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Spectrophotometric and potentiometric determination of the stability constant of pyrazinamide-Fe(III) binary complex

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ABSTRACT

The present study offers valuable comparison of two techniques used for determination of stability constant of coordination complex of Pyrazinamide with Fe (III). The stoichiometry of the complex formation was examined applying the continuous variation (Job plot) method and a 1:1 molar ratio has been identified, at the examined concentrations. Stability constant Kst of the formed complex was determined spectrophotometrically according to the spectral changes of PZA absorbance due to complexation and potentiometrically using the technique adopted by Irving and Rossotti.

KEYWORDS

Pyrazinamide;
Fe (III);
UV/Visible spectroscopy;
Potentiometry;
Coordination complexation.

INTRODUCTION

Pyrazinamide (PZA) (Figure 1) is an important front-line anti- tuberculosis (TB) drug that forms the most effective TB chemotherapy along with isoniazid, rifampicin and ethambutol[1]. Pyrazinamide plays a key role in shortening the TB therapy from previously 9–12 months to 6 months[2]. Despite its high in vivo sterilizing activity[3,4] pyrazinamide has poor or practically no activity under normal culture conditions at close to neutral pH[5]. This discrepancy between the in vitro and in vivo activity of pyrazinamide reflects possible differences between in vivo and in vitro conditions that affect the drug activity. The well-known acid pH requirement for pyrazinamide activity was discovered previously based on such reasoning but does not completely explain the discrepancy between in vivo and in vitro activity of pyrazinamide[6].

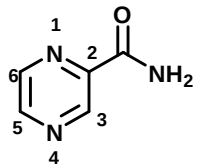

Figure 1 : Structure of PZA

The extent of complexation is an important factor, especially in therapeutics, where the pharmacological effect of a drug is directly related to its nature (meaning in free or complexed form)[7].

In a study[8], which examined the effect of iron (which could potentially be elevated in local inflammatory lesions), on pyrazinamide activity in vitro, ferric iron (ferric chloride) was found to enhance the activity of pyrazinoic acid (active metabolite of PZA) and pyrazinamide while other metal ions such as magnesium, calcium and zinc did not exhibit the same effect. The mechanism of such an effect is not yet known.

The aim of this work was to study the complex-

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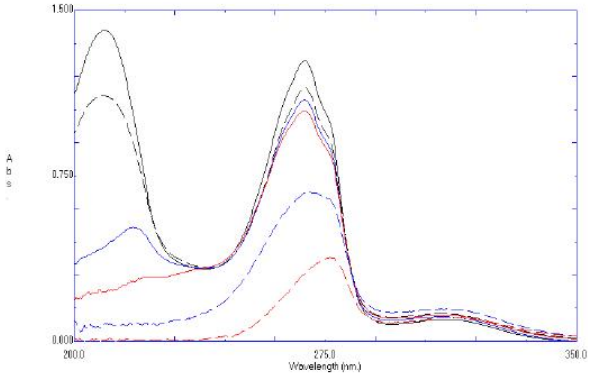

Figure 2 : UV spectra of 1.6x10^{-4}M aqueous solution of PZA in the absence (-) and presence (- - -) of increasing concentrations of $FeCl_3$ solution

ation of PZA with Fe (III) ions which is of interest because, on the one hand, the biological effect of PZA might be profoundly enhanced by complexation with such ions and on the other hand, the complex stability should be of such an order to ensure the transport of the complex through the cell membrane. These findings may have implications on the study of mechanism of action of PZA and its iron complex for improving the activity of the drug. To that end, pH measurements were used for evaluating the stoichiometry of the complex formed between the metal and the chelating agent and for computing its stability constant., using the potentiometric technique adopted by Irving and Rossotti[9,10], while the Benesi–Hildebrand linear approach was used for the spectrophotometric determination of the stability constant using the spectral shift method.

MATERIALS AND METHODS

Chemicals and reagents

(a) PZA was kindly supplied by Amoun Pharmaceutical Co.S.A.E.El-Obour City, Cairo, Egypt, and was used as received. Under license from: LUPIN CHEMICALS (THAILAND) LTD. (Assay: 99.91% according to the official USP 23 method)[11].

(b) Ferric Chloride (Sigma) 5×10^{-3} M aqueous solution. The solution was standardized by reduction to ferrous and titration with 0.1 N potassium permanganate solution in presence of Zimmermann's reagent[12].

(c) Sodium Chloride (Adwic), 0.1 M aqueous solution.

(d) Standard Buffer pH 4 and pH 10 (Panreac, Spain).

(e) Sodium Hydroxide (Fluka), 0.5 and 0.05 M aqueous solutions.

(f) Hydrochloric acid (Sigma), 1 and 0.1 M aqueous solutions.

(g) De-ionized water was used as solvent.

All chemical and reagents used through this work are of spectroscopic analytical grade. De-ionized water is used throughout the whole work and is indicated by the word 'water'.

Instruments

(a) Absorption spectra were recorded on SHIMADZU Dual-beam (Kyoto/Japan) UV - Visible spectrophotometer model UV-1601 PC with 1-cm quartz cuvettes connected to IBM compatible computer fitted with UV – PC personal spectroscopy software version 3.7 (SHIMADZU).

(b) pH meter model 3510 equipped with a combined pH electrode (Jenway).

Hot plate with magnetic stirrer

Standard solutions

Solutions of 4×10^{-3} M and 10^{-2} M of PZA were prepared by weighing and dissolving the appropriate amounts in water.

Procedures

Spectrophotometric method

Examination of the changes in PZA ultraviolet absorption spectra in the absence and in presence of Fe (III) ion

The examination of the changes in 1.6×10^{-4} M PZA spectra, in the absence and in presence of increasing $FeCl_3$ concentrations (1.25 to 7.5 times that of PZA), was achieved by scanning the wavelengths between 200 and 350 nm, using water and an equimolar aqueous solution of $FeCl_3$ as reference, respectively.

Determination of stoichiometry by continuous variation method[13]

The coordination complex of PZA with Fe(III) ion was prepared by making solutions having certain molar ratios through mixing different volumes of equimolar aqueous solutions(10^{-3}M) of PZA and $FeCl_3$ and the total concentration was kept constant ([PZA] + [Fe (III)] =M = 1.2×10^{-4} M) by completing to a constant final volume.

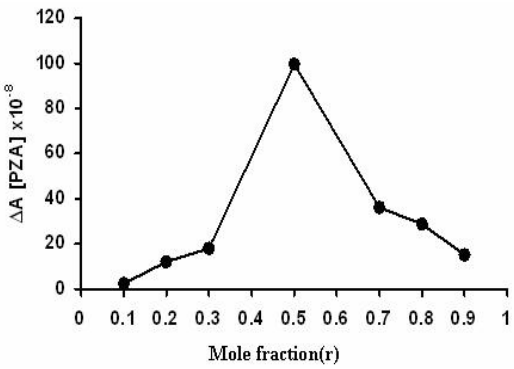

Figure 3 : Continuous variation plot (Job plot) of PZA-Fe (III) complex at 269 nm

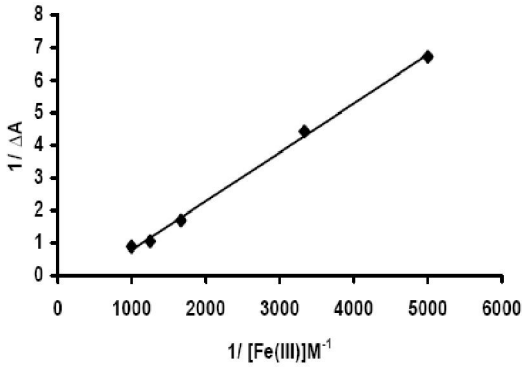

Figure 4 : Benesi-Hildebrand plot for the effect of Fe(III) on PZA absorbance at 269 nm

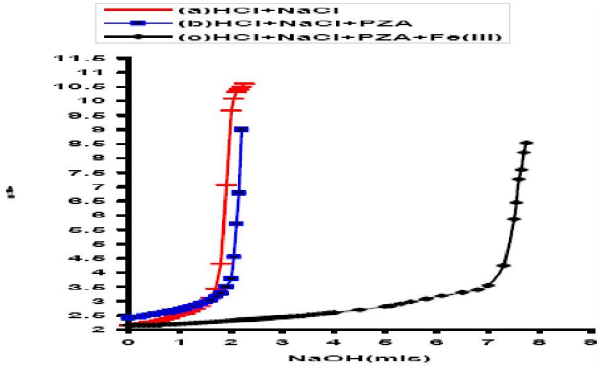

Figure 5 : PZA (1×10^{-3} M)-Fe (III) pH-titration curves using 0.5 M NaOH as a titrant, at ionic strength adjusted using 0.1 M NaCl

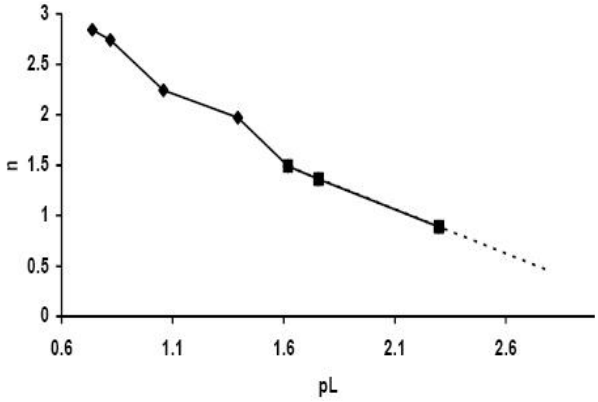

Figure 6 : Continuous variation plot (Job plot) of PZA-Fe (III) complex at 269 nm. n-pL relationship for PZA (1×10^{-3} M)-Fe (III) binary complex

Subsequently, ΔA [PZA] was plotted against r which varied between 0 and 1{r = [PZA]/ ([PZA] + [Fe (III)])}. ΔA values were calculated by measuring the absorbance of PZA in the absence (A_0) and presence (A) of the corresponding concentration of the Fe (III). The quantities ΔA [PZA] are proportional to the concentrations of the complexes. The ratio (r) corresponding to the maximum absolute complex concentration does not depend on M or the binding constant[14].

The method was repeated, using water instead of aqueous solutions of $FeCl_3$ at the same volume proportion and the solutions were analyzed against a blank reference cell containing water.

Determination of stability constant

Aliquots of 1 ml of Pyrazinamide stock solution (4×10^{-3} M) were accurately transferred into 25-ml volumetric flasks containing increasing amounts of standard $FeCl_3$ solutions (5×10^{-3} M) and diluted to volume with water (where the final concentrations of $FeCl_3$ ranged from 1.25 to 7.5 times that of PZA). The absorbance of each solution was recorded at 269 nm, using equimolar aqueous solutions of $FeCl_3$ as reference.

Potentiometric method

All the potentiometric measurements were performed at 25°C ±1 in a 50-ml beaker with continuous stirring with a magnetic stirrer. The ionic strength was adjusted using 0.1 M sodium chloride and the total volume was completed to 25 ml using water. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities so that all the constants determined in this work are concentration constants. The electrode system is calibrated before and after each series of pH measurements under the same conditions using standard buffers pH 4 and 10.

The following solutions were titrated potentiometrically against standard carbon dioxide free 0.05 M NaOH. Increments of NaOH were added and stirred magnetically till equilibrium.

(a) 1ml 0.1 M HCI + 5 ml 0.1 M NaCl.

(b) 1ml 0.1 M HCI + 5 ml 0.1 M NaCl + 2.5 ml 10^{-2} M Pyrazinamide

(c) 1ml 0.1 M HCI + 5 ml 0.1 M NaCl + 2.5 ml 10^{-2} M Pyrazinamide + 2.5 ml 5×10^{-3} M $FeCl_3$ solution.

RESULTS AND DISCUSSION

Spectrophotometric method

The ultraviolet absorption spectrum of PZA in water showed three absorption maximum wavelengths at 209, 269 and 310 nm which were slightly modified due to Fe (III) presence (Figure 2). While hypochromic and bathochromic shifts at the absorption maximum wavelengths of PZA were intensified as the concentrations of Fe (III) increased, no new peaks were detected. These results suggested that PZA formed a stable complex with Fe (III).

By adding Fe (III), there was a bathochromic shift for the absorption maximum at 269 nm reaching 278 nm at the maximum concentration of Fe (III) used. As for the peak at 310 nm, the bathochromic shift observed in the presence of Fe (III) reached 315 nm. Finally, the peak at 209 nm disappeared completely at the maximum concentration of Fe (III) used.

Determination of complex formation stoichiometry

Job[13] described the continuous variation technique that can provide a reliable determination of the complex stoichiometry, based on the difference in a physical parameter, for example, the difference in absorbance ΔA ($\Delta A=A-A_0$) of PZA in the presence of Fe (III). On applying this technique, the resulting plots (Figure 3) demonstrate that since the ΔA [PZA] maximum has an r value of 0.5, therefore both PZA- Fe (III) complex has1:1 stoichiometry.

Determination of stability constant

The interactions of ligands with metal ions may modify the absorbance at the absorption maximum wavelengths without the detection of new peaks[15]. The difference in absorbance 'ΔA' at 269 nm as a function of Fe (III) concentration, [Fe (III)] follows the typical binding isotherm of 1:1 complexes:

$$\frac{\Delta A}{b} = \frac{[PZA]K_{st}\Delta\varepsilon[Fe(III)]}{1+K_{st}[Fe(III)]} \quad (1)$$

When a high excess of [Fe (III)]t is being used compared to[PZA], the assumption that [Fe (III)] = [Fe (III)]t is employed and through this, eq. (1) is transformed, according to Benesi and Hildebrand[16] into the more widely utilized double reciprocal linear equation (Eq. 2).

$$\frac{1}{\Delta A} = \frac{1}{[PZA]K_{st}\Delta\varepsilon[Fe(III)]} + \frac{1}{[PZA]_t\Delta\varepsilon} \quad (2)$$

Where ΔA is the difference between the absorbance of PZA in the presence and absence of Fe (III), $\Delta\varepsilon$ is the difference between the molar absorption coefficient of PZA and the complex and Kst is the stability constant.

The stability constant of PZA-Fe (III) complex was determined by UV spectrophotometry according to the spectral shift method[17], which is based on the decrease in absorbance of PZA due to complexation and calculation of (ΔA) of the absorbance of PZA in the presence and absence of Fe (III). The measurement was carried out at 269 nm, the main of the three absorption maximum wavelengths, in order to achieve more reliable values of ΔA. The complex stability constant Kst was calculated from the ratio of intercept to slope using the Benesi–Hildebrand linear method for 1:1 host–guest complex which is solved graphically, using the linear least-squares regression analysis applied to known mathematical models such as Benesi–Hildebrand, Scatchard etc.

A linear plot is obtained according to (Eq. 2) (Figure 4) which suggests, together with the data obtained from Job's plot, that PZA- Fe (III) complex has a 1:1 stoichiometry and the complex stability constant Kst was found to be 624 ± 25.

The formation of Fe (III)–PZA complex was demonstrated by the significant hypochromic effect and the bathochromic shift observed in the UV-spectra of PZA upon increasing of the added Fe (III) concentration.

Determination of the stability constant using Irving and Rossotti Potentiometric technique

Potentiometric study on drugs with metal ions supplies us with useful knowledge about whether and how drug–metal interactions may affect drug delivery to target cell[18,19].

Potentiometric titration curves for the free and complexed ligand at adjusted ionic strength using 0.1M NaCl are shown in figure 5, which represent the pH titration curves of Fe-Pyrazinamide 0.05 M sodium

hydroxide in presence of 5 ml 0.1 M NaCl. By comparing the pH titration curves of the free ligand to that of the complex solution, a drop in pH was observed indicating the complex formation where n can be calculated from the following equation.

$$\bar{n} = \frac{(V_3 - V_2)(N^\circ + E^\circ)}{(V_o + V_2)(n_a T_{oCM})} \quad (3)$$

Where, V_2 and V_3 are the volumes of the alkali required to reach the same pH for the free ligand (drug) and metal complex titration curves.

T_{oCM} = Total concentration of the metal.

$\bar{n}$ = Average number of lingands attached per complex ion

In the determination of the formation constants of the binary complexes some notes must be taken in consideration:

1 The concentration of the metal must not exceed the concentration of the ligand for neglecting hydrolysis of metal ions.
2 The ionic strength must be controlled at values not exceeding 0.2 M for preventing the formation or ion-pair between the anionic species and the cationic species or strong electrolytes.

The formation constants of the formed binary complexes were computed from the following equation: n=1

$$pL = \log\left\{\left[\frac{1}{\sum_{n=0}^{n=1} {}^{H}_{n}\beta \frac{1}{(\text{anti}\log\beta)^n}}{T_{oCL} - (\bar{n}C_M)}\right] \times \left(\frac{(V_o + V_3)}{V_o}\right)\right\} \quad (4)$$

Where, C_M is the initial concentration of the metal, ${}^{H}_{n}\beta$ = is the proton ligand stability constant.

By plotting $\bar{n}$ versus pL, (Figure 6), the formation constant or the pL value at n= 0.5 (indicates a 1:1 metal: ligand complex) is 2.75 for Pza-Fe (III) while the pL value at n = 1.5 (indicates a 1:2 metal: ligand less stable complex) is 1.6.

CONCLUSION

The proposed structure of the formed complex as predicted from the potentiometric study agrees with the suggested structure deduced from Job's plot and the value of the stability constant calculated using the potentiometric method agrees with the corresponding value calculated using the spectrophotometric method.

Since pharmacologically active PZA derivatives have recently been designed and shown to be clinically effective drugs[20] and iron has been proven to enhance the activity of PZA thus the results of this study could be used as basis for further investigation of the effect of the complexation of PZA with Fe (III) on the efficacy, stability, permeability and bioavailability of PZA.

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