

Physico-Chemical and Bacteriological Study of Anti-Tuberculosis Drug with Alkaline Earth Metals

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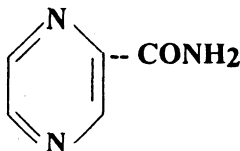
The avidity of pyrazinamide (pyrazine-carboxamide, PZA) for the ions of alkaline earth metals, have been measured and recorded in the form of stability constants and free energy changes, using Bjerrum Calvin pH titration technique as adopted by Irving-Rossotti at 30°C and 0.1 M ionic strength with KNO₃. The order of stability obtained for the alkaline earth metal ion follows the order of e^2/r ratio, electronegativities, second ionisation potential and sum of the first and second ionisation potentials of the metal ions.

INTRODUCTION

Coordination complexes play a role in bio-chemical reactions^{1,2}. Winberg³ pointed out that all antimicrobial agents have chelation tendency. Further, it was pointed out, many drugs function as metal complexes. Probably with these considerations, in the last few years, chelation tendency of various drugs has been worked out⁴.

Metals present in traces in the body change the behaviour of the enzyme system by binding with them and alter the structures and functions of genetic materials by acting with nucleic acids.

Pyrazinamide (pyrazine carboxamide) (PZA) was discovered in 1982.⁵ Pyrazinamide is several times more active than *p*-aminosalicylic acid and approximately seven times as active as niacinamide⁶. Clinically, pyrazinamide is



an extremely effective and a mildly toxic compound.

Since some metal chelates are used as antituberculosis compounds, the metal chelates formed under physiological pH conditions may be used for the inhibition of tuberculosis. This communication reports the thermodynamic stability constants and free energy change (ΔG) for the interaction of pyrazinamide with the alkaline earth metals Be(II), Mg(II), and Ca(II) using Bjerrum-Calvin pH titration

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-technique⁷ as adopted by Irving and Rossotti⁸ at 30°C and 0.1 M ionic strength with KNO_3 . Probable structure of isolated complex is determined by elemental analysis and spectral data. Bactericidal activity is also done against mycotuberculosis, an acid-fast bacteria, giving valuable information about antituberculosis activity of metal chelates.

EXPERIMENTAL

Conductometric and potentiometric (pH) titration employing the monovariation method⁹ were done to study the preliminary stoichiometry shown in Fig. 1. All the solutions were prepared by dissolving high purity chemicals in doubly distilled CO_2 -free water. Pyrazinamide was dissolved in hot water while NaOH solution was prepared in CO_2 -free water and standardised against a standard solution of oxalic acid.

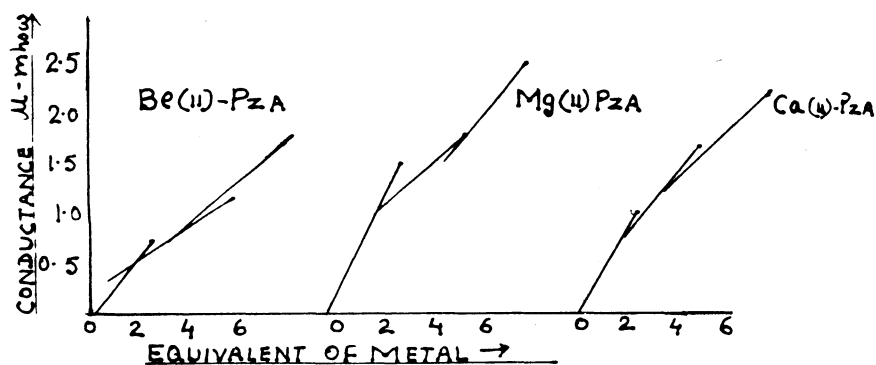


Fig. 1.

The Bjerrum-Calvin pH titration technique was used to determine proton-ligand constant of pyrazinamide (PZA). The following mixtures (total volume 50 mL) were titrated against 0.1 M NaOH as shown in Fig. 2.

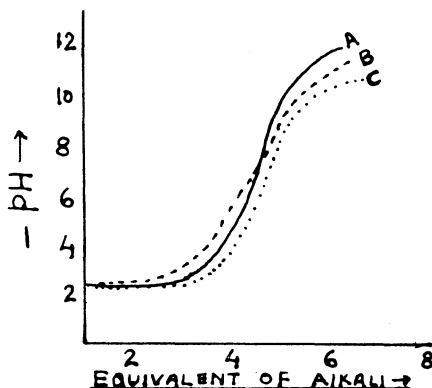


Fig. 2

- (A) 5 mL of 1.0×10^{-2} M HNO_3
 (B) A + 10 mL of 5×10^{-3} M PZA solution
 (C) B + 5 mL of 10^{-3} M metal ion solution.

The ionic strength of the solution was maintained at 0.1 M by KNO_3 solution keeping the total volume 50 mL. Further, with the use of very dilute solution of metal ions possibilities of the formation of polynuclear complexes were minimised. The formation constants were obtained from the analysis of the formation curve drawn between n (average number of ligands attached per metal ion) and PL (free ligand component). The values of n and PL were calculated from Irving-Rossotti equation given below:

$$\bar{n} = \frac{(V^{\text{III}} - V^{\text{II}})(N + E^0)}{(V^0 + V^{\text{II}})n_A T_{\text{CM}_0}}$$

$$\text{PL} = \log_{10} \left[\frac{\sum_{n=0}^{n=j} \beta^{\text{H}} \left(\frac{1}{\text{antilog } \beta} \right) \frac{V^0 V^{\text{II}}}{T_{\text{CL}_0} - nAT_{\text{CM}_0}}}{V^0} \right]$$

where T_{CM_0} and T_{CL_0} are total concentrations of metal and ligand respectively, B is the pH in acid ligand and metal titration curves respectively, while V^0 , N^0 and E^0 are the initial volume, the normality of alkali and initial concentration of the acid respectively.

The values of thermodynamic stability constants are recorded in Table-1. The values of $\log K^1$ and $\log K^2$ were recorded directly from the formation curves and refined by the method of least squares as shown in Fig. 3.

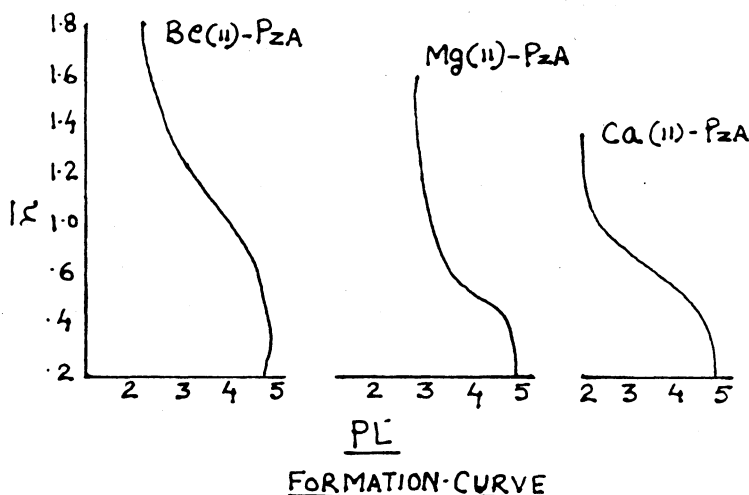


Fig. 3

TABLE-1
STABILITY CONSTANTS OF ALKALINE EARTH METAL COMPLEXES OF
PYRAZINAMIDE AT 30°C AND 0.1 M IONIC CONCENTRATION

log K _n	Be(II)	Mg(II)	Ca(II)
log K ¹	4.60	3.75	4.00
log K ²	2.16	2.80	2.00
log β	6.76	6.55	6.00
ΔG ₁	-6.37	-5.19	-5.54
ΔG ₂	-2.99	-3.80	-2.77
ΔG	-9.59	-1.07	-8.31

For the study of the attachment site of ligand (PZA) with metal ions Ca(II), Be(II), Mg(II), chelates were isolated by refluxing aqueous solutions of the metal salt and ligand in 1 : 2 molar ratio for 3 to 4 h. The insoluble complex separated out on cooling was washed dried in vacuum and stored in air-tight bottles. The composition of the chelates was ascertained by estimating metals, carbon, hydrogen and nitrogen in Table-2.

TABLE-2
ANALYTICAL DATA AND IR FREQUENCIES (cm⁻¹) OF PZA COMPLEX

Analysis (%)	PZA	PZA-Be(II)	PZA-Mg(II)	PZA-Ca(II)
C	49.48	46.60	42.23	48.61
H	3.86	3.57	3.21	4.47
N	34.00	38.10	28.26	26.25
—NH (primary) stretching	3413	3400	3400	3400
—NH (primary) binding	1610	1600	1610	1600
—NH (secondary) binding	1578	1580	1580	1580
—Tert. N aromatic	1377	1377	1380	1300
—C—H benzene	3161	3000	3100	3200
v(C=O)	1715	1700	1700	1684
v(C—O)	1165	1160	1180	1100
Coordinate H ₂ O	869	860	860	860

For the study of chemotherapeutic activity of alkaline earth metal complex. the technique used to determine the chemotherapeutic activity the pyrazinamide and its alkaline earth metal complexes were screened *in vivo* for the sensitivity test. The method used was the disc colonies test due to Bauer *et al.*⁹

Methodology

10.5 g 7H11 media was dissolved in 200 mL of CO₂-free water and stirred properly and 2.5 mL of glycerol was added. The volume was make up to 500 mL

and the media was autoclaved for 15 min at 121°C; after autoclaving 2 h and 20 mL was poured into each plate under sterile conditions. Serial solutions were prepared so as to obtain the desired concentration 0.1 µg/mL, 0.01 µg/mL, 0.001 µg/mL in autoclaved distilled water.

Bacteria H-37 RV was added to serially diluted compound so that the final concentrations become 0.1 µg, 0.01 µg and 0.001 µg and the plates were spread with the help of spreader. The observations are presented in Table-3.

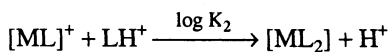
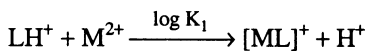
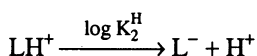
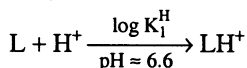
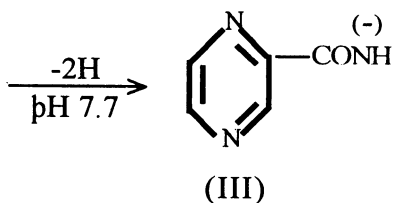
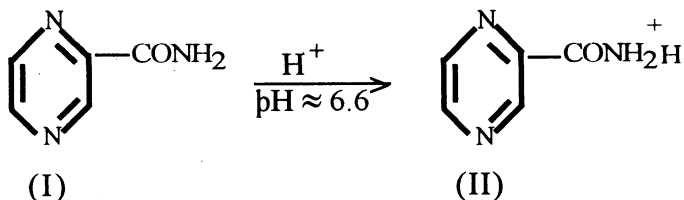
TABLE-3

S. No.	Complex	Colour of crystal	m.p. (°C)	C.F.U. obtained
1.	PZA	White	176	30 colonies
2.	PZA-Be(II)	White	180	07 colonies
3.	PZA-Mg(II)	White	180	29 colonies
4.	PZA-Ca(II)	White	190	09 colonies

RESULTS AND DISCUSSION

Metal-drug interaction: The conductometric titration indicates the formation of 1 : 1 and 1 : 2 (M : L) complexes in the system studied (L = pyrazinamide).

The nature of the pH titration curves for the mixtures B and C indicates that stepwise complex formation takes place through deprotonation of amino moiety.



Stability constants: The metal ligand formation constants are given in Table-1. The values were directly obtained from the formation curve while the refined values were obtained by the method of least squares. The energy change ΔG was calculated by the formula

$$\Delta G = -2.303 RT \log K$$

The observed order given above for the alkaline metals, shows that the variation is normal in the light of the fact that the strength of complexation should increase with increasing ionic charge 'e' and decreasing ionic radius 'r' of the metal, *i.e.*, the effective nuclear charge, generally expressed in terms of e^2/r ratio or ionization potential^{11, 12}. The present investigation for the alkaline earth metal ions follows the order e^2/r ratio, and the sum of the first and second ionization potential of the metal ions $Be > Mg > Ca$.

Structure of the complexes

The analytical data in confirmation with the stoichiometric studies indicate 1 : 2 (M : L) stoichiometry of the isolated complexes. These complexes have characteristic colour and melting point. The infrared data of the isolated complexes shows that ligand has been acting as bidentate ligand, *i.e.*, during formation of chelate using tertiary nitrogen and deprotonation of amide moiety.

Most of the bands of the pyrazinamide remain unchanged on chelation while it shows change in the $\nu(N-H)$ stretching frequency of pyrazinamide and shows two bands at 3413 cm^{-1} and 3161 cm^{-1} and $-N-H$ bending frequencies at 1610 cm^{-1} but in complexes one broad band at *ca.* $3400-3000 \text{ cm}^{-1}$ occurs. Tertiary nitrogen aromatic frequency in pyrazinamide in complex about *ca.* 1300 cm^{-1} because of electrons from nitrogen atom to the metal ion which in turn weakens the $-N-$ bands. The absorption in the *ca.* 1600 cm^{-1} and 1300 cm^{-1} regions is due to asymmetric and symmetric $-NH$ deformation vibrations. The presence of co-ordinated water finds absorption band at 869 cm^{-1} . A respectively broad band near $3600-3200 \text{ cm}^{-1}$ in the complexes further indicates the presence of coordinated water.

Antitubercular activity: It has shown that specific metals are required for genetic regulation. The results of Table-3 show that antitubercular activity of alkaline earth metal complexes with that of pyrazinamide is in the following order.



The order shows that Mg(II) complex has less tendency than Ca(II) and Be(II). This is due to the fact that Mg(II) acts as nutrition for mycobacteria whereas Be(II) complex has more antitubercular activity because Be(II) complex has high stability constant in comparison to Ca(II) and Mg(II). The high stability constant and dissociation constant are also in favour of drug action.

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