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Short Communication

Potentiometric and Conductometric Studies on Complexes of Folic Acid with some Metal Ions

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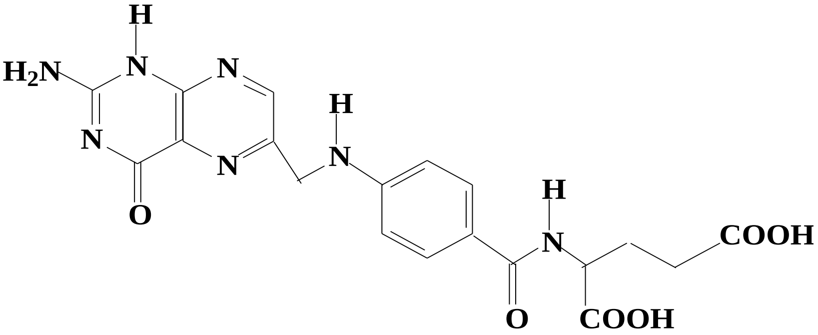
Sixteen metal ions viz; Li (I), Mg (II), Sr (II), Ca (II), Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Ba (II), Pb (II), Al (III), Cr (III), Fe (III) and Th (IV) were selected to elucidate their interaction with folic acid using potentiometric method. The protonation and stability constants of formed complexes have been calculated using ionic strength, I = 0.2 M $NaNO_3$ in aqueous solutions at 25±0.1 °C. Complexes of 1:1, 1:2 and/or 1:3 metal to ligand ratios were formed depending on the nature of the ligand or metal ions. The order of stability constants of the binary complexes was examined. The stiochiometry of the formed complexes was confirmed by conductometric method. Also, the ionic equilibria of ligand and its complexes with different metal ions in solution were investigated.

Keywords: Folic acid, metal complexes, potentiometry, conductometry

1. INTRODUCTION

Folic acid ((2S)–2–[[4–[(2–amino–4–oxo–1H–pteridin–6–yl)methylamino]benzoyl]amino] pentanedioic acid; (FA)) is a water–soluble compound of Vit. B9 group and it is added to many food products to prevent folate deficiency in individuals [1,2]. It is used in the treatment of megaloblastic anemia associated with FA deficiency of pregnancy and lactation; folate deficiency due to: inadequate intake, malabsorption syndrome, excessive utilization and increased excretion or need as in sprue [3]. Requirement of FA may be increased in alcoholism, cirrhosis, hepatic disease, hemolytic anemia, prolonged diarrhea and use of oral contraceptives or anticonvulsant therapy. Folate deficiency is the most common cause of anemia after iron deficiency and is thought to increase the likelihood of heart attacks and strokes [4]. Several review articles gives detailed account of FA history, design, chemistry and pharmacology [5–7]. FA structure can be shown as follow:



The equilibrium and kinetics of the complexation of Co (II) by FA have been studied at 25 ºC, the ionic strength being regulated with KNO_3 in the 5.6–7.3 pH range [8]. In unbuffered solutions, different electrochemical techniques were used to elucidate the complexation reaction between FA and Cd (II) [9], Cu (II) [10] and Pb (II) [11]. Also, different spectroscopic techniques were used to study complexes between FA and different metals [12–17].

The potentiometric method has been used extensively in many branches of solution chemistry. Great attention has been paid to use potentiometric methods in study of binary and ternary complexes of transition metals with molecules of biological and pharmaceutical interest [16–24]. The significance of using potentiometric methods is studies related to such complexes that it is most accurate and widely applicable technique currently available for the study of ionic equilibria in solutions [25].

In current work, we report for the first time on metal complexes of FA with the type of M^{+n}–FA using potentiometric methods, such as: Li (I), Mg (II), Sr (II), Ca (II), Mn (II), Co (II), Ni (II), Zn (II), Ba (II), Al (III), Cr (III), Fe (III) and Th (IV) nitrates ions. The dissociation constants of FA and stability constants of their complexes will be determined. It should be noted that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of such organic compounds [26].

2. EXPERIMENTAL

2.1. Apparatus:

All pH measurements were carried out on pH–meter model ELE international, using combined glass electrode (accurate total 0.01 pH units). Conductometric titration measurements were carried out using conductivity meter model 4320, Jenway, using an immersion cell. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities; thus all constants determined in this work are concentration constants.

Calvin–Bjerrum is technique as adopted by Irving and Rossoti [27, 28] or Kather and Munshi [29] were used to determine the dissociation constants of the ligand (FA) and the formation constants of FA–metal complexes at 25±0.1 ºC in dilute acidic solution.

2.2. Chemicals and Materials:

The solutions of Li (I), Mg (II), Sr (II), Ca (II), Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Ba (II), Pb (II), Al (III), Cr (III), Fe (III) and Th (IV) ions (Merck, BDH) as nitrates were prepared and titrated complexometrically by EDTA [18]. Sodium hydroxide (NaOH) and Folic acid (FA) was purchased from (AL–Maya Pharmaceutical Factory, Libya). FA was prepared by dissolving the appropriate amount of FA in dilute acidic solution.

2.3. Procedure

Generally, three sets of solutions; (a) 0.02 M HNO_3, (b) a + 0.001 M FA and finally (c) b + 0.001 M metal nitrate solution were prepared and titrated potentiometrically with standard carbonate free–NaOH solution standardized against standard potassium hydrogen phthalate. The total volume was adjusted to 50 mL by adding doubly–distilled water in each case. The titration's were performed at 25±0.1 ºC and different ionic strengths of I = 0.2 M $NaNO_3$.

Conductometric titration were carried out at room temperature (25±0.1 ºC) by titrating 25 mL of 1×10^{-3} M of each metal ion solution with 1×10^{-2} M of FA solution in 0.5 mL increments. Correction for the dilution effect is performed by multiplying the values of specific conductance by factor $\left(\frac{25+V}{25}\right)$, where *V* is volume of titrant added.

3. RESULTS AND DISCUSSION

3.1. Proton–FA system

The titration curves are shown in Fig. 1. The average number of proton attached per ligand ($\bar{n}H$) was calculated using Eq. 1 [27, 28]:

$$\bar{n}H = Y + \frac{(V_1 - V_2)(N^{\circ} + E^{\circ})}{(V_{\circ} + V_1)T_C L^{\circ}} \qquad (1)$$

Where Y = 2 (number of dissociable protons in the ligand), V_0 is the initial volume, V_1 and V_2 are the volume of alkali required to reach the same pH in mineral acid (HNO_3) and (HNO_3+FA), respectively. $T_C L^{\circ}$ is the total concentration of ligand, N° is the normality of the alkali and E° is the initial concentration of free acid. Calculation of proton ligand association constants were carried out by plotting ($\bar{n}H$) against pH at 0.1 M HNO_3 ionic strength is shown in Fig. 2. The values of $LogK_1^H$ and $LogK_2^H$, (the first and second proton association constants of the FA) are the pH values corresponding to $\bar{n}H$= 0.5 and 1.5, respectively. It is worth mentioning that the ligand do not hydrolyses under the experimental conditions. This is indicated by the rapid attainment of equilibrium during the titration time. Fig. 1 shows that titration curves of the metal–ligand solutions (c) are well separated from the ligand solution (b). Thus replacement of H^+ ion is due to complexation. From these data, the proton–ligand and metal–ligand stability constants were obtained as shown in Table 1.

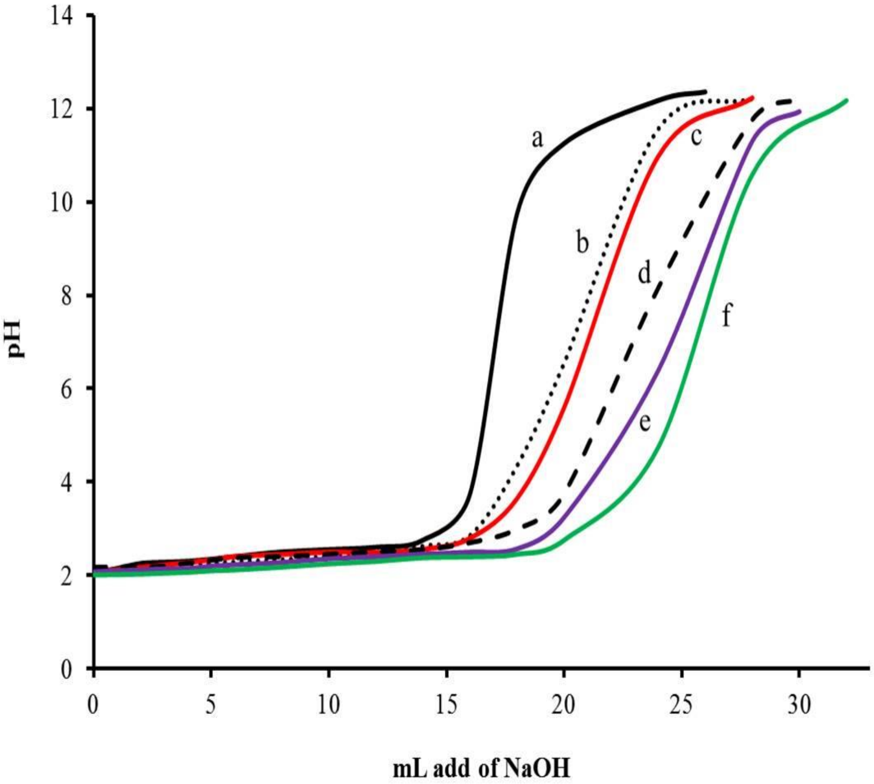


Figure 1. Representative potentiometric titration curves of FA in 0.2 M $NaNO_3$ at 25±0.1ºC: **(a)** 0.01 M HNO_3, **(b)** a + 0.001 M FA, **(c)** b + 0.001 M Ca (II), **(d)** b + 0.001M Cu (II), **(e)** b + 0.001 M Fe (III) and **(f)** b + 0.001M Co (II).

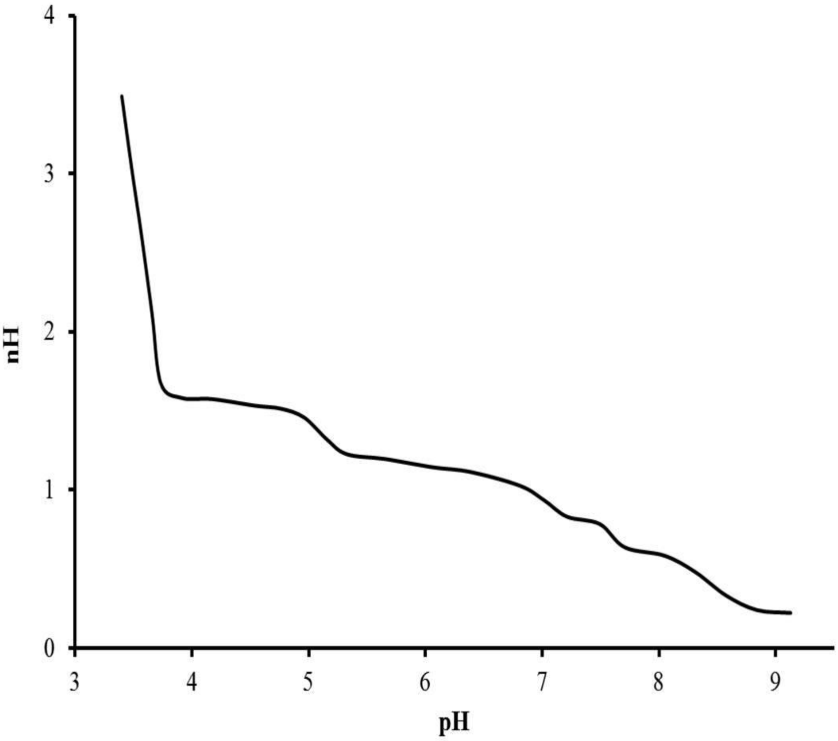


Figure 2. Protonation constant curve of FA in 0.2 M $NaNO_3$ at 25±0.1ºC.

Table 1. Protonation constants of FA and stability constants of metal ion complexes at 0.2 M $NaNO_3$ and 25±0.1 °C.

Metal ion	Log K_1(M:L)*	Log K_2 (M:L)*	Log K_3 (M:L)*
H^+	8.6	5.1	3.63
Al (III)	9.20 (1:1)	7.66 (1:2)	5.93 (1:3)
Mn (II)	13.19 (1:1)	11.26 (1:2)	8.11 (1:3)
Pb (II)	-------	14.83 (1:2) 5.78 [11]**	13.22 (1:3) 6.99 [11]**
Ba (II)	-------	14.37 (1:2)	12.97 (1:3)
Ca (II)	7.10 (1:1)	-------	-------
Cd (II)	12.71 (1:1) 6.76 [9]**	-------	-------
Co (II)	14.81 (1:1)	13.93 (1:2)	12.24 (1:3)
Fe (III)	14.70 (1:1)	13.54 (1:2)	12.04 (1:3)
Li (I)	-------	-------	2.50 (1:3)
Mg (II)	14.05 (1:1)	12.76 (1:2)	10.01 (1:3)
Ni (II)	14.37 (1:1)	13.60 (1:2)	11.85 (1:3)
Cr (III)	8.12 (1:1)	-------	-------
Sr (II)	-------	14.42 (1:2)	12.92 (1:3)
Th (IV)	12.92 (1:1)	9.53 (1:2)	7.14 (1:3)
Zn (II)	-------	-------	-------
Cu (II)	14.06 (1:1)	11.59 (1:2) 11.57 [10]**	8.62 (1:3)

* These ratios are from potentiometric and conductometric methods
** Data compared from literature survey

On titration with dilute base (0.2 M), the maximum number of protons can be released from FA is three protons in the pH range 2.8–12. FA behaves as triprotic acid [H_3–FA], the protonation centers are the first carboxyl group, second carboxyl group and imino group of glutamic acid. The acid–base properties of FA in 0.2 M acid at ionic strength (I = 0.2 M $NaNO_3$) indicate that one proton from the first carboxyl group was ionized in the pH range 3.63–4.95. The second proton is released from the second carboxylic one in pH range 5.10–8.40. The third proton is from the imino group within the pH range 8.60–9.20. The values of $LogK_1^H$, $LogK_2^H$ and $LogK_3^H$ (the first, second and third proton dissociation constants of FA, respectively) are the pH values corresponding to $\bar{n}_A$ = 0.5, 1.5 and 2.5, respectively. The values of $LogK_1^H$ (8.6), $LogK_2^H$ (5.1) and $LogK_3^H$ (3.63) are tabulated in Table 1.

The equilibria established from this study can be shown in the following forms Eqs. (2, 3 and 4):

$$H_3\text{-}FA \leftrightarrow H_2\text{-}FA^- + H^+ \qquad (pH = 3.63 – 4.95) \qquad (2)$$

$$H_2\text{-}FA^- \leftrightarrow H\text{-}FA^{-2} + H^+ \qquad (pH = 5.10 – 8.40) \qquad (3)$$

$$H\text{-}FA^{-2} \leftrightarrow FA^{-3} + H^+ \qquad (pH = 8.60 – 9.20) \qquad (4)$$

3.2. Metal ion–FA Complexes

In Fig. 3, the titration curves were used to calculate $\bar{n}$ (average number of ligand molecules attached per metal ion) and *pL* (free ligand exponent) using Irving and Rossotti equation [27, 28]:

$$\bar{n} = \frac{(V_3 - V_2)(N^{\circ} + E^{\circ})}{(V^{\circ} + V_2)\bar{n}H T_c M^{\circ}} \quad (5)$$

$$pL = Log\left[\frac{1 + K_1^H[H^+] + K_2^H[H^+]^2 + K_3^H[H^+]^3 + ------}{(T_c l^{\circ} - \bar{n} T_c M^{\circ})} \times \frac{V^{\circ} + V_3}{V^{\circ}}\right] \quad (6)$$

Where V_1, V_2 andV_3 are the amounts of alkali to reach the same pH in the free acid, free acid + ligand and free acid + ligand + metal, respectively.$T_c M^{\circ}$denotes the total concentration of metal present in the solution. The $\bar{n}$ values were plotted against the corresponding pL values to get the formation curves of the metal complexation equilibria.

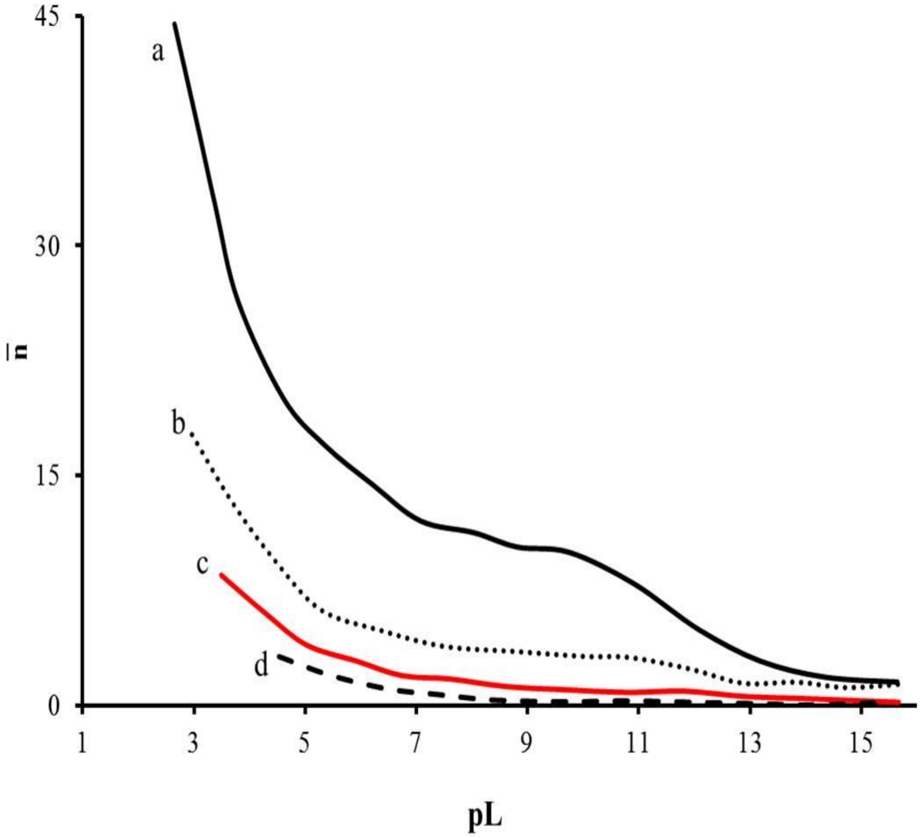


Figure 3. Representative formation curves of binary metal ion complexes with FA at I = 0.1 M: **(a)** Pb (II), **(b)** Ni (II), **(c)**Th (IV) and **(d)** Cr (III).

The formation curves are shown in Fig. 3. From these formation curves, the values of stability constants at 0.2 M $NaNO_3$ ionic strength listed in Table 1 were determined using the half–integral method [30, 31].

From Table 1 we can observe that some metal ions viz; Al (III), Fe (III), Th (IV), Cu (II), Mn (II), Mg (II), Co (II) and Ni (II) ions form (1:1), (1:2) and (1:3) metal to ligand complexes. Also, Sr (II), Ba (II) and Pb (II) ions form (1:2) and (1:3) metal to ligand complexes, but in case of Ca (II) and Cd (II) only one complex (1:1) metal to ligand was formed. The formation constants of the first complex was only obtained since the formation of the second one is disturbed by hydrolyses and precipitation of the metal ion. Therefore, the experimental data in this pH range would not be useful in the numerical calculations. Moreover, these data cannot be considered to be at equilibrium, since the pH readings at this stage showed unsteady drift. Furthermore, Li (I) form only one complex (1:3) metal to ligand. This is may be due to the nature of metal ion, concentration of ligand and ionic strength.

The stability constants of the complexes formed with FA listed in Table 1 are in the order of stability of the different binary complexes formed between FA and transition metal ions investigated in this study is in the expected Irving–Williams order [27, 28].

Co (II) > Ni (II) > Cu (II) > Mg (II) > Mn (II) > Cd (II) > Ca (II)

FA behavior may be based on the tridentate nature which coordinates through the first and second carboxyl group of glutamic acid and imino group of glutamic acid, forming stable six–membered chelate rings. It is well known that tri–valence metal ions tends to form six–membered rings in their chelates, as well as the bi–valence metal ions tends to form five membered rings.

In Fig. 1, reverses to the distance between c and b curves corresponding to stepwise formation of ML, ML_2 and ML_3 complex species. The results obtained for the formation of the binary complexes investigated are shown in Table 1. From our literature survey, we noticed that, between sixteen metal ions studied in current work, only Cu (II), Pb (II) and Cd (II) were previously investigated with FA using voltammetric methods [9–11]. As shown in Table 1, in case of Cu (II), there is a good agreement with the literature [10] while in case of Cd (II) and Pb (II) we found that there is a disagreement between current work results and the previous data [9, 11], which can be attributed to the difference in both experimental conditions (I = 0.15 M $NaClO_4$) and the used methods (polarographic method).

3.3. Conductometric Titration of FA

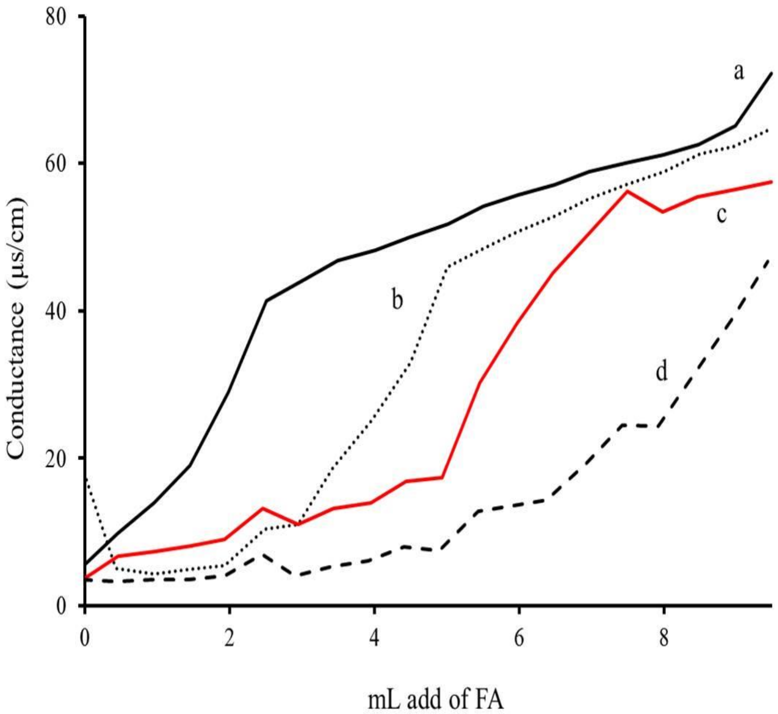


Figure 4. Representative conductometric titration of 25 mL metal ions (1×10^{-3} M) with 1×10^{-2} M of FA: **(a)** Ca (II), **(b)** Co (II), **(c)** Cu (II) and **(d)** Fe (III).

Greater use in tracing the complex formation, a conductometric analysis is used. This method finds its useful application as a sensitive tool to test for decimal variations in ionic radii of transition metal ions investigated are shown in Fig. 4. The conductometric analysis is based on changes in the electrical conductivity values of solutions as a result of complex formation. These changes depend upon the number of ions present and their mobility's. Conductivity measurements are employed to trace the different types of chelate species formed between metal ions and the ligand.

The conductometric titration curve was established for the binary ligand system containing Li (I), Mg (II), Sr (II), Ca (II), Mn (II), Mg (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Ba (II), Pb (II), Al (III), Cr (III), Fe (III) and Th (IV) ions. Fig. 4 shows an initial decrease in conductance and a minimum at (1:1). This may be due to the neutralization of H^+ ions resulting from the formation of the [M–(FA)] complex. Further, the conductance increases slightly between 1:1, 1:2 and/or 1:3, probably due to the formation of the binary ligand complex and the release of H^+ ions from FA. From the data tabulated in Table 1, we found that there is a good agreement between potentiometric and conductometric methods of all metal:ligand stoichiometric ratios for the complexes under investigations.

3.4. Species Distribution Diagrams of FA

Distribution curves of FA at I = 0.02 M $NaNO_3$ (Fig. 5) shows that in the pH range (2.8 – 6.8) the major species is α_o (H_3–FA) while in the pH range (3.4–6.8) the major one is α_1 (H_2–FA^-), with no significant α_2 (H–FA^{-2}) and α_3 (FA^{-3}) species is the major one in pH range from (5.0–12).

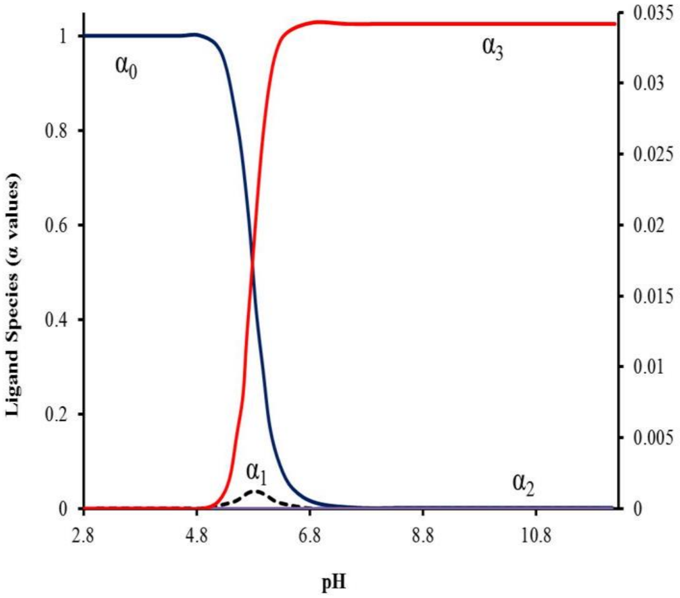


Figure 5. Ionic equilibria of FA in different pH's range

The species distribution curves can be obtained by plotting metal species and pH as shown in curves which depicted in Fig. 6. The analysis of these diagrams reveals that at low pH value, most of the metal ions are often present as free ions. This indicated that no complex occur in the acidic medium. On increasing the concentration of the ligand by increasing the pH of the solution during the titration, the mole fraction of the free metal ion tends to decrease, while that of ML species tend to develop at moderately acidic media. However, the value of $LogK_1^H > LogK_2^H$ indicates that there will be an appreciable concentration of ML species in this pH region. Further increasing in the pH of the solution, the essential change is the increase in the concentration of ML_2 with decrease in ML. Above this region almost the entire metal ion remains in the form of ML or ML_2 species on increasing the pH of the solution. Some fraction species at intersection points and maximum pH, complexes are represented. The values of –Log [H] at the intersection points (αM, αML) and (αML, αML_2) are in close with the values of stability constants for each of metal–ligand complex under studies as recorded in Table 1.

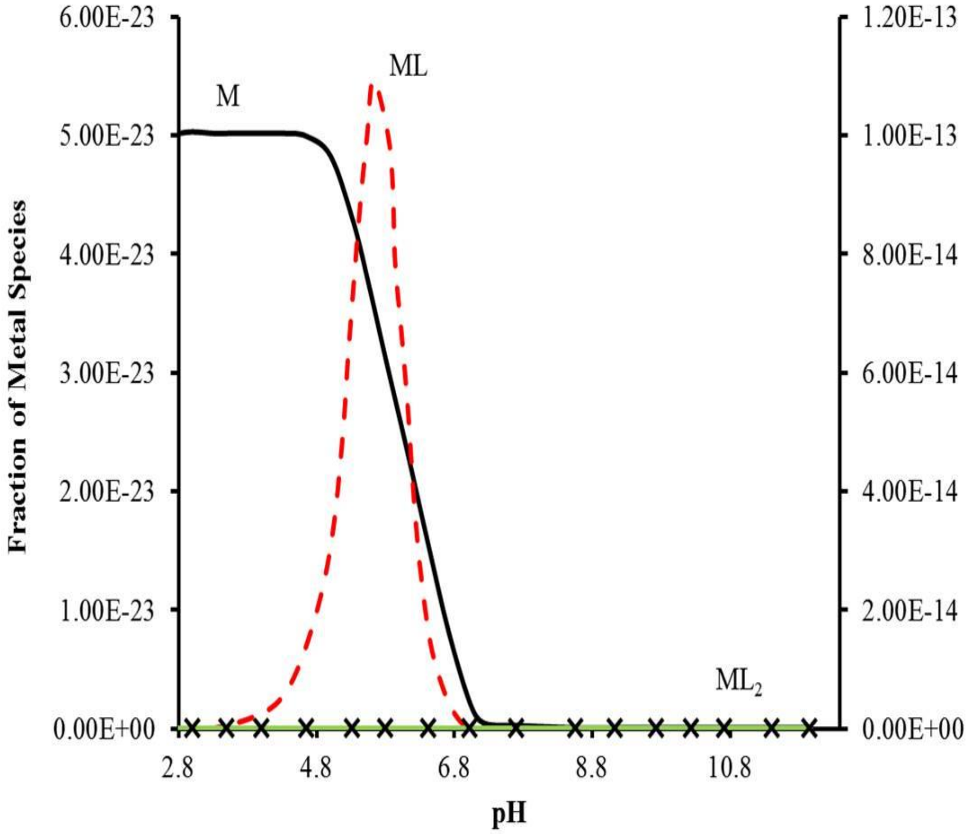


Figure 6. Representative ionic equilibria of Th–FA complexes in different pH's ranges.

4. CONCLUSION

In this article, a potentiometric studies were carried out to elucidate the interaction between folic acid and sixteen metal ions viz; Li (I), Mg (II), Sr (II), Ca (II), Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Ba (II), Pb (II), Al (III), Cr (III), Fe (III) and Th (IV) using ionic strength I = 0.2 M $NaNO_3$ in aqueous solutions at 25±0.1 ºC. The protonation constants of the ligands ($LogK_1^H$ = 8.6, $LogK_2^H$ = 5.1 and $LogK_3^H$ = 3.63) and formation constants of the resulting complexes were calculated and tabulated in Table 1. The formation constants of the resulting complexes possess the stoichiometric ratios of 1:1, 1:2 and/or 1:3 metal to ligand ratios, depending on the nature of the ligand or metal ions. The overall stability constant of the formed complexes was found to follow the order:

Co (II) > Ni (II) > Cu (II) > Mg (II) > Mn (II) > Cd (II) > Ca (II)

This order comes in accordance with Irving–Williams order. Based on conductometric measurements and tridentate nature of FA, the chelation can take place through the first and second carboxyl group of glutamic acid and imino group of glutamic acid, forming stable six–membered chelate rings.

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