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In vitro Study on the Interaction of Caffeine with Gliclazide and Metformin in the Aqueous Media

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Abstract: An *in vitro* study of interaction of caffeine with gliclazide and metformin HCl has been studied at room temperature and at different pH. It has been found that caffeine forms stable 1:1 molecular complexes with gliclazide and metformin HCl. The studies have been carried out by various UV spectrophotometric and conductometric methods. Observation of the UV spectra of the two molecules in presence of caffeine has indication that it reacts with the anti-diabetic agents. The conductometric method was used to further ascertain about the nature of interaction and stoichiometries. 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-metformin HCl system in all pH conditions.

Key words: Caffeine, gliclazide, metformin, Ardon's plots, complexation

INTRODUCTION

Caffeine is a methylxanthine that inhibits the enzyme phosphodiesterase and has an antagonistic effect at central adenosine receptors. It is a stimulant of the CNS, particularly the higher centres 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 (Sawynok, 1995). Gliclazide is a sulfonylurea antidiabetic. It is given by mouth in the treatment of type 2 diabetes mellitus. Metformin Hydrochloride is a biguanide antidiabetic. It is also given by mouth in the treatment of type 2 diabetes mellitus and is the drug of first choice in obese patients. 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 (Hansten and Horn, 1989). Takanohashi *et al.* (2007) showed that nateglinide (an antidiabetic agent) metabolism would hardly be affected by the 18 drugs that may be prescribed together with nateglinide (metformin, buformin, aspirin, gemfibrozil, simvastatin, pioglitazone, rosiglitazone, carbamazepine, clarithromycin, gliclazide, clofibrate, fluconazole, bezafibrate, phenylbutazone, nifedipine, famotidine, ibuprofen and miconazole), except for miconazole and fluconazole. Repaglinide (a new class of oral antidiabetic agents) showed additive effects when used in combination with other oral antidiabetic agents including metformin, troglitazone, rosiglitazone and pioglitazone and intermediate-acting insulin (NPH) given at bedtime (Culy and Jarvis, 2001). In type 2 diabetic patients, the vasodilating response to forearm ischemia was the same whether patients were treated with diet treatment alone or with glibenclamide or glimepiride at blood glucose-lowering equipotent closes (Spallarossa *et al.*, 2001). Adverse effects due to drug-drug interactions are

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not expected in diabetic patients receiving oral antidiabetic agents that are not metabolized through the CYP 3A4 system (e.g., tolbutamide, gliclazide, glibenclamide, glipizide and metformin) (Verspeelt *et al*., 1999). Therefore, itraconazole can be used safely and efficiently for the treatment of dermatological disorders in diabetic patients. Koyama *et al*. (1997) suggested that the extensive glycosylation of plasma proteins in diabetic patients complicates drug-drug interactions (glibenclamide, acetohexamide, tolbutamide, glicrazide, metfolmin) beyond those seen in normal people.

In the last twenty years, Hossain and co-workers have been carrying out research on drug-drug interactions, drug-metal interactions and drug-food interactions (Amran *et al*., 2006a,b; Amran *et al*., 2008; Bari *et al*., 2000). In this work to observe the fate of multiple drug-uses, we studied the effects of gliclazide and metformin on the plasma concentration of caffeine. The aim of this study was to investigate *in vitro* complex formation and to examine the nature and strength of complexes which could be formed due to interaction of caffeine with gliclazide and metformin hydrochloride.

MATERIALS AND METHODS

Materials

Caffeine, gliclazide and metformin HCl have been collected from The Orion Laboratories Ltd., Dhaka, Bangladesh and have been used without further purification. Potassium chloride (reagent grade), potassium dihydrogen orthophosphate (reagent grade, Merck), disodium hydrogen orthophosphate (reagent grade, Merck), orthophospharic acid (reagent grade), potassium hydroxide (reagent grade), sodium hydroxide (reagent grade, Merck) and formic acid (98-100%, reagent grade) have been used for the preparation of the buffer solution.

Equipments

UV-Visible Spectrometer (Model No. UV-1601, Shimadzu, Japan), pH Meter (Mettler Toledo, Switzerland), Conductometer (Mettler Toledo, Switzerland), Power Sonic (Model No. 510, Seoul, Korea) have been utilized.

Preparation of Standard Solutions (Amran *et al*., 2006a, b, 2008)

The 0.02 M Caffeine, gliclazide and metformin HCl solution were dissolved in demineralized water separately. These stock solutions were diluted to desired strengths by buffer solution to get the working standard solution.

Preparation of Standard Curve (Amran *et al*., 2006a, b, 2008; Zivanovic *et al*., 2005)

The 2, 4, 6, 8, 10 and 12 μg mL^{-1} working standard solutions of caffeine were prepared. Then, a standard curve was prepared by plotting absorbance (measured at 273 nm) VS concentration of caffeine (Fig. 1).

The *in vitro* interaction study (Hossain and Amran, 2000) of caffeine with gliclazide and metformin HCl has been performed by observing absorption spectra, conductometric data and Ardon's spectrophotometric curves.

Spectrophotometric Analysis (Amran *et al*., 2006a, b, 2008; Hassan *et al*., 1999; Ulvi and Keski-Hynnila, 1994; Skoog *et al*., 2004)

In observation of the Spectra, the absorption characteristics of caffeine, gliclazide and metformin HCl and their 1:1 mixtures in the solutions of buffers (Perrin and Dempsey, 1974; Bates, 1964) of pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 and 7.4 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

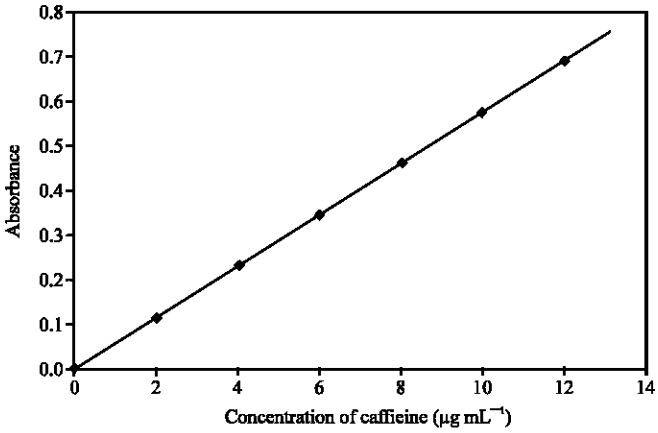

Fig. 1: Standard curve of caffeine

and automatic recording unit. The stock solutions of the samples were diluted to appropriate levels, diluted with buffers at the desired pH and the spectra were recorded between 400-200 nm. The spectra were compared with the pure sample in each case.

In Spectrophotometric continuous-variation analysis (Skoog *et al.*, 2004), cation (gliclazide and metformin HCl) and ligand (Caffeine) solutions with identical analytical concentrations were mixed in such a way that the total volume and the total moles of reactants in each mixture remained constant but the molar ratio of reactants varied systematically. The absorbance of each solution was then measured at a suitable wavelength and corrected for any absorbance the mixture might exhibit if no reaction had occurred. The corrected absorbance was plotted against the volume fraction of one reactant. Here, solution of different concentrations of caffeine, gliclazide and metformin HCl were prepared by using water and a continuous-variation plots were prepared by plotting corrected absorbance (measured at 273 nm) against the volume fraction of one reactant.

In Spectrophotometric mole-ratio analysis (Skoog *et al.*, 2004), a series of solutions was prepared in which the analytical concentration of one reactant (usually the cation) was held constant, while that of the other is varied. 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.

Conductometric titration in demineralized water system at different pH were carried out to find out the molar ratios at which complexation occurred (Nabi *et al.*, 1974), Here, 40 mL of 0.001 M caffeine solution was taken in a 100 mL beaker and was titrated individually with gradual addition of 0.01 M solution of gliclazide and metformin HCl from a burette. Reversely 40 mL each of 0.001 M gliclazide and metformin HCl were titrated with gradual addition of 0.01 M caffeine under similar conditions. The conductance values (ms) were plotted against 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 pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 and 7.4 using a pH meter.

In the Ardon's Spectrophotometric method (Ardon, 1957), concentrations of gliclazide and metformin HCl were varied while the concentrations of the caffeine was kept fixed. The absorbance of solutions having pH 1.4, 2.4, 3.4, 4.4, 5.4, 6.4 and 7.4 were measured at 273 nm using UV-VIS. recording 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 $1/(D - E_A C)$ was plotted versus 1/[B] to get the straight lines. The concentration of caffeine was kept constant at 5×10^{-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:

$$K = \text{intercept/slope}$$

It should be mentioned that this method is valid only for the systems where 1:1 complexes are found.

Statistical Analysis (Daniel, 2004)

The results were expressed as Mean±SEM values for each experiment. Differences in mean values between experimental groups were analyzed by unpaired t-test. A probability values less than 0.05 ($p<0.05$) was defined to be significant.

RESULTS

In Spectral observation analysis, each of the drugs studied shows absorption in UV-VIS region. The molecular species of gliclazide and metformin HCl when separately mixed with caffeine showed some changes in absorption characteristics of this drug molecule including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction of drugs. In this study, the UV absorption of the drug and drug-drug interaction mixtures was measured at 200-400 nm. The spectra of caffeine alone at different pH showed a sharp absorption maximum at 273 nm (Fig. 2).

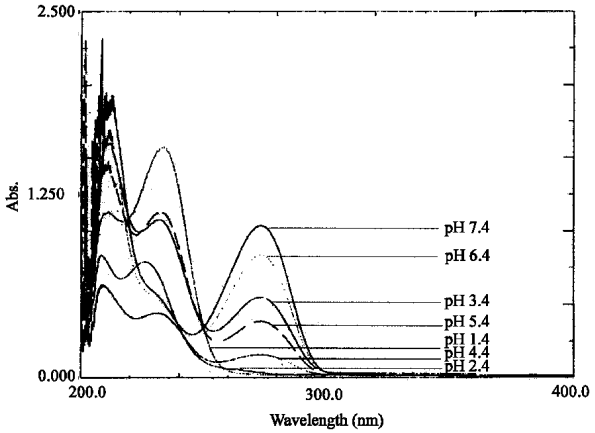

Fig. 2: UV spectra of Caffeine at different pH (Conc. of caffeine = 0.0001 M)

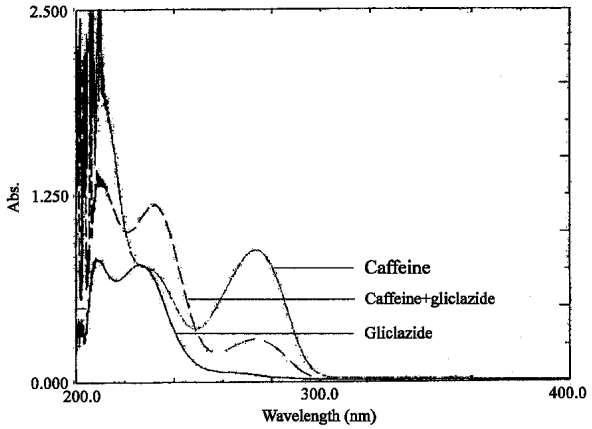

Fig. 3: UV spectra of caffeine-gliclazide systems. (Conc. of caffeine = Conc. of Gliclazide = 0.0001 M)

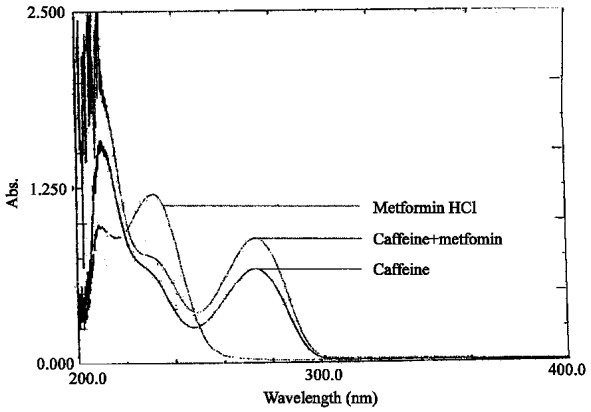

Fig. 4: UV spectra of caffeine-metformin HCl systems. (Conc. of Caffeine = Conc. of metformin HCl = 0.0001 M)

The intensities of these peaks vary with different pH. 1:1 mixtures of caffeine with gliclazide and metformin HCl showed noticeable changes in the absorption intensities at pH 3.4 due to interaction (Fig. 3, 4).

Continuous-variation plots (Skoog *et al.*, 2004) conformed the formation of 1:1 complexes of caffeine with gliclazide and metformin HCl. In this method, solution of different concentrations of caffeine, gliclazide and metformin HCl were the prepared by plotting corrected absorbance (measured at 273 nm) against the volume fraction of one reactant (Fig. 5, 6). It may be mentioned that cation and ligand solutions with identical analytical concentrations are mixed in such a way that total volume and the total moles of reactants in each mixture is constant but the mole ratio of the reactants varies systematically.

Mole-ratio plots (Skoog *et al.*, 2004) conformed the formation of 1:1 complexes of caffeine with gliclazide and metformin HCl. In this method, a series of solutions were prepared in which the analytical concentration of reactant was held constant while that of others was varied. The absorbance

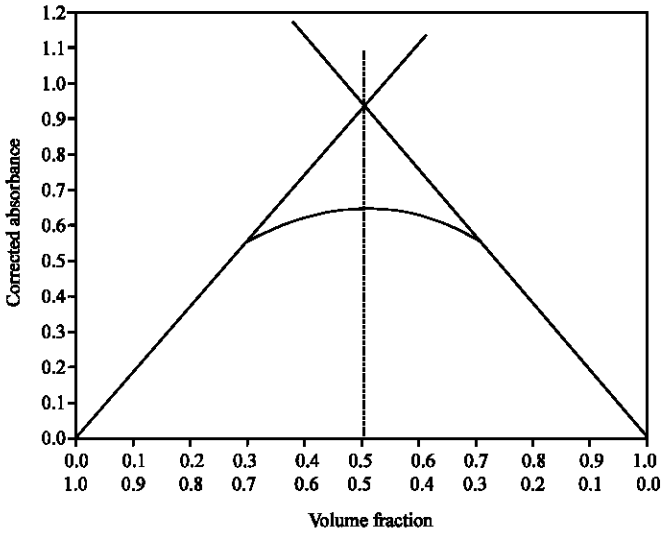

Fig. 5: Continuous-variation plot caffeine-gliclazide system

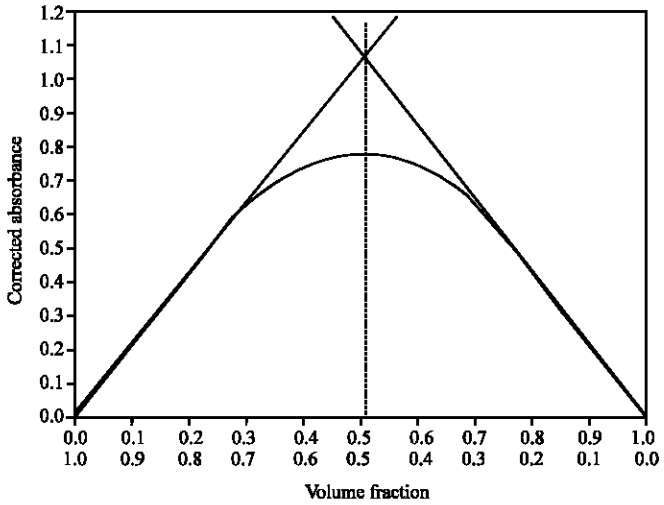

Fig. 6: Continuous-variation plot for caffeine-metformin HCl system

of different solutions (at 273 nm) was plotted against mole-ratio i.e., mole ligand per mole cation (Fig. 7, 8).

In this study, Conductometric titrations were carried out to detect the complexation of caffeine with Gliclazide and metformin HCl as well as to find the molar ratios of the interacting species to the drug molecule in the complex (Nabi *et al.*, 1974). For each combination, two titrations were performed one was titrated against the other and vice-versa. 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. This method conformed that caffeine forms a stable 1:1 complexes with gliclazide and metformin HCl at different pH. Conductometric data

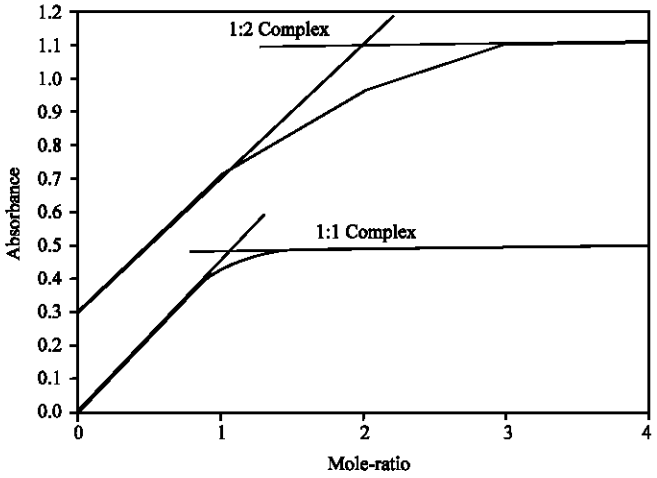

Fig. 7: Mole-ratio plot caffeine-gliclazide system

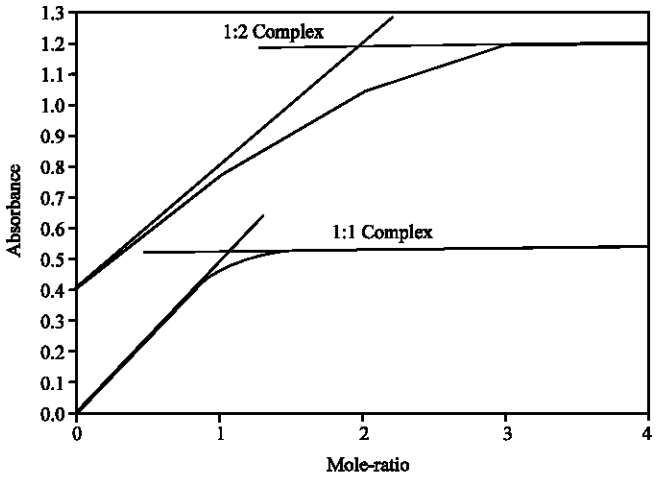

Fig. 8: Mole-ratio plot for caffeine-metformin HCl system

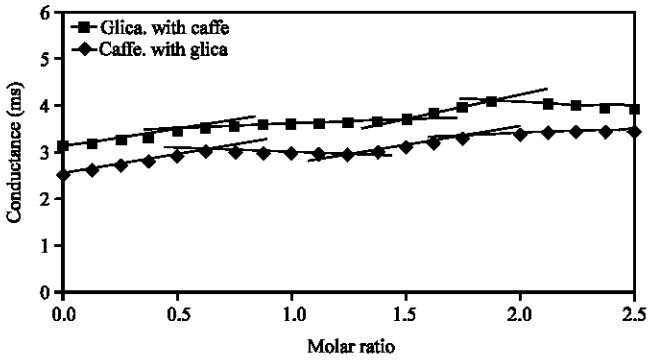

Fig. 9: Conductometric titration of caffeine with gliclazide and gliclazide with Caffeine

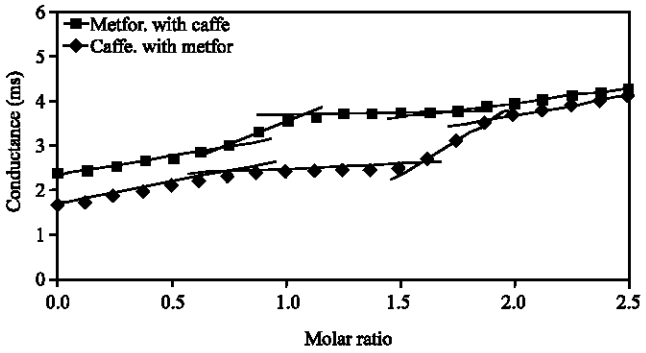

Fig. 10: Conductometric titration of caffeine with metformin HCl and metformin HCl with caffeine

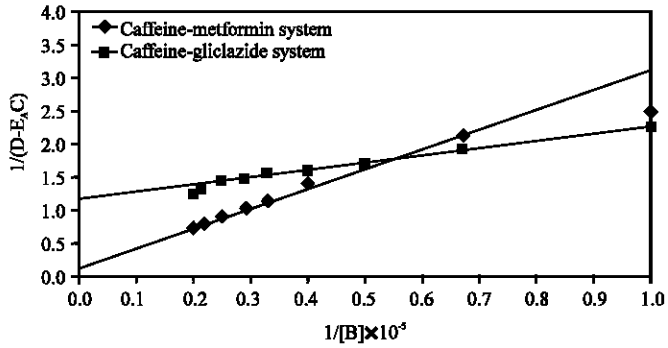

Fig. 11: Ardon's plot for complexation of caffeine with Gliclazide and Metformin HCl

Table 1:The stability constants for different systems

	Stability constants K×10^{-3}/mole						
Systems	pH 1.4	pH 2.4	pH 3.4	pH 4.4	pH 5.4	pH 6.4	pH 7.4
Caffeine-gliclazide	2.57	2.48	35.67	3.78	3.75	3.45	2.35
Caffeine-metformin	5.48	5.42	3.65	2.72	2.45	2.33	2.23

is shown at pH 3.4 (Fig. 9, 10). When caffeine was titrated with gliclazide at pH 3.4 three distinct breaks corresponding to caffeine-gliclazide molar ratios of 1:1, 2:1 and 3:1 were found in the conductivity curve. The reverse titration showed breaks at 1:1, 2:1 and 4:1 molar-ratio. These indicate that caffeine forms stable complex with gliclazide at 1:1 molar ratio.

Ardon's plots conformed the formation of 1:1 complexes of caffeine with gliclazide and metformin HCl. The data for Ardon's plots was given straight lines with different intercepts, indicating the formation of 1:1 complexes for all systems. Stability constants for caffeine-gliclazide and caffeine-metformin HCl systems were obtained from the Ardon's plots, which are straight lines. The stability constants were calculated from the slopes and intercepts of these plots and shown in Table 1. The Ardon's plot for caffeine–gliclazide and caffeine-metformin HCl systems is shown at pH 3.4 (Fig. 11).

DISCUSSION

The spectra of target molecules alone and the mixture (1:1) of caffeine and gliclazide or metformin HCl showed significant changes in their absorption intensities. This may be due to the interaction of

caffeine with the drugs that alter the absorption intensities as donor-acceptor complexation occurs. This finding was similar to that of Amran and Hossain (1999), who studied the evaluation of *in vitro* interaction of diltiazem HCl with iron (II) in the aqueous media and Mahmood *et al*. (2000), who studied the evaluation of *in vitro* interaction of nifedipine with copper (II) in the aqueous media.

Spectrophotometric continuous variation plots showed straight lines which cross each other at 0.5 volume fraction indicating the formation of 1:1 complexes of the systems. Therefore, this analysis confirmed the formation of 1:1 complexes of caffeine with gliclazide and metformin HCl.

The mole-ratio plots produced straight lines with points of intersection, which indicated the formation of 1:1 complexes for all the systems. It should be noted that the ligand of the 1:1 complex absorbs at the wavelength selected (at 273 nm) so that the slope beyond the equivalence point is greater than zero. Therefore, we deduced that the uncomplexed cation involved in the 1:2 complex absorptions, because the initial point has an absorbance greater than zero. This observation was similar to that of Hossain and Amran (2000), who studied the interaction of omeprazole and ranitidine with diltiazem in the aqueous medium and found that omeprazole and ranitidine formed 1:1 complex with diltiazem specially at lower pH values (at acidic environment).

In the conductometric titration analysis, it was observed that when caffeine was titrated with gliclazide at pH 3.4 three distinct breaks corresponding to caffeine-gliclazide molar ratios of 1:1, 2:1 and 3:1 were found in the conductivity curve. The reverse titration showed breaks at 1:1, 2:1 and 4:1 molar-ratio. These indicate that caffeine forms stable complex with gliclazide at 1:1 molar ratio. Similar fashioned conductivity curve was observed in the case of caffeine-metformin HCl system indicating the formation of stable 1:1 complex (Fig. 9, 10). Conductometric titrations were performed to detect complexation as well as possible molar ratios of the caffeine and two oral antidiabetic agents. The results obtained were similar to that of Amran *et al*. (2006a, b), who studied the *in vitro* and *in vivo* interaction of diltiazem with ibuprofen and naproxen in aqueous medium and rabbits. During conductometric study they found that diltiazem formed 1:1 stable complexes with some other intermediary products with ibuprofen and naproxen.

The stability constants obtained from the Ardon's plot for the both systems (caffeine-gliclazide and caffeine-metformin HCl) were found to 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 both the systems while at other pH conditions relatively weak complexes are formed.

The studies of interaction between oral anti-diabetic drugs and other agents have been carried out (Milon and Hossain, 2009; Rahman and Hossain, 2008; Salam and Hossain, 2001). The aim of the present study was to investigate *in vitro* complex formation and also to study the nature and strength of complexes which could be formed due to interaction of caffeine with gliclazide and metformin hydrochloride. The results of the present study indicated that caffeine formed 1:1 complexes with some other intermediate products. The stability constants that are the strength of the complexes were summarized in Table 1. From this table we observe that the stability constant values for caffeine-metformin system at lower pH values are higher and the value of stability constant 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.

In vitro studies sometimes contradict with *in vivo* studies by the same method and in the same experimental models. In a recent study Amran *et al*. (2009), it was found that in the *in vitro* study ibuprofen decreased 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.

If we compare the results of the present study with our previous studies (Amran, 2006a, b, 2008), 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 form hydrogen bonding network between these two compounds.

CONCLUSION

Observation of spectral data has revealed the possibility of interaction of caffeine with gliclazide and metformin HCl. Continuous-variation plots, Mole-ratio plots and Conductrometric titrations have showed that 1:1 complexes are formed between caffeine and each of the interacting species. The Ardon spectrophotometric plots confirmed the phenomenon of 1:1 complexation in all cases. 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-metformin HCl system in all pH conditions. It has indicated a comparatively weak interaction in the caffeine-metformin HCl system.

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