Spectroscopic and Conductometric Investigation of the Interaction of Azithromycin with Iron (II) Ion

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Abstract

The interaction of the antibiotic azithromycin with ferrous sulfate and ferrous perchlorate in methanol has been investigated by spectroscopic and conductometric methods under equilibrium conditions. The interaction of azithromycin with both salts has been found to form one complex with metal to ligand composition of 1:1. The value of log K_f calculated from the absorption spectra for $FeSO_4$-azithromycin complex at 25°C was 5.16 ± 0.07 (K_f = 1.4 x 10^5 M^{-1}). The value of log K_f for $Fe(ClO_4)_2$-azithromycin complex calculated from the conductivity measurements at 25°C was 5.20 ± 0.08 (K_f = 1.6 x 10^5 M^{-1}). The enthalpy and entropy of complexation were determined from the temperature dependence of the complexation constants. The ΔH° and ΔS° were 103.8 kJ/mol and 486.9 J/mol.K, respectively. The calculated molar conductivity of the $Fe(ClO_4)_2$-azithromycin complex at 25°C was 99.7 S cm^2 mol^{-1}. This high molar conductivity has been considered indicative of the formation of a charged complex formed from the coordination of the neutral azithromycin ligand to the iron (II) ion without being accompanied by loss of protons.

Keywords: Azithromycin; Iron; Spectroscopic titration; Conductometric titration; Drug-metal ion interaction.

Introduction

Azithromycin (Figure 1) is a 15-membered azalide antibiotic with a broad spectrum of activity that has been used in treating a wide variety of infections caused by susceptible organisms [1]. It is derived from erythromycin by the incorporation of methyl substituted nitrogen atom in the lactone ring. This substitution gives azithromycin (Azi) several advantages over erythromycin. These advantages include: rapid absorption from the stomach and gastrointestinal tract, high tissue and serum or plasma concentration, wide distribution throughout the body and a high activity against a wide range of gram positive and gram negative pathogens [2-4]. Azithromycin serum elimination half-life is 44.1 hours [4]. This long serum elimination half-life and the high tissue concentration allow the single-dose daily treatment to be used for a wide variety of infections.

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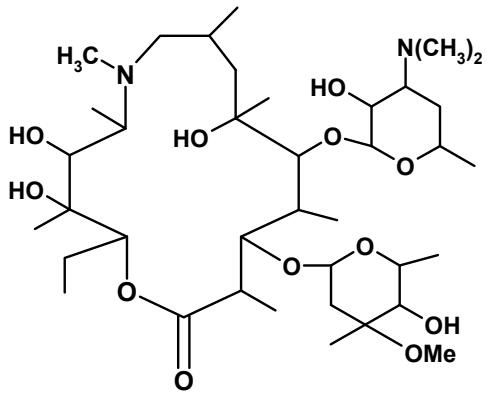

Figure 1: The chemical structure of azithromycin.

Iron is one of the essential elements for the survival of living organisms. The average adult contains 4 to 5 grams of iron and the human body maintains a meticulous balance between the loss and the absorption of this vital element [5]. The iron needs of the body are met by the dietary intake, which provides 5-20 mg of iron per day. Although this quantity is generally adequate to satisfy the normal body needs, iron supplements that normally contain iron as ferrous salts, are among the most prescribed medications. Furthermore, these iron preparations are used by many as over-the-counter medications. Iron sulfate, which is commonly used in iron and multivitamin supplements, is known to interfere with absorption of several drugs such as thyroxin [6], methyldopa [7], levodopa [8], carbidopa [9], tetracycline and its derivatives [10], ciprofloxacin and ofloxacin [11]. This interference is presumed to occur through the complexation of these drugs by ferrous ion and consequently reducing the extent of their absorptions [12]. For example, clinical work showed that the concurrent ingestion of tetracycline and its derivatives with drugs containing multivalent cations (i.e. iron, aluminum, magnesium, and calcium) could reduce the bioavailability by up to 90% [10]. Another study revealed significative modifications of the dissolution profiles of ciprofloxacin and ofloxacin as a consequence of the presence of cations, especially for Fe^{2+} which decreases 34.7% the maximum amount dissolved for ciprofloxacin and 29.1% for ofloxacin [11]. In other cases it has found that the iron can catalyze the redox destruction of some drugs (e.g., carbidopa) and rendering it inactive [9].

The lactone ring in azithromycin is substituted with a number of hydroxyl and amine functional groups. These groups are positioned in suitable configuration for interaction with metal ions [13]. The bioavailability of azithromycin can be affected by concurrent ingestion of medications containing multivalent cations. The concurrent use of aluminum- and magnesium- containing antiacids decreases the maximum concentration of azithromycin by approximately 24%, but has no effect on the area under the plasma concentration-time curve. Oral azithromycin should be administered

at least 1 hour before or 2 hours after aluminum- and magnesium- containing antiacids [14]. In this work, we studied the interaction of azithromycin with iron sulfate and iron perchlorate salts to characterize and determine the formation constants of the possible complexes that can be produced by these interactions. Furthermore, we used both spectroscopic and electrochemical methods to elucidate the structural details of azithromycin that contribute to these interactions.

Experimental

Materials

The azithromycin dihydrate used in this investigation was a gift from Al-Hikma Pharmaceuticals (Amman, Jordan) and Advanced Pharmaceuticals Industries Co. Ltd (Amman, Jordan) and was purified by crystallization from a mixture of water and methanol. The iron salts $FeSO_4.7H_2O$ (BDH, England) and $Fe(ClO_4)_2$ (Aldrich, U.S.A) where of the highest purity and were used without any further purification. All the experiments were conducted in HPLC grade methanol (Scharlau, Spain).

The optical Spectra were measured using a Unicam Helios α or Jasco 7800 uv-vis. Spectrophotometer. The conductivity measurements were carried out using WTW conductivity meter at different temperatures. The cell constant (1.002 cm^{-1}) was determined by measuring the conductivity of a series of solutions of KCl.

Spectroscopic Method

The interaction of azithromycin with iron (II) salts was studied by the preparation of a series of methanolic solutions that contain a fixed concentration of $FeSO_4$ (1.00 $x10^{-4}$ M) and a variable concentration of azithromycin. In these solutions, the concentration of azithromycin was varied in a way that makes the total azithromycin to $FeSO_4$ concentration ratio range from 0 to 8. The solution was allowed to stand at 25˚C for 1 hour to equilibrate and the optical spectra were measured. The optical spectra were analyzed to determine the coordination number of the complexes formed and to calculate their formation constants using the Specfit and Opium Softwares [15,16]. The two softwares produced almost identical results. The coordination numbers of the complexes formed were further confirmed using Job's method of continuous variations[17].

Conductometric Method

The interaction of azithromycin with Fe^{2+} was also investigated using the conductivity measurements to determine the number of complexes formed and to calculate their formation constants. In these experiments $Fe(ClO_4)_2$ was used instead of $FeSO_4$ to reduce the effect of ion association on the measured conductivities. In a typical experiment, 50.00 mL of $Fe(ClO_4)_2$ solution (2.00 x 10^{-4} M) was titrated with a standard solution of azithromycin. The dilution of the $Fe(ClO_4)_2$ during the titration can seriously complicate the calculation of the formation constant [18]. This problem has

been eliminated by titrating the $Fe(ClO_4)_2$ solution with a solution that contains a known concentration of azithromycin (2.50×10^{-3} M) in addition to an equal concentration of $Fe(CLO_4)_2$ to that of the solution to be titrated. Thus, the concentration of $Fe(ClO_4)_2$ remains constant during the titration. The titration was continued until the desired ligand to metal total concentration ratio is attained. The formation constant at various temperatures was calculated from conductivity data by the method of Buschmann [18]. This method is based on assuming an initial value for the formation constant and using it to calculate a value for the molar conductivity of the complex. With this value, the molar conductivities of the solutions at various molar ligand to metal ratios were calculated. By symmetrical variation of the value of the formation constant, the value of the molar conductivity of the complex that minimizes the error square sum $\Sigma(\Lambda_{obs}-\Lambda_{cal})^2$ is selected. A transform program that runs within the environment of the SigmaPlot Software was used to perform the calculations of the formation constant.

Results and Discussion

Spectroscopic Investigation

Figure (2) shows the spectra of a series of solutions of fixed concentration of $FeSO_4$ (1.00×10^{-4} M) and a variable concentration of azithromycin. In these solutions, the azithromycin to Fe^{2+} ion total concentration ratio was varied between 0 and 8 folds. It can be seen that increasing azithromycin to Fe^{2+} concentration ratio causes an increase in the absorbance at all wavelengths. However, this absorbance increase starts to levels off when the azithromycin to Fe^{2+} total concentration ratio reaches a certain point indicating that it is caused by the reaction of azithromycin and the Fe^{2+} ion. To determine the number of complexes formed from these interactions, we examined the patterns of absorbance changes at selected wavelengths. Figure (3) shows the observed changes in the absorbance at the wavelengths 352 nm, 308 nm and 208 nm upon increasing the azithromycin to Fe^{2+} total concentration ratio. It can be seen that increasing the azithromycin concentration causes an increase in absorbance at all of these wavelengths until the azithromycin to Fe^{2+} concentration ratio of 1:1 is reached. After reaching this ratio the absorbance remains practically constant. This behavior is indicative of the formation of only one complex with molar metal to ligand ratio of 1:1. Furthermore, it can be seen that the leveling off produces a sharp titration end point when the 1:1 ratio is attained indicating that the complex formed possess a relatively high degree of stability. These conclusions were further confirmed using Job's method of continuous variations [17]. Figure (4) shows the changes in absorbance at the wavelengths 360, 320, 260, 240 and 220 nm for a set of solutions in which the total concentration of azithromycin plus the concentration of Fe^{2+} is constant while the molar metal to ligand ratio varies from one solution to another. In Figure (4), it can be seen that the change in absorbance at all the wavelengths shown exhibits a relatively sharp maximum at a ligand mole fraction of 0.5. This behavior

confirms the conclusion that interaction of azithromycin with Fe^{2+} produces one relatively stable complex with a metal to ligand ratio is 1:1.

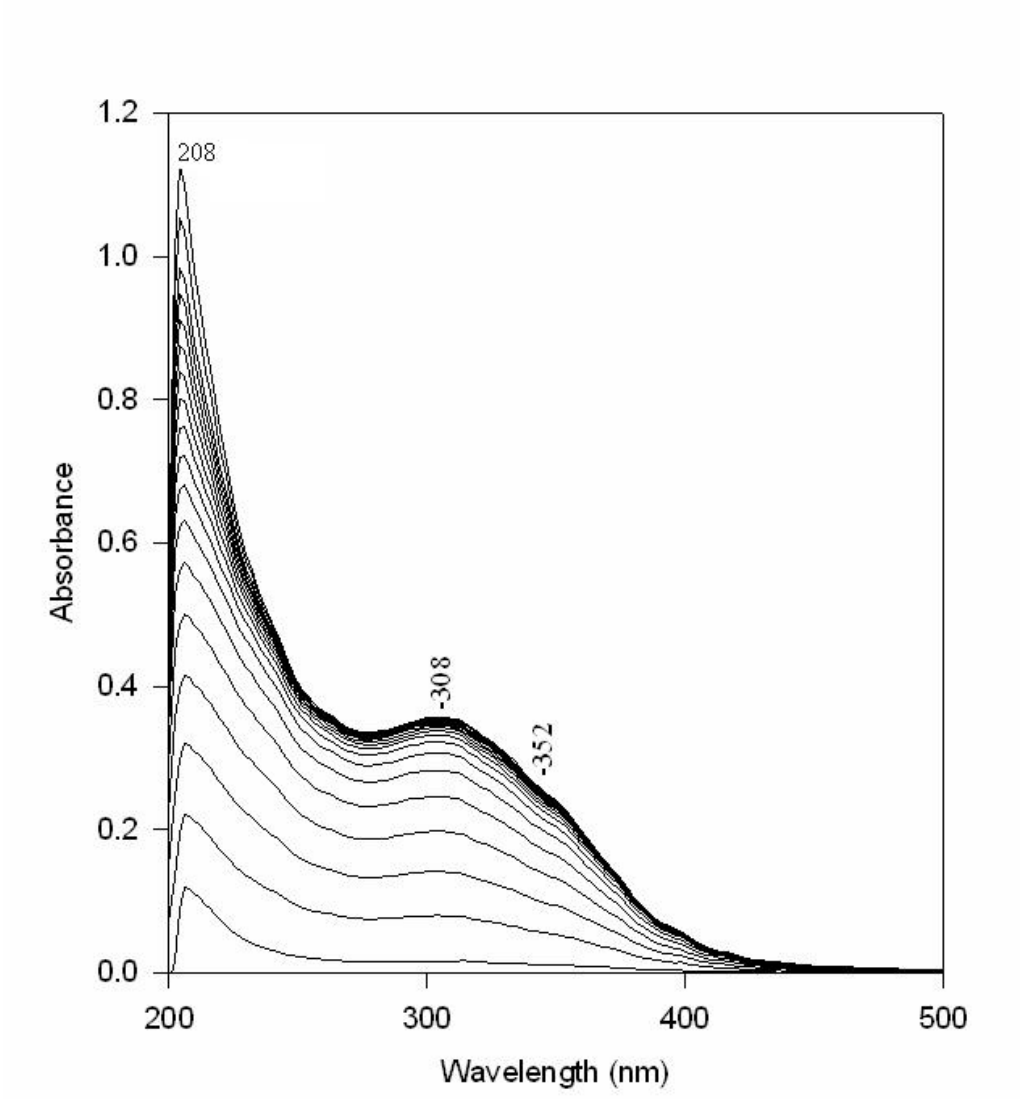

Figure 2: The optical spectra of a series of methanolic solutions containing a fixed concentration of $FeSO_4$ (1.00×10^{-4} M) and a varying concentration of azithromycin.

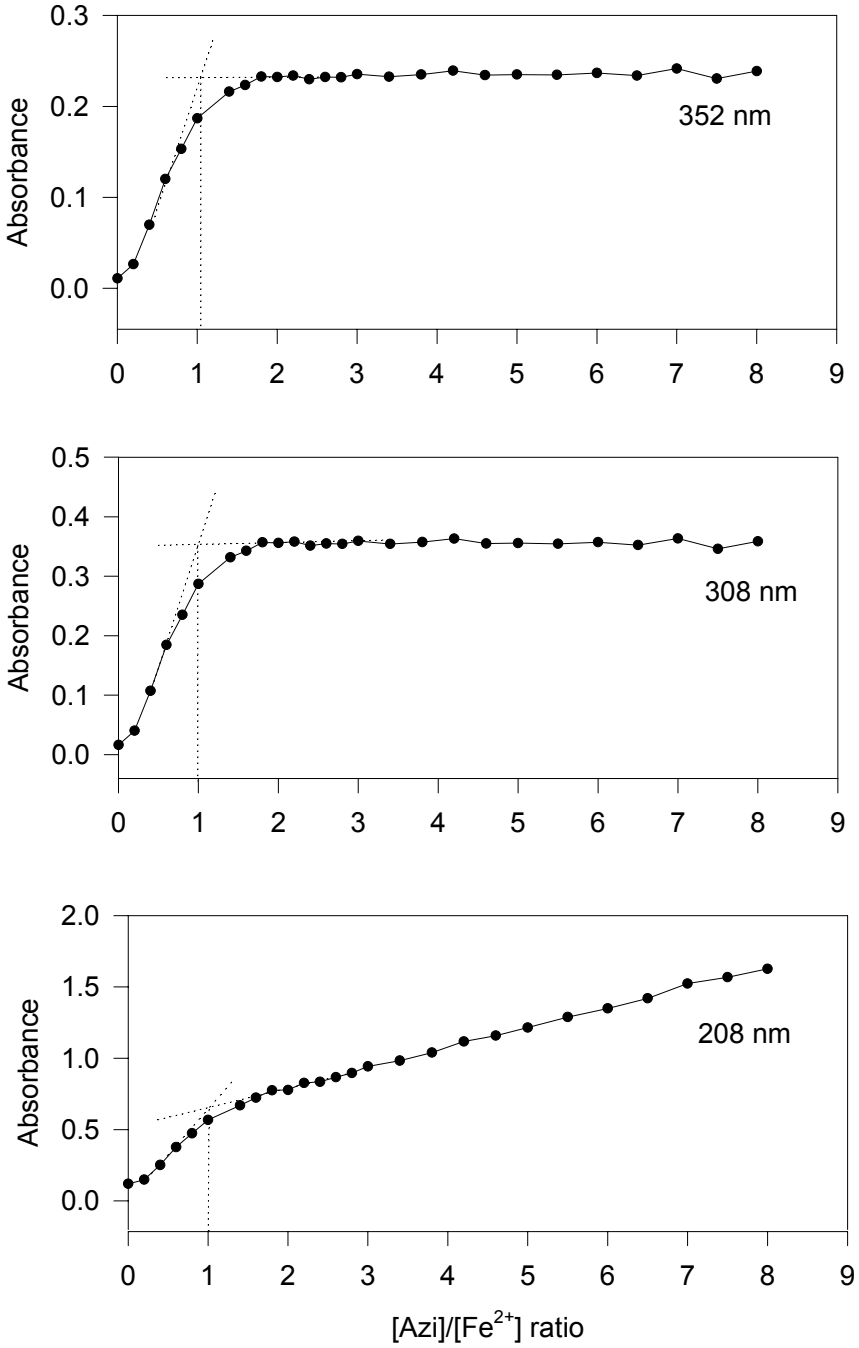

Figure 3: The observed variation in absorbance at the wavelengths 352 nm (upper), 308 nm (middle), and 208 nm (lower). The absorbance was obtained from the spectra in Figure 2.

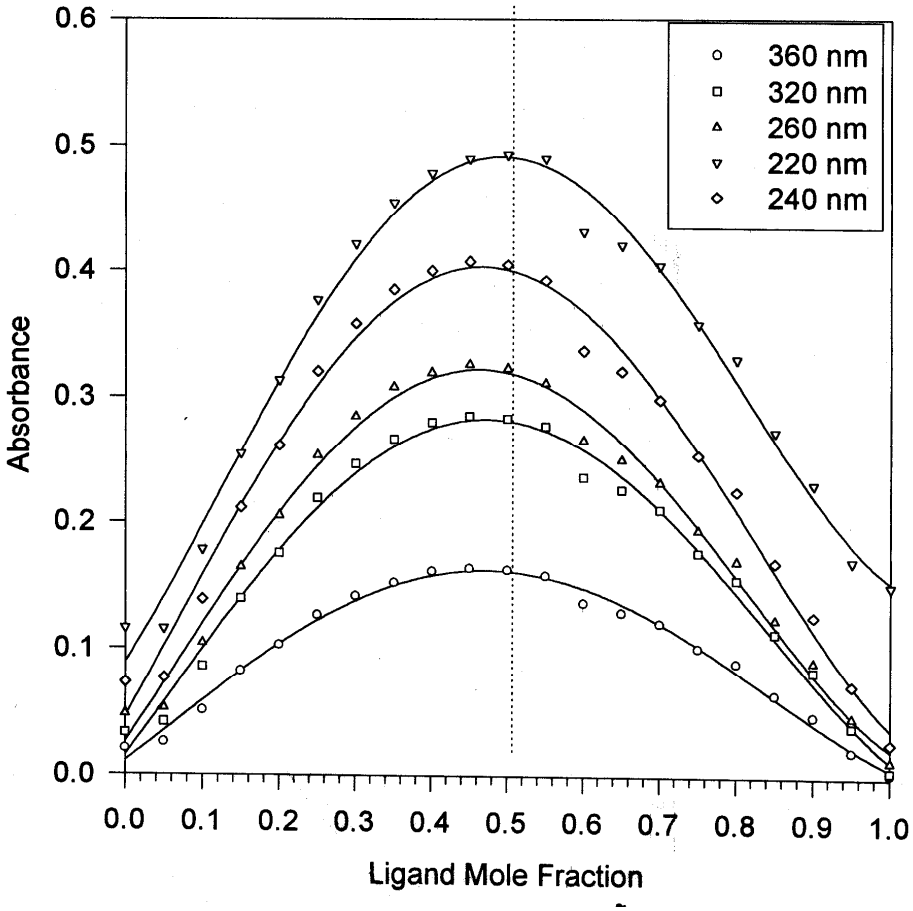

Figure 4: Jobs plots for the reaction of azithromycin with iron sulfate at selected wavelengths.

The calculation of the equilibrium constant from the spectra shown in Figure (2) was done using the Specfit computer program which uses a Levenberg-Marquardt least square fitting procedure [15,16]. The spectra in Figure (2) were fitted to a model consisting of three colored species namely the $FeSO_4$, azithromycin and the complex formed from their interactions. The optimized log K_f returned from the fit was 5.16 $\pm$ 0.07 corresponding to formation constant of 1.4 x 10^5 M^{-1}. Determination of the equilibrium constant from the spectra in Figure (2) using the Opium computer program returned a value that is in excellent agreement with that obtained from Specfit.

The Specfit and the Opium computer programs were used to determine the spectra of the various components present in the solutions using the least squares fit. The calculated spectra are shown in Figure (5). The calculated spectrum iron(II)-azithromycin complex was in excellent agreement with that obtained experimentally from a sample prepared by mixing stoichiometric amount of azithromycin and $FeSO_4$ in methanol and evaporating the solvent with a stream of nitrogen. Also by using these two programs we were able to calculate the percentage concentration profiles for $FeSO_4$, azithromycin and complex formed from their interactions. These profiles are shown in Figure (6). These profiles reveal that at azithromycin to Fe^{2+} concentration

ratio of 1:1, around 80% of the iron is present in the complexed form while about 20% of the azithromycin is present in the free form.

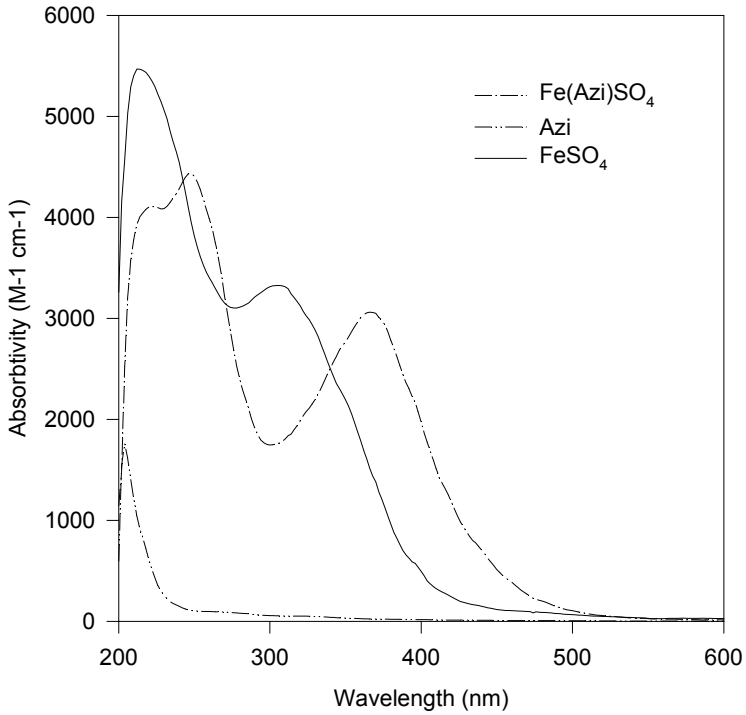

Figure 5: The calculated absorption spectra of $FeSO_4$, azithromycin and their complex.

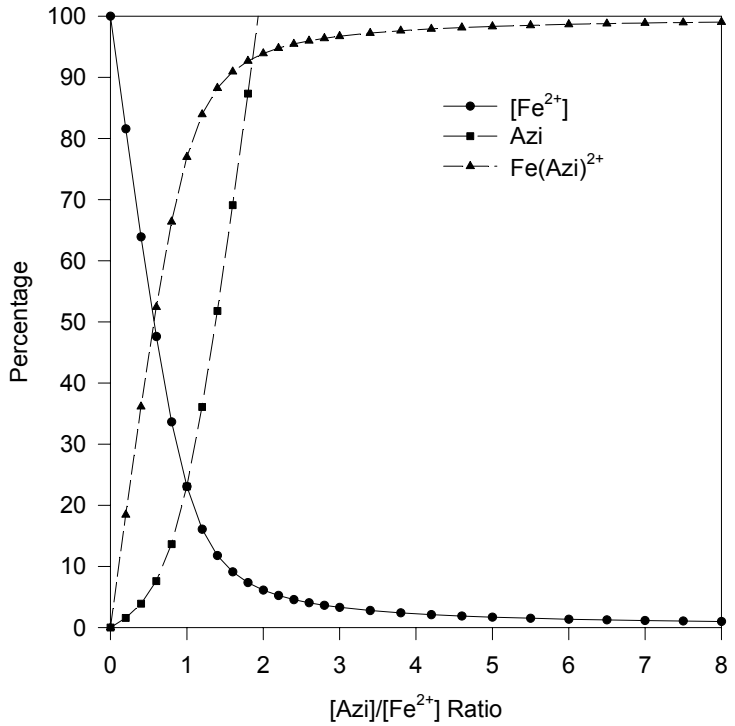

Figure 6: The percentage molar concentration of $FeSO_4$, azithromycin, and $Fe(Azi)^{2+}$ complex at different ligand to metal molar ratios.

Conductometric Investigation

In general, the free cation and the complex have different ionic mobilities. Therefore, it is expected that the addition of a ligand to a metal ion solution to change its electrical conductivity if a complex is formed. A conductance study of the interaction of $Fe(ClO_4)_2$ and azithromycin in methanol has been carried out at different temperatures (5, 15, 25, and 35˚C). The results of the titration of 2.00 x 10^{-4} M solution of $Fe(ClO_4)_2$ with a solution of 2.50 x 10^{-3} M in azithromycin and 2.00 x 10^{-4} M in $Fe(ClO_4)_2$ are shown in Figure (7). This figure demonstrates that the addition of azithromycin to a solution of $Fe(ClO_4)_2$ causes a continuous decrease in the molar conductance of the $Fe(ClO_4)_2$ solution, indicating that the mobility of $Fe(ClO_4)_2$-azithromycin complex is lower than the mobility of the solvated Fe^{2+} cation. This decrease in the molar conductance begins to level off at a ligand to metal molar ratio of about one. This behavior is also indicative of the formation of a complex with 1:1 Fe^{2+} to azithromycin ratio and the sharpness of the titration end point demonstrates the relative stability of this complex. The formation of a stable complex from the interaction of azithromycin with Fe^2 means the efficiency of azithromycin could be reduced due to the concurrent ingestion of azithromycin with drugs containing Fe^{2+}. The formation constants of the 1:1 complexes formed from the reaction of azithromycin with $Fe(ClO_4)_2$ at the various temperatures were calculated using the method of Buschmann [18]. The stability constants obtained at various temperatures are listed in Table (1). It is obvious that the stability of the complex decreases rapidly with decreasing the temperature. This indicates that the binding of Fe^{2+} and azithromycin is an endothermic process. The thermodynamic quantities ΔH° and ΔS° were derived by a linear curve-fitting of the plot of log K versus 1000/T (Figure 8). The values of ΔH° and ΔS° obtained were 103.8 ± 0.5 kJ/mol and 486.9 ± 0.9 J/mol.K, respectively. These values indicate that the complex formation is enthalpy destabilized but it is entropy stabilized. The unfavorable enthalpic contribution is compensated by the large value of the entropic contribution resulting in large values of log K. The calculated value of log K_f (5.20 ± 0.08) at 25˚C from the conductometric method was in good agreement with the value calculated from the spectroscopic study (5.16 ± 0.07). The calculated molar conductivity of the $Fe(ClO_4)_2$-azithromycin complex at 25˚C was 99.7 S cm^2 mol^{-1}. This high molar conductivity is indicative of the charged nature of the formed complex. This means that the neutral azithromycin ligand reacts with Fe(II) ion without accompanying deprotonation reactions of the ligand.

Table 1. Stability constants, enthalpy and entropy for $Fe(ClO_4)_2$- azithromycin complex in methanol.

Temperature K	Log K	Δ H^o kJ mol^{-1}	Δ S^o J mol^{-1} K^{-1}
278.15	3.69	103.8 ± 0.5	486.9 ± 0.9
288.15	4.46		
298.15	5.20		
308.15	5.68		

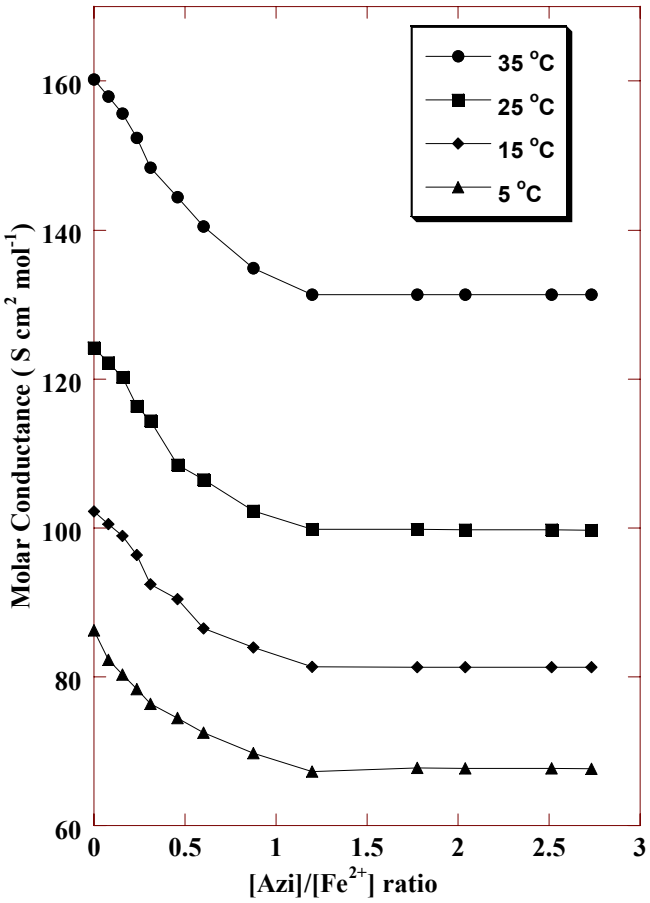

Figure 7: The variation in the molar conductivity of iron (II) perchlorate with the concentration of azithromycin at various temperatures.

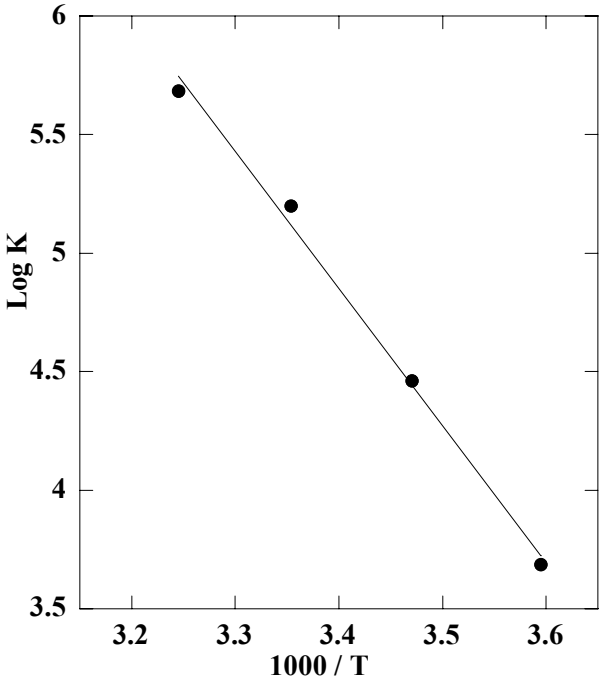

Figure 8: The plot of log K versus 1000/T for the $Fe(ClO_4)_2$-azithromycin complex.

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