Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 9, 2015

Original Article

COMPLEXATION STUDY OF GLIMEPIRIDE WITH Mg^{2+}, Ca^{2+}, Cu^{2+}AND Zn^{2+}CATIONS IN METHANOL BY CONDUCTOMETRY, SPECTROPHOTOMETRY AND LC-MS

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Received: 31 May 2015 Revised and Accepted: 06 Jul 2015

ABSTRACT

Objective: The present work describes complexation study of sulfonylurea based drug, glimepiride with divalent metal ions (Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}) in methanol by conductometry, spectrophotometry and liquid chromatography-mass spectrometry.

Methods: The stoichiometry of resulting metal ion-glimepiride complexes were ascertained by molar conductance vs. mole ratio of glimepiride/metal ion plots, Job's method of continuous variation and liquid chromatography-mass spectrometric analysis. The values of enthalpy and entropy of complexation reactions in methanol were obtained from van't Hoff plots.

Results: The formation constants of 1:1 (M^{2+}: glimepiride) complexes at different temperatures followed the order Mg^{2+}>Cu^{2+}>Zn^{2+}>$Ca^{2+}$by conductometry as well as spectrophotometry. High molar conductivities were observed for all the complexes indicating formation of charged complex and the results were supported by the presence of protonated precursor complex ions in the mass spectral study.

Conclusion: The stability of complexes increased with temperature suggesting endothermic nature of complexation reactions. The thermodynamic data showed that all the complexes formed were entropy stabilized and enthalpy destabilized. A good linear relationship betweenΔH and $T\Delta S$ values suggests existence of entropy–enthalpy compensation in the complexation of these four cations with glimepiride.

Keywords: Glimepiride, Conductometry, Spectrophotometry, Liquid chromatography-mass spectrometry, Formation constant, Thermodynamic parameters.

INTRODUCTION

Diabetes mellitus is one of the most serious metabolic disorders characterized by high blood glucose (blood sugar) and disrupted insulin secretion. Diabetic patients have an increased prevalence of hypertension, sexual dysfunction, atherosclerotic cardiovascular, peripheral arterial, cerebrovascular and kidney diseases [1]. Glimepiride (GMP), a second generation sulfonylurea is well tolerated and offers an efficacious option for the treatment and management of Type 2 (non insulin dependent) diabetes [2]. It reduces blood glucose levels without deleterious alterations in plasma lipoproteins in Type 2 diabetic patients. GMP increases insulin levels in blood by binding to sulfonylurea receptor on the adenosine triphosphate (ATP) sensitive potassium ion channel of pancreatic β-cell and thus facilitates outflow of potassium ions and influx of calcium ions to promote insulin secretion from pancreas [3]. Its binding with 65-kDa protein regulates release of insulin and lowers threat of hypoglycaemia [4]. GMP is administered orally in a once daily dose of 1-2 mg which can extend up to 8 mg. It gets completely absorbed after 1 h, showing maximum glucose lowering effects within 4 h after administration [5].

Metal ions play a vital role in growth, overall health and maintenance of human body but at the same time can induce various diseases due to their imbalance. Electron rich ligands or drugs effectively bind and interact with metal ions to give so called "metallodrugs" and "metallo pharmaceuticals". They offer promising and unique therapeutic applications as they possess multi-functional properties without the threat of drug-resistant strain [6]. Several drugs and potential pharmaceuticals possess metal-binding sites and can influence their biological activity and sometimes can inflict damage to the target biomolecules [7]. As GMP has an extensive use with fewer side effects for long term therapy, co-administration with number of other drugs and various dietary supplements may enhance or reduce its effects. For instance, magnesium oxide is taken as an antacid that increases GMP absorption rate throughout the body while lithium supplement with GMP reduces blood glucose level leading to increased risk of hypoglycaemia [8]. Chromium supplements reduce blood glucose level effectively however; effect on glucose metabolism has not been observed [9]. Further, higher intake of zinc is associated with somewhat lower risk of Type 2 diabetes especially in women [10]. In a 24 weeks therapy, GMP taken with insulin glargine (containing zinc chloride) showed improved glycaemic control without any incidence of hypoglycaemic episodes [11]. In this context it is essential and useful to investigate the effect of metal ion on the therapeutic efficacy and mode of action of GMP.

Literature survey revealed few studies on metal ion-GMP binding with different transition and inner transition metal ions. GMP complexation with mercury and lanthanum metal ions have been studied by spectral and thermogravimetric analysis [12, 13] have synthesized lanthanide (Nd^{3+}, $Tb^{3+}$and Er^{3+})-GMP complexes and evaluated their antimicrobial activity.

Metal complexes of $Mn^{2+}$and $Co^{2+}$with GMP have been synthesized and characterized by IR spectra, electronic spectra and molar conductance data [14]. In another report, GMP complexes with Cu^{2+}, Mg^{2+}, $Ni^{2+}$and Cd^{2+}metal ions were characterized by their physical and analytical data [15]. Similarly, spectral studies have been carried for GMP complexes with $Hg^{2+}$and molybdenum [16, 17]. Furthermore, $Nd^{3+}$and Sm^{3+}complexes of GMP are also studied for their hypoglycemic activity [18, 19].

Conductometry and spectrophotometry are two versatile and widely used tools in the field of co-ordination chemistry to investigate the phenomena of complexation [20, 21]. In the present study, binding of GMP with bivalent metal ions Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}has been investigated by conductometry and spectrophotometry. Formation constants of the complexes have been evaluated by both these techniques. The stoichiometry of complexes is verified through molar conductance-mole ratio plots, Job's method of continuous variation and confirmed by liquid chromatography-mass spectrometric (LC-MS) analysis. Thermodynamic parameters of

complexes are estimated to gain further insight into the nature and strength of binding.

MATERIALS AND METHODS

Chemicals and reagents

Reference standard of glimepiride (99.27 %) was purchased from Clearsynth Labs Pvt. Ltd. (Mumbai, India). Analytical grade nitrate salts of bivalent metal ions, Mg $(NO_3)_2 \cdot 6H_2O$, Ca $(NO_3)_2 \cdot 4H_2O$, Cu $(NO_3)_2 \cdot 3H_2O$ and Zn$(NO_3)_2 \cdot 6H_2O$ were procured from Central Drug House Pvt. Ltd. (New Delhi, India) having purity ≥ 99.8 %. HPLC grade methanol and acetonitrile were purchased from Mallinckrodt Baker, S. A. de C. V. (Estado de Mexico, Mexico). Analytical grade ammonium acetate was obtained from Merck Specialties Pvt. Ltd. (Mumbai, India). Water was purified using Milli-Q water purification system from Millipore (Bangalore, India). All standard stock and working solutions of GMP and metal ions were prepared by accurately weighing known amounts of the compounds in methanol having conductivity less than 1.0×10^{-7} S/cm.

Instrumentation and conditions

The conductance measurements were carried out using 856 Conductivity Module with touch control from METROHM AG (Herisau, Switzerland). A dip-type conductivity cell with a cell constant of 0.59 cm^{-1} was used. The cell constant of the conductivity cell was determined by measuring the conductivity of aqueous potassium chloride solutions of different concentrations [22]. A thermo stated water bath was used to maintain a constant solution temperature at the desired value having an accuracy of±0.01 °C. Spectrophotometric measurements were performed on Shimadzu UV-1700 double beam spectrophotometer (Kyoto, Japan) with matched 10 mm quartz cells.

The wavelength accuracy was kept within±0.5 nm and a bandwidth was set at 1 nm. For data processing, Shimadzu UV PC software version 2.0 was used. The spectra were recorded at a scan speed of 400 nm/min. Sartorius GD503 (Bradford, MA, USA) analytical balance having a readability of 0.0001 g was employed for weighing of samples.

Chromatographic analysis of metal ion-GMP complexes was carried out on Shimadzu LC-VP HPLC system (Kyoto, Japan) using ACE C18 (100 mm length × 4.6 mm internal diameter, 5 µ particle size) analytical column (Advanced Chromatography Technologies, Aberdeen, Scotland) maintained at 35 °C in a column oven. A mobile phase consisting of acetonitrile: 1.0 mM ammonium acetate pH 4.0 (75:25, *v/v*) was employed for isocratic elution which was delivered at a flow rate of 1.0 mL/min. The auto sampler temperature was maintained at 5 °C and the pressure of the system was held at 1100 psi. Mass analysis was performed on a triple quadrupole mass spectrometer MDS SCIEX API-2000 (Toronto, Canada), equipped with electro spray ionization operating in positive ionization mode. The optimized parameters set were turbo heater temperature: 400 °C; ion spray voltage: 5500 V; entrance potential: 10 V; declustering potential: 65 V; curtain gas pressure: 20 psig; Gas 1 (nebulizer gas): 30 psig; and dwell time: 200 ms. Analyst classic software version 1.5.1 was used to control all parameters of HPLC and MS.

Conductometric titration

In order to evaluate the formation constants (log K_f), 50 mL (2.0×10^{-4}M) solution of the desired metal salt solution in methanol was placed in specially designed water jacketed titration cell. To maintain a constant temperature it was connected to a thermo stated circulator water bath and the conductance of the solution was measured. A 2.0×10^{-3}M solution of GMP was added in a stepwise manner using a pre-calibrated micro-burette and the conductance of the solution was measured after each addition and stirring the mixture at the desired temperature. Addition of the drug solution was continued until its concentration was four times that of the metal ions. The same procedure was followed at 25, 35 and 45 °C and the conductivity data was used for the calculation of the formation constant of the complexes at the desired temperatures. The conductivity data was analyzed by a nonlinear least-squares curve fitting program as reported previously [23, 24].

Spectrophotometric measurements

Molar ratio method [25] and Job's method of continuous variation [26] were used to determine the stoichiometry of metal ion-GMP complexes. For mole ratio method, series of solutions containing fixed amounts of cations (1.0×10^{-4} M) and varying concentration of GMP in methanol were prepared. For Job's method, the absorbance of complex of a series of solutions containing metal ions (1.0×10^{-4} M) and GMP (1.0×10^{-4} M) in different mole fractions but constant total concentration was measured. Prior to spectrophotometric measurements all the solutions were equilibrated at 25 °C at the respective wavelength maxima.

Liquid chromatography and mass spectrometric (LC-MS) analysis

Standard solutions of GMP and metal ions (2×10^{-4} M each) were prepared in methanol and mixed in 1:1, 1:2, 1:3 and 1:4 (M: GMP) stoichiometry ratio respectively. Aliquots of 10 µl were then injected into the LC-MS system and the mass spectra were recorded from *m/z* 100-1200.

RESULTS AND DISCUSSION

Conductometric study

To study the interaction of GMP with nitrate salts of Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}ions in methanol, the molar conductance (Λ_M) of the solution was monitored as a function of mole ratio of GMP/metal ion by titration of metal ion solutions (2×10^{-4} M) with 2×10^{-3} M solution of GMP. The stoichiometry of the complexes formed was evaluated by these mole ratio plots at different temperatures (25 °C, 35 °C and 45 °C). As evident from fig. 1 (A-D) there is a gradual decrease inΛ_M with increase in GMP concentration for all four cations. This indicates that M^{2+}-GMP complexes have less ionic mobility compared to solvated cations. Further, the slope of each molar conductance-mole ratio plots changes at a point where GMP/cation ratio is about 2, which is an evidence for the formation of 1:2 complexes [M^{2+}:$(GMP)_2$]. Further addition of GMP resulted in no significant change in molar conductance.

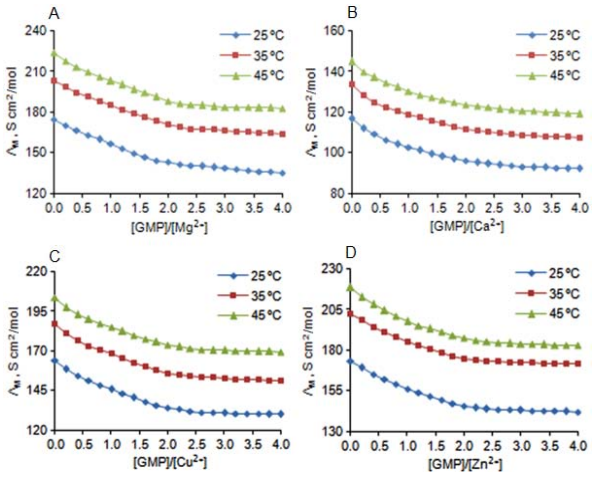


Fig. 1: Molar conductance vs. mole ratio [GMP]/[M^{2+}] plots for (A) Mg^{2+}, (B) Ca^{2+}, (C) $Cu^{2+}$and (D) Zn^{2+}in methanol at 25 °C, 35 °C and 45 °C

The formation constant values for all four cations were evaluated using a nonlinear least-squares curve fitting program from the corresponding molar conductance vs. mole ratio plots at three different temperatures (table 1).

From the results it is evident that the stability of the ML_2 complexes vary in the order Mg^{2+}>Cu^{2+}>Zn^{2+}>Ca^{2+}at all temperatures. The higher stability of Mg^{2+}compared to $Cu^{2+}$and $Zn^{2+}$with almost identical ionic size can be reasonably ascribed to hard soft acid base (HSAB) principle. The hard oxygen donor atoms in GMP prefer to

bind with hard Lewis acid Mg^{2+}more strongly compared to soft Lewis acids $Cu^{2+}$and Zn^{2+}. However, contrary to this interpretation the stability of $Ca^{2+}$was lower compared to both the transition metal ions. A possible reason for this deviation could be due to the large difference in ionic size of the hard Ca^{2+}ion (1.00Å) compared to Cu^{2+}(0.73 Å), Zn^{2+}(0.74 Å) ions. Besides the large size of GMP and Ca^{+2}cation may cause a steric hindrance during complexation, which can affect the complex stability. The changes in log K_f values of GMP complexes with the ionic radii of the metal cations is shown in fig. 2. Thus the stability of complexes was dependent on the ionic size in addition to the hardness/softness of the cations. Further the higher log K_f value for Cu^{2+}over Zn^{2+}observes the Irving Williams order for first transition series complexes with some other ligands [27]. The probable structure of the complex is depicted in fig. 3. The formation of charged complexes is evident from the high molar conductivity as shown in table 2.

Table 1: Formation constants for M^{2+}-GMP complexes in methanol at different temperatures, *n* = 3

Cation (Ionic radii, Å)	Conductometry Log K_f±Standard deviation			Spectrophotometry	
	25 °C	35 °C	45 °C	λ_{max}	Log K_f±SD at 25 °C
Mg^{2+}(0.72)	4.071±0.031	4.249±0.041	4.460±0.063	222.0	4.015±0.042
Ca^{2+}(1.00)	3.876±0.022	4.025±0.019	4.208±0.045	219.0	3.830±0.028
Cu^{+2}(0.73)	3.953±0.053	4.219±0.027	4.409±0.029	217.0	3.943±0.047
Zn^{2+}(0.74)	3.938±0.034	4.182±0.035	4.321±0.032	221.0	3.931±0.029

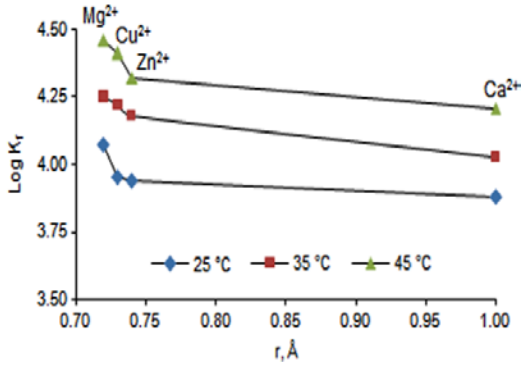


Fig. 2: Variation of log K_f values for M^{2+}-GMP (M: Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}) complexes versus the ionic radii in methanol at 25 °C, 35 °C and 45 °C

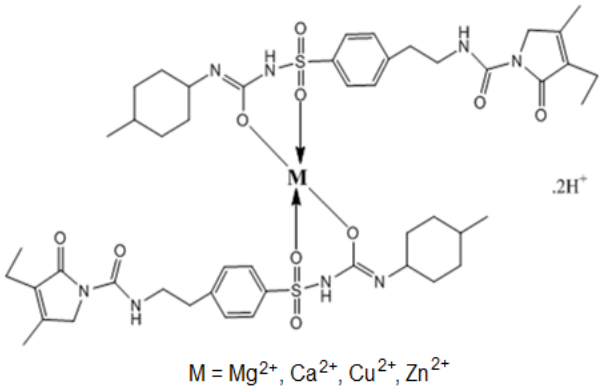


Fig. 3: Probable structure of glimepiride complexes with Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}in methanol

Table 2: Molar conductance of M^{2+}-$(GMP)_2$ complexes in methanol at different temperatures

Temperature (°C)	Molar conductance of M: L_2 complex (S/cm² mol)			
	$Mg(GMP)_2$	$Ca(GMP)_2$	$Cu(GMP)_2$	$Zn(GMP)_2$
25	142.9	95.8	145.5	134.0
35	170.8	111.7	174.9	156.0
45	188.0	123.7	187.5	173.6

In order to have a better understanding of the thermodynamic behavior of M^{2+}-$(GMP)_2$ complexes, it is useful to consider the contribution of enthalpy (ΔH) and entropy (ΔS) to these reactions in methanol. The thermodynamic data for all cations are summarized in table 3. The negative values of free energy (ΔG) indicate that the complexation process is spontaneous.

The influence of temperature on log K_f values implies that the stability of the complexes increases with increase in temperature. This dependence indicates that the complexation of these cations with GMP is an endothermic process. The values for thermodynamic quantities ΔH and ΔS were evaluated from the corresponding ln K_f vs temperature plots by applying a linear least square analysis according to the van't Hoff equation 1 [28],

$$2.303\log K_f = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \text{..... (1)}$$

The plots of ln K_f vs. 1000/T for all the complexes were linear as evident from fig. 4. The values of ΔH and ΔS were computed from the slopes and intercepts of the plots respectively. The thermodynamic data given in table 3 shows that all cation complexes were enthalpy destabilized and entropy stabilized. However, the unfavorable contribution of enthalpy was adequately compensated by the magnitude of TΔS values, which favors the process of complexation. Fig. 5 shows a good linear relationship between ΔH and TΔS values that suggests existence of entropy–enthalpy compensation in the complexation of these four cations with GMP.

Table 3: Gibbs free energy, enthalpy and entropy for M^{2+}-GMP complexes in methanol by conductometry, *n* = 3

Cation	ΔG±SD (kJ/mol)	ΔH±SD (kJ/mol)	ΔS±SD (J/mol. K)
Mg^{+2}	-23.23±0.151	35.19±0.301	195.70±2.75
Ca^{+2}	-22.12±0.102	30.08±0.404	175.00±2.98
Cu^{+2}	-22.56±0.214	41.48±0.463	215.28±2.56
Zn^{+2}	-22.47±0.142	34.84±0.443	192.59±1.95

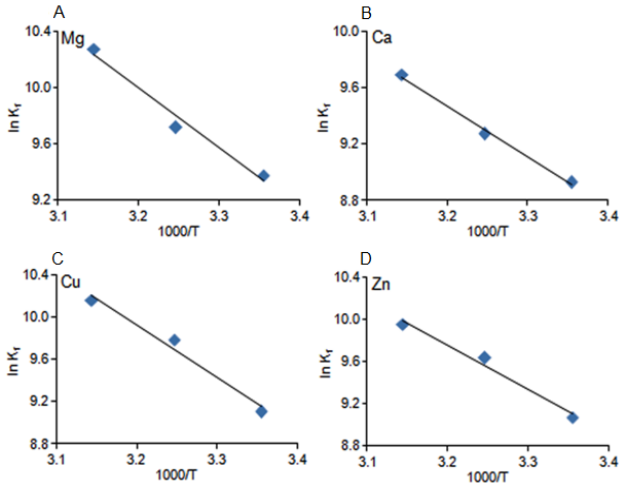


Fig. 4: van't Hoff's plot of ln K_f vs. 1000/T for the metal complexes with glimepiride (A) Mg^{2+}, (B) Ca^{2+}, (C) $Cu^{2+}$and (D) Zn^{2+}in methanol

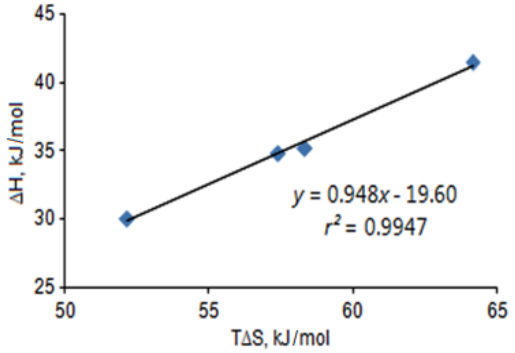


Fig. 5: Plot of ΔH (kJ/mol) versus TΔS (kJ/mol) for 1:2 complexation of Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}ions with GMP in methanol

Spectrophotometric study

The absorption spectra of GMP and its Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}complexes in methanol was recorded in the wavelength range from 200-300 nm and are shown in fig 6. A wavelength shift of the complexes from GMP spectra of about 10 nm wavelength was observed in all the cases. The mole ratio plots of absorbance vs. [GMP]/[M^{2+}] at respective wavelength maxima are presented in fig. 7.

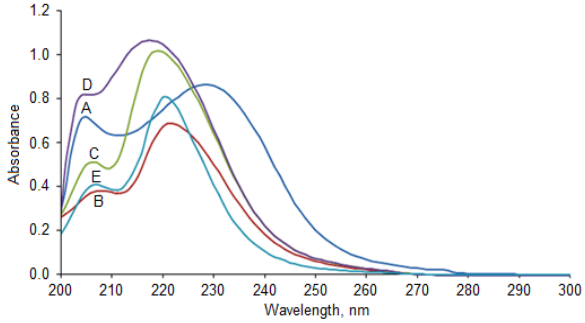


Fig. 6: Absorption spectra of (A) glimepiride (1.0×10^{-4} M) and its complexes with (B) Mg^{2+}, (C) Ca^{2+}, (D) $Cu^{2+}$and (E) Zn^{2+}in methanol

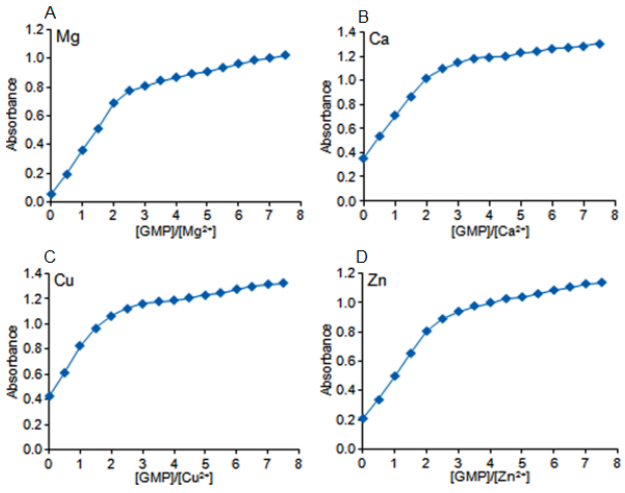


Fig. 7: Mole ratio [GMP]/[M^{2+}] plots for (A) Mg^{2+}, (B) Ca^{2+}, (C) $Cu^{2+}$and (D) Zn^{2+}in methanol at their respective wavelength maxima at 25 °C

It can be seen that increasing [GMP]/[M^{2+}] concentration ratio causes an increase in absorbance until this ratio reaches 1:2. Beyond this ratio the absorbance tends to level off for all four cations. This indicates that the stoichiometry of the complexes formed is 1:2 (M^{2+}:GMP). These findings were further confirmed using Job's method of continuous variation. The change in absorbance at the corresponding wavelengths was recorded for a set of solutions in which the total concentration of GMP plus metal ion was constant while their individual molar concentration varied from one solution to another. Fig. 8 shows that the change in absorbance exhibits maxima at a GMP mole fraction of about 0.67. This confirms formation of relatively stable metal ion-GMP complexes with 1:2 stoichiometries.

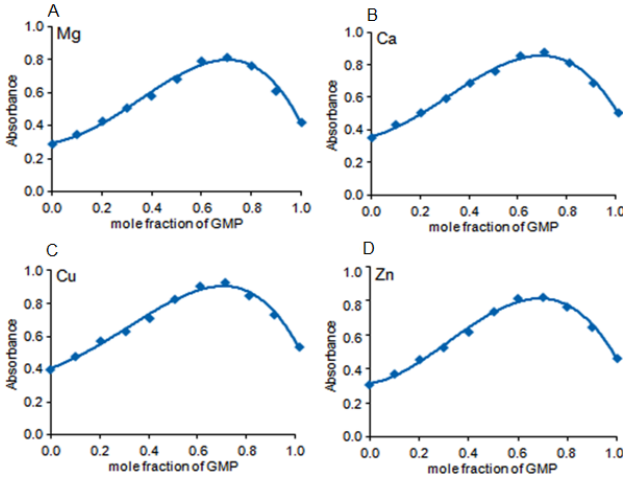


Fig. 8: Job's plots for the reaction between glimepiride and cations at respective wavelength maxima, (A) Mg^{2+}, (B) Ca^{2+}, (C) $Cu^{2+}$and (D) Zn^{2+}in methanol

The formation constants K_f was also determined using the Rose-Drago method for UV-visible spectroscopy using equation 2 below as described previously [29].

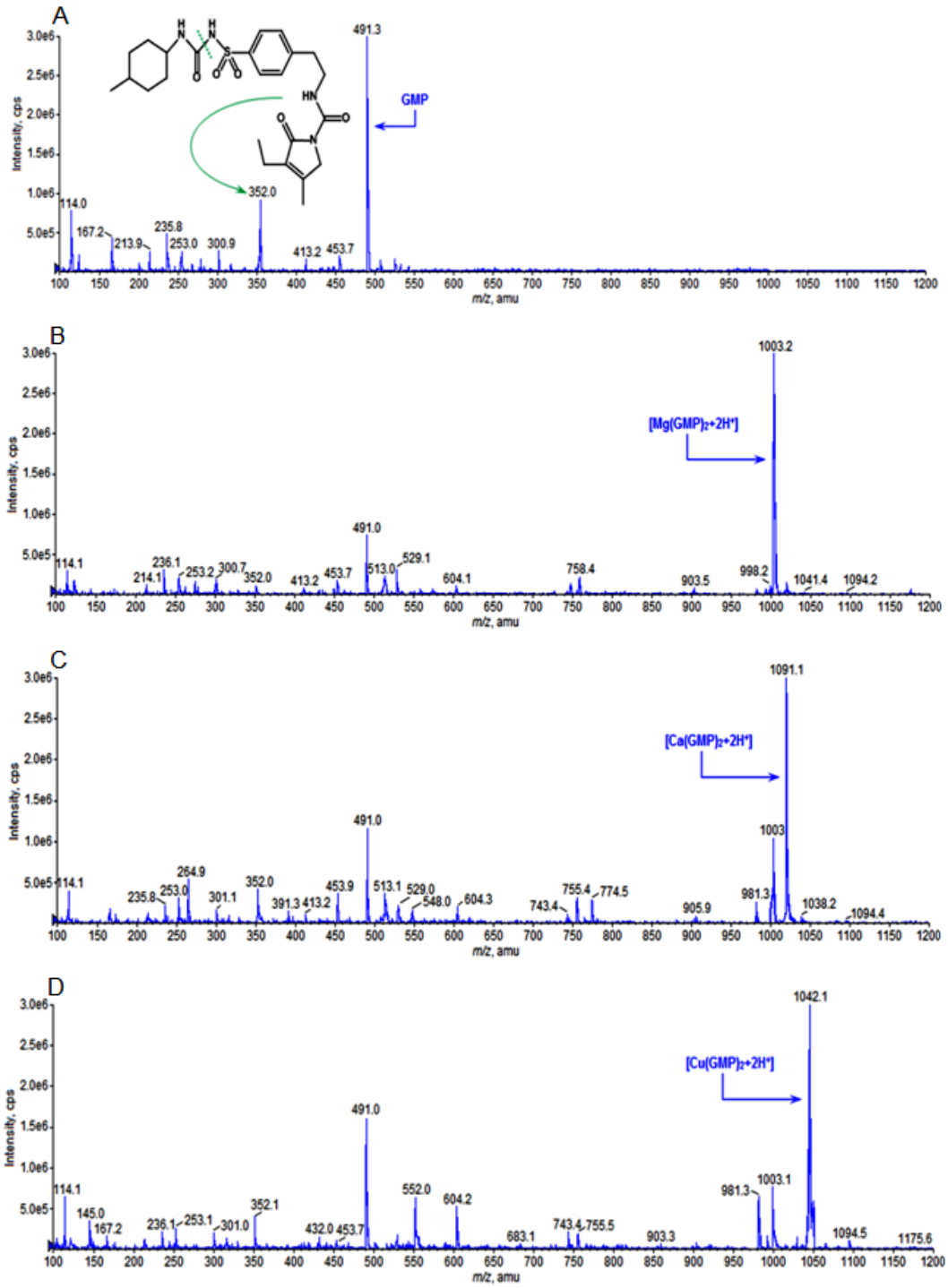
$$\frac{1}{K} = \frac{A_{obs} - \epsilon_M \cdot [M]_t - \epsilon_{GMP} \cdot [GMP]_t}{\epsilon_c - \epsilon_M - \epsilon_{GMP}} - ([M]_t + [GMP]_t)$$

$$+ \frac{\epsilon_c - \epsilon_M - \epsilon_{GMP}}{A_{obs} - \epsilon_M \cdot [M]_t - \epsilon_{GMP} \cdot [GMP]_t} \cdot [M]_t \cdot [GMP]_t \quad ..(1)$$

Where $\in_c$, $\in_M$ and $\in_{GMP}$ are the molar absorptivity of the complex, metal ion and GMP respectively; A_{obs} is the absorbance value for the mixture of standard mixtures containing $[M]_t$ and $[GMP]_t$ (concentration of metal ion and GMP respectively). The calculated values were in good agreement with those found by the conductometric method as shown in table 1. Further, the stability order of complexes $Mg^{2+}>Cu^{2+}>Zn^{2+}>Ca^{2+}$was also identical with that obtained by conductometry.

LC-MS analysis

LC-MS analysis of solutions containing cations and GMP in methanol showed clear evidence of 1:2 (M: L) complex formation. Fig. 9(a-e) presents Q1 full scan mass spectra (100-1200 amu) for GMP and its complexes with Mg^{2+}, Ca^{2+}, $Cu^{2+}$and Zn^{2+}cations respectively in the positive ionization mode. All fig. (fig. 9b-e) contain protonated precursor ions corresponding to the formation of 1:2 complexes, parent drug (*m/z* 491) and a major fragmentation product of GMP (*m/z* 352) due to elimination of methylcyclohexyl carbamoyl group (fig. 9a). Further, all protonated precursor complex ions were found at 2 mass units higher [M $(GMP)_2$+2H$^+$] compared to their excepted masses. This further confirms formation of charged complexes and is supported by the high conductance value for the complexes as shown previously in table 2. Further, an extended mass scan up to 1700 amu revealed no fragments corresponding to higher order complex formations (fig. not shown). All the chromatograms showed overlapping peaks corresponding to the protonated complex ion and parent ion. However, it was not possible to resolve the peaks by subtle changes in the mobile phase composition on ACE column.



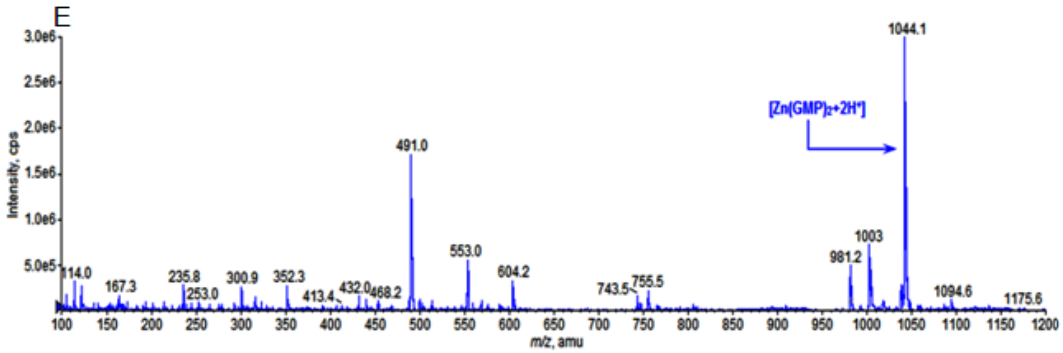


Fig. 9: Q1 mass spectra of (A) glimepiride and its complexes with (B) Mg^{2+}, (C) Ca^{2+}, (D) $Cu^{2+}$and (E) Zn^{2+}cations

CONCLUSION

In summary we have successfully demonstrated the binding ability of GMP with Mg^{2+}, Ca^{2+}, $Cu^{2+}$and $Zn^{2+}$by conductometry and spectrophotometric techniques. The study revealed formation of charged metal ion-GMP complexes as evident from their high molar conductivities. This observation was well supported by LC-MS analysis which showed presence of protonated complex $[M(GMP)_2+2H^+]$ ions in the mass spectra. Further, the 1:2 (metal: GMP) complex stoichiometry as evaluated by all the three techniques were in good agreement. The stability order of the complexes showed that ionic size and hard soft acid base (HSAB) concept plays a role in the process of complexation in methanol. The thermodynamic data revealed that the process of complexation was endothermic and that the cation complexes were enthalpy destabilized and entropy stabilized. However the higher magnitude of TΔS values compared to ΔH favored complex formation.

ACKNOWLEDGEMENT

One of the authors, Priyanka A. Shah wishes to thank DST, New Delhi for INSPIRE Fellowship (INSPIR Code IF 140410) and to the Department of Chemistry, Gujarat University for providing necessary facilities to carry out this work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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