Asian Journal of Chemistry; Vol. 25, No. 3 (2013), 1237-1240

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.12660

Spectrophotometric and Conductometric Investigation of Azithromycin with Ca^{2+} Cation and Determination of Azithromycin in Dosage Form by Spectrophotometric Method

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(*Received*: 30 August 2011; *Accepted*: 5 September 2012) AJC-12087

The interaction of the azithromycin with $Ca(NO_3)_2.4H_2O$ in methanol has been investigated by conductometric method to determine the number of complexes formed. The conductance data show that the stoichiometry of complex between azithromycin and Ca^{2+} is 2:1 [M:L_2] in pure MeOH. The interaction of the azithromycin with Ca^{2+} was studied by spectrophotometric method under equilibrium conditions. Calcium is a biometal in human body. The interaction of azithromycin with Ca^{2+} salt has been found to form one complex with metal to ligand composition of 1:2. The value of ($K_f = 3.7 \times 10^4$ M^{-2}) was calculated from the absorption spectra for Ca^{2+}- azithromycin complex at room temperature. Spectrophotometric method was developed and used to determine azithromycin- calcium at a λ_{max} of 260 nm. The influence of pH, time, temperature and interferences has been tested. Also, the calibration graph of complex at the presence of HCl was linear ($R^2 = 0.980$) over the range of (10^{-4}-10^{-2} M). This simple method has been applied to the determination of azithromycin in dosage form.

Key Words: Azithromycin, Ca^{2+} cation, Spectrophotometric, Drug interaction, Conductometric.

INTRODUCTION

Azithromycin (Fig. 1) is a novel of the family of 15-membered macrolide antibiotics named azalides[1]. Azithromycin plays a leading role in the treatment of respiratory tract infections, toxoplasmosis, nonclassical pathogens and oprtunistic infection in AIDS[2]. Several HPLC methods have also been described for the determination of azithromycin in human plasma using UV[3,4], electrochemical[5-13] and fluoresceen after derivation[14-17]. Clinical work showed that the concurrent ingestion of antibiotic with drugs containing multivalent cations could reduce the bioavailability by up to 90 %[18]. Experimental studies showed that the fraction of the drug not bound to protein is found in the complexed from with Ca^{2+} and Mg^{2+}[19]. The complexes involving Fe^{2+}, Zn^{2+} and Al^{3+} are also present in the biological medium and have been considered to be responsible for a decrease in the bioavailability of the drug and some observed side effects[20-24]. Also the belief that antibiotic activity is related to the formation of complexes with cations has simulated a great deal of investigation. The complexes of ampicilin[25] and amoxycilin[26] with different cations expected to increase their effectiveness for a broad antibacterial spectrum[27]. It has been proposed that catalysis occurs by means of an intermediate 1:1 metal ionantibiotic complex[28]. This work describes a spectrophotometric method exploiting azithromycin to form with the $Ca(NO_3)_2.4H_2O$ complex in non aqueous solvent.

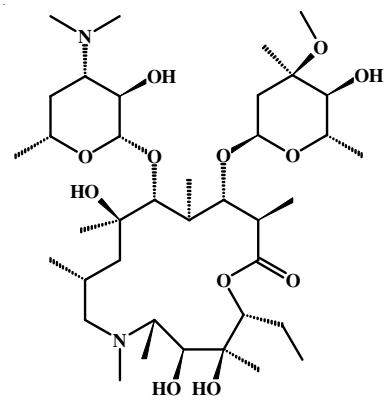

Fig. 1. Azithromycin structure

EXPERIMENTAL

Azithromycin dihydrate used in this investigation was purchased from SciENcelab, inc and was used without further purification. The methanol (Merck) and $Ca(NO_3)_2.4H_2O$ (Merck) were used with the highest purity.

The experimental procedure to obtain the formation constant of complex and the influence of the pH, time, temperature and interference has been tested. Also, this paper led us to study the reaction of azithromycin and $Ca(NO_3)_2.4H_2O$ in methanol solvent in an attempt to develop a simple method for determination of azithromycin in dosage forms.

The conductance measurements were performed on Methrohm conductivity apparatus, in a water bath thermostated with a constant temperature. The electrolytic conductance was measured using a cell consisting of two platinum electrodes to which an alternating potential was applied. A conductometric cell with a cell constant of 1.061 cm^{-1} was used throughout the study.

Conductometric method: The experimental procedure to determine the number of complexes formed by conductometric method is as follows: a solution of metal salt (5×10^{-4} M) was placed in a titration cell and the conductance of the solution was measured, then step by step increase in azithromycin ligand concentration was performed by a rapid transfer from azithromycin solution prepared in the same solvent (5×10^{-3} M) to the titration cell using a microburet and the conductance of the solution in the cell was measured after each transfer at the desired temperatures[29]. The variations of molar conductance (Λ) *versus* the ligand to the cation molar ratio [L]/[M] for complexation of azithromycin and Ca^{2+} in methanol were studied at different temperatures (Fig. 2).

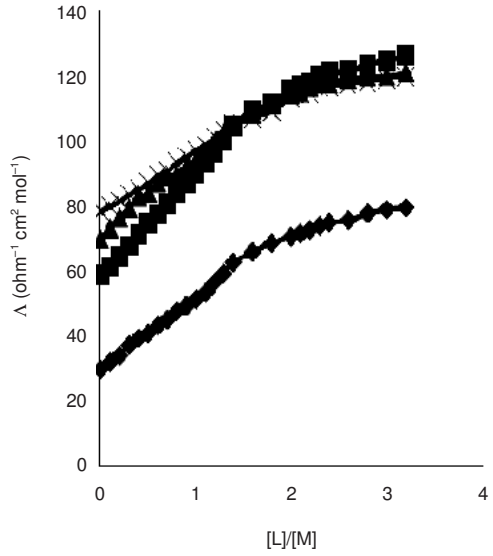

Fig. 2. Variation in the molar conductivity of Ca^{2+} with azithromycin in pure; MeOH at 15 ºC (◇), 25°C (●), 35°C (△), 45 ºC (×)

All spectrophotometric measurements were performed on a Cary 50 Bio spectrophotometer equipped with Cary Win UV software and using a 1 cm quartz cell. The pH was checked by Metrohm 780. All measurements were carried out at room temperature.

Spectrophotometric method: In this method, to obtain the maximum absorbance of complex, the stock solutions of azithromycin and Ca^{2+} cation with (2×10^{-4} M) concentration was prepared. At least, 10 fold of azithromycin were added to Ca^{2+}. Azithromycin solution was placed at room temperature for 15 min so that complex formation reaction can be completed. Complex absorbance spectra were taken and the maximum absorbance of complex came from by spectrophotometer set at 260 nm. The maximum absorbance is shown in Fig. 3.

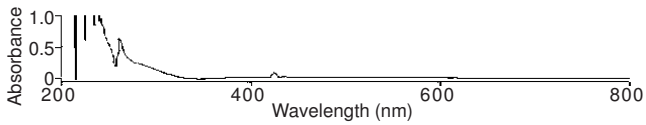

Fig. 3. Calculated absorption spectra of Ca-azithromycin complex

Preparation of standard solution: To draw calibration curve, standard solution with equal concentration of Ca^{2+} and different concentration of azithromycin was prepared. After dissolving it in 5 mL methanol, it was evened by sonication and then was transported to a 25 mL flask after filtering by whatman. As much as 1 mL from HCl was added to the whole solution to get optimum pH equal 4. Afterwards, they were diluted by deionized water. If HCl increases, the rate of absorption will go up. After adjusting pH, the rate of absorption at a λ_{max} 260 nm was measured. Absorption variation is drawn on the basis of concentration one. The results are drawn in Figs. 4 and 5.

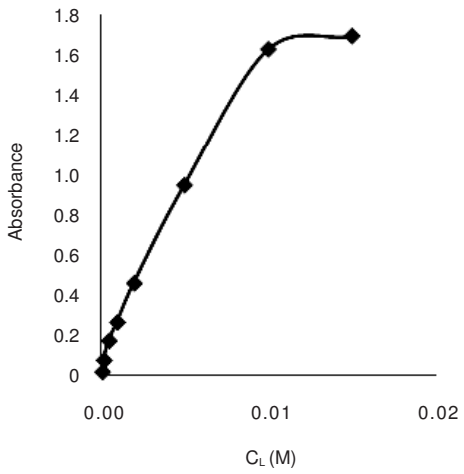

Fig. 4. Calibration curve for azithromycin complex at selected wavelength and pH

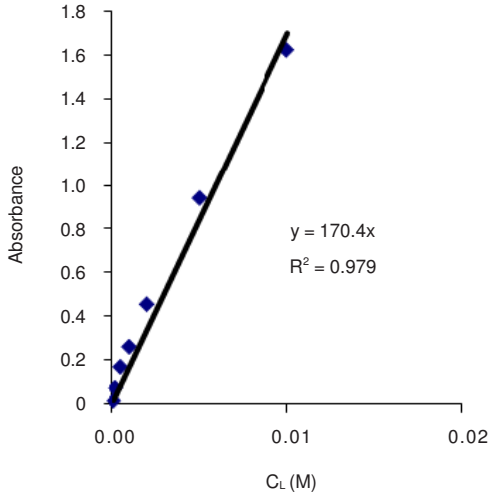

Fig. 5. Linear regression of azithromycin complex at selected wavelength and pH

Assay of azithromycin capsule: To measure the capsule sample concentration azithromycin, six capsules of azithromycin, claiming 250 mg, were mixed and weighted, then as much as 392.5 mg of that was transferred to a 5 mL flask. After diluting qualitatively up to the mark with methanol, the solution was sonicated for 15 min to have an even one. Having taken 1 mL of the above solution, transferred into 10 mL flask and added 1 mL of HCl, it would be diluted up to the mark by deionized water[30,31].

RESULTS AND DISCUSSION

In case of conductometric method, it is shown that molar conductivity in methanol system increases with the molar ratio increase of the ligand to cation. The slope of molar conductance mole ratio curves change at the point ([L]/[M] = 2) which indicates the formation of complex 2:1 (ligand: cation). The investigations of the curves show that raising azithromycin concentration will cause the increase of molar conductivity so the alterative slope will depend on solvent. The relevant curve slope can be a criterion of stable complex qualitatively. On increasing the temperature curve slope also increases and shows it is an endothermic reaction. In methanol with a relatively high Guttman donor number (DN = 20), the solvation of the Ca^{2+} ion should be strong[32]. Although the solvation of the cation is an important factor in complexation reactions, solvation of the ligand and resulting complex has also been documented to contribute to the overall free energy of complex formation[33].

In case of spectrophotometric method using constant variations, solutions with (0.1 M) concentration were prepared from dense ligand and cation solution. Then they were mixed with different volumetric ratio so that the final volume could be equal to 10 mL. After adjusting the optimum pH, the absorption of solution was measured at λ = 260 nm. It was found that increasing the azithromycin concentration caused an increase in absorption until the azithromycin to Ca^{2+} concentration ratio 2:1 was reached at. The results are shown in Fig. 6.

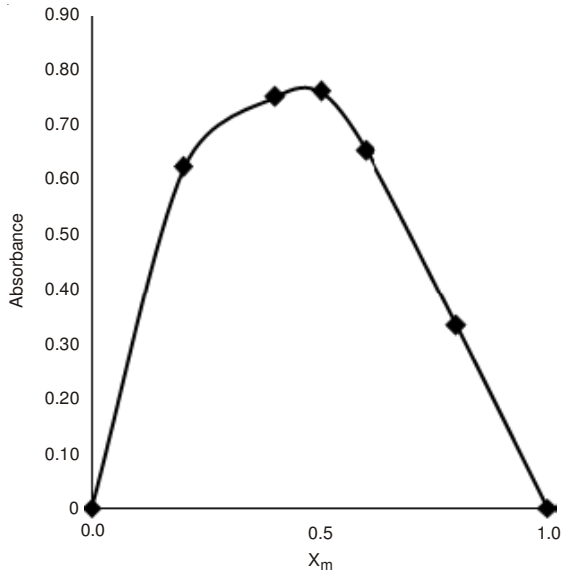

Fig. 6. Jobs plot for the reaction of azithromycin ligand with calcium ion at selected wavelength

Effect of pH: Complex absorbance spectra are considerably under the influence of acid media. To investigate the effect of pH, cation Ca^{2+} and azithromycin ligand with concentration of 1×10^{-3} M were prepared. Ligand was added ten times more than cation concentration so that the complex with any stochiometric ratio could be completely formed and finally complex absorbance on the basic of pH variation at λ = 260 nm was measured. In Fig. 7 the diagram of maximum absorption variations on the basic of pH at λ = 260 nm is shown.

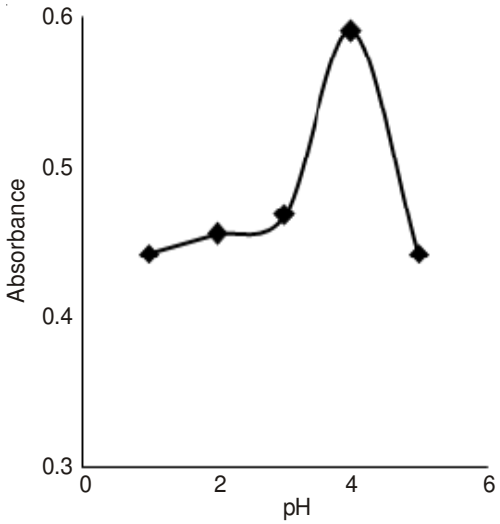

Fig. 7. Variation of absorbance *vs*. pH of complex at selected wavelength at room temperature

As it illustrated, the optimum pH equals 4 and by increasing pH above 4 the rate of absorption reduces. In pH higher than 5, the complex solution Ca^{2+} and azithromycin lost its transparency and formed sediment, hence the determination of pH in acid media continued just to pH = 5.

Effect of time and temperature: To find out the effect of temperature on forming complex, a series of solutions with 10^{-3} M from ligand and cation at 10 to 1 volumetric ratio was prepared and the absorbance of complex in various temperatures was measured. The results showed that the raising temperature increased the absorption complex so that complex formation was done better at higher temperatures (Fig. 8).

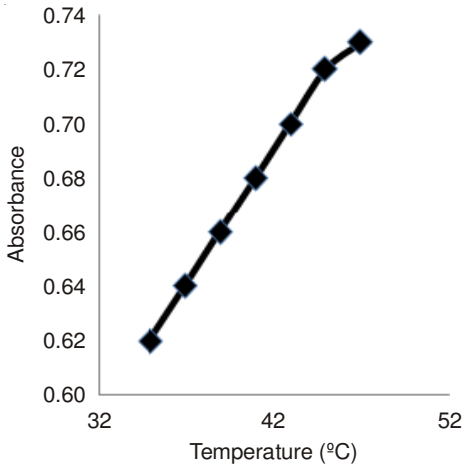

Fig. 8. Variation of absorbance *vs*. temperature of complex at selected wavelength

Also, to measure the necessary time to conduct complex production reaction a series of solvents with 10^{-3} M concentration from ligand and cation were prepared. Then they were mixed at 10 to 1 ratio to form the required complex. After that, the absorbance of complex at λ = 260 nm and optimum pH was calculated. It was observed that the formed complex absorption remained stable with the passing of time (Fig. 9).

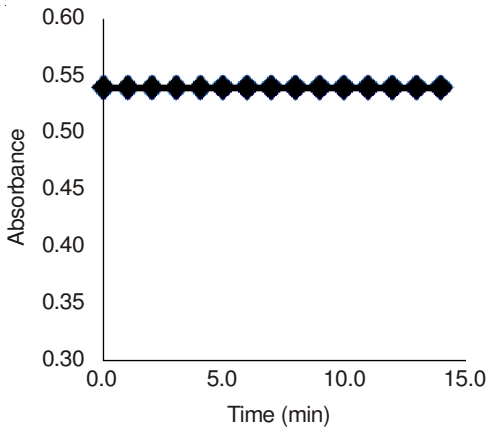

Fig. 9. Variation of absorbance *vs*. time of complex at selected wavelength

Interference: To investigate the effect of the interfering ions, solutions of Mg^{2+}, Cu^{2+}, Na^{+}, Fe^{2+} were prepared. These cations were added 100 times concentration to Ca^{2+} having 10^{-3} M concentration. The complex absorption equaled 0.5400 at the absorbance of interfering ion. As is obvious in Table-1, interfering ions were Cu^{2+} and Fe^{2+} cations.

TABLE-1
EFFECT OF INTERFERING IONS

Interfering ions	Concentration ratio of interfering ions to Ca^{2+}	Abs	RSD (%) N = 3
Mg^{2+}	100	0.5400	1.9
Na^{+}	100	0.5300	3.7
Cu^{2+}	100	0.3800	6.8
Fe^{2+}	100	0.1900	4.2

Results from analyzing azithromycin complex in dosage form: The rate of the capsule sample concentration equaled 0.2210. The linear calibration curve was used to determine the amount of indefinite concentration of azithromycin capsules equaling 10^{-3} M.

Limit of detection: To determine limit of detection, the formula (LOD = 3S/m) was used, where, S represents standard deviation and m curve slope. The amount of standard deviation equaled 0.0093 and the curve slope 170.46. Therefore, LOD equaled 10^{-4} M.

Dynamic range: As calibration curve shows linear range will be from 10^{-4} to 10^{-2} M.

Conclusion

The experiments illustrated that azithromycin is stable at room temperature for a certain time. That is because azithromycin is found in the forms of monohydrate and dihydrate. Monohydrate in the presence of moisture can convert to the more stable dihydrate form[34]. In addition, the experiments show that formation of this complex is influenced by pH and temperature. If acid media and temperature increase, the rate of complex formation will also increase. The reason of complex absorption in acid media is that macrolides are stable in neutral solution, but their glycoside relations are hydrolyzed, hence it will be more possible to form complex with multivalent ions and the increasing of temperature helps it. This fact shows that complex formation of this kind will have a growing increase in body condition with approximate temperature of 37 ºC. Spectroscopic investigation can be used to calculate K_f.

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