COMPLEXATION STUDY OF GLIMEPIRIDE WITH Mg²⁺, Ca²⁺, Cu²⁺ AND Zn²⁺ CATIONS IN METHANOL BY CONDUCTOMETRY, SPECTROPHOTOMETRY AND LC-MS

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ABSTRACT

Objective: The present work describes complexation study of sulfonylurea based drug, glimepiride with divalent metal ions (Mg²⁺, Ca²⁺, Cu²⁺ and Zn²⁺) 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 (M²⁺; glimepiride) complexes at different temperatures followed the order Mg²⁺>Cu²⁺>Zn²⁺>Ca²⁺ 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 between ΔH and ΔS 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 “metallo drugs” and “metallo pharmaceuticals”. They offer promising 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 with no 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.

Metal complexes of Mn²⁺ and Co²⁺ with GMP have been synthesized and characterized by IR spectra, electronic spectra and molar conductance data [14]. In another report, GMP complexes with Cu²⁺, Mg²⁺, Ni²⁺ and Cd²⁺ metal ions were characterized by their physical and analytical data [15]. Similarly, spectral studies have been carried for GMP complexes with Hg²⁺ and molybdenum [16, 17]. Furthermore, Nd³⁺ and Sm³⁺ 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 Mg²⁺, Ca²⁺, Cu²⁺ and Zn²⁺ 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
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 (NO₃)₂·6H₂O, Ca (NO₃)₂·4H₂O, Cu (NO₃)₂·3H₂O and Zn(NO₃)₂·6H₂O 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 GDS03 (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 Kᵢ.), 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 × 10⁻⁴ 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 mixture at the desired temperature. The conductance 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 (2.0 × 10⁻⁴ M each) were prepared in methanol and mixed in 1:1, 1:2, 1:3 and 1:4 (M:GMP) stoichiometric 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 Mg²⁺, Ca²⁺, Cu²⁺ and Zn²⁺ ions in methanol, the molar conductance (Λm) of the solution was monitored as a function of molar ratio of GMP/metal ion by titration of metal ion solutions (2.0 × 10⁻⁴ M) with 2.0 × 10⁻⁴ M solution of GMP. The stoichiometry of the complexes formed was evaluated by these molar ratio plots at different temperatures (25 °C, 35 °C and 45 °C). As evident from fig. 1 (A-D) there is a gradual decrease in the molar conductance with increase in GMP concentration for all four cations. This indicates that metal-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 [M²⁺·(GMP)₂]. Further addition of GMP resulted in no significant change in molar conductance.

**Fig. 1**: Molar conductance vs. mole ratio [GMP]/[M²⁺] plots for (A) Mg²⁺, (B) Ca²⁺, (C) Cu²⁺ and (D) Zn²⁺ 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 ML₂ complexes vary in the order Mg²⁺>Ca²⁺>Zn²⁺>Cu²⁺ at all temperatures. The higher stability of Mg²⁺ compared to Cu²⁺ and Zn²⁺ 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 Mg²⁺ more strongly compared to soft Lewis acids Ca²⁺ and Zn²⁺. However, contrary to this interpretation the stability of Ca²⁺ 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 Ca²⁺ ion (1.00 Å) compared to Cu²⁺ (0.73 Å), Zn²⁺ (0.74 Å) ions. Besides the large size of GMP and Ca²⁺ cation may cause a steric hindrance during complexation, which can affect the complex stability. The changes in log Kf 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 Kf value for Cu²⁺ over Zn²⁺ 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 M²⁺-GMP complexes in methanol at different temperatures, n = 3**

<table>
<thead>
<tr>
<th>Cation (Ionic radii, Å)</th>
<th>Conductometry Log Kf, Standard deviation</th>
<th>Spectrophotometry λ_max</th>
<th>Log Kf ±SD at 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg²⁺ (0.72)</td>
<td>4.07±0.031 4.24±0.041</td>
<td>4.46±0.063</td>
<td>222.0 4.01±0.042</td>
</tr>
<tr>
<td>Ca²⁺ (1.00)</td>
<td>3.97±0.022 4.05±0.019</td>
<td>4.20±0.045</td>
<td>219.0 3.80±0.029</td>
</tr>
<tr>
<td>Cu²⁺ (0.73)</td>
<td>3.95±0.053 4.21±0.027</td>
<td>4.40±0.029</td>
<td>217.0 3.94±0.047</td>
</tr>
<tr>
<td>Zn²⁺ (0.74)</td>
<td>3.93±0.034 4.18±0.035</td>
<td>4.32±0.032</td>
<td>221.0 3.93±0.029</td>
</tr>
</tbody>
</table>

**Table 2: Molar conductance of M²⁺-(GMP)₂ complexes in methanol at different temperatures**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Molar conductance of M²⁺ complex (S/cm² mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg(GMP)₂⁺</td>
</tr>
<tr>
<td>25</td>
<td>142.9</td>
</tr>
<tr>
<td>35</td>
<td>170.8</td>
</tr>
<tr>
<td>45</td>
<td>188.0</td>
</tr>
</tbody>
</table>

In order to have a better understanding of the thermodynamic behavior of M²⁺-(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 Kf 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 Kf vs temperature plots by applying a linear least square analysis according to the van’t Hoff equation 1 [28].

\[
2.303 \log K_f = \frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (1)
\]

The plots of ln Kf 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 ΔS values, which favors the process of complexation.

**Table 3: Gibbs free energy, enthalpy and entropy for M²⁺-GMP complexes in methanol by conductometry, n = 3**

<table>
<thead>
<tr>
<th>Cation</th>
<th>ΔG±SD (kJ/mol)</th>
<th>ΔH±SD (kJ/mol)</th>
<th>ΔS±SD (J/mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg²⁺</td>
<td>-23.23±0.151</td>
<td>35.19±0.301</td>
<td>195.70±2.75</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>-22.12±0.102</td>
<td>30.08±0.040</td>
<td>175.00±2.98</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>-22.56±0.214</td>
<td>41.49±0.463</td>
<td>215.28±2.56</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>-22.47±0.142</td>
<td>34.84±0.443</td>
<td>192.59±1.05</td>
</tr>
</tbody>
</table>
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 $\Delta H$ (kJ/mol) versus $T\Delta S$ (kJ/mol) for 1:2 complexation of Mg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ ions with GMP in methanol.

Fig. 6: Absorption spectra of (A) glimepiride ($1.0\times10^{-4}$ 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.

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.

Spectrophotometric study

The absorption spectra of GMP and its Mg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ complexes in methanol were 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.

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.

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].

$$\frac{1}{K} = \frac{A_{obs} - \varepsilon_M [M]_t - \varepsilon_{GMP} [GMP]_t}{\varepsilon_o - \varepsilon_M - \varepsilon_{GMP}} - \frac{([M]_t + [GMP]_t)\varepsilon_M [M]_t - \varepsilon_{GMP} [GMP]_t}{\varepsilon_o - \varepsilon_M - \varepsilon_{GMP}} \cdot \frac{[M]_t [GMP]_t}{[M]_t + [GMP]_t}.$$  (1)
Where $\varepsilon_c$, $\varepsilon_M$ and $\varepsilon_{GMP}$ 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 $[M]$ and $[GMP]$, (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 expected 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.
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 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 complexes showed 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 $\Delta$S values compared to $\Delta$H favored complex formation.

ACKNOWLEDGEMENT
One of the authors, Priyanka A. Shah wishes to thank DST, New Delhi for INSPIRE Fellowship (INSPIR Code 140110) 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

REFERENCES

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
