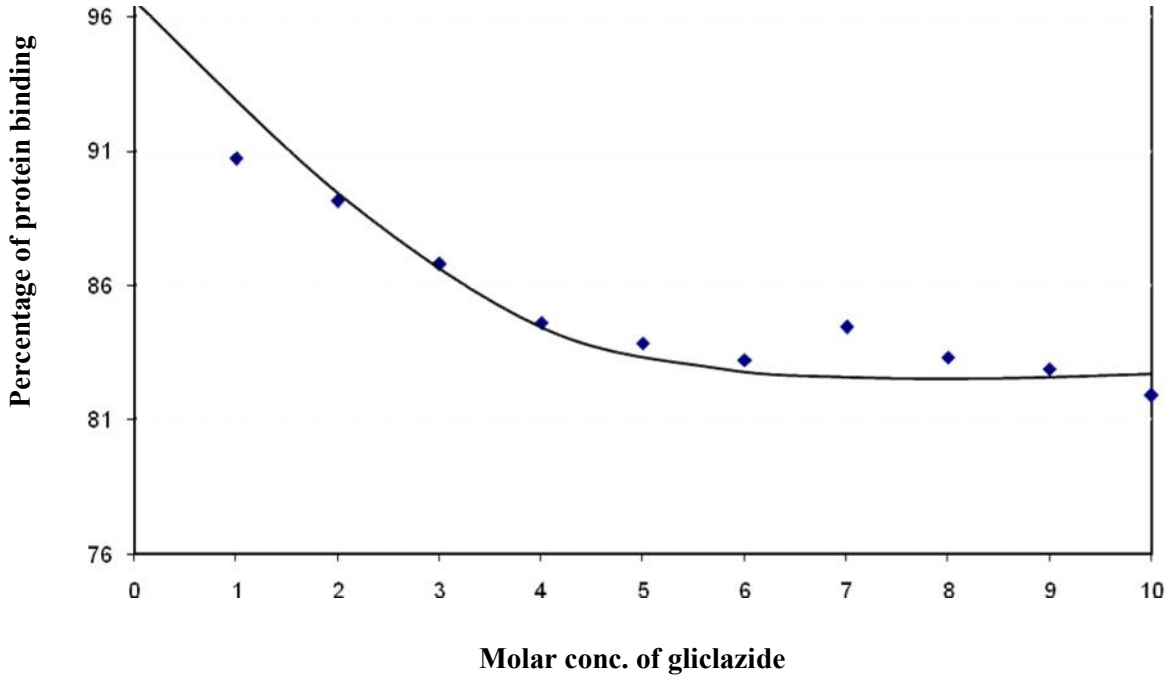


Fig. 7.2.1.3: Protein binding of gliclazide in presence of theophylline (1:1 mixture)

Table 7.2.1.4: Data for percentage of protein binding of glipizide alone

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.125

Conc. of glipizide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glipizide in buffer, $A = A^o - a$	Conc. of free glipizide in buffer [A] x 10^5 M	Conc. of glipizide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.004	0.03	0.98	96.93
2	0.009	0.07	1.85	96.21
3	0.022	0.10	2.80	96.43
4	0.047	0.23	3.68	93.75
5	0.071	0.35	4.57	92.34
6	0.100	0.49	5.44	90.99
7	0.119	0.58	6.33	90.84
8	0.131	0.61	7.31	91.66
9	0.139	0.70	8.41	91.68
10	0.152	0.73	9.31	92.16

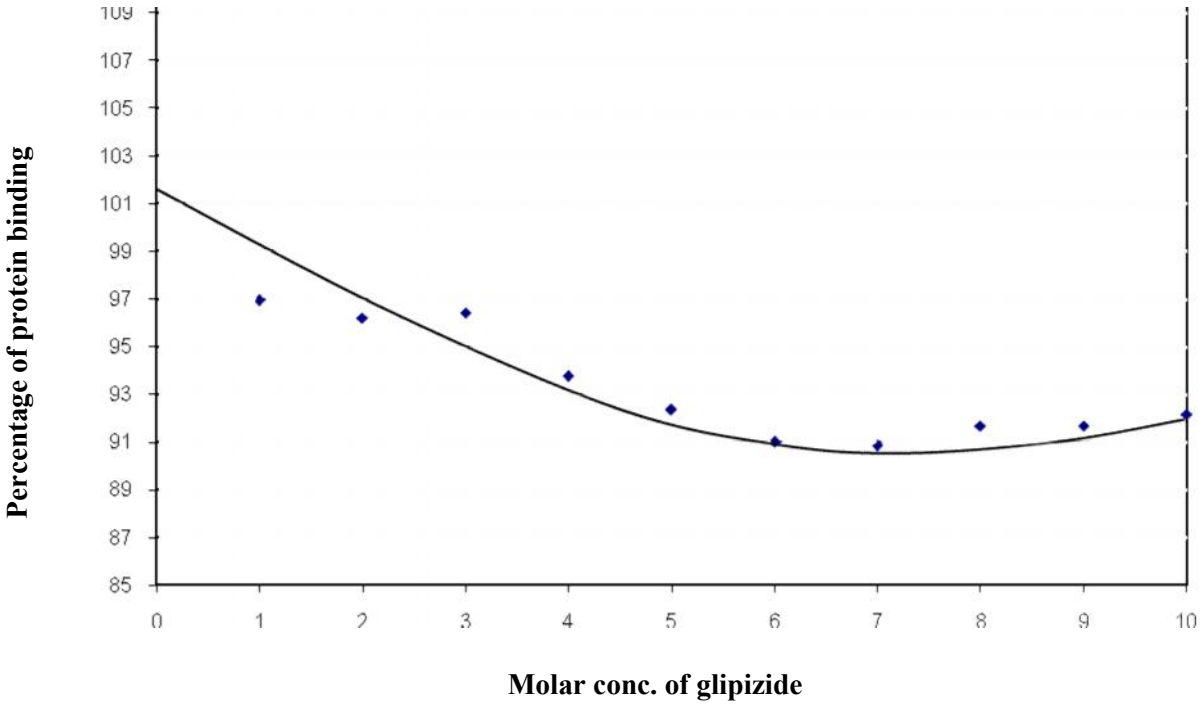


Fig. 7.2.1.4: Protein binding of glipizide alone

Table 7.2.1.5: Data for percentage of protein binding of 1:1 mixture of glipizide with caffeine

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.125

Conc. of glipizide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glipizide in buffer, $A = A^o - a$	Conc. of free glipizide in buffer [A] x 10^5 M	Conc. of glipizide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.019	0.10	0.97	89.69
2	0.045	0.23	1.88	87.77
3	0.079	0.37	2.75	86.55
4	0.124	0.61	3.48	82.47
5	0.156	0.75	4.32	82.64
6	0.191	0.92	5.15	82.14
7	0.211	1.01	6.12	83.66
8	0.245	1.20	6.92	82.66
9	0.282	1.41	7.82	81.97
10	0.316	1.62	8.74	81.46

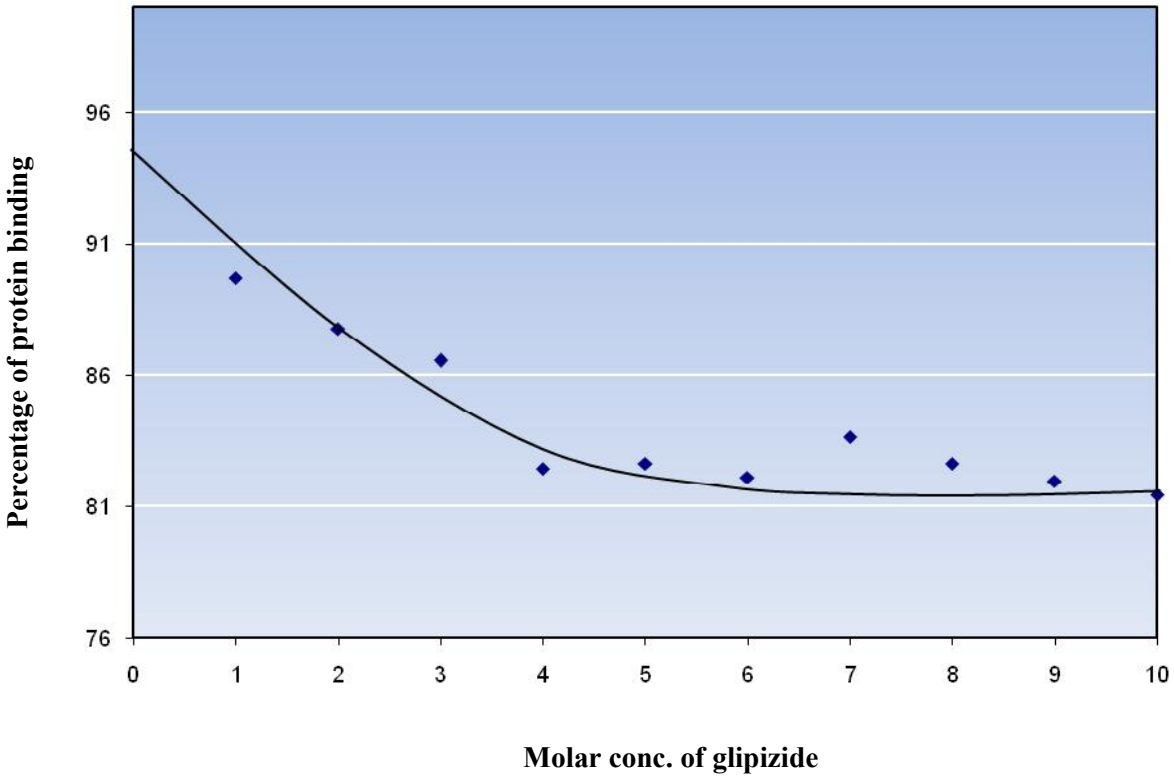


Fig. 7.2.1.5: Protein binding of glipizide in presence of caffeine (1:1 mixture)

Table 7.2.1.6: Data for percentage of protein binding of 1:1 mixture of glipizide with theophylline

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.125

Conc. of glipizide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glipizide in buffer, $A = A^o - a$	Conc. of free glipizide in buffer [A] x 10^5 M	Conc. of glipizide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.019	0.11	1.18	90.68
2	0.042	0.25	1.91	86.91
3	0.077	0.34	2.78	87.77
4	0.123	0.59	3.55	83.38
5	0.154	0.73	4.78	84.73
6	0.188	0.87	5.38	83.83
7	0.208	0.99	5.95	83.36
8	0.241	1.15	6.95	83.45
9	0.276	1.38	7.88	82.49
10	0.311	1.58	8.81	82.07

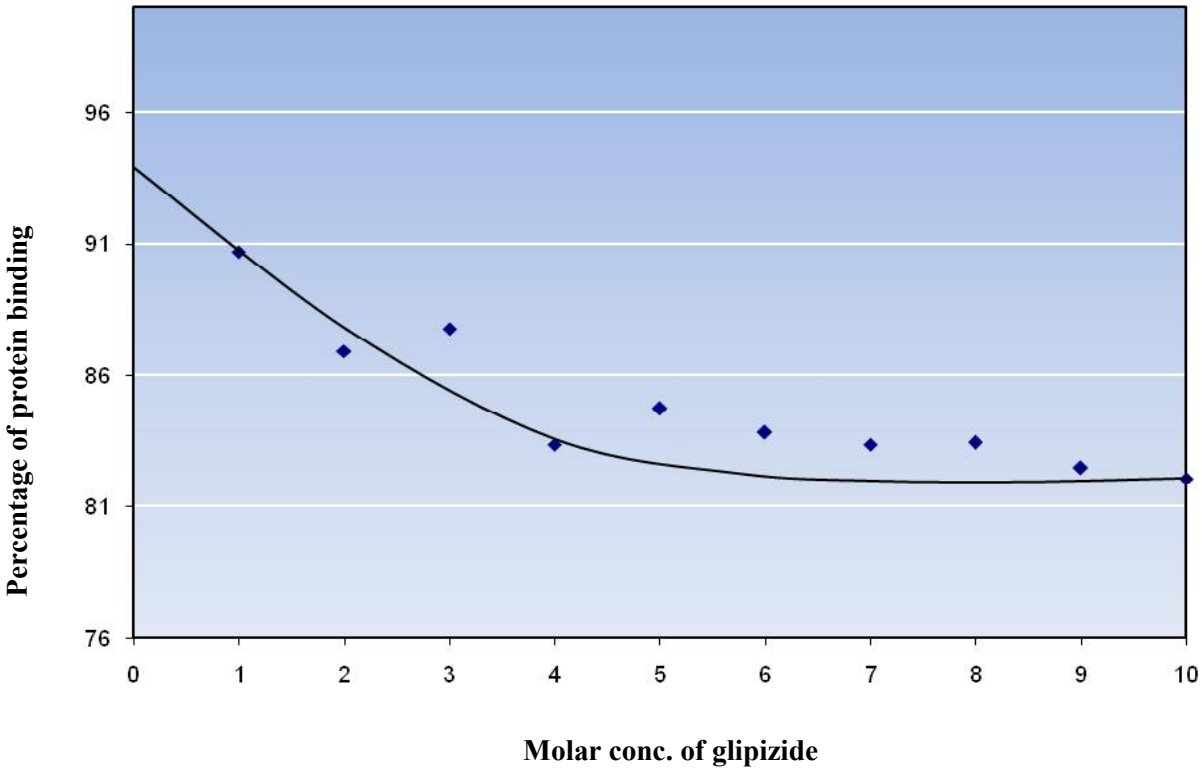


Fig. 7.2.1.6: Protein binding of glipizide in presence of theophylline (1:1 mixture)

Table 7.2.1.7: Data for percentage of protein binding of glyburide alone

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.115

Conc. of glyburide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glyburide in buffer, $A = A^o - a$	Conc. of free glyburide in buffer [A] x 10^5 M	Conc. of glyburide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.003	0.04	0.93	95.70
2	0.012	0.09	1.85	95.14
3	0.027	0.14	2.17	93.55
4	0.051	0.26	3.72	93.01
5	0.075	0.38	4.61	91.76
6	0.103	0.52	5.48	90.51
7	0.126	0.63	6.55	90.38
8	0.137	0.65	7.37	91.18
9	0.143	0.73	8.45	91.36
10	0.157	0.76	9.24	91.77

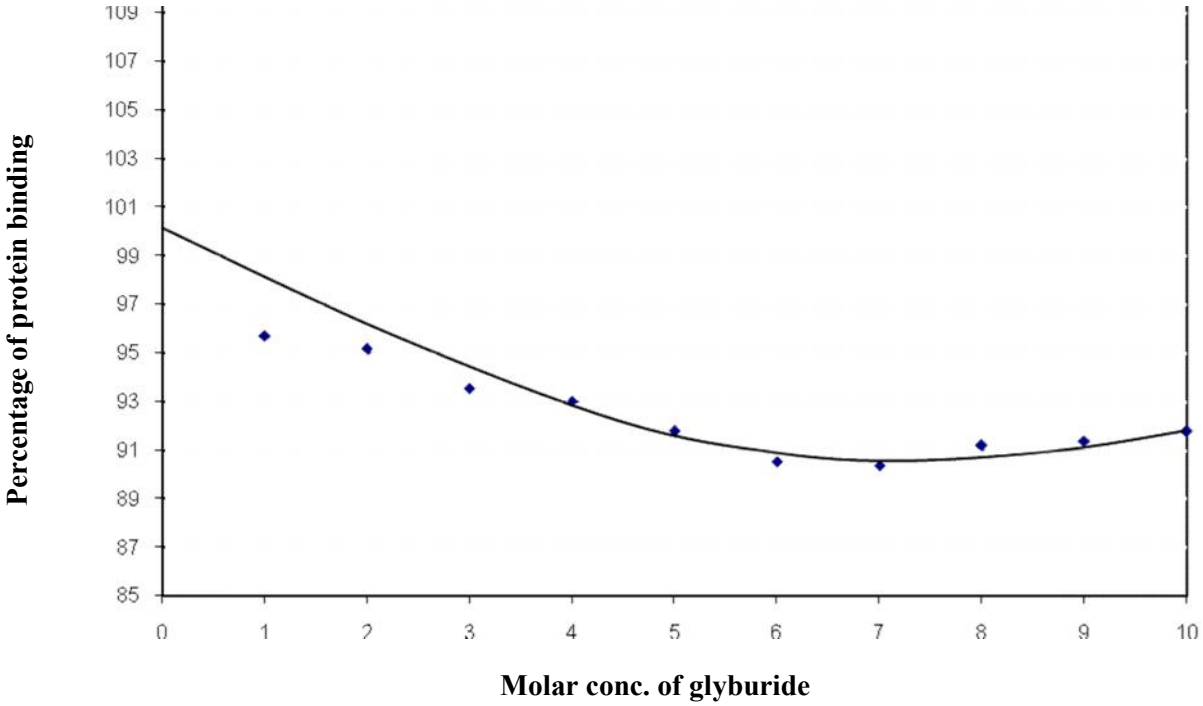


Fig. 7.2.1.7: Protein binding of glyburide alone

Table 7.2.1.8: Data for percentage of protein binding of 1:1 mixture of glyburide with caffeine

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.115

Conc. of glyburide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glyburide in buffer, $A = A^o - a$	Conc. of free glyburide in buffer [A] x 10^5 M	Conc. of glyburide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.009	0.05	0.84	94.05
2	0.028	0.15	1.69	91.12
3	0.067	0.28	2.58	89.15
4	0.111	0.48	3.35	85.67
5	0.143	0.65	4.18	84.45
6	0.178	0.81	5.01	83.83
7	0.198	0.89	5.93	84.99
8	0.231	1.08	6.74	83.98
9	0.268	1.29	7.69	83.22
10	3.102	1.49	8.58	82.63

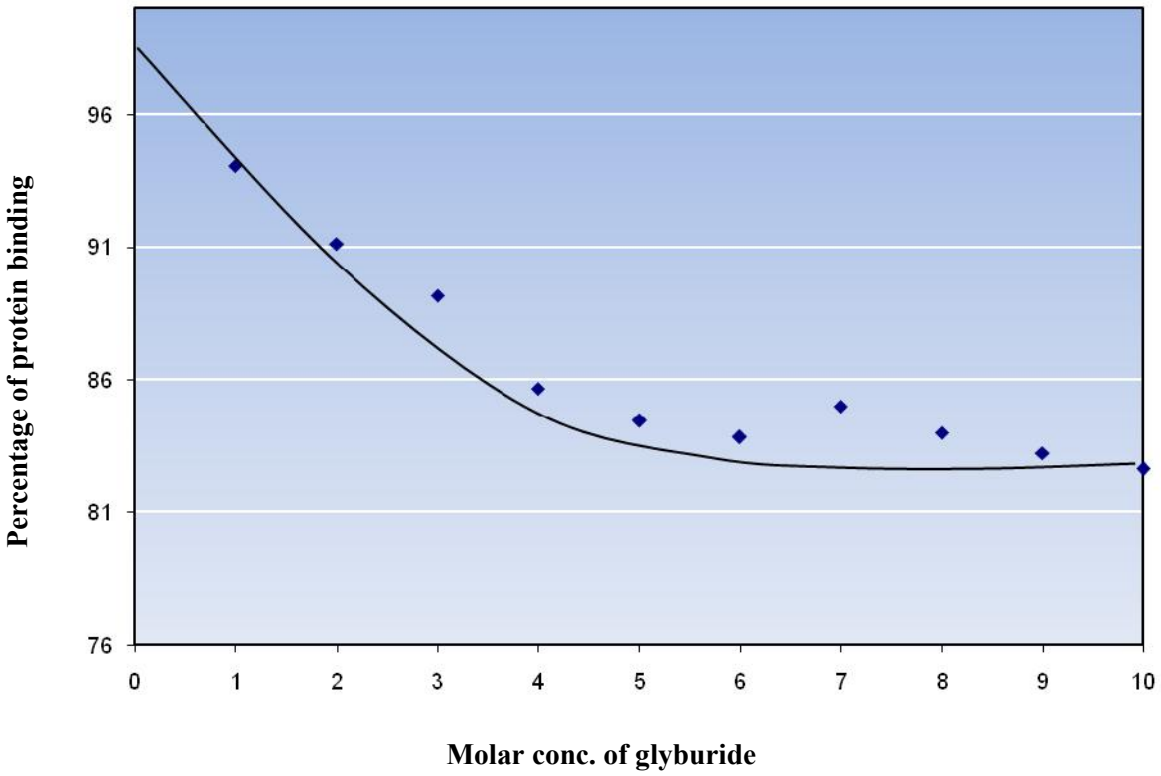


Fig. 7.2.1.8: Protein binding of gyburide in presence of caffeine (1:1 mixture)

Table 7.2.1.9: Data for percentage of protein binding of 1:1 mixture of glyburide with theophylline

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.115

Conc. of glyburide in protein sol. M x 10^5	After equilibrium			
	Abs. of free glyburide in buffer, $A = A^o - a$	Conc. of free glyburide in buffer [A] x 10^5 M	Conc. of glyburide in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.015	0.08	1.15	93.04
2	0.036	0.21	1.82	88.46
3	0.070	0.27	2.70	90.00
4	0.118	0.53	3.48	84.77
5	0.148	0.67	4.71	85.77
6	0.180	0.80	5.30	82.90
7	0.200	0.92	5.88	84.35
8	0.235	1.08	6.88	84.30
9	0.270	1.31	7.79	83.18
10	0.305	1.52	8.75	82.63

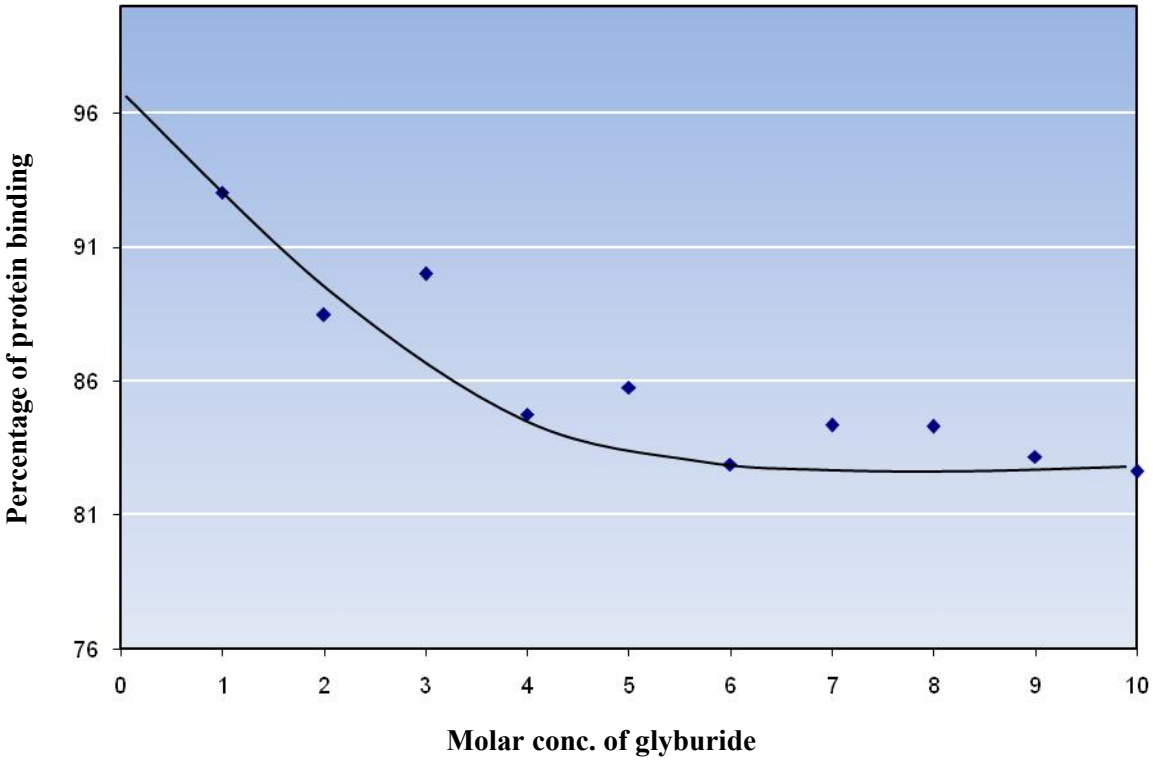


Fig. 7.2.1.9: Protein binding of theophylline in presence of gyburide (1:1 mixture)

Table 7.2.1.10: Data for percentage of protein binding of metformin alone

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.132

Conc. of metformin in protein sol. M x 10^5	After equilibrium			
	Abs. of free metformin in buffer, $A = A^o - a$	Conc. of free metformin in buffer [A] x 10^5 M	Conc. of metformin in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.007	0.07	0.98	93.87
2	0.014	0.08	1.21	94.07
3	0.029	0.19	2.13	91.07
4	0.053	0.25	3.23	92.26
5	0.074	0.36	4.32	91.67
6	0.107	0.50	5.23	90.44
7	0.123	0.62	6.25	90.08
8	0.136	0.67	6.88	90.26
9	0.141	0.72	7.78	90.75
10	0.155	0.77	8.33	90.76

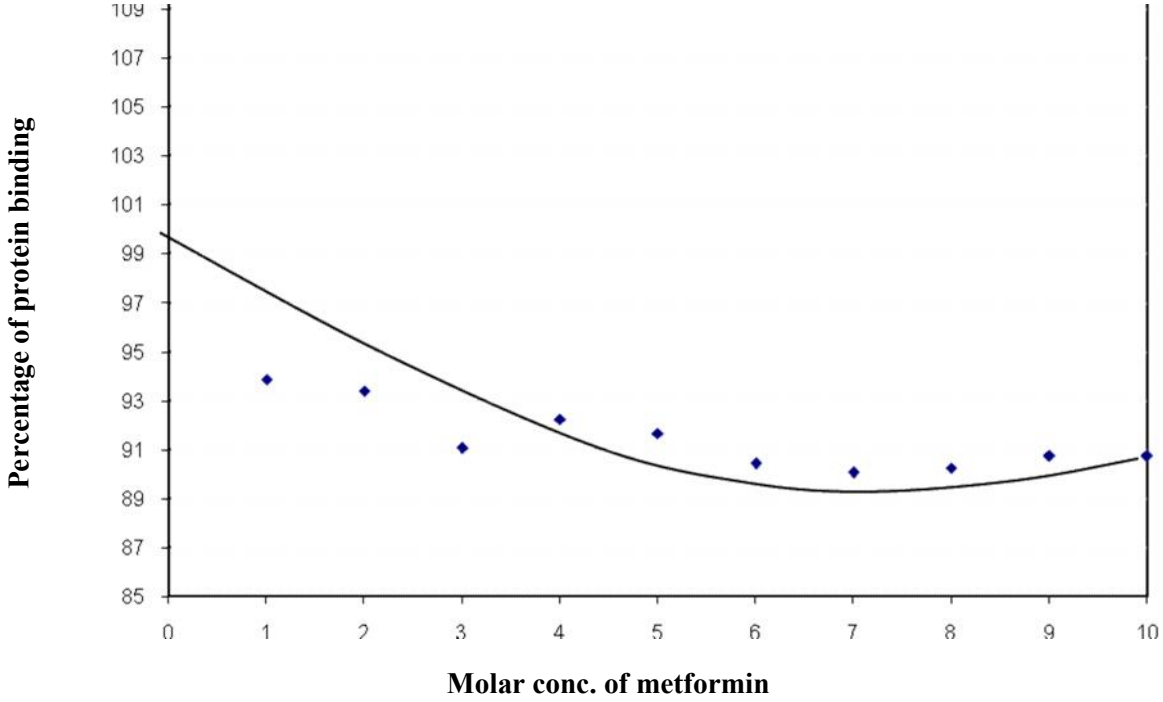


Fig. 7.2.1.10: Protein binding of metformin alone

Table 7.2.1.11: Data for percentage of protein binding of 1:1 mixture of metformin with caffeine

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.132

Conc. of metformin in protein sol. M x 10^5	After equilibrium			
	Abs. of free metformin in buffer, $A = A^o - a$	Conc. of free metformin in buffer [A] x 10^5 M	Conc. of metformin in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.023	0.12	0.96	87.50
2	0.051	0.28	1.78	84.27
3	0.091	0.47	2.57	81.71
4	0.142	0.64	3.42	81.28
5	0.179	0.83	4.24	80.42
6	0.218	0.97	5.08	80.91
7	0.236	1.04	5.38	80.67
8	0.280	1.17	6.18	81.07
9	0.369	1.53	7.68	80.08
10	0.478	1.69	8.65	80.46

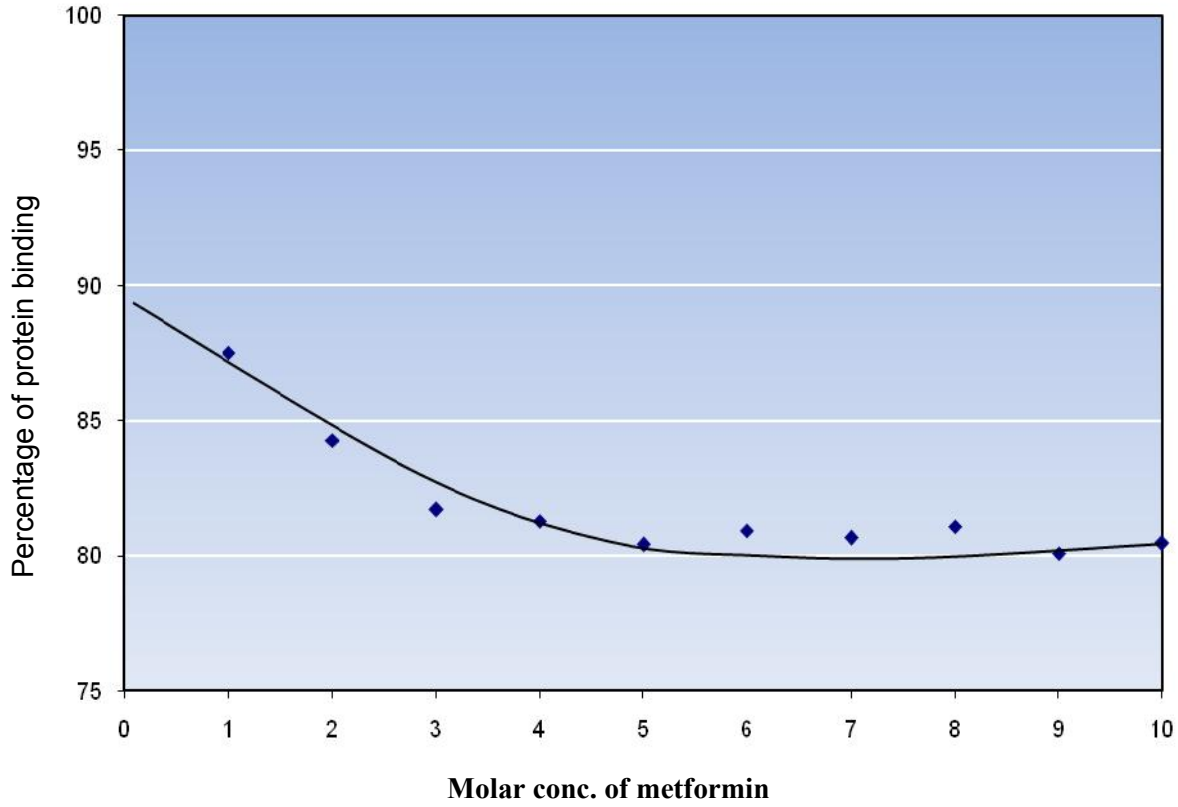


Fig. 7.2.1.11: Protein binding of metformin in presence of caffeine (1:1 mixture)

Table 7.2.1.12: Data for percentage of protein binding of 1:1 mixture of metformin with theophylline

Amount of phosphate buffer (pH 7.4) = 30 ml

Molar concentration of B.S.A = 5 x 10^{-5} M

(Drug + Protein + Buffer) in dialysis bag = 3 ml

Absorbance of buffer (after dialysis of protein), a = 0.132

Conc. of metformin in protein sol. M x 10^5	After equilibrium			
	Abs. of free metformin in buffer, A = A^o – a	Conc. of free metformin in buffer [A] x 10^5 M	Conc. of metformin in B.S.A [B] x 10^5 M	Percentage of protein binding = {[B]-[A]}/[B] x100
1	0.019	0.09	0.84	89.29
2	0.045	0.22	1.68	86.90
3	0.083	0.40	2.48	83.87
4	0.132	0.62	3.29	81.16
5	0.169	0.76	4.15	81.69
6	0.211	0.95	4.96	80.85
7	0.232	1.02	5.91	82.74
8	0.273	1.12	6.65	83.16
9	0.363	1.48	7.61	80.55
10	0.467	1.66	8.51	80.49

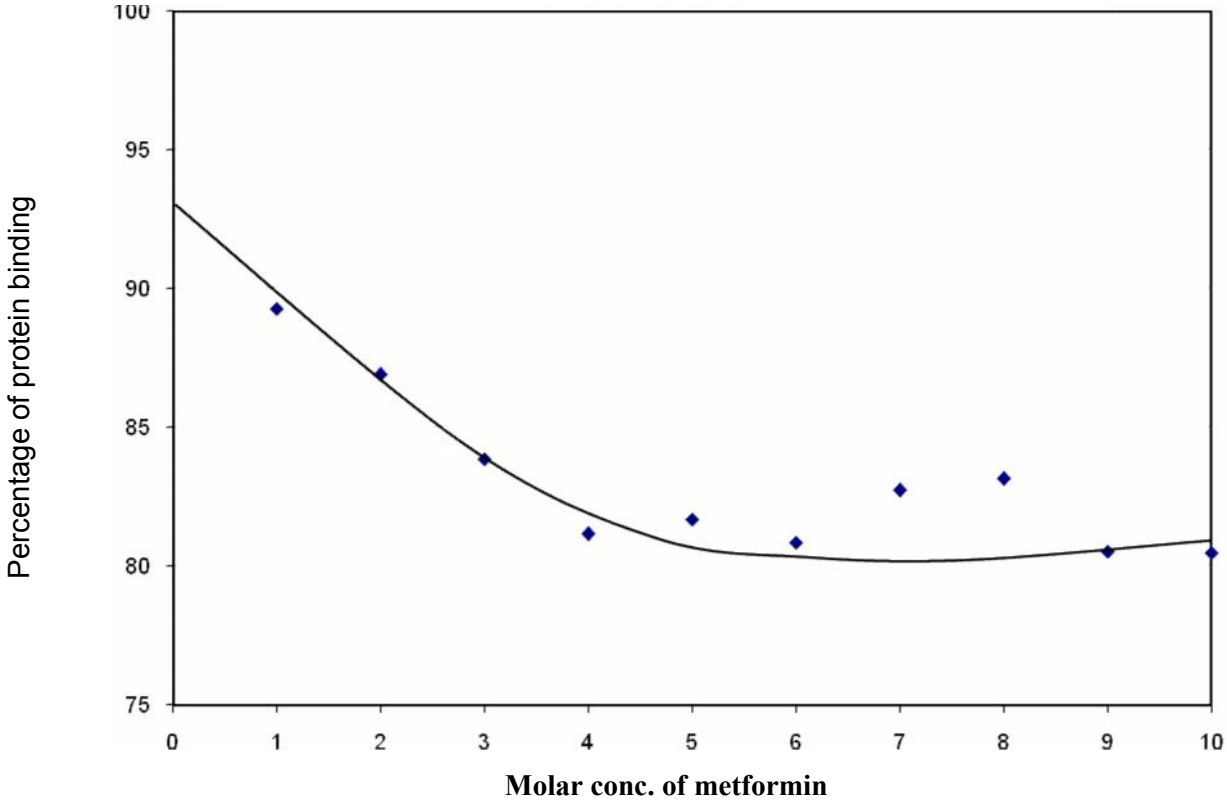


Fig. 7.2.1.12: Protein binding of metformin in presence of theophylline (1:1 mixture)

The table given below shows a comparative picture of number of protein binding of caffeine & theophylline alone and their 1:1 mixtures with gliclazide, glipizide, glyburide and metformin.

The values for number of protein binding

System	Protein Binding (%)
Gliclazide alone	92.46
Gliclazide – Caffeine System	83.99
Gliclazide - Theophylline System	85.09
Glipizide alone	93.30
Glipizide – Caffeine System	84.10
Glipizide – Theophylline System	84.88
Glyburide alone	92.44
Glyburide – Caffeine System	86.31
Glyburide – Theophylline System	85.94
Metformin alone	91.52
Metformin – Caffeine System	81.84
Metformin – Theophylline System	83.07

From above table, we observe that by comparing this with that of gliclazide, glipizide, glyburide and metformin alone, it can be inferred that both caffeine and theophylline have significant effect on the protein binding of gliclazide, glipizide, glyburide and metformin. This is obviously due to a good affinity of the complex and also caffeine and theophylline for the protein. The significant lowering of protein binding of gliclazide, glipizide, glyburide and metformin due to caffeine and theophylline interference indicates that binding of caffeine and theophylline are also site specific.

As caffeine and theophylline decrease the percentage of protein binding of gliclazide, glipizide, glyburide and metformin, they increase the free plasma concentration of gliclazide, glipizide, glyburide and metformin which may give toxic effects.

7.2.2 The number of protein binding sites and values of affinity constants

The Scatchard plots show at least two classes of binding sites (class I and class II, the warfarin and the diazepam sites, respectively). The number of binding sites n_1 and n_2 for class I and class II, and affinity constants k_1 and k_2 for these classes have been calculated from Scatchard plots. Numbers of binding sites were obtained by dividing the intercept (nk) by slope (k) of the straight lines. The values for

affinity constants associated with respective class of binding sites were obtained directly from the slope of the straight lines.[81]

Number of binding sites and affinity constants for

Gliclazide alone

From the table 7.2.2.1 and Scatchard plot (fig.7.2.2.1), the number of binding sites for gliclazide alone in B.S.A is found to be 0.73 and 1.35 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 17.35 and 3.67 respectively.

Caffeine–gliclazide system

From the table 7.2.2.2 and Scatchard plot (fig.7.2.2.2), the number of binding sites for Caffeine–gliclazide system in B.S.A is found to be 1.04 and 6.25 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.41 and 0.17 respectively.

Theophylline–gliclazide system

From the table 7.2.2.3 and Scatchard plot (fig.7.2.2.3), the number of binding sites for Caffeine–gliclazide system in B.S.A is found to be 1.01 and 4.76 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.17 and 0.14 respectively.

Glipizide alone

From the table 7.2.2.4 and Scatchard plot (fig.7.2.2.4), the number of binding sites for glipizide alone in B.S.A is found to be 0.79 and 1.44 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 18.21 and 3.76 respectively.

Caffeine–glipizide system

From the table 7.2.2.5 and Scatchard plot (fig.7.2.2.5), the number of binding sites for Caffeine–gliclazide system in B.S.A is found to be 1.04 and 5.78 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.36 and 0.17 respectively.

Theophylline–glipizide system

From the table 7.2.2.6 and Scatchard plot (fig.7.2.2.6), the number of binding sites for theophylline–glipizide system in B.S.A is found to be 1.00 and 4.65 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.15 and 0.13 respectively.

Glyburide alone

From the table 7.2.2.7 and Scatchard plot (fig.7.2.2.7), the number of binding sites for glyburide alone in B.S.A is found to be 0.77 and 1.39 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 17.85 and 3.71 respectively.

Caffeine–glyburide system

From the table 7.2.2.8 and Scatchard plot (fig.7.2.2.8), the number of binding sites for Caffeine–glyburide system in B.S.A is found to be 1.02 and 5.69 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.33 and 0.15 respectively.

Theophylline–glyburide system

From the table 7.2.2.9 and Scatchard plot (fig.7.2.2.9), the number of binding sites for theophylline–glyburide system in B.S.A is found to be 1.02 and 5.23 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 2.21 and 0.16 respectively.

Metformin alone

From the table 7.2.2.10 and Scatchard plot (fig.7.2.2.10), the number of binding sites for metformin alone in B.S.A is found to be 0.73 and 1.32 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 16.82 and 3.23 respectively.

Caffeine–metformin system

From the table 7.2.2.11 and Scatchard plot (fig.7.2.2.11), the number of binding sites for Caffeine–metformin system in B.S.A is found to be 3.19 and 2.21 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 0.37 and 0.53 respectively.

Theophylline–metformin system

From the table 7.2.2.12 and Scatchard plot (fig.7.2.2.12), the number of binding sites for theophylline–metformin system in B.S.A is found to be 3.13 and 2.03 for class I and class II respectively. The affinity constants k_1 and k_2 associated with class I and class II were 0.37 and 0.52 respectively.

Table 7.2.2.1: Data for scatchard plot for protein binding of gliclazide alone

Here, Molar conc. of B.S.A = 5×10^{-5} **M**

Conc. of	After equilibrium

Unbound gliclazide [A] x 10^5 M	Conc. of gliclazide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	r = $\frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.05	1.02	0.97	0.194	3.880	38.80
0.10	1.98	1.88	0.376	3.760	37.60
0.15	2.83	2.68	0.536	3.573	35.73
0.27	3.75	3.48	0.696	2.578	25.78
0.39	4.59	4.20	0.840	2.154	21.54
0.51	5.48	4.97	0.994	1.949	19.49
0.63	6.36	5.73	1.115	1.819	18.19
0.65	7.32	6.67	1.334	2.052	20.52
0.71	8.23	7.52	1.504	2.118	21.18
0.78	9.36	8.58	1.716	2.200	22.00

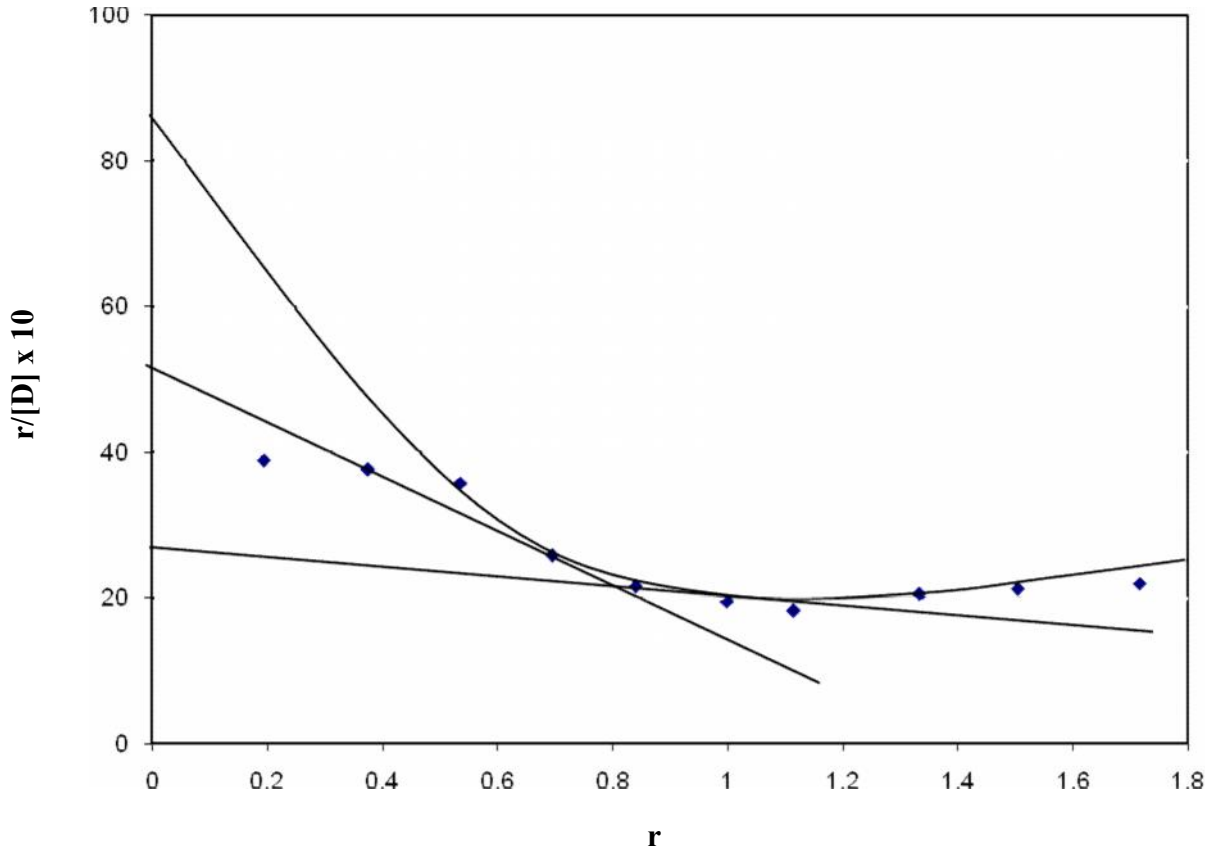


Fig. 7.2.2.1: Scatchard plot for protein binding of gliclazide alone

Table 7.2.2.2: Data for scatchard plot for protein binding of gliclazide in a 1:1 mixture with caffeine

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound gliclazide [A] x 10^5 M	After equilibrium				
	Conc. of gliclazide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.05	0.89	0.84	0.168	3.360	33.60
0.17	1.77	1.60	0.320	1.882	18.82
0.31	2.63	2.32	0.464	1.497	14.97
0.53	3.43	2.90	0.580	1.094	10.94
0.73	4.28	3.55	0.710	0.973	9.73
0.85	5.07	4.22	0.844	0.993	9.93
0.91	5.98	5.07	1.014	1.114	11.14
1.12	6.78	5.66	1.132	1.011	10.11
1.20	7.73	6.53	1.306	1.088	10.88
1.23	8.65	7.42	1.484	1.207	12.07

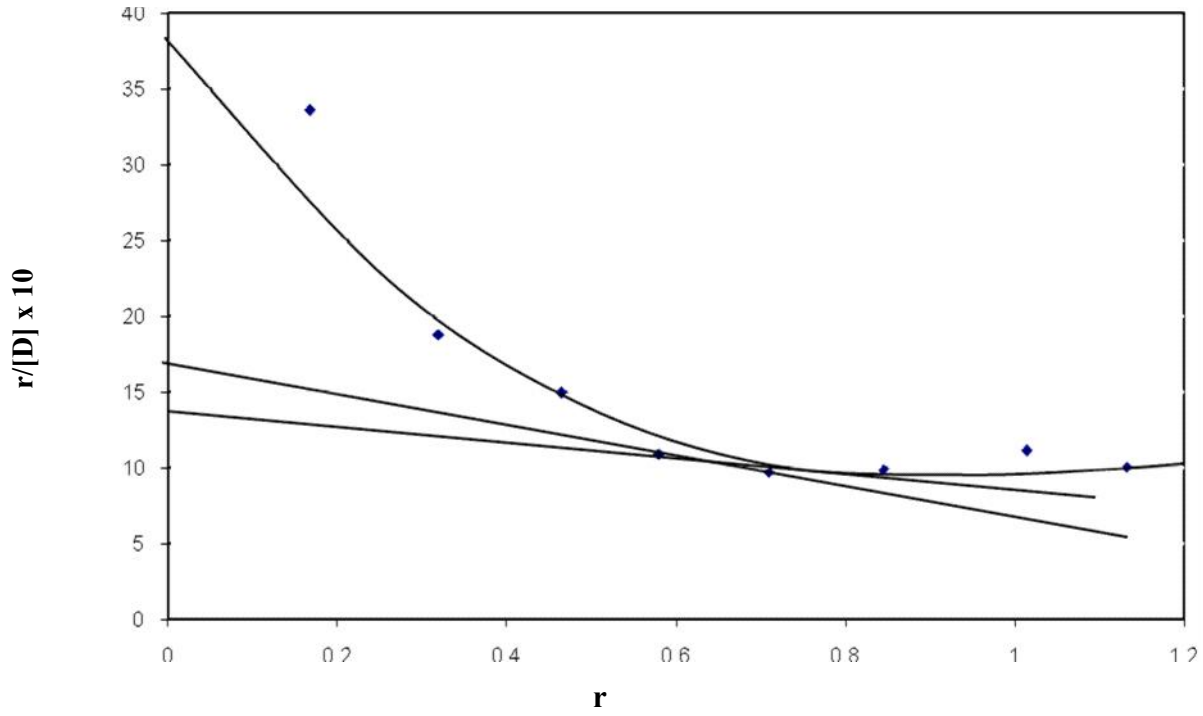


Fig. 7.2.2.2: Scatchard plot for protein binding of gliclazide in presence of caffeine (1:1 mixture)

Table 7.2.2.3: Data for scatchard plot for protein binding of gliclazide in a 1:1 mixture with theophylline

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound gliclazide [A] x 10^5 M	After equilibrium				
	Conc. of gliclazide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.10	0.86	0.76	0.152	1.520	15.20
0.22	1.73	1.51	0.302	1.373	13.73
0.35	2.57	2.22	0.444	1.268	12.68
0.58	3.37	2.79	0.558	0.962	9.62
0.74	4.21	3.47	0.694	0.938	9.38
0.90	5.00	4.10	0.820	0.911	9.11
0.99	5.95	4.96	0.992	1.002	10.02
1.18	6.75	5.57	1.114	0.944	9.44
1.23	7.69	6.46	1.292	1.050	10.50
1.25	8.60	7.35	1.470	1.176	11.76

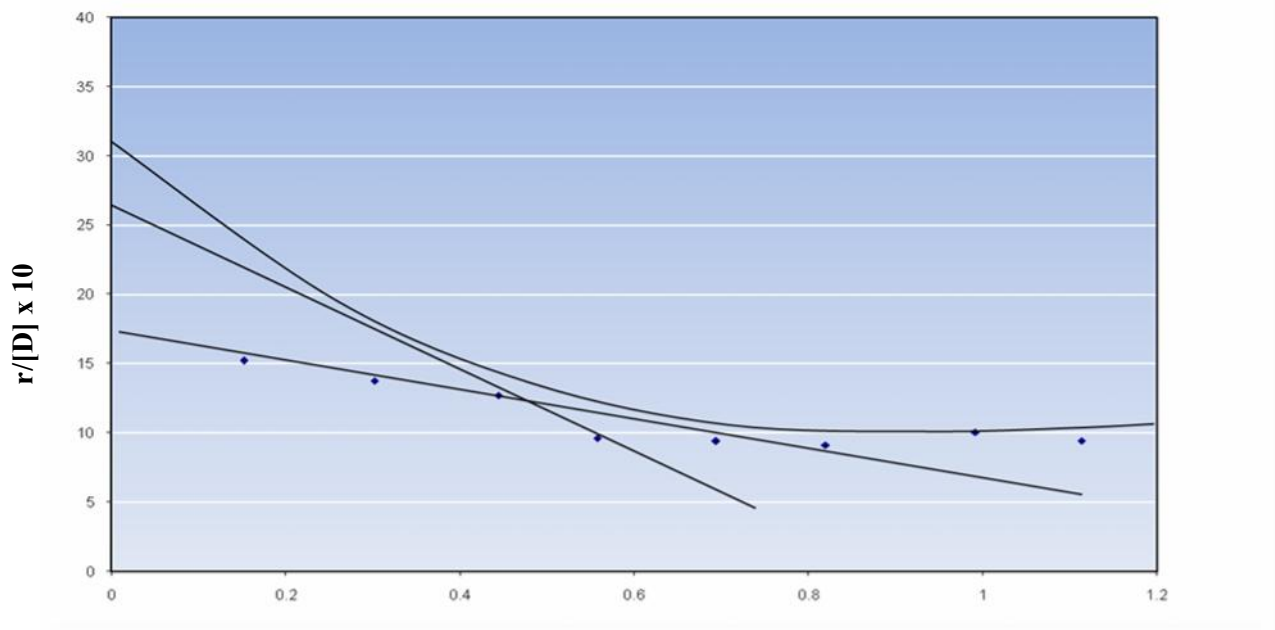


Fig.7.2.2.3: Scatchard plot for protein binding of gliclazide in presence of theophylline (1:1 mixture)

Table 7.2.2.4: Data for scatchard plot for protein binding of glipizide alone

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of	After equilibrium

Unbound glipizide [A] x 10^5 M	Conc. of glipizide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.07	0.98	0.91	0.182	2.600	26.00
0.17	1.81	1.64	0.328	1.929	19.29
0.27	2.75	2.48	0.496	1.837	18.37
0.39	3.63	3.24	0.648	1.662	16.62
0.51	4.50	3.99	0.798	1.565	15.65
0.65	5.38	4.73	0.946	1.455	14.55
0.75	6.26	5.51	1.102	1.469	14.69
0.78	7.26	6.68	1.296	1.662	16.62
0.82	8.15	7.33	1.466	1.788	17.88
0.88	9.21	8.33	1.666	1.893	18.93

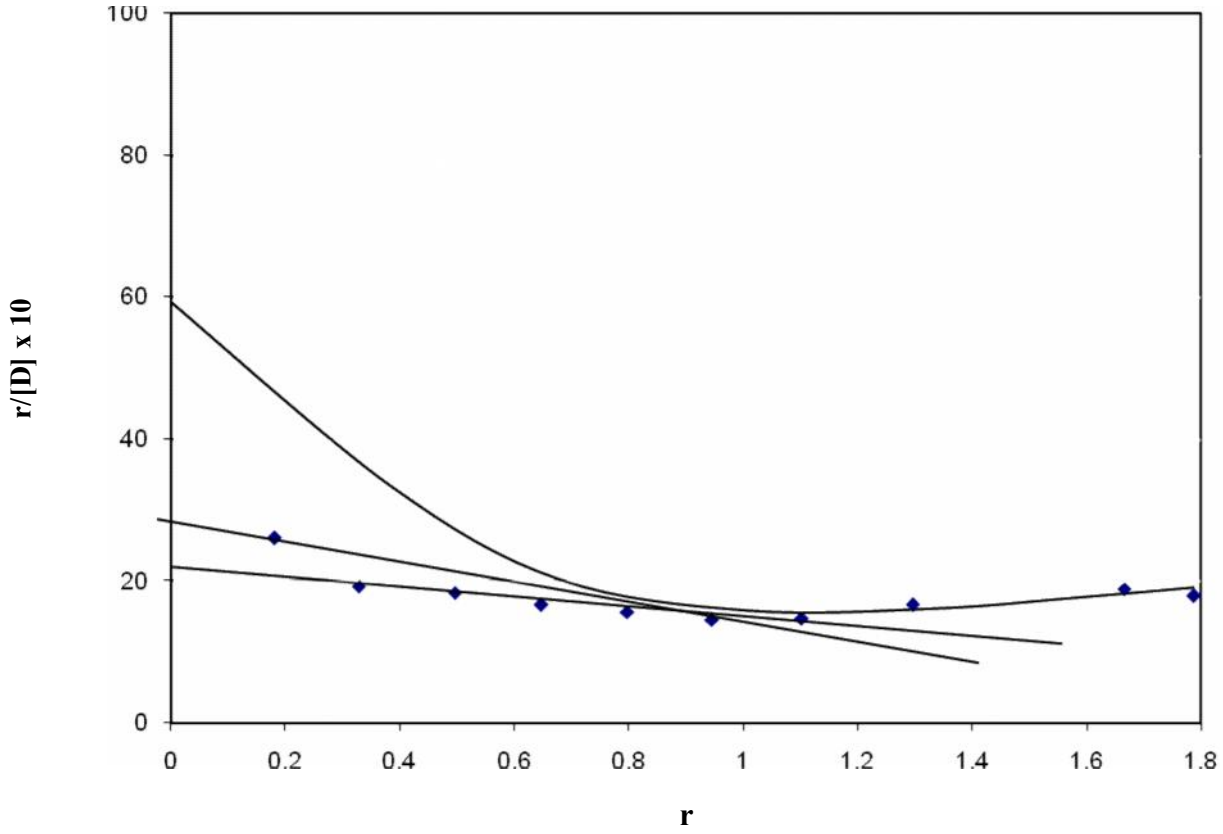


Fig. 7.2.2.4: Scatchard plot for protein binding of glipizide alone

Table 7.2.2.5: Data for scatchard plot for protein binding of glipizide in a 1:1 mixture with caffeine

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound glipizide [A] x 10^5 M	After equilibrium				
	Conc. of glipizide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.08	0.88	0.80	0.160	2.000	20.00
0.23	1.72	1.49	0.298	1.296	12.96
0.38	2.55	2.29	0.434	1.142	11.42
0.59	3.36	2.77	0.554	0.939	9.39
0.77	4.19	3.42	0.684	0.888	8.88
0.92	5.02	4.10	0.822	0.891	8.91
1.03	5.95	4.92	0.984	0.955	9.55
1.19	6.76	5.57	1.114	0.936	9.36
1.25	7.71	6.46	1.292	1.034	10.34
1.28	8.59	7.31	1.462	1.142	11.42

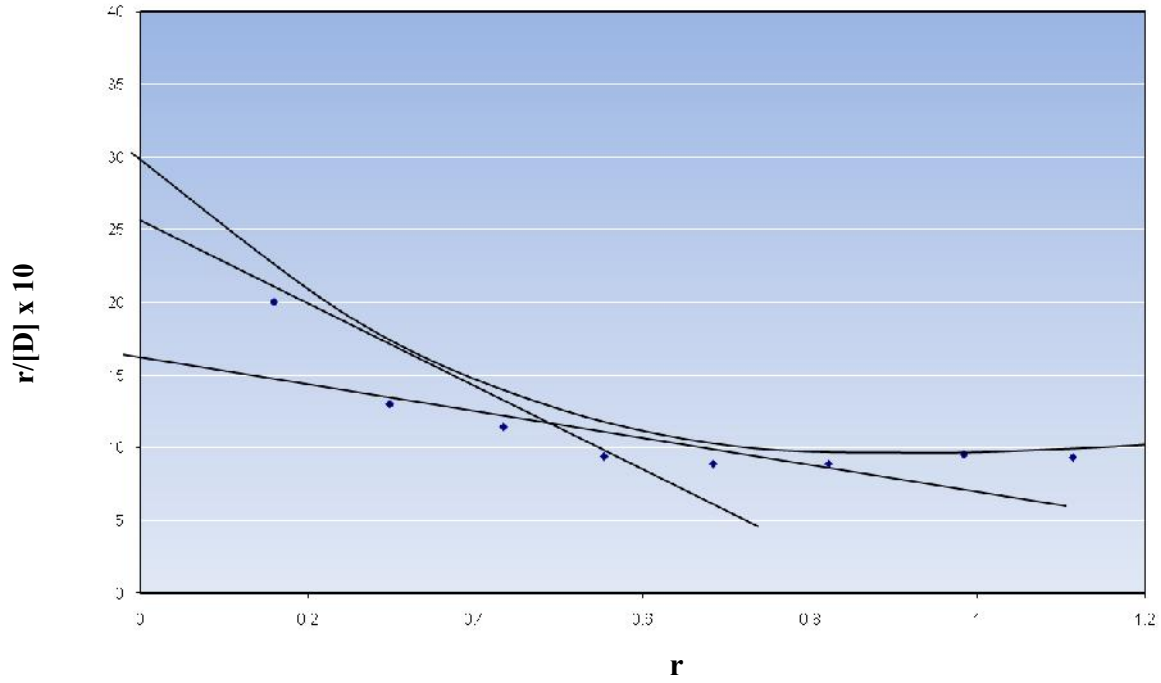


Fig. 7.2.2.5: Scatchard plot for protein binding of glipizide in presence of caffeine (1:1 mixture)

Table 7.2.2.6: Data for scatchard plot for protein binding of glipizide in a 1:1 mixture with theophylline

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound glipizide [A] x 10^5 M	After equilibrium				
	Conc. of glipizide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.09	0.93	0.84	0.168	1.867	18.67
0.23	1.91	1.68	0.336	1.461	14.61
0.37	2.73	2.36	0.472	1.276	12.76
0.59	3.48	2.89	0.578	0.980	9.80
0.76	4.33	3.57	0.714	0.939	9.39
0.95	5.15	4.20	0.840	0.840	8.40
1.05	6.12	5.07	1.014	0.966	9.66
1.18	6.85	5.67	1.134	0.961	9.61
1.25	7.82	6.57	1.314	1.051	10.51
1.28	8.75	7.47	1.494	1.167	11.67

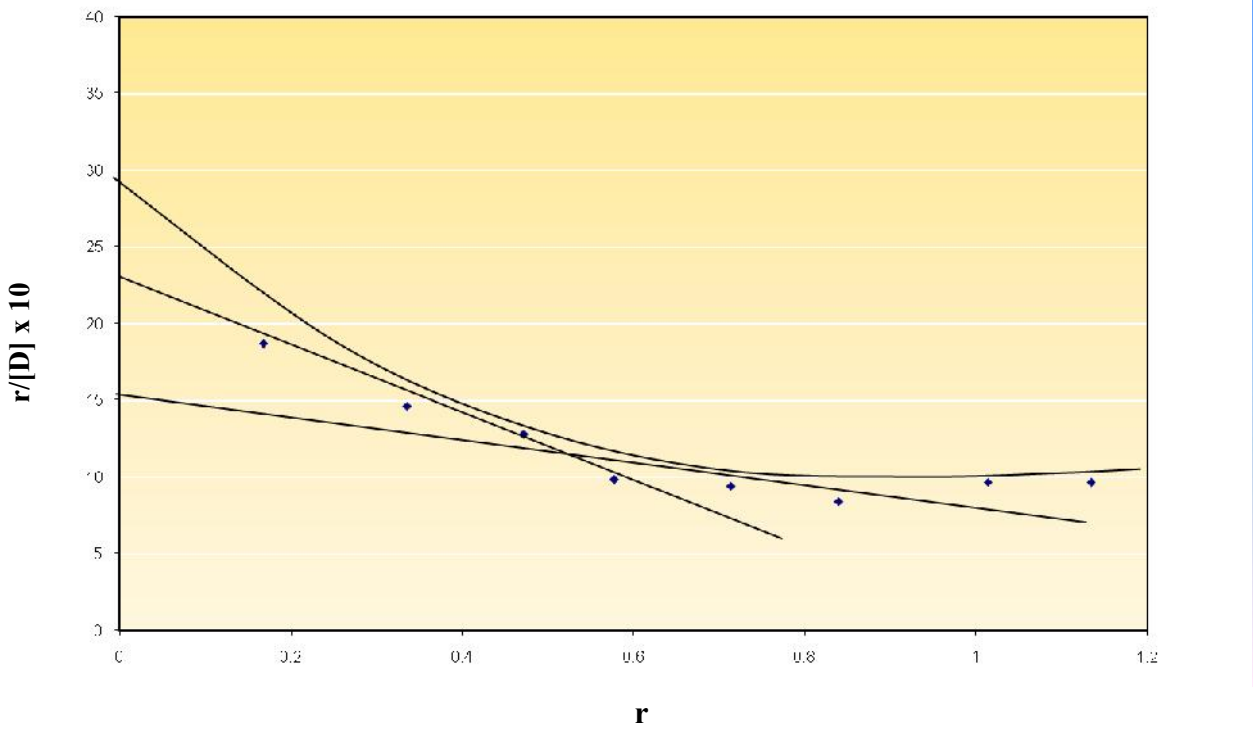


Fig. 7.2.2.6: Scatchard plot for protein binding of glipizide in presence of theophylline (1:1 mixture)

Table 7.2.2.7: Data for scatchard plot for protein binding of glyburide alone

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of	After equilibrium

Unbound glyburide [A] x 10^5 M	Conc. of glyburide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	r = $\frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.09	0.97	0.88	0.176	1.956	19.56
0.18	1.76	1.58	0.316	1.756	17.56
0.25	2.65	2.40	0.480	1.920	19.20
0.35	3.69	3.34	0.668	1.909	19.09
0.49	4.39	3.90	0.780	1.592	15.92
0.62	5.48	4.86	0.972	1.568	15.68
0.72	6.36	5.64	1.128	1.611	16.11
0.75	7.42	6.67	1.334	1.779	17.79
0.83	8.19	7.36	1.472	1.773	17.73
0.87	9.23	8.36	1.672	1.922	19.22

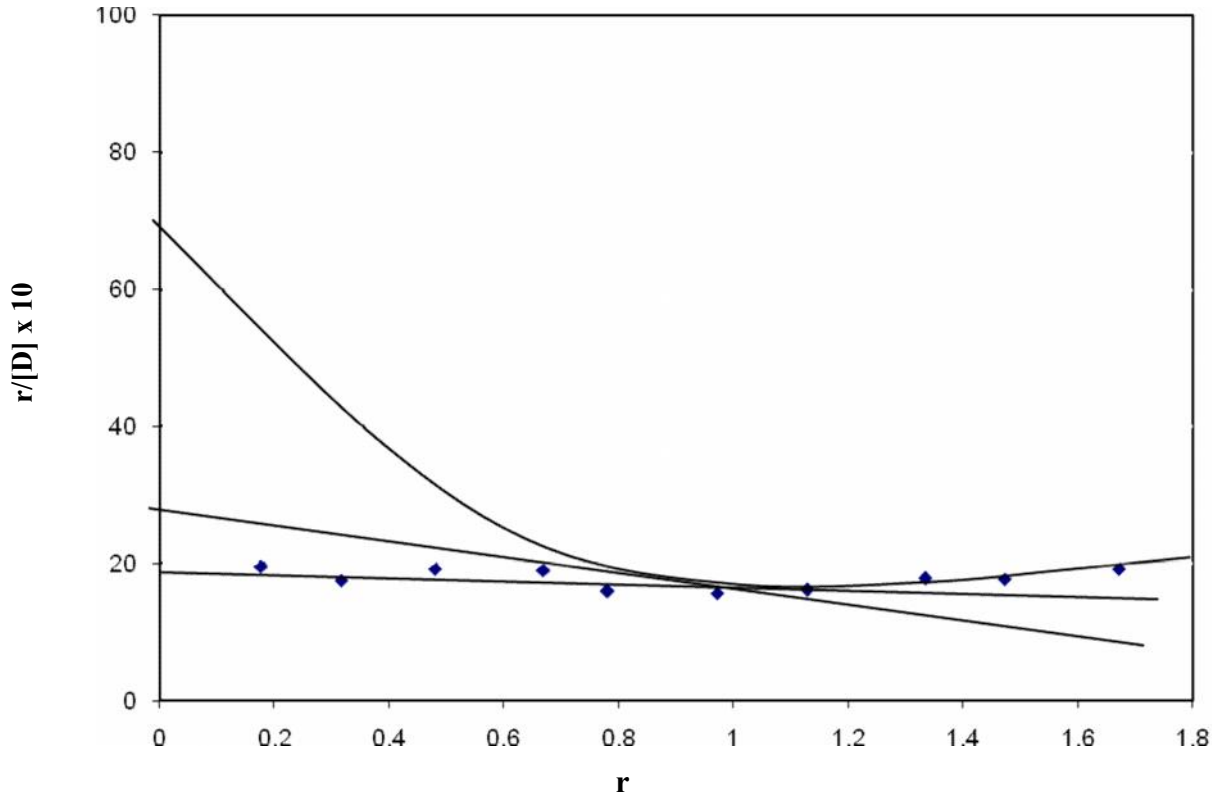


Fig. 7.2.2.7: Scatchard plot for protein binding of glyburide alone

Table 7.2.2.8: Data for scatchard plot for protein binding of glyburide in a 1:1 mixture with caffeine

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound glyburide [A] x 10^5 M	After equilibrium				
	Conc. of glyburide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.11	0.85	0.74	0.148	1.345	13.45
0.25	1.71	1.46	0.292	1.168	11.68
0.39	2.55	2.16	0.432	1.108	11.08
0.62	3.35	2.73	0.546	0.881	8.81
0.76	4.19	3.43	0.686	0.903	9.03
0.94	5.01	4.07	0.814	0.866	8.66
1.02	5.93	4.91	0.982	0.963	9.63
1.21	6.75	5.54	1.108	0.916	9.16
1.27	7.68	6.41	1.282	1.009	10.09
1.30	8.58	7.28	1.456	1.120	11.20

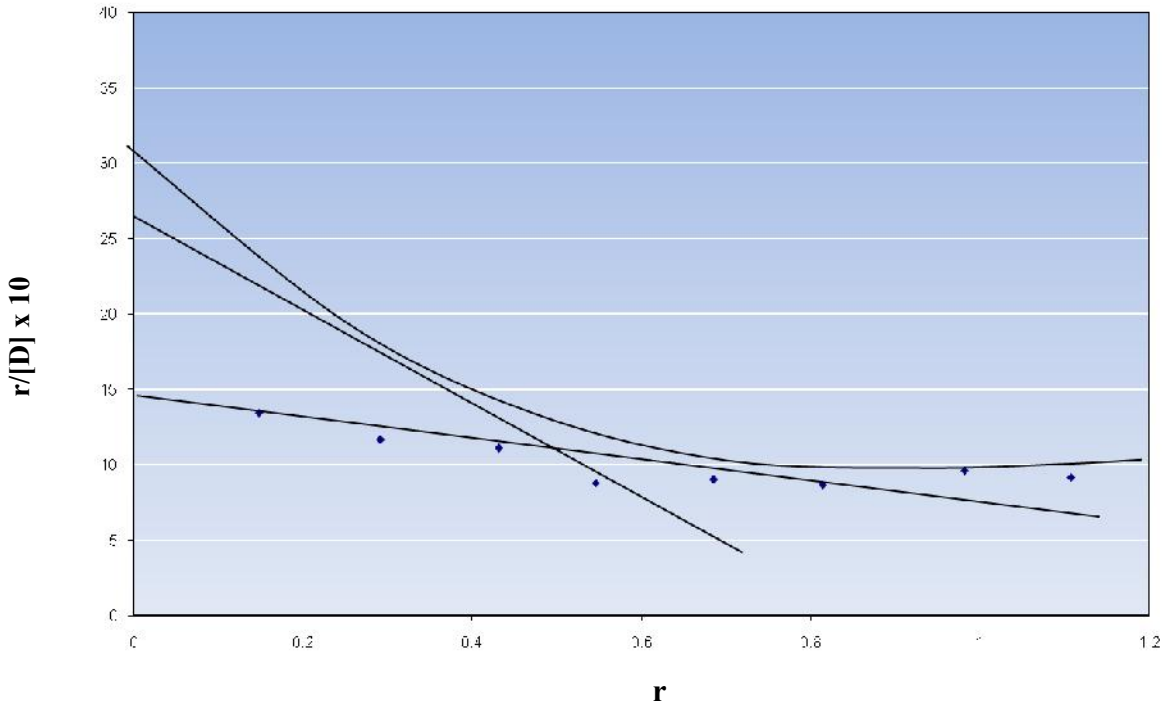


Fig. 7.2.2.8: Scatchard plot for protein binding of glyburide in presence of caffeine (1:1 mixture)

Table 7.2.2.9: Data for scatchard plot for protein binding of glyburide in a 1:1 mixture with theophylline

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound glyburide [A] x 10^5 M	After equilibrium				
	Conc. of glyburide in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.08	0.89	0.81	0.162	2.025	20.25
0.18	1.74	1.56	0.312	1.733	17.33
0.30	2.58	2.28	0.456	1.520	15.20
0.58	3.37	2.79	0.558	0.962	9.62
0.68	4.21	3.53	0.706	1.038	10.38
0.84	5.03	4.19	0.838	0.998	9.98
0.91	5.93	5.02	1.004	1.103	11.03
1.11	6.79	5.68	1.136	1.023	10.23
1.23	7.71	6.48	1.296	1.054	10.54
1.25	8.61	7.36	1.472	1.178	11.78

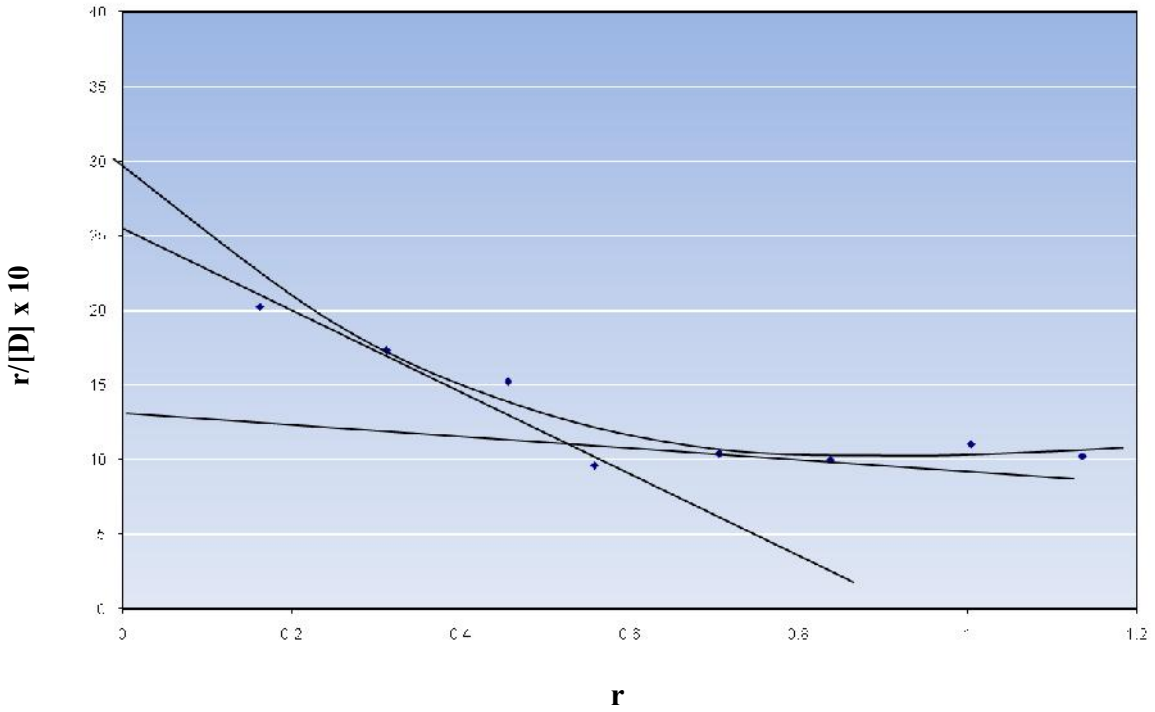


Fig. 7.2.2.9: Scatchard plot for protein binding of glyburide in presence of theophylline (1:1 mixture)

Table 7.2.2.10: Data for scatchard plot for protein binding of metformin alone

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound metformin [A] x 10^5 M	After equilibrium				
	Conc. of metformin in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	r = $\frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.06	1.07	1.01	0.202	3.880	33.67
0.12	1.88	1.76	0.352	2.933	29.33
0.13	2.38	2.25	0.450	3.462	34.62
0.23	3.71	3.48	0.696	3.026	30.26
0.41	4.63	4.22	0.844	2.059	20.59
0.46	5.54	5.08	1.016	2.209	22.09
0.56	6.41	5.85	1.170	2.089	20.89
0.63	7.35	6.72	1.344	2.133	21.33
0.72	8.24	7.52	1.504	2.089	20.89
0.77	9.34	8.57	1.714	2.226	22.26

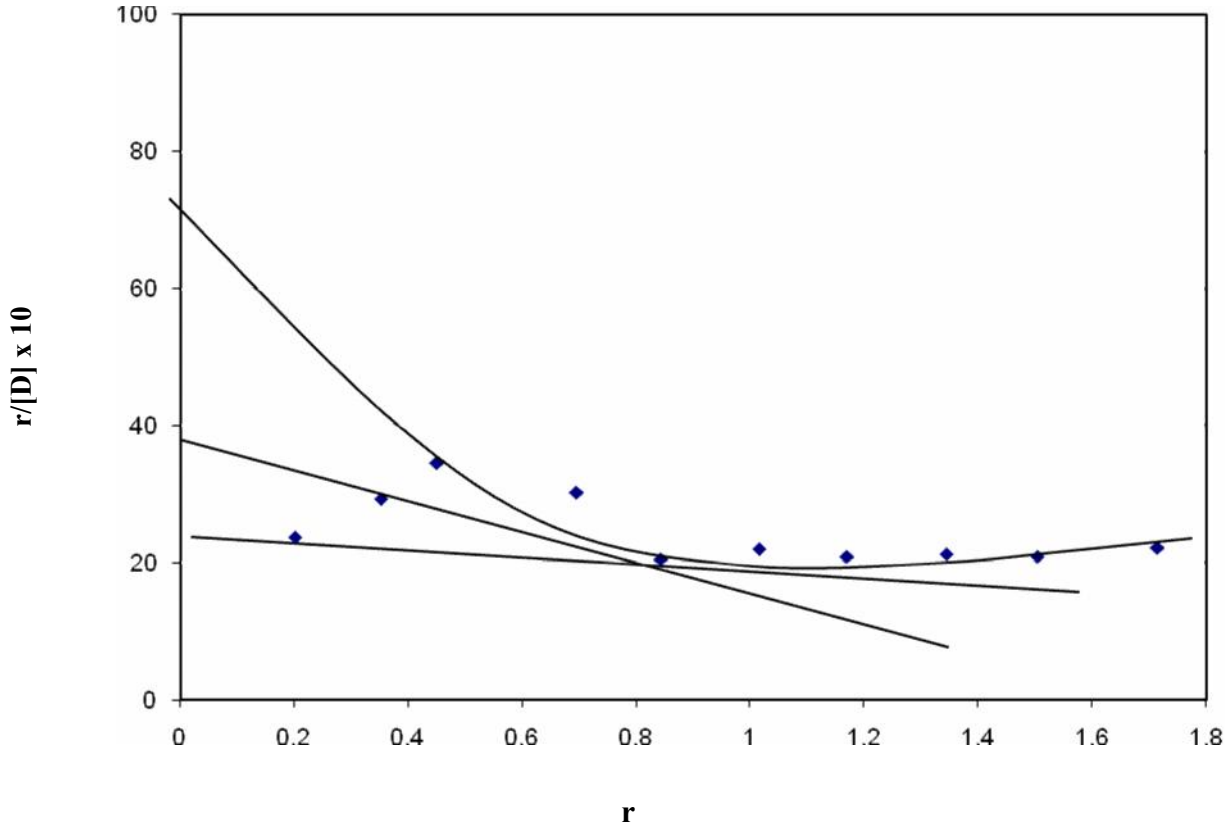


Fig. 7.2.2.10: Scatchard plot for protein binding of metformin alone

Table 7.2.2.11: Data for scatchard plot for protein binding of metformin in a 1:1 mixture with caffeine

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound metformin [A] x 10^5 M	After equilibrium				
	Conc. of metformin in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	r = $\frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.13	1.03	0.90	0.180	1.385	13.85
0.29	1.77	1.48	0.296	1.021	10.21
0.45	2.62	2.17	0.434	0.964	9.64
0.69	3.39	2.70	0.540	0.783	7.83
0.88	4.23	3.35	0.670	0.7614	7.61
0.99	4.93	3.94	0.788	0.796	7.96
1.11	5.78	4.67	0.934	0.841	8.41
1.33	6.65	5.32	1.064	0.800	8.00
1.51	7.53	6.02	1.204	0.797	7.97
1.71	8.49	6.78	1.356	0.793	7.93

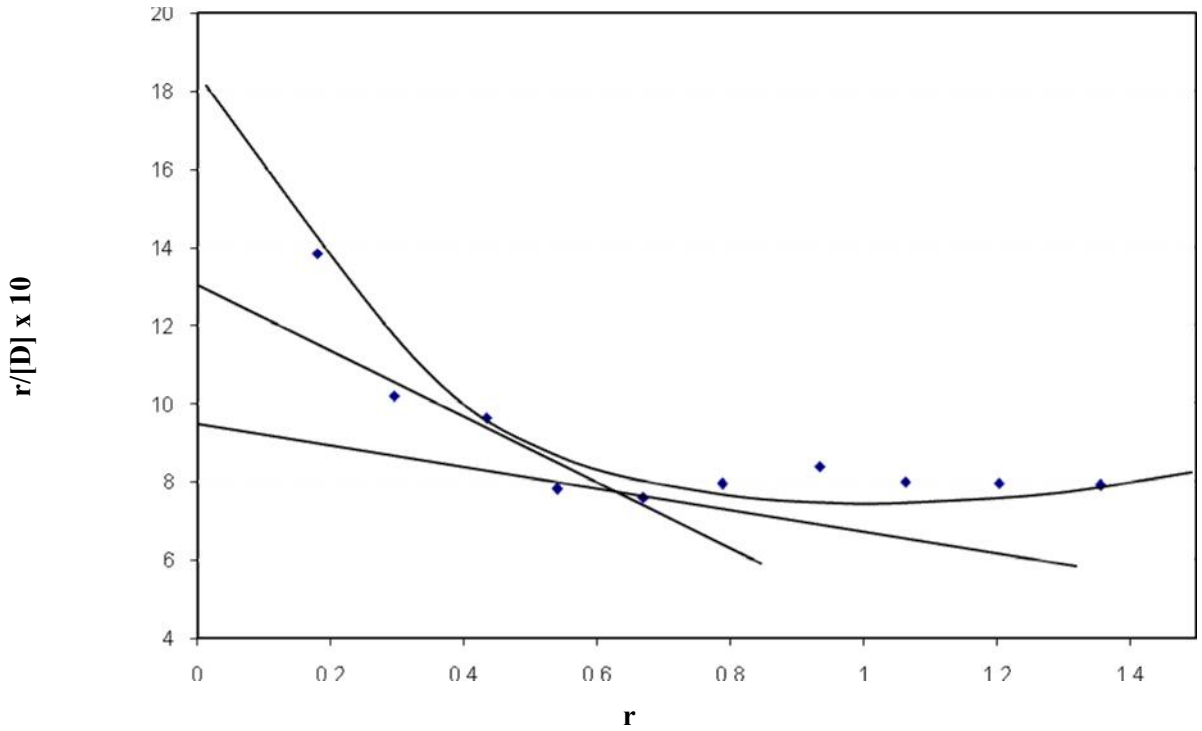


Fig. 7.2.2.11: Scatchard plot for protein binding of metformin in presence of caffeine (1:1 mixture)

Table 7.2.2.12: Data for scatchard plot for protein binding of metformin in a 1:1 mixture with theophylline

Here, Molar conc. of B.S.A = 5 x 10^{-5} **M**

Conc. of Unbound theophylline [A] x 10^5 M	After equilibrium				
	Conc. of theophylline in plasma, [B] x 10^5 M	{[B]-[A]} x 10^5 M	$r = \frac{[B]-[A]}{5x10^{-5}}$	r/[D]=r/[A]	r/[D] x10
0.11	0.97	0.86	0.172	1.564	15.64
0.29	1.82	1.53	0.306	1.055	10.55
0.45	2.57	2.12	0.424	0.942	9.42
0.63	3.28	2.65	0.530	0.841	8.41
0.78	4.18	3.34	0.680	0.872	8.72
0.95	4.93	3.98	0.796	0.838	8.38
1.10	5.95	4.85	0.970	0.882	8.82
1.35	6.63	5.28	1.056	0.782	7.82
1.47	7.47	6.02	1.204	0.819	8.19
1.73	8.32	6.59	1.318	0.762	7.62

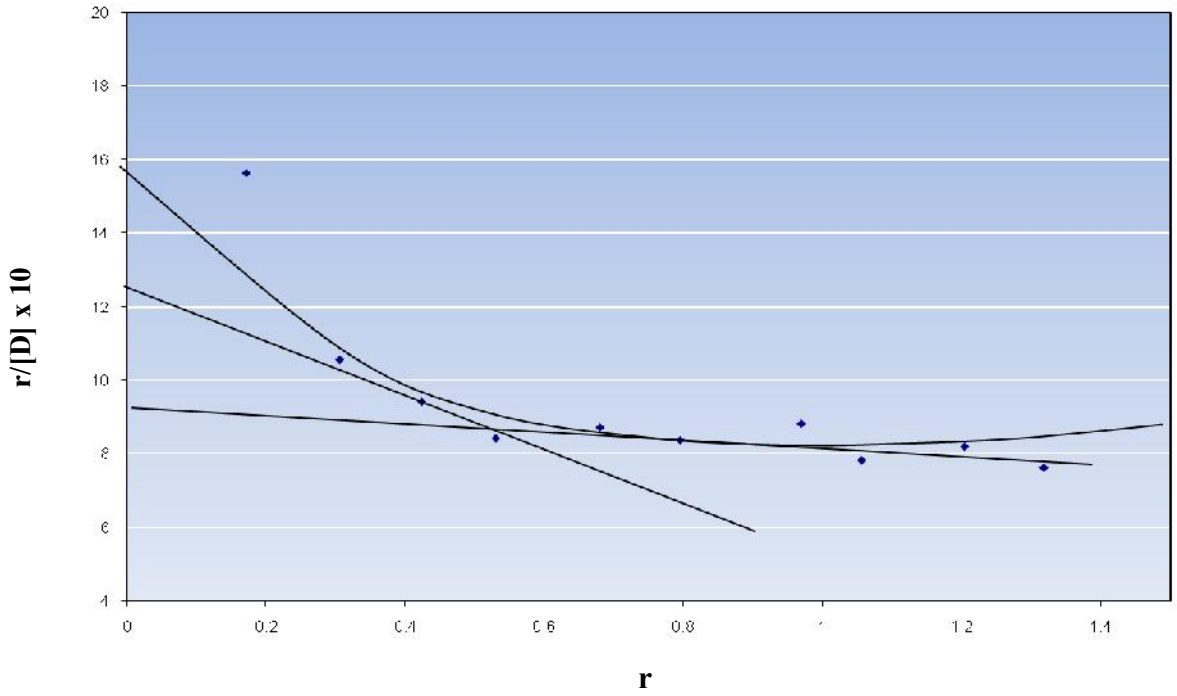


Fig. 7.2.2.12: Scatchard plot for protein binding of metformin in presence of theophylline (1:1 mixture)

The table given below shows a comparative picture of number of protein binding sites and associated affinity constants of caffeine & theophylline and their 1:1 mixtures with gliclazide, glipizide, glyburide and metformin.

The values for number of binding sites and affinity constants

System	Class I binding sites			Class II binding sites		
	$n_1 K_1$	$K_1 M^{-1}$	n_1	$n_2 K_1$	$K_2 M^{-1}$	n_2
Gliclazide alone	13.78	17.35	0.73	4.68	3.67	1.35
Gliclazide – Caffeine System	2.52	2.41	1.04	1.11	0.17	6.25
Gliclazide – Theophylline System	2.22	2.17	1.01	1.03	0.14	4.76
Glipizide alone	14.12	18.21	0.79	4.93	3.76	1.44
Glipizide - Caffeine System	2.48	2.36	1.04	1.08	0.17	5.78
Glipizide – Theophylline System	2.19	2.15	1.00	1.01	0.13	4.65
Glyburide alone	13.92	17.85	0.77	4.78	3.71	1.39
Glyburide – Caffeine System	2.41	2.33	1.02	1.06	0.15	5.69
Glyburide – Theophylline System	2.26	2.21	1.02	1.04	0.16	5.23
Metformin alone	12.66	16.82	0.73	3.92	3.23	1.32
Metformin – Caffeine System	2.12	0.37	3.19	0.98	0.53	2.21
Metformin – Theophylline System	2.10	0.37	3.13	0.95	0.52	2.03

From this table, it is found that caffeine and theophylline cause a decrease in associated affinity constants but increases the number of binding sites in the mixed condition with gliclazide, glipizide and glyburide. They lower the associated affinity constants of metformin for both class I and class II binding sites. It increases the number of binding sites largely for class I binding sites and shortly for class II binding sites of metformin.

Due to decrease in affinity to plasma protein binding, there is an increase in the apparent volume of distribution (V_d) of the drug because affinity of a drug for protein binding is a limiting factor of the distribution of the drug**.**

In other words due to increase in affinity, the V_d is decreased. V_d can be calculated by dividing the amount of drug in the body by the plasma concentration and is expressed as,

$$V_d = \frac{\text{Amount of drug in body (A)}}{\text{Conc. of drug in plasma}}$$

Since the apparent volume of distribution increases in both the cases it is a matter of great concern that a concurrent application of caffeine theophylline with gliclazide, glipizide, glyburide & metformin should be considered only after thorough in-vivo studies.

The present study indicates that caffeine and theophylline decrease the percentage of protein binding of gliclazide, glipizide, glyburide and metformin i.e., increase the free plasma concentration of gliclazide, glipizide, glyburide and metformin which may give toxic effects. Therefore, we infer that the combination therapy of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin

may not be safe. Because such type of combination therapy may change the pharmacokinetic and pharmacodynamic properties of gliclazide, glipizide, glyburide and metformin.

From this study, we can infer that the concurrent therapy of caffeine and theophylline with either gliclazide or glipizide or glyburide or metformin may increase hepatic first pass effect. Thus renal clearance of the drug and its therapy may alter the half life ($t_{1/2}$) of the drug.This can be understood from the relation between clearance and half life.

$$t_{1/2} = \frac{0.693 \times \text{volume of distribution } (V_d)}{\text{Clearance}}$$

Thus due to increase in concentration of free drug or decrease in affinity for protein, the pharmacological effects of drug will increase if the concentration of the drug remains within MEC and MTC, through $t_{1/2}$ is shortened. But if the concentration exceeds the MTC then toxicity appears. [78]

Diminished protein binding is not always accompanied by increased effect. Sulfadimethoxine displaces tolbutamide from its plasma binding and increases its V_d. But the hypoglycemic effect of tolbutamide is not increased**.**

In the study of interaction between oral anti-diabetic drugs and other agents, most of the agents used did not interact strongly with the oral anti-diabetic agents. [86] [87] [88] But in the present study, caffeine and theophylline decreases the percentage of protein binding of metformin. This will increase the free plasma concentration of the metformin which may affect pharmacokinetic and pharmacodynamic activities of drug. Such a change in the pharmacokinetic and pharmacodynamic behavior might not be beneficial to the patients.

In-vitro studies sometimes contradict with *in-vivo* studies by the same method and in the same experimental models.In a recent study, it was found that in the *in-vitro* study ibuprofen decrease the protein binding of glipizide and gliclazide but when studied *in-vivo* in rat model the plasma concentration of glipizide and gliclazide did not changed significantly. [77]

7.3 IN-VIVO EFFECTS OF CAFFEINE AND THEOPHYLLINE ON PLASMA CONCENTRATION OF GLICLAZIDE, GLIPIZIDE, GLYBURIDE AND METFORMIN IN RAT

The in-vivo effects of caffeine and theophylline on plasma concentration of gliclazide, glipizide, glyburide and metformin have been studied by observing the change in plasma concentration of gliclazide, glipizide, glyburide and metformin in rat.

In this study, the plasma concentration of each gliclazide, glipizide, glyburide and metformin were determined by UV method after oral single administration of gliclazide, glipizide, glyburide and metformin alone and with caffeine & theophylline in rat. [82]

7.3.1 Determination of plasma concentration after single oral administration of gliclazide alone and its mixture with caffeine and theophylline in rat

From the table 7.3.1.1 and the figure 7.3.1.1, it has been found that the peak plasma concentration of gliclazide was 100 ng/ml, which was obtained after 1 hour of oral administration of gliclazide alone. But it has been observed from the table 7.3.1.2 and the same figure 7.3.1.1 that the oral concomitant administration of gliclazide and caffeine did not make a noticeable change in plasma concentration of gliclazide. In this case the peak plasma concentration of gliclazide was 101 ng/ml. And, it has been observed from the table 7.3.1.3 and the same figure 7.3.1.1 that the oral concomitant administration of gliclazide and theophylline did not make a noticeable change in plasma concentration of gliclazide. In this case the peak plasma concentration of gliclazide was 103 ng/ml.

Table 7.3.1.1: Plasma concentration of gliclazide after single oral administration of gliclazide alone

Time (hour)	Absorbance				Plasma conc. of gliclazide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.017	0.018	0.016	0.017	46	49	44	47	47
1.0	0.035	0.036	0.037	0.036	97	100	103	100	100
2.0	0.013	0.014	0.015	0.016	35	37	40	43	39
3.0	0.007	0.006	0.008	0.007	16	14	19	15	16
4.0	0.006	0.005	0.007	0.006	14	11	16	14	14
5.0	0.005	0.004	0.006	0.004	11	8	14	9	11

Table 7.3.1.2: Plasma concentration of gliclazide after single oral administration of gliclazide with caffeine

Time (hour)	Absorbance				Plasma conc. of gliclazide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.020	0.019	0.018	0.020	55	52	50	56	53
1.0	0.037	0.036	0.035	0.037	104	100	97	103	101
2.0	0.017	0.016	0.018	0.019	46	44	49	53	48
3.0	0.006	0.007	0.006	0.005	13	15	13	11	13
4.0	0.005	0.006	0.005	0.004	11	13	11	8	11
5.0	0.004	0.005	0.003	0.003	8	11	6	6	8

Table 7.3.1.3: Plasma concentration of gliclazide after single oral administration of gliclazide with theophylline

Time	Absorbance	Plasma conc. of gliclazide	Avg. plasma

(hour)					(ng/ml)				conc. (ng/ml)
0.5	0.017	0.018	0.019	0.017	45	48	52	46	48
1.0	0.038	0.037	0.036	0.037	105	103	100	103	103
2.0	0.015	0.014	0.013	0.015	41	38	36	41	39
3.0	0.007	0.005	0.006	0.005	19	12	13	11	14
4.0	0.005	0.004	0.005	0.004	11	8	12	8	10
5.0	0.003	0.004	0.003	0.005	6	8	6	11	8

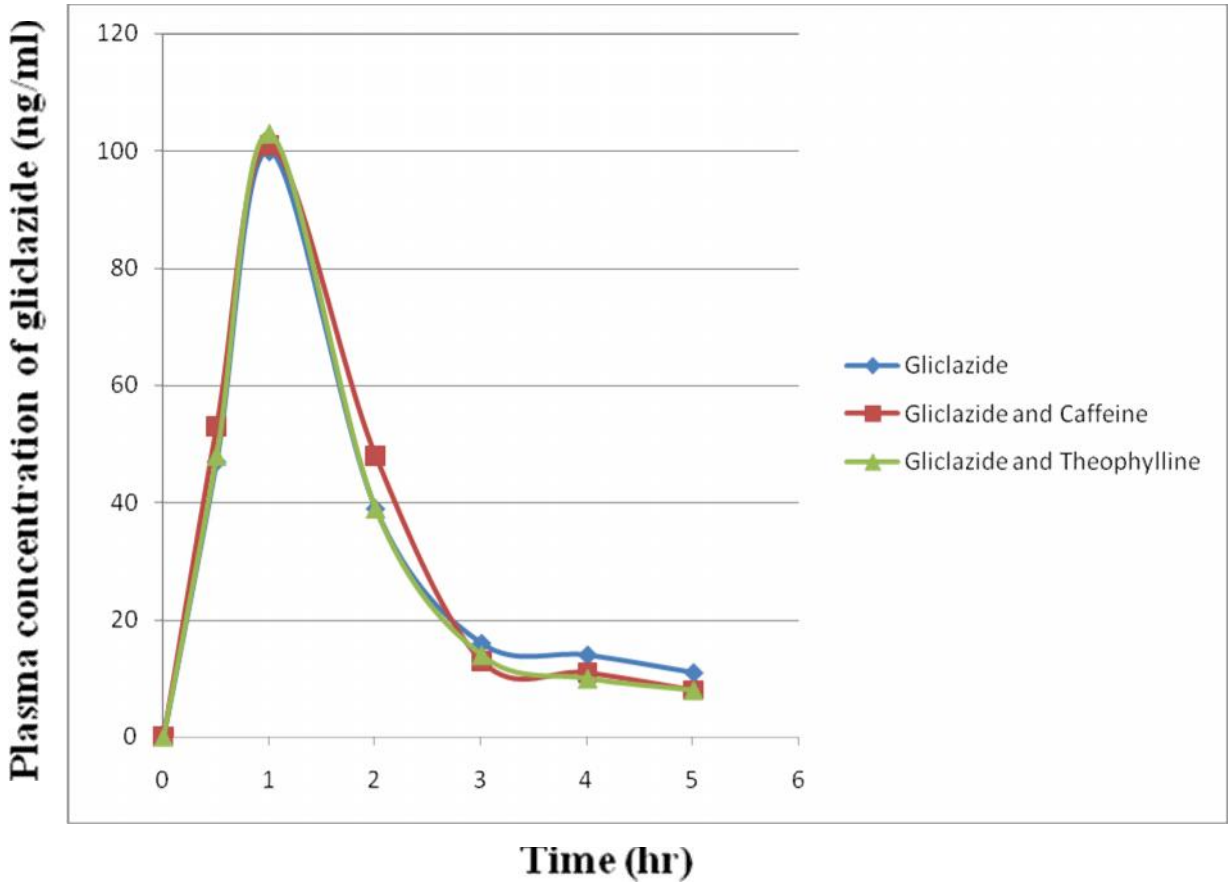


Fig.7.3.1.1: Plasma concentration after single oral administration of gliclazide alone and its mixture with caffeine and theophylline in rat

7.3.2 Determination of plasma concentration after single oral administration of glipizide alone and its mixture with caffeine and theophylline in rat

From the table 7.3.2.1 and the figure 7.3.2.1, it has been found that the peak plasma concentration of glipizide was 99 ng/ml, which was obtained after 1 hour of oral administration of glipizide alone.But it

has been observed from the table 7.3.2.2 and the same figure 7.3.2.1 that the oral concomitant administration of glipizide and caffeine made a significant change in plasma concentration of glipizide. In this case the peak plasma concentration of glipizide was 108 ng/ml, which is significantly greater than that of glipizide when administered alone. This may be due to higher affinity of glipizide for the plasma protein. Again, it has been observed from the table 7.3.2.3 and the same figure 7.3.2.1 that the oral concomitant administration of glipizide and theophylline did not make a noticeable change in plasma concentration of glipizide. In this case the peak plasma concentration of glipizide was 104 ng/ml.

Table 7.3.2.1: Plasma concentration of glipizide after single oral administration of glipizide alone

Time (hour)	Absorbance				Plasma conc. of glipizide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.017	0.019	0.020	0.018	45	50	52	48	49
1.0	0.039	0.041	0.040	0.038	98	102	100	96	99
2.0	0.014	0.015	0.013	0.014	39	40	36	39	39
3.0	0.007	0.008	0.009	0.006	20	22	25	18	21
4.0	0.004	0.006	0.004	0.005	12	16	12	15	14
5.0	0.002	0.003	0.002	0.004	7	8	7	12	9

Table 7.3.2.2: Plasma concentration of glipizide after single oral administration of glipizide with caffeine

Time (hour)	Absorbance				Plasma conc. of glipizide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.021	0.019	0.017	0.020	55	50	45	53	51
1.0	0.042	0.041	0.045	0.043	105	102	115	109	108
2.0	0.013	0.015	0.014	0.015	36	40	39	40	39
3.0	0.007	0.008	0.009	0.006	20	22	25	18	21
4.0	0.005	0.004	0.006	0.005	15	12	18	15	15
5.0	0.002	0.003	0.002	0.004	6	8	7	12	8

Table 7.3.2.3: Plasma concentration of glipizide after single oral administration of glipizide with theophylline

Time (hour)	Absorbance				Plasma conc. of glipizide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.018	0.017	0.021	0.019	48	46	55	50	50
1.0	0.040	0.041	0.042	0.043	100	102	105	109	104
2.0	0.013	0.015	0.016	0.014	36	40	42	39	39
3.0	0.006	0.009	0.008	0.006	18	25	22	18	21
4.0	0.005	0.006	0.007	0.005	15	16	20	15	17
5.0	0.003	0.004	0.002	0.004	8	7	7	12	9

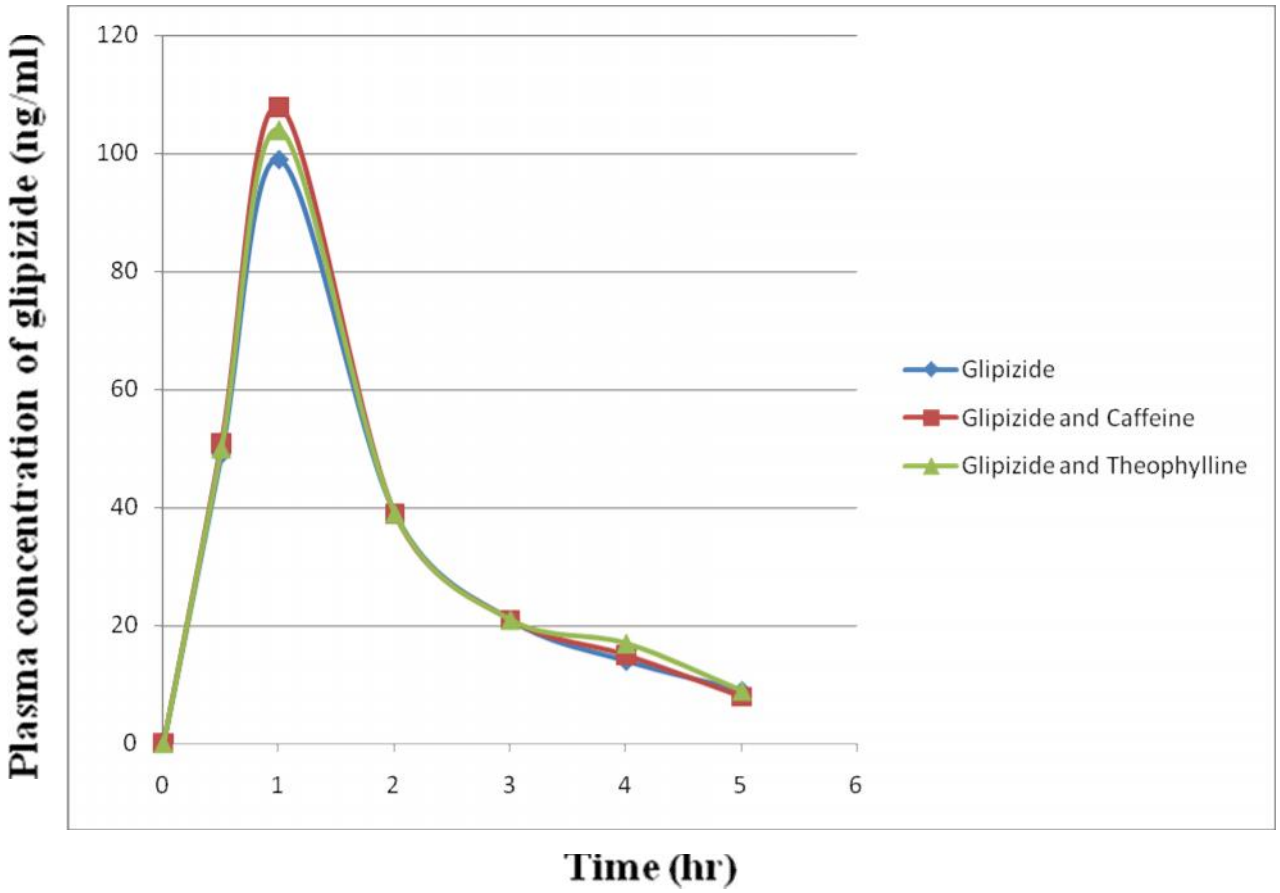


Fig.7.3.2.1: Plasma concentration after single oral administration of glipizide alone and its mixture with caffeine and theophylline in rat

7.3.3 Determination of plasma concentration after single oral administration of glyburide alone and its mixture with caffeine and theophylline in rat

From the table 7.3.3.1 and the figure 7.3.3.1, it has been found that the peak plasma concentration of glyburide was 96 ng/ml, which was obtained after 1 hour of oral administration of glyburide alone.But it has been observed from the table 7.3.3.2 and the same figure 7.3.3.1 that the oral concomitant administration of glyburide and caffeine did not make a noticeable change in plasma concentration of glyburide. In this case the peak plasma concentration of glyburide was 98 ng/ml. Again, it has been observed from the table 7.3.3.3 and the same figure 7.3.3.1 that the oral concomitant administration of glyburide and theophylline did not make a noticeable change in plasma concentration of glyburide. In this case the peak plasma concentration of glyburide was 97 ng/ml.

Table 7.3.3.1: Plasma concentration of glyburide after single oral administration alone

Time (hour)	Absorbance				Plasma conc. of glyburide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.017	0.016	0.015	0.017	54	51	48	53	52
1.0	0.031	0.030	0.032	0.033	94	91	98	100	96
2.0	0.016	0.018	0.017	0.016	51	56	53	51	53
3.0	0.007	0.006	0.008	0.007	28	23	31	28	28
4.0	0.005	0.004	0.006	0.005	20	16	23	20	20
5.0	0.003	0.002	0.004	0.003	12	8	16	12	12

Table 7.3.3.2: Plasma concentration of glyburide after single oral administration of glyburide with caffeine

Time (hour)	Absorbance				Plasma conc. of glyburide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.018	0.016	0.017	0.015	57	51	54	48	53
1.0	0.033	0.031	0.033	0.032	100	94	100	98	98
2.0	0.015	0.017	0.017	0.016	45	52	53	49	50
3.0	0.006	0.007	0.008	0.007	23	26	31	27	27
4.0	0.005	0.004	0.007	0.005	20	16	27	20	21
5.0	0.003	0.002	0.002	0.003	12	8	8	12	10

Table 7.3.3.3: Plasma concentration of glyburide after single oral administration of glyburide with theophylline

Time (hour)	Absorbance				Plasma conc. of glyburide (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.016	0.018	0.015	0.017	51	56	48	53	52
1.0	0.032	0.032	0.031	0.033	97	97	94	100	97
2.0	0.015	0.017	0.017	0.018	50	56	53	55	54
3.0	0.007	0.006	0.007	0.006	28	23	28	24	26
4.0	0.004	0.005	0.006	0.007	16	20	23	28	22
5.0	0.002	0.003	0.004	0.005	8	12	16	20	14

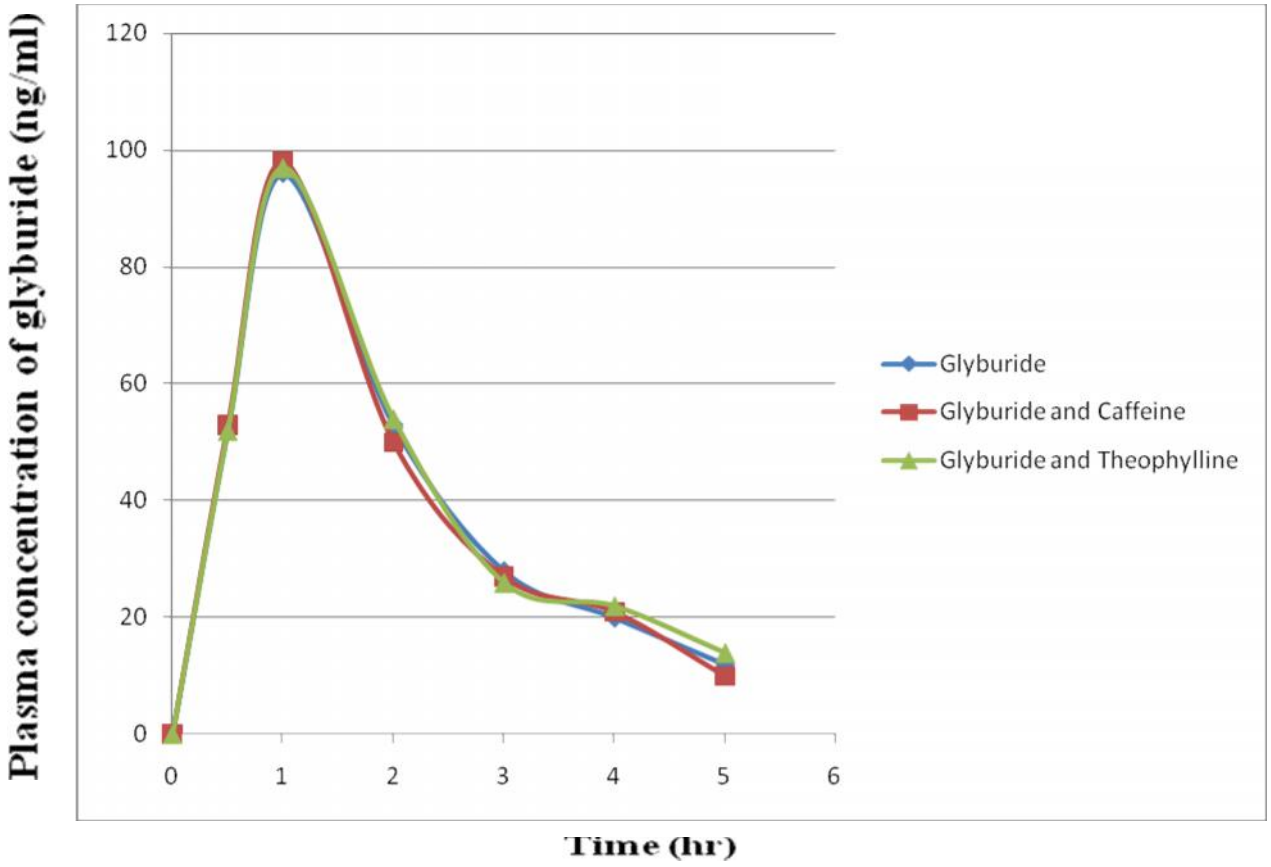


Fig. 7.3.3.1: Plasma concentration after single oral administration of glyburide alone and its mixture with caffeine and theophylline in rat

7.3.4 Determination of plasma concentration after single oral administration of metformin alone and its mixture with caffeine and theophylline in rat

From the table 7.3.4.1 and the figure 7.3.4.1, it has been found that the peak plasma concentration of metformin was 99 ng/ml, which was obtained after 1 hour of oral administration of metformin alone.But it has been observed from the table 7.3.4.2 and the same figure 7.3.4.1 that the oral concomitant administration of metformin and caffeine made a noticeable change in plasma concentration of metformin. In this case the peak plasma concentration of metformin was 106 ng/ml, which is significantly greater than that of metformin when administered alone. This may be due to higher affinity of caffeine for the plasma protein. Again, it has been observed from the table 7.3.4.3 and the same figure 7.3.4.1 that the oral concomitant administration of metformin and theophylline also made a noticeable change in plasma concentration of metformin. In this case the peak plasma concentration of metformin was 104 ng/ml, which is significantly greater than that of metformin when administered alone. This may be due to higher affinity of theophylline for the plasma protein.

Table7.3.4.1: Plasma concentration of metformin after single oral administration of metformin alone

Time (hour)	Absorbance				Plasma conc. of metformin (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.017	0.018	0.019	0.018	38	40	42	41	40
1.0	0.043	0.042	0.041	0.044	100	98	95	103	99
2.0	0.014	0.015	0.014	0.015	33	35	32	35	34
3.0	0.006	0.007	0.007	0.008	16	18	17	22	18
4.0	0.004	0.005	0.006	0.004	12	13	16	12	13
5.0	0.003	0.002	0.003	0.002	8	6	8	6	7

Table 7.3.4.2 Plasma concentration of metformin after single oral administration of metformin with caffeine

Time (hour)	Absorbance				Plasma conc. of metformin (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.020	0.018	0.019	0.018	45	40	42	41	42
1.0	0.048	0.046	0.043	0.044	112	108	101	103	106
2.0	0.015	0.013	0.014	0.015	36	35	32	35	35
3.0	0.006	0.008	0.007	0.008	16	20	18	22	19
4.0	0.005	0.004	0.006	0.004	13	12	16	12	13
5.0	0.004	0.002	0.003	0.002	12	6	8	6	8

Table7.3.4.3: Plasma concentration of metformin after single oral administration of metformin with theophylline

Time (hour)	Absorbance				Plasma conc. of metformin (ng/ml)				Avg. plasma conc. (ng/ml)
0.5	0.019	0.018	0.017	0.018	42	40	38	41	40
1.0	0.044	0.045	0.046	0.044	102	104	106	103	104
2.0	0.016	0.015	0.014	0.015	36	34	33	35	35
3.0	0.008	0.007	0.008	0.007	22	18	22	18	20
4.0	0.004	0.005	0.006	0.006	12	13	16	16	14
5.0	0.002	0.002	0.005	0.002	8	8	13	6	9

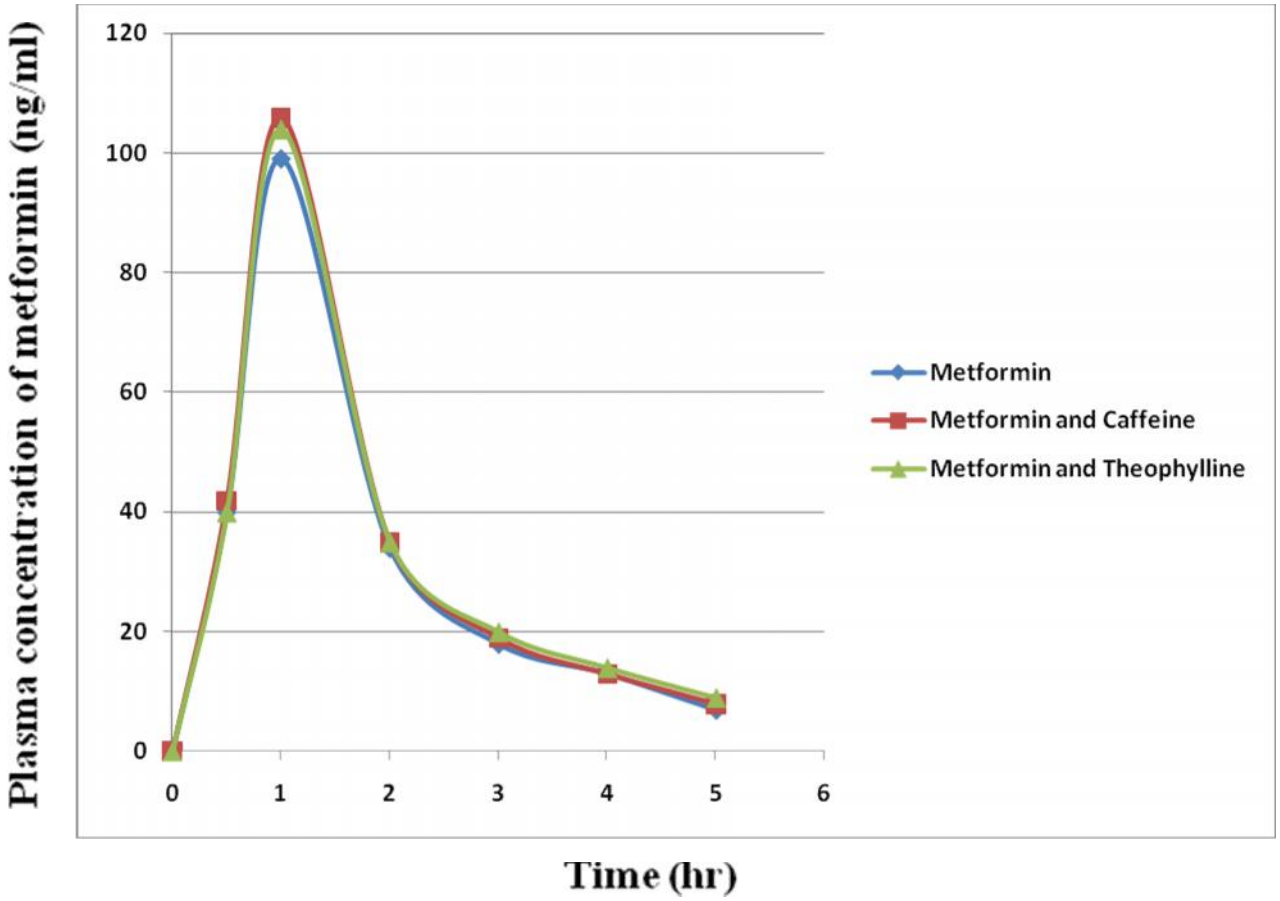


Fig.7.3.4.1: Plasma concentration after single oral administration of metformin alone and its mixture with caffeine and theophylline in rat

From the above tables and figures for each system, it has been observed that the oral concomitant administration of caffeine and theophylline each with glipizide and metformin made a noticeable change in plasma concentration of glipizide and metformin.This may be due to higher affinity of caffeine and theophylline for the plasma protein.On the other hand, it has been also observed that the oral concomitant administration of caffeine and theophylline each with gliclazide and glyburide did not make a noticeable change in plasma concentration of gliclazide and glyburide.This may be due to lower affinity of caffeine and theophylline for the plasma protein.

The study indicates that caffeine and theophylline make a noticeable change in plasma concentration of glipizide and metformin but do not make a noticeable change in plasma concentration of gliclazide and glyburide .

A competitive inhibition of the binding to plasma proteins in the mixed condition can lead hazardous consequences i.e, a competitive inhibition of the binding to plasma protein by caffeine and theophylline increases the plasma concentration of glipizide and metformin.Such interactions of the drugs that affect the binding of plasma protein and subsequently that change the plasma concentration of the drugs are very vital to be given priority before formulating drug therapy. Since, drug displaced from plasma protein will redistribute into its full potential volume of distribution, the concentration of free drug in plasma and tissues after re-distribution may be increased slightly. But this may changed the pharmacokinetics properties of the drug and thereby may affect its pharmacological and toxic effects. **[83]**

In the study of interaction between oral anti-diabetic drugs and other agents, most of the agents used did not interact strongly with the oral anti-diabetic sgents but in the present study, caffeine and theophylline increase the plasma concentration of oral anti-diabetic drugs, particularly glipizide and metformin. This is due to competitive protein binding between CNS stimulant molecules and oral anti-diabetic drugs. **[85] [86] [90]**

Coffee consumption has been extensively studied in relation to various diseases, but not until recently has it been examined in relation to risk of type 2 diabetes. A study shows that higher coffee consumption was associated with subsequently lower risk of type 2 diabetes. But in this study found a significant change in plasma concentration of oral anti-diabetic drugs when glipizide and metformin administered concurrently with caffeine and theophylline. Therefore, the diabetic patients who are taking metformin should avoid excessive consumption of coffee or tea that contained caffeine and care and monitoring might be necessary as well. **[84]**

7.4 IN-VIVO EFFECT OF CAFFEINE AND THEOPHYLLINE ON THE HYPOGLYCEMIC ACTIVITY OF GLICLAZIDE, GLIPIZIDE, GLYBURIDE AND METFORMIN IN RAT

To observe the effect of caffeine and theophylline on hypoglycemic activity of gliclazide, glipizide, glyburide and metformin, healthy rats weighing about 250 ± 25 g were used. The blood sugar levels of animals were measured after administration of a drug (gliclazide, glipizide, glyburide and metformin) alone and in combination (with caffeine or theophylline). The blood sugar levels were estimated in two stages; firstly, after 2 weeks and secondly, after 4 weeks of the administration of drug. [89] [91] [92] [93]

The treated rats showed values of 253± 3.1 mg/100 ml and 304 ± 2.7 mg/100 ml of sugar respectively after 2 week and 4 week of alloxan application. The affected rats showed values of 263 ± 25 g and 272 ± 25 g of weight respectively after 2 week and 4 week of alloxan application.

7.4.1 Observation of blood sugar level in rats after 2 week of the administration of drugs

From the table 7.4.1.1 and figure 7.4.1.1, normal blood sugar level was 72 ± 2.9 mg/100 ml. After induction of diabetes by administration of alloxan, the blood sugar level raised to 253 ± 3.1 mg/100 ml. At this stage, when gliclazide alone was administered, the blood sugar level decreased to 136 ± 5.1 mg/100 ml and when gliclazide was administered with caffeine, the blood sugar level decreased to 122 ± 4.5 mg/100 ml and when gliclazide was administered with theophylline, the blood sugar level decreased to 129 ± 5.4 mg/100 ml.

When glipizide alone was administered, the blood sugar level decreased to 144 ± 5.5 mg/100 ml and when glipizide was administered with caffeine, the blood sugar level decreased to 124 ± 4.6 mg/100 ml and when glipizide was administered with theophylline, the blood sugar level decreased to 135 ± 6.1 mg/100 ml.

When glyburide alone was administered, the blood sugar level decreased to 140 ± 5.3 mg/100 ml and when glyburide was administered with caffeine the blood sugar level decreased to 118 ± 4.3 mg/100 ml and when glyburide was administered with theophylline the blood sugar level decreased to 115 ± 4.8 mg/100 ml.

On the other hand, when metformin alone was administered, the blood sugar level decreased to 128 ± 5.6 mg/100ml and when metformin was administered with caffeine the blood sugar level decreased to 116 ± 5.0 mg/100 ml and when metformin was administered with theophylline the blood sugar level decreased to 107 ± 5.1 mg/100 ml after two weeks of treatment.

Table 7.4.1.1: Data for blood sugar level after 2 weeks in different groups of experimental rats

Groups	Absorbance	Molar Absorbity (A)	Conc. of sugar mg/100 ml blood	Average conc. of blood sugar mg/100 ml blood
Group I (control)	0.042	36.2	74	72
	0.043	36.7	75	
	0.041	35.7	73	
	0.040	34.8	70	
	0.038	33.2	68	
Group II (Alloxan)	0.149	131.2	263	253
	0.146	126.7	255	
	0.144	125.7	253	
	0.140	122.2	246	
	0.142	123.7	248	
Group III (Gliclazide)	0.082	69.1	140	136
	0.078	67.7	137	
	0.076	66.2	136	
	0.075	65.2	135	
	0.074	64.2	132	
Group IVA (Caffeine+Gliclazide)	0.073	63.2	128	122
	0.071	61.2	125	
	0.069	60.5	124	
	0.068	58.2	118	
	0.067	57.2	115	
Group IVB (Theophylline+Gliclazide)	0.077	68.2	136	129
	0.072	63.3	130	
	0.068	59.6	123	
	0.069	60.8	125	
	0.073	63.4	129	
Group V (Glipizide)	0.076	66.4	136	144
	0.080	69.6	140	
	0.082	71.2	146	
	0.086	73.4	150	
	0.084	72.4	148	
Group VIA (Caffeine+Glipizide)	0.075	65.3	130	124
	0.073	63.3	127	
	0.071	61.8	125	
	0.070	59.5	121	
	0.069	59.0	118	
Group VI (Theophylline+Glipizide)	0.081	68.6	138	135
	0.078	67.7	137	
	0.077	68.2	136	
	0.075	65.2	135	
	0.074	64.4	131	

Groups	Absorbance	Molar Absorbity (A)	Conc. of sugar mg/100 ml blood	Average conc. of blood sugar mg/100 ml blood
Group VII (Gliyburide)	0.074	64.2	132	140
	0.078	67.5	136	
	0.081	70.1	144	
	0.082	71.5	146	
	0.081	69.6	142	
Group VIIIA (Caffeine+Glyburide)	0.072	62.4	126	118
	0.071	61.8	125	
	0.068	58.6	116	
	0.067	57.4	113	
	0.066	56.5	111	
Group VIIIB (Theophylline+Glyburide)	0.067	58.8	118	115
	0.066	57.2	122	
	0.062	53.6	116	
	0.060	51.8	112	
	0.058	50.1	109	
Group IX (Metformin)	0.076	65.2	134	128
	0.074	63.7	128	
	0.072	62.7	128	
	0.071	62.2	126	
	0.069	61.3	124	
Group XA (Caffeine+Metformin)	0.070	59.2	120	116
	0.068	58.7	118	
	0.066	57.5	117	
	0.065	56.7	115	
	0.063	54.8	110	
Group XB (Theophylline+Metformin)	0.062	52.5	114	107
	0.058	50.1	109	
	0.056	48.8	106	
	0.054	46.8	104	
	0.053	46.0	102	

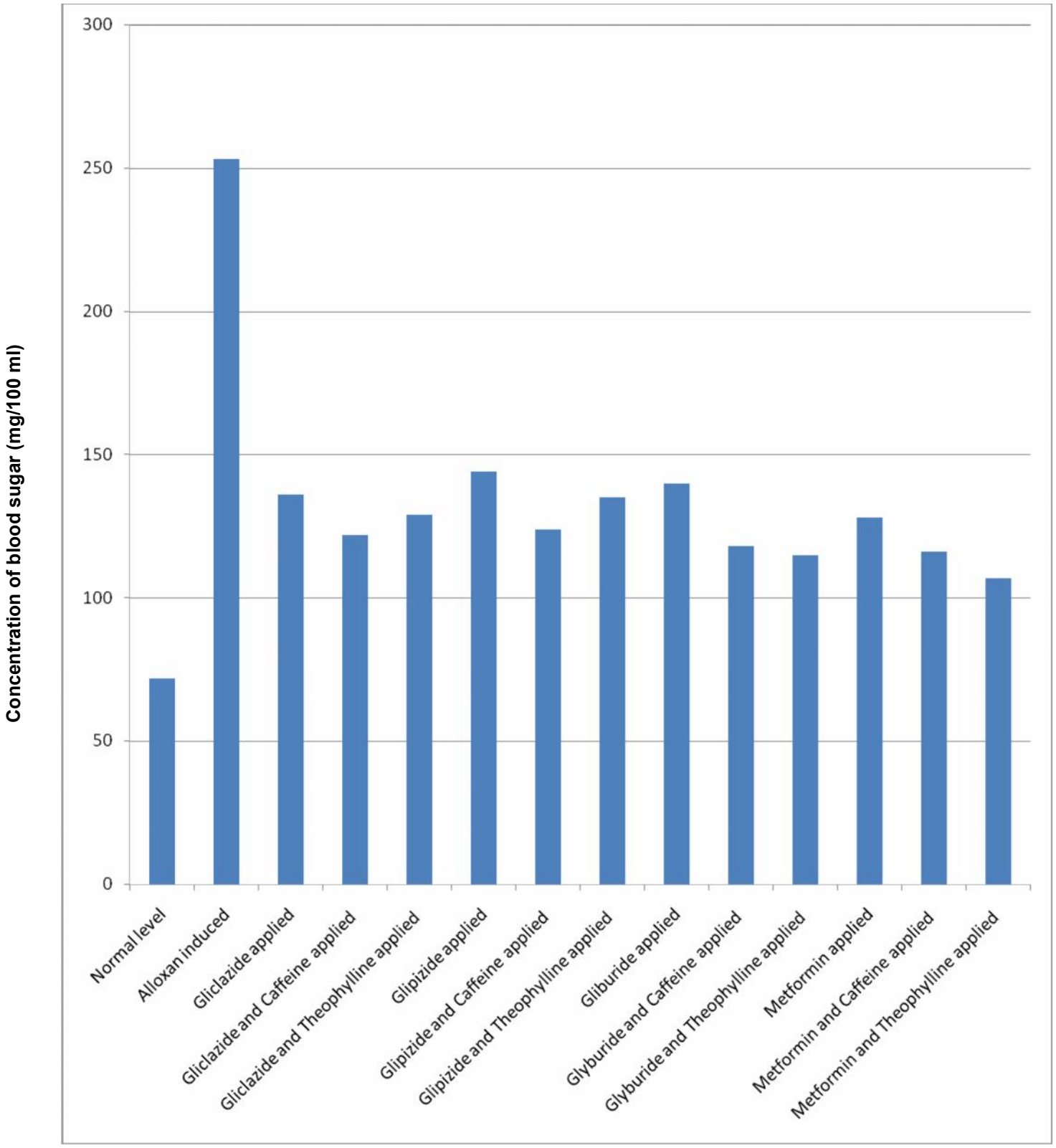


Fig.7.4.1.1: Comparison of blood sugar level observed in rats after 2 weeks of the administration of drugs

7.4.2 Observation of blood sugar level in rats after 4 weeks of the administration of drugs

Again in the other experiment, from the table 7.4.2.1 and figure 7.4.2.1, normal blood sugar level was 74 ± 2.6 mg/100ml. After induction of diabetes by administration of alloxan, the blood sugar level raised to 304 ± 2.7 mg/100 ml. At this stage, when gliclazide alone was administered, the blood sugar level decreased to 130 ± 6.4 mg/100 ml and when gliclazide was administered with caffeine and theophylline, the blood sugar level decreased to 114 ± 3.4 mg/100 ml and 117 ± 5.4 mg/100 ml respectively; when glipizide alone was administered, the blood sugar level decreased to 118 ± 5.3 mg/100 ml and when glipizide was administered with caffeine and theophylline, the blood sugar level decreased to 107 ± 4.3 mg/100 ml and 111 ± 5.8 mg/100 ml respectively; when glyburide alone was administered, the blood sugar level decreased to 127 ± 5.4 mg/100 ml and when glyburide was administered with caffeine and theophylline, the blood sugar level decreased to 112 ± 4.5 mg/100 ml and 118 ± 5.6 mg/100 ml respectively. On the other hand, when metformin alone was administered, the blood sugar level decreased to 122 ± 5.1 mg/100 ml and when metformin was administered with caffeine and theophylline, the blood sugar level decreased to 98 ± 5.4 mg/100 ml and 116 ± 6.3 mg/100 ml after four weeks of treatment.

Table 7.4.2.1: Data for blood sugar level after 4 weeks in different groups of experimental rats

Groups	Absorbance	Molar Absorbity (A)	Conc. of sugar mg/100 ml blood	Average conc. of blood sugar mg/100 ml blood
Group I (control)	0.048	40.4	82	74
	0.043	37.7	75	
	0.042	36.3	73	
	0.041	34.5	72	
	0.038	31.7	68	
Group II (Alloxan)	0.180	158.2	318	304
	0.174	152.5	307	
	0.173	151.7	305	
	0.168	147.6	297	
	0.167	145.5	293	
Group III (Gliclazide)	0.079	68.3	139	130
	0.076	65.5	132	
	0.074	63.6	129	
	0.072	62.7	128	
	0.071	60.2	122	
Group IVA (Caffeine+Gliclazide)	0.068	57.0	118	114
	0.067	56.1	114	
	0.066	56.5	115	
	0.063	55.7	113	
	0.062	54.5	110	
Group IVB (Theophylline+Gliclazide)	0.070	62.1	123	117
	0.069	61.2	121	
	0.067	58.8	118	
	0.065	56.2	115	
	0.062	54.6	110	

Groups	Absorbance	Molar Absorbity (A)	Conc. of sugar mg/100 ml blood	Average conc. of blood sugar mg/100 ml blood
Group V (Glipizide)	0.073	63.0	128	118
	0.071	60.2	122	
	0.069	57.8	120	
	0.064	56.1	114	
	0.061	53.6	108	
Group VIA (Caffeine+Glipizide)	0.063	55.7	113	107
	0.061	53.6	108	
	0.059	51.5	105	
	0.058	50.8	103	
	0.060	52.5	106	
Group VIB (Theophylline+Glipizide)	0.066	58.1	118	111
	0.065	57.2	115	
	0.064	56.0	113	
	0.061	51.8	108	
	0.060	50.2	102	
Group VII (Gliyburide)	0.077	66.2	135	127
	0.076	65.5	132	
	0.073	63.0	128	
	0.070	59.5	121	
	0.068	57.0	118	
Group VIIIA (Caffeine+Glyburide)	0.065	56.2	116	112
	0.062	54.5	110	
	0.067	55.8	113	
	0.066	56.5	115	
	0.061	53.6	108	
Group VIIIB (Theophylline+Glyburide)	0.071	62.2	126	118
	0.069	61.8	122	
	0.067	59.2	117	
	0.064	56.0	113	
	0.063	55.5	111	
Group IX (Metformin)	0.074	63.5	129	122
	0.072	62.2	125	
	0.070	61.5	122	
	0.068	58.2	118	
	0.067	57.5	116	
Group XA (Caffeine+Metformin)	0.061	52.5	108	98
	0.056	47.3	100	
	0.056	46.5	96	
	0.054	45.5	94	
	0.052	44.2	92	
Group XB (Theophylline+Metformin)	0.069	61.8	121	116
	0.068	60.2	119	
	0.067	59.2	118	
	0.066	57.4	113	
	0.065	56.2	111	

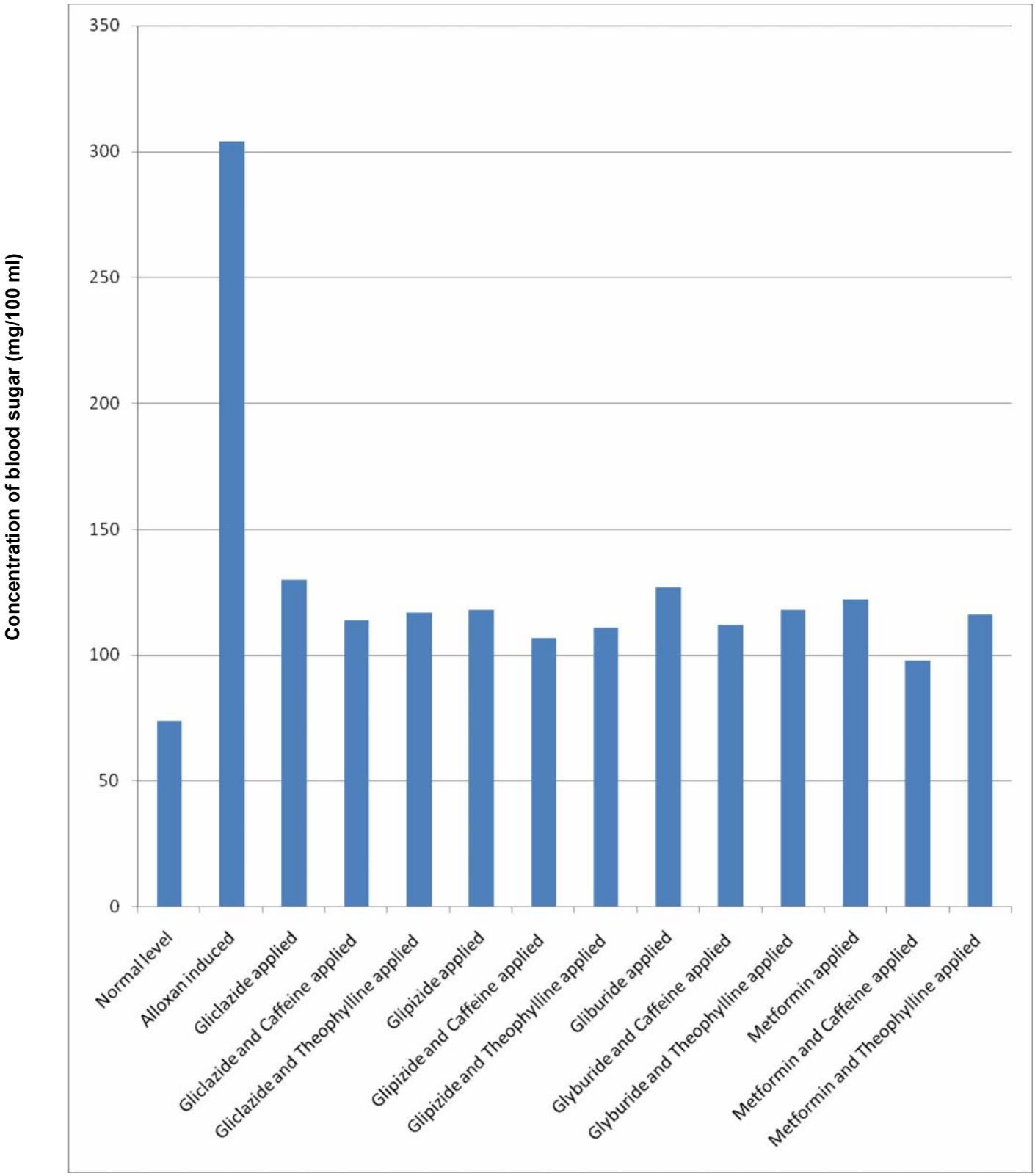


Fig. 7.4.2.1: Comparison of blood sugar level observed in rats after 4 weeks of the administration of drugs

From the above tables and figures, we observe that by comparing this with that of gliclazide, glipizide, glyburide and metformin alone, it can be inferred that both caffeine and theophylline have significant effect on the hypoglycemic effect of gliclazide, glipizide, glyburide and metformin after 2 weeks and 4 weeks of concurrent administration of the drugs.It has been found that caffeine and theophylline can enhance hypoglycemic effect of gliclazide, glipizide, glyburide and metformin in rats. It is further found that the influence of caffeine on metformin is stronger than on gliclazide, glipizide and glyburide in respect of hypoglycemic activity.

The present study indicates that caffeine and theophylline can enhance hypoglycemic activity of gliclazide, glipizide, glyburide and metformin in rats. It can be infered that the hypoglycemic activities of gliclazide, glipizide, glyburide and metformin are potentiated and broadened by caffeine and theophylline concurrent application. It can be infered that the hypoglycemic activities of gliclazide, glipizide, glyburide and metformin are potentiated by caffeine and theophylline concurrent application.The results in this study have shown that both caffeine and theophylline can enhance hypoglycemic effect of gliclazide, glipizide, glyburide and metformin in rats.

So, it can be concluded that the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin are potentiated by concurrent application of both caffeine and theophylline. However, it was observed that the influence of caffeine on the hypoglycemic activity of metformin was stronger than that on gliclazide, glipizide and glyburide. It may be mentioned that caffeine and theophylline do not possess any hypoglycemic property. The potentiation of the antidiabetic properties of gliclazide, glipizide, glyburide and metformin may be due to relaxation effect of caffeine and theophylline on smooth muscles of the rat as well as some sort of modification of the molecular conformations of the antidiabetic agents.

CHAPTER 8:

CONCLUSION

CONCLUSION

A number of analytical methods have been applied in this study. These are Infrared spectroscopy method, UV-Visible spectrophotometric method, continuous-variation method, mole-ratio method, conductometric method and Ardon's spectrophometric method, equilibrium dialysis method and some in-vivo experiments by using spectrophotometric methods. The physicochemical parameters, which have been studied in this work, can explain the pharmacokinetic changes in biological systems, particularly in man.

The in-vitro study on the interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin have been studied at room temperature and at different pHs followed by Infrared spectroscopy method, UV-Visible spectrophotometric method, continuous-variation method, mole-ratio method, conductometric method and Ardon's spectrophometric methods.Observations of infrared and ultraviolat spectral data have revealed the possibility of interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin. The spectra of target molecules alone and the mixture of (1:1) of caffeine and theophylline with gliclazide, glipizide, glyburide or metformin showed significant changes in their absorption intensities. This may be due to interaction of caffeine and theophylline with the drugs that alter the absorption intensities.

Continuous-variation plots have conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide, the formation of 2:1 complexes of theophylline with gliclazide & glipizide, 1:2 complexes of theophylline with glyburide and 1:1 complexes of theophylline with metformin. But Job's method of continuous-variation plots conformed the formation of 1:1 complexes of both caffeine & theophylline with metformin.Mole-ratio plots conformed the formation of 1:1 complexes of caffeine with gliclazide, glipizide & metformin and 1:2 complexes of caffeine with glyburide, the formation of 1:2 complexes of theophylline with gliclazide & glipizide and 1:2 complexes of theophylline with glyburide & metformin.The conductometric method was used to further ascertain about the nature of interaction and stoichiometries.Conductrometric titrations have showed that 1:1 complexes are formed between caffeine & each of the interacting species. It has also showed that 1:1 complexes are formed between theophylline & each of the interacting species. It has been found that along with stable complexes some unstable intermediates are formed.

The Ardon spectrophotometric plots confirmed the phenomenon of 1:1 complexation in all cases since straight lines have been obtained in these plots. The stability constants of the complexes are estimated from these straight lines using the Ardon equation. It has been observed that the stability constants for caffeine-gliclazide system were higher than that of caffeine-glipizide, caffeine-glyburide, caffeine-metformin, theophylline-gliclazide, theophylline-glipizide, theophylline-glyburide and theophylline-metformin in all pHs conditions. It has indicated a comparatively weak interaction in the all other systems. From the stability constants, it has been observed that the stability constant values for caffeine-metformin system at lower pH values are higher and the stability constants of caffeine-gliclazide system at pH 3.4 is very high indicating a stronger complex formation between these two drugs at this pH. However it might be inferred that such complex formation at lower pH values might affect the absorption of drugs from stomach after oral administration. As a result optimal plasma concentration would not be achieved and hence desired therapeutic effect would be hampered. Therefore, during administration of combination therapy of such drugs, plasma concentration monitoring might be necessary.

An in-vitro study for the effect of caffeine and theophylline on the protein binding of gliclazide, glipizide, glyburide and metformin has been carried out by equilibrium dialysis method to evaluate the influence of caffeine and theophylline on the percentage of protein binding of gliclazide, glipizide, glyburide and metformin hydrochloride. The in-vitro study of protein binding has showed that caffeine and theophylline cause lowering the affinity and percentage of binding of gliclazide, glipizide, glyburide and metformin to bovine serum albumin; hence an increase in volume of distribution of gliclazide, glipizide, glyburide and metformin might be occurred i.e, present finding indicates that may increase the free plasma concentration of gliclazide, glipizide, glyburide and metformin which may give toxic effects. Because such type of combination therapy may change the pharmacokinetic and pharmacodynamic properties of gliclazide, glipizide, glyburide and metformin.Therefore, it can be inferred that care and monitoring should be practiced during administration of combination therapy or concurrent administration of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin.

The in-vivo study for determination of plasma concentration of gliclazide, glipizide, glyburide and metformin in rat by UV-Visible spectrophotometric method showed that concurrent administration of caffeine and theophylline with gliclazide and glyburide have not made noticeable changes in plasma concentration of gliclazide and glyburide. But administration of caffeine and theophylline with glipizide and metformin in rats has showed a significant change in plasma concentration of glipizide and metformin.Any change in plasma concentration may affect the pharmacological or toxic effects of the drug.Thus, the interaction of caffeine and theophylline with gliclazide, glipizide, glyburide and metformin can increase the free drug concentration of gliclazide, glipizide, glyburide and metformin in blood plasma. This may change the pharmacokinetic and pharmacodynamic properties of the drugs. So, there is prospect for a combination therapy of both caffeine and theophylline with gliclazide, glipizide, glyburide or metformin, particularly glipizide and metformin but some more in-vivo studies are necessary to avoid any consequence of untoward incidents or harmful interactrions.

An in-vivo study has been carried out by UV-Visible spectrophotometric method to observe the effect of caffeine and theophylline on the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin under various conditions. The present study indicates that caffeine and theophylline can enhance hypoglycemic activity of gliclazide, glipizide, glyburide and metformin in rats. It can be infered that the hypoglycemic activities of gliclazide, glipizide, glyburide and metformin are potentiated and broadened by caffeine and theophylline concurrent application. It is further found that the influence of caffeine on metformin is stronger than on gliclazide, glipizide and glyburide in respect of hypoglycemic activity.

So, it can be concluded that the hypoglycemic activity of gliclazide, glipizide, glyburide and metformin are potentiated by concurrent application of both caffeine and theophylline. However, it was observed that the influence of caffeine on the hypoglycemic activity of metformin was stronger than that on gliclazide, glipizide and glyburide. It may be mentioned that caffeine and theophylline do not possess any hypoglycemic property. The potentiation and broadening of the antidiabetic properties of gliclazide, glipizide, glyburide and metformin may be due to relaxation effect of caffeine and theophylline on smooth muscles of the rat as well as some sort of modification of the molecular conformations of the antidiabetic agents.

To arrive at a conclusive idea about the pharmacokinetic, pharmacological and toxic effects of gliclazide, glipizide, glyburide and metformin due to simultaneous administration of caffeine and theophylline more extensive work will be needed. Similarly further study is needed to establish the fact that the vitally important CNS stimulant drug caffeine or theophylline can substantially potentiate the antidiabetic properties of gliclazide, glipizide, glyburide and metformin.This type of potentiation of properties of such important drugs should be a matter of interest for various reasons. Foremost of these are drug safety and development of more effective drugs. It believed that more and more interest should be focused on improving the effectivity of already proven safe drugs in essential areas of drug application.

Multiple drug therapy is a useful common practice in modern medical science, where two or more drugs are given at the same time or concurrently. The drugs may exert effects independently, or may interact or interfere with each other. The interaction may be the potentiation or antagonism of one drug by another. Thus, the study of interaction is significant both with respect to bio-pharmaceutics and pharmacology.The data obtained from the present study would help us to suggest that low molecular hypoglycemic drugs gliclazide, glipizide, glyburide as well as metformin may result into compatible combination therapies with CNS stimulant molecules caffeine and theophylline. However trials in higher animals and human are necessary.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Hansten, P. D. and J. R. Horn: **Drug interactions: Clinical significance of drug- drug interaction**, 6th edition, Philadelphia, 1989, P. 5-13.
2. Kristensen M: **Drug interaction and clinical pharmacokinetics,** Clin Pharmacokin, 1986.
3. Cadwallader, D.E., 1985. **Biopharmaceutics and drug interactions.** 3rd Edn., Raven Press, Ney York.
4. Alicia Gutierrez Valanvia y Luis F. López-Cortés *Interacciones farmacológicas entre fármacos antirretrovirales y fármacos usados para ciertos transtornos gastrointestinales.* on [4] Consulted 24 September 2008
5. Elizabeth Lipp (2008-06-15). "Tackling Drug-Interaction Issues Early On". *Genetic Engineering & Biotechnology News*
6. Goldstein A; Aronow L; Kalman S: **Principles of drug action: the basis of pharmacology,** 2nd edition, John Wiley and Sons, 1974.
7. Gago Bádenas, F. *Curso de Farmacología General. Tema 6.- Excreción de los*
8. Christophr, J.C: **Molecular mechanism of drug action,** Taylor and Francis, 1988.
9. *National Prescribing Service*, 2009. Available at http://nps.org.au/news.*fármacos*. En
10. S Gonzalez. "Interacciones Farmacológicas" (in Spanish). Retrieved 2009.
11. [a] [b] *Curso de Farmacología Clínica Aplicada*, in El Médico Interactivo
12. Baños Díez, J.E.; March Pujol, M (2002). *Farmacología ocular* (in spanish) (2da ed.). Edicions UPC. p. 87. ISBN 8483016478. Retrieved 23 May 2009.
13. Merle L, Laroche M-L, Dantoine T y Charmes J-P *Predicting and Preventing Adverse Drug Reactions in the Very Old* Drugs and Aging 22(5):375-392, 2005
14. [a] [b] García Morillo, J.S. *Optimización del tratamiento de enfermos pluripatológicos en atención primaria* UCAMI HHUU Virgen del Rocio. Sevilla. Spain. Available for members of SEMI at: ponencias de la II Reunión de Paciente Pluripatológico y Edad Avanzada
15. Castells Molina,S.; Castells,S. y Hernández Pérez, M. *Farmacología en enfermería* Published by Elsevier Spain, 2007 ISBN 84-8174-993-1, 9788481749939 Available from [1]
16."Definition of a molecular complex". *Compendium of Chemical Terminology: Gold Book*. International Union of Pure and Applied Chemistry. 2012-08-19.
17. D.G. Grahame-smith, J.K-Aronson**: Oxford Textbook of clinical Pharmacologyand drug therapy,** 2nd edition, P. 122-136.
18. Roger walker, Clive Edwards : **Clinical Pharmacy and Therapeutics,** P. 21-29.
19. Ringbom (1963), Complexation in analytical chemistry, New York, Inter Science.
20. Lodish, H.; Berk, A.; Kaiser, C. (2008). *Molecular Cell Biology*. ISBN 978-0-7167-7601-7.
21. Guadhah MW, Guth EP: **Complexation of drug with excipients**, J Pharm Sci, 1985, Vol 54.
22. Joseph J: **Protein binding of drugs,** 3rd edition, Wiley Inter. Science, 1982.
23. Singlas E: **Protein binding of drugs, F. Hoffman-La-Roche and Co.Ltd,** Basle, Switzerland, 1987.
24. Tillement, J.P, Lhoste F, Giudichelle JF: **Disease and Drug Protein Binding, Clinical Pharmacokinetic,** 1978, 3: 144-154.
25. Gibaldi M : **Biopharmaceutics and Clinical Pharmacokinetics,** P. 305-338.
26. British Pharmacopoeia 2009
27. U.S. Pharmacopoeia - National Formulary 2009
28. European Pharmacopoeia 2009
29. Martindale**: 2009.** The Complete Drug Reference: 36th edition; Pharmaceutical press.
30. Remington**: The Sciences and Practice of Pharmacy,** Mack publishing company, USA, 19th edition, 1995.

31. ***Influence of nicorandil on the pharmacodynamics and pharmacokinetics of gliclazide in rats and rabbits.***Satyanarayana S, Kilari EK.*Pharmacology Division, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, 530 003, Andhra Pradesh, India. profssn79@rediffmail.com*

32. ***Effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide.***Park JY, Kim KA, Park PW, Park CW, Shin JG.*Department of Pharmacology, Ghil Medical Center, Gachon Medical School, Incheon, South Korea.*

33. ***The in vivo interaction between gliclazide and glibenclamide and insulin on glucose disposal in the rat.***Tanira MO, Furman BL*Department of Pharmacology, College of Medicine, Sultan Qaboos University, Muscat-123, Oman.*

34. ***Sulfonylurea sensitivity of adenosine triphosphate-sensitive potassium channels from beta cells and extrapancreatic tissues.***Gribble FM, Ashcroft FM.*Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, United Kingdom.*

35. A study on drug-drug interaction between anti-hypertensive drug (propranolol) and anti-diabetic drug (glipizide). B. Parameshappa1, N. Venkat Rao1, T. Shivaraj Gouda1, Saikat Sen*2, Raja Chakraborty2, Md Ali Basha2, S. Ashok Reddy1 and S.M. Shanta Kumar1*1 V. L. College of Pharmacy, Raichur, Karnataka, INDIA, 2 CES College of Pharmacy, Kurnool, Andhra Pradesh, INDIA*

36. Glipizide amlodipine drug interactions - glipizide swelling - difference between glipizide and glyburide.**M.D Martins Health Blog** quicolhovsmi.

37. Glipizide and Metformin (Oral Route). Drug Information provided by: Micromedex US Brand Names Metaglip.

38. Anti-infectives and risk of severe hypoglycemia in glipizide and glyburide users Hedi Schelleman, PhD,[1] Warren B. Bilker, PhD,[1,2] Colleen M. Brensinger, MS,[1] Fei Wan, MS,[1] and Sean Hennessy, PharmD, PhD[1,2]

39. ***Differential effects of sulphonylureas on the vasodilatory response evoked by K(ATP) channel openers.***Ravel D, Levens N, Félétou M, Néliat G, Auclair J, Bouskela E.*Institut de Recherches Internationales Servier, Courbevoie, France.*

40. ***Differential selectivity of insulin secretagogues: mechanisms, clinical implications, and drug interactions.***Gribble FM, Reimann F.*Department of Clinical Biochemistry, University of Cambridge, Addenbrookes Hospital, Box 232, Hills Road, CB2 2QR, Cambridge, UK.*

41. ***Heterogeneity of the inhibitory influence of sulfonylureas on prostanoid-induced smooth muscle contraction.***Delaey C, Van de Voorde J.*Department of Physiology and Physiopathology, University of Ghent, Belgium.*

42. GLYBURIDE/GLIBENCLAMIDE-METFORMIN.*Internatinal drugmart.com.*

43. Investigation of the pharmacokinetic and pharmacodynamic interactions between memantine and glyburide/metformin in healthy young subjects: a single-center, multiple-dose, open-label study.**Rao N**, **Chou T**, **Ventura D**, **Abramowitz W**.Forest Research Institute, Forest Laboratories, Inc., Jersey City, New Jersey, USA. rao.niranjan@kyowa-kpi.com

44. ***Metformin hydrochloride: an antihyperglycemic agent.***

Klepser TB, **Kelly MW**.College of Pharmacy, University of Iowa, Iowa City 52242, USA. teresa-klepser@uiowa.edu

45. ***Effect of altered gastric emptying and gastrointestinal motility on metformin absorption.***Marathe PH, Wen Y, Norton J, Greene DS, Barbhaiya RH, Wilding IR.*Department of Metabolism and Pharmacokinetics, Bristol-Myers Squibb Company, Princeton, NJ 08543, USA. marathep@bms.com*

46. Metformin hydrochloride in the treatment of type 2 diabetes mellitus: a clinical review with a focus on dual therapy.Setter SM, Iltz JL, Thams J, Campbell RK.Department of Pharmacotherapy, College of Pharmacy, Washington State University, Spokane 99217-6131, USA. ssetter@smhca.org

47. *Caffeine and psychiatric medication interactions:* *Broderick PJ*, *Benjamin AB*, *Dennis LW*. ***University of Oklahoma, USA.***

48. *Caffeine and the dopaminergic system.* *Cauli O*, *Morelli M*. *Department of Toxicology and Centre of Excellence for Neurobiology of Dependence, University of Cagliari, Cagliari, Italy.*

49. *A primer on caffeine pharmacology and its drug interactions in clinical psychopharmacology.* *Donovan JL*, *DeVane CL*.*Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, 67 President Street, Charleston, SC, 29425-0742, USA.*

50. *Interactions of caffeine with smoking.* *Kroon LA*.*Department of Clinical Pharmacy, School of Pharmacy, University of California San Francisco, 3333 California Street, Suite 420, Box 0613, San Francisco, CA 94118, USA. kroonl@pharmacy.ucsf.edu*

51. *Caffeine and headaches.* *Shapiro RE*. *University of Vermont, College of Medicine, Department of Neurology, Given C219B, 89 Beaumont Avenue, 05405 Burlington, VT, USA. robert.shapiro@uvm.edu*

52. The theophylline-erythromycin interaction. Rieder MJ, Spino M.Division of Clinical Pharmacology, Hospital for Sick Children, Toronto, Ontario, Canada.

53. Drug interaction studies with repaglinide: repaglinide on digoxin or theophylline pharmacokinetics and cimetidine on repaglinide pharmacokinetics. Hatorp V, Thomsen MS. Novo Nordisk A/S, Bagsvaerd, Denmark.

54. Evaluation of drug-drug interaction study of zabofloxacin in vivo. Abstract number: P701; Ku S.K., Seong S.K., Lee S.H., Choi D.R., Rock J., Ryu J.M.

55. Drug interactions with azithromycin and the macrolides: M. Nahata, *Colleges of Pharmacy and Medicine, Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA*

56. Overview of drug interactions with the quinolones. Davey PG., Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee, Scotland. J Antimicrob Chemother. 1988 Sep;22 Suppl C:97-107.

57. Amran, M.S., A.H.M.R. Bari and M.A. Hossain, 2006b. ***In vitro* and *in vivo* interactions of diltiazem with ibuprofen and naproxane in aqueous medium and rabbits.** *Dhaka Univ. J. Pharm. Sci., 5: 25-28*

58. Amran, M.S., S.N. Morshed, M.J.A. Khandakar, M.M. Rahman and M.A. Hossain, 2008. **The *In vitro* effects of atenolol and zinc chloride on the protein binding of amlodipine in aqueous medium.** *Dhaka Univ. J. Pharm. Sci., 7: 15-21*

59. Amran, M.S., A.H.M.R. Bari and M.A. Hossain, 2006a. ***In vitro* and *in vivo* interactions of diltiazem with ibuprofen and naproxane in aqueous medium and rabbits.** *Dhaka Univ. J. Pharm. Sci., 5: 25-28*

60. Bates, R.G., 1964. **Determination of $p^{H,}$ Theory and Practice,** 2nd Edition, John Wiley and Sons, Inc., New York, pp: 50-67

61. Perrin D.D, Boyed D: **Buffer for P^{H} and Metal ion control,** Science Papers Back, 1974, P. 44-50, 62.

62. **Griffiths, P.; de Hasseth, J.A. (18 May 2007).** *Fourier Transform Infrared Spectrometry* (2nd ed.). Wiley-Blackwell. ISBN 0-471-19404-2.

63. Skoog, D.A., D.M. West, F.J.Holler and S.R. Crouch, 2004. **Fundamentals of Analytical Chemistry,** 8th edition, Brooks/Cole, U.K., pp: 337-349.

64. Schwarzenbach (1957), Complexation titration, New York, Inter. Sciences.

65. Ardon, M., 1957.**Oxidation of ethanol by ceric perchlorate**. *J. Chem. Soc., 1957: 1811-1815.*

66. Rahat, M., M.S. Amran and M.A.Hossain, 1999. ***In vivo*** **study of effect of nifedipine, ketotifen fumarate and potassium nitrate on the plasma concentration of diltiazem in rabbits.** *Pak.J. Pharmacol*., 16: 57-61

67. Kobayashi K, Kimura M, Sakoguchi T, Hase A, Matsuoka A, KanekoS: **Pharmacokinetics of Gliclazide in healthy and diabetic subjects,** J Pharm Sci1984 Dec; 73(12): 1684-7.

68. Kristensen M: **Drug induced changes of the blood glucose lowering effect of oral hypoglycemic agent,** Acta Diabetica Latina.

69. Zaman, M.R., Amran, M.S. and Hossain, M.A. 1999. ***In vivo*** **study of effects of nifedipine, Ketotifen fumerate and potassium nitrate on plasma concentration of dilitazem in rabbits.** *Pakistan J. Pharmacol*. **16**, 57-61.

70. Akhter, B., Amran, M.S. and Hossain, M.A. 2001. ***In vivo*** **evalution of famotidine and prochlorperazine maleate on the serum concentration of nifedipine in rabbits**. *Pakistan J. Pharmacol*. **18,** 23-29.

71. Amran, M.S., Hashimoto, K. and Homma, N. 2004. **Effects of sodium-calcium exchange inhibitors, KB-R7943 and SEA0400, on aconotine-induced arrhythmias in guinea pigs *in vivo*, *in vitro* and in computer simulation studies.** *J.Pharmacol*. Exp. Ther. **310**, 83-89.

72. Amran, M.S. and M.A. Hossain, 1999. **Evaluation of *in vitro interaction* of diltiazem hydrochloride with iron (II) in the aqueous media.** *Rajshahi Univ. Stud. Part B, 26: 35-43.*

73. Mahmood, A.E., A.T.M.Z. Azam, M.S. Amran and M.A.Hossain, 2000. **Evaluation of *in vitro interaction* of nifedipine with copper (II) in the aqueous medium.** *J. Asiat.Soc. Bangladesh Sci., 26: 53-58.*

74. Nabi, S.N., A. Hussain and N.N. Ahamed, 1974. **Eletrolyte conductance of solutions of thionyl chloride and its reaction with silver salts and lewis acids in acetone.** *J. Chem. Soc., 1: 1199-1205.*

75. Milon .A. and M.A.Hossain, 2009. **A study on the interaction of metformin and glimepiride with copper chloride and cobalt chloride**. ..M.Pharma. Thesis. Department of pharmaceutical chemistry, Faculty of pharmacy, University of Dhaka, Bangladesh.

76. Rahman, M.M. and M.A.Hossain, 2008. **A study on the interaction of glipizide with Mg (II), Al (III), Ca (II) and Zn (II) ions in the aqueous medium.** M.Sc. Thesis. Department of chemistry, Faculty of Sceinces, University of Dhaka, Bangladesh.

77. Salam, A. and M.A.Hossain, 2001. **The influence of ibuprofen on the activity of glibenclamide, glipizide and gliclazide in vitro and in vivo.** M.Sc. Thesis. Department of chemistry, Faculty of Sceinces, University of Dhaka, Bangladesh.

78. Tillement, JP, Zini R, Vassent G: **Binding of certain acidic drugs to human albumin: Theoretical and practical estimation of fundamental parameters, Eur J Chem Pharmacol,** 1974.

79. Amran, M.S. and M.A. Hossain, 1998. **Study of protein binding of diltiazem in presence of omeprazole and ranitidine.** *J. Biol.Sci., **6:** 115-121.*

80. Amran, M.S., M.R. Zaman and M.A. Hossain, 1999. **Study of protein binding of diltiazem in presence of nifedipine and kitotifen fumarate.** *Rajshahi Univ. Studies, Part B, 27: 45-54.*

81. Scatchard, G., 1949. **The attractions of proteins for small molecules and ions**. Ann. N.y. Acad. Sci., 51: 660-672.

82. Bari, A.H.M.R., A.T.M.Z. Azam, M.S. Amran and M.A.Hossain, 2000. ***In vivo*** **effects of ibuprofen and naproxen on the plasma concentration of diltiazem in rabbits.** *Pak.J. Pharmacol*., 3: 555-557.

83. Gilman, A.G., l.s. Goodman, R.W. Rally and F. Mural, 1991. **The Pharmacological Basis of Therapeutica.** 8th Edn., Vol. I-II, Maxwell Macmillan, New York, ISBN: 0-07-135469-7,pp: 1018-1200.

84. Van Dam, R.M. and F.B. Hu, 2005. **Coffee consumption and risk of type 2 diabetes:** A systematic review. *J.Am.Med. Assoc*., 294: 97-104.

85. Noguchi H, Tomita N, Naruto S, Nakano S: **Determination of Gliclazide in serum by high-performance liquid chromatography using solid-phase extraction,** J chromatogr 1992 Dec 2; 583(2): 266-9.

86. Emilson H: **High- performance liquid chromatographic determination of Gliclazide in human plasma and urine,** J chromatogr 1987 Oct 30; 421(2): P.319- 326.
87. Sawynok, J. 1995. **Pharmacological rationales for the clinical use of caffeine**. **49**, 37-50.
88. Scheen, A. J. 1996. **Clinical pharmacokinetics of metformin**. *Clin Pharmacokinet.* **30**, 359-371.
89. Sambol, N.C. *et al.* 1996.**Pharmacokinetics and Pharmacodynamics of metformin in healthy subjects and patients with non-insulin-dependent-diabetes- mellitus**. *J.Clin. Pharmacol.* **36,** 1012-1021.
90. Amran, M.S., Baki, M.A. and Hossain, M.A. 2009. ***In vitro*** **and** ***in vivo*** **effects of glipizide and gliclazide on the protein binding and plasma concentration of ibuprofen.** Presented at the 31st Annual Conference of Bangladesh Chemical Society, University of Dhaka, Dhaka, Bangladesh. 30 January-01 February, 2009.
91. Solomon J., Bulkley R.J., Mayer J., 1974. **Effect of alloxan on blood glucose, islet beta cell granulation, body weight and insulin resistance of ob/ob mice.** *Diabetologia* **10**, 709-714.
92. Amran, M.S., M. Rahatuzzaman and M.A. Hossain, 2006a. **The *synergim of diltiazem and nifedipine in their antihypertensive functions in the animal model and in case of coadministration of ketotifen fumarate and potassium nitrate with them*.** *J. Biol.Sci., **14:** 61-67*
93. Amran, M.S., M. Rahatuzzaman and M.A. Hossain, 2006b. **The *synergim of diltiazem and nifedipine in their antihypertensive functions in the animal model and in case of coadministration of ketotifen fumarate and potassium nitrate with them*.** *J. Biol.Sci., **14:** 61-67*
94. Hossain M.A. and M.S. Amran, 2000. **The *interactions*** **of omeprazole and ranitidine with diltiazem in the aqueous media.** *J. Bangladesh Chem. Soc., 13: 7-13.*
95. Daniel, W.W., 2004. **Biostatistics: A foundation for analysis in the Health Sciences.** *8th Edn., John Wiley and Sons Inc., USA*

LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

1. Mohiuddin, M., Azam, A.T.M. Z., Amran, M.S. and Hossain, M.A., 2009. ***In vitro*** **Effects of Gliclazide and Metformin on the Protein Binding of Caffeine in the Aqueous Media.** *J. Biol. Sci., 9 (5):476-481.*

2. Mohiuddin, M., Azam, A.T.M. Z., Amran, M.S. and Hossain, M.A., 2009. ***In vivo*** **Effects of Gliclazide and Metformin on the Plasma Concentration of Caffeine in Healthy Rats.** *Pak. J. Biol. Sci., 12 (9):734-737.*

3. Mohiuddin, M., Amran, M.S. and Hossain, M.A., 2009.**The *In vivo* Effects of Caffeine on the Hypoglycemic Activity of Gliclazide and Metformin in Healthy Rats.** *Dhaka Univ. J. Pharm. Sci., 8 (1):47-51.*

4. Mohiuddin, M., Azam, A.T.M. Z., Amran, M.S. and Hossain, M.A., 2009. ***In vitro*** **Study on the Interaction of Caffeine with Gliclazide and Metformin in the Aqueous Media.** *J. Pharmacol. Toxicol. , 4 (5):194-204.*