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Application of Drug–Metal Ion Interaction Principle in Potentiometric and Conductometric Determination of Folic Acid

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Abstract:

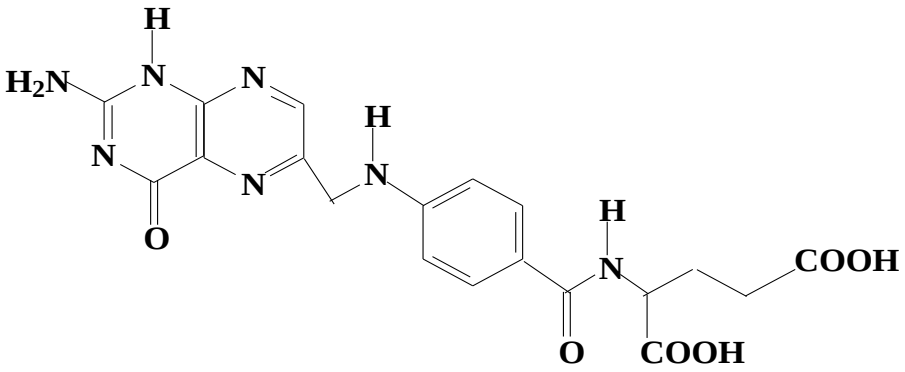
This study includes carefully selecting nine metal ions: magnesium, calcium, cobalt, nickel, copper, lead, chromium, iron, and thorium to clarify their interaction with folic acid (FA) by potentiometric technique. The association constant of folic acid and the stability constants of the complexes made at ionic strength, I = 0.1M $NaClO_4$ in an aquatic medium at room temperature were calculated. Complexes were obtained with metal - FA ratios according to the nature of the metallic ions and folic acid. However, for iron(III), Thorium(IV), Cupper (II), Magnesium (II), Cobalt (II) and Nickel (II), form (1:1), (1:2) and (1:3) , for Ca(II) and Cr(III) form only one (1:1) ,but in the case of lead (II) ions which form (1:2) and (1:3) metallic-ligand complexes. Also, the formation of stability constants of the binary complexes was tested. The connection of the complexes was determined through the conductivity measurement method. The ionic equilibria of the FA and its complexes with different metallic elements in the medium were examined. Simple, accurate, quick, low-cost potentiometric and conductivity procedures are used to determine folic acid (FA) in pure pharmaceutical samples. The potentiometric technique is depend on the direct titration of folic acid in aqueous medium 0.1 M NaOH at μ=0.5$NaClO_4$ and 25±1.0ºC in the presence of a composite glass electrode and using the standard addition method, the detection and quantitation limits were established to be 0.005 mg/ml, with a standard deviation, SD=0.001, and a correlation coefficient, R=0.9943 (n=5), and the linear concentration ranges were 0.005 to 0.07 mg/ml. This method was wide used for the estimation of FA in pure solutions and tablets and satisfactory results were obtained. No interventions were detected in the occurrence of public constituents of the samples under study. The recovery of folic acid from different dosage tablets ranged from 97.3 to 98.6%.

Keywords: Folic acid, metallic complexes, potentiometric method, conductometric method, and tablets

1. INTRODUCTION

The structure of folic acid is: (2S)-2-[(4-{[(2-Amino-4-oxo-1,4-dihydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioic acid.

The construction is as follows:



Folic acid (FA) stands out as a molecule having biological importance in recent years. The name folate usually outlines a class of compounds with chemical structures related to pteroylmonoglutamic acid and generally recognized as folic acid (FA, vitamin M, B9 or B11). There are more than 100 compounds that can be defined as folates and folic acid is the simplest synthetic and oxidized form of folates. Folate is present in legumes, egg, kidney, liver, tongue, citrus fruits, leafy green vegetables, beans, wheat germ, and yeast. Folate is synthesized solely by microorganisms and plants so that humans need to intake the vitamin from different dietary sources as a natural form or from supplements and enriched foods as a folic acid. Since FA is more stable, cheap and easily absorbable synthetic form and it reveals higher bioavailability than naturally occurring food folate, this water soluble B group vitamin is increasingly utilizing for food fortification purposes. The recommend daily intakes (RDI) for folate change from 150 to 600 µg per day depending on the age and sex of the individuals and also vary notably from country to country. The United States Public Health Service suggests that all women of childbearing age should use 400 µg daily dose of folic acid for the prevention of spina bifida or other neural tube defects. Currently, some European countries recommend that intake 500 µg of daily folic acid for women who are breastfeeding [1].

Folic acid is useful in treating megaloblastic anemia resulting from folic acid deficiency during lactation and pregnancy; folic acid lack is owing to malabsorption disease, insufficient intake and improved excretion, and excessive use or need as in the case of celiac disease [2]. In addition, folic acid is very useful in cases of liver disease, alcoholism, hemolytic anemia, cirrhosis, use of oral contraceptives, treatment with anticonvulsants, or diarrhea for long periods. Also, recently it has been said that folic acid can be used in the treatment of methanol poisoning, but this has not been proven [3]. The equilibrium and kinetics of the formation of complexes for Cobalt (II) with folic acid were studied at room temperature, in the pH range of 5.6–7.3 after adjusting the ionic strength with KNO_3 [4]. Voltammetric determination of folic acid with a multi-walled carbon nanotube-modified gold electrode[5] and advanced nanostructure amplified strategy[6], were used for voltammetric determination of folic acid. In general, there are several critical articles that provide an exhaustive description of the history, proposal, chemistry and pharmaceutical of folic acid [7-11].

The potentiometric method is one of the most precise and extensively techniques for studying ionic equilibria and is widely used in several branches of solution chemistry [12]. Recently, there has been a great interest in studying the binary complex of transitional elements with molecules of biological and pharmaceutical importance [13-18]. In addition, the occurrence of metallic ions in biological fluids can have an important impact on the biological activity of drugs [18-22]. Furthermore,the use of potentiometric and conductometric methods to analyze binary and trinary complexes formed between metals and biological and pharmacological compounds has attracted a lot of interest as analytical[18-22]

According to the human need for the application of folic acid, it has been investigated and estimated by many analytical procedures like spectrophotometry, thermogravimetry, chromatography, ultra-performance fluid chromatography-tandem mass spectrometry, fluorescence, and electroanalytical methods [22-26]. Despite the growing importance of folic acid (FA) in biological and pharmaceutical contexts, limited information is available regarding its interactions with metal ions to form coordination complexes. Therefore, the objective of this study is to investigate the formation of metallic complexes of folic acid with various metal ions, including magnesium, calcium, cobalt, nickel, copper, lead, iron, chromium, and thorium, specifically of the type M^{+n} -folic acid. The ionization constant (pKa) of folic acid will be determined, and the stability constants of its complexes with these metal ions will be calculated.

Given that the potentiometric method is widely regarded for its simplicity, cost-effectiveness, high precision, and accuracy, it has been chosen as the primary analytical technique for the quantification and analysis of folic acid. Accordingly, this research also extends to the accurate determination of folic acid in pharmaceutical tablet formulations using the potentiometric method.

2. EXPERIMENTAL

2.1. Reagents and Instruments

Folic acid was obtained by Sigma-Aldrich (Stenheim, Germany) and used without further cleansing. Standard solutions were equipped daily by liquefying a definite amount of the drugs in twice-distilled H_2O. A series of dilutions in water were prepared to obtain the appropriate concentrations. The solutions of Mg (II), Ca(II), Co(II), Ni(II), Cu(II), Pb (II), Cr(III), Fe(III), and Th (IV) ions (Merck, BDH) as nitrates were prepared and titrated complexometrically by EDTA [19].Sodium hydroxide, potassium hydrogen phthalate, perchloric acid, and sodium perchlorate (BDH and Merck) were prepared by dissolving the appropriate amount of each compound in bidistilled water. Also, Pharmaceutical tablets: Folvit (folic acid) tablets in different doses (400, 800, and 1000 μg, injectable solution: 5 mg/ml) were purchased from Sedico Pharmaceuticals, 6th of October City, Egypt. All these pharmacological samples were performed for the detection of folic acid. pH trials were performed by a pH meter (Janeway), containing a composite glass electrode (total precision unit 0.01 pH). Conductivity titration trials were performed by a conductivity measurement 4320, Janeway, using an immersion cell. The electrode structure was calibrated in terms of hydrogen ion focuses rather than actions; Therefore, all the constants estimated in this study are in the form of concentration constants. The solution was kept under continuous stirring at room temperature in mutual pH and conductivity titration curvatures.

2.2. Procedures

2.2.1. Metallic ion complexed with FA

Calvin-Bjerrum, method as assumed by Irving and Rossoti [27] or Kather and Munshi [28] stayed charity to determine the ionization constant of the Folic acid and the association constant of their metallic ion complexes with FA at room temperature in dilute acidic solution.

The following solutions were titrated by potentiometric method with 0.1 M NaOH solution neutralized versus stock potassium hydrogen phthalate a = 0.01 M perchloric acid ($HClO_4$), b = a + 0.001 M FA and c = b + 0.001 M metallic ion solution. The entire volume was completed to 50 ml by the addition of doubly-distilling H_2O in individual cases. The titrations were achieved at room temperature (25 ± 0.1ºC) and different ionic strength of I = 0.2 M $NaClO_4$. Conductometric titration was permitted at room temperature by titrating 25.0 ml of 0.001 M of each metallic ion with 0.01 M of FA solution in 0.5 ml increments. Modification for the dilution outcome is done using swelling the values of definite conductivity by the factor, (25 + V)/25, where V is the volume of the additional reagent.

2.2.2. Detection of FA

2.2.2.1. Pure Formula

Stock solutions (25 ml) of FA (0.01 M) (adopted to 0.5 M by $NaClO_4$) were provided by watering down the real typical solution with twice-distilled H_2O. In this regard, 15 ml of FA solution was neutralized by the voltage-conductivity method with drops of NaOH solution (I = 0.1 M) in a heat-stabilized beaker cubicle (25 ± 0.1 °C).

2.2.2.2. Tablets Formula

Ten of tablets were balanced and finely ground to get the regular tablets weight. A part of the residue containing around 25 mg of FA was weighed accurately and added to 10 ml of twice-distilled H_2O. The resulting compounds were filtered, and their ionic strength was reduced to 0.5 M by sodium perchlorate. A part of this solution was then diluted by twice-distilling H_2O in a 10 ml bottle and investigated to obtain the pure form of FA, as previously stated. By means of the typical calibration curvature, the amount of FA per tablet could be computed.

2.2.3. Statistical Analysis

All statistical parameters, stoichiometric constant, and Log K were calculated by Microsoft Excel. To evaluate FA, a Gran chart was used and from there lower limit of detection, lower quantitative, and standard deviation were computed using social accounting matrix (SAM). Beforehand, Microsoft Excel and SAM have been effectively used for the same purpose [16,29-32].

3. RESULTS AND DISCUSSION

3-A. Complexes of folic acid

3.A.1. Proton-folic acid system

Figure 1 shows the titration curves. The mean number of hydrogen ions bound to each ligand was detected [27].

$$\bar{n}H = Y + \frac{(V_1 - V_2)(N^0 + E^0)}{(V_0 - V_1)(Tcl^0)} \quad \text{----------} \rightarrow (1)$$

It was found that Y equals 2 (the number of hydrogen ions dissociated in folic acid), V_0 represents the primary volume, V_1 and V_2 represent the volume of the alkaline that gives the similar pH in the $HClO_4$ and ($HClO_4$ + FA) one-to-one. Tcl^0 denotes the entire concentration of the FA, N^0 represents the alkaline base and E^0 expresses the primary concentration of the acidic ions. The proton FA binding constants were calculated by plotting the graph with the pH at an ionic strength of $HClO_4$ of 0.1 M as shown in Figure (2). The values of $\log K_1^H$ and $\log K_2^H$ (represent the binding constants of the first and second protons to folic acid) represent the pH values equivalent to 0.5 and 1.5 one-to-one. It means noting that the FA does not hydrolyze under the practical conditions. Which leads to the quick arrival of equilibrium through titration. Also, the titration curves for the solutions of the metallic folic acid (c) appear well separated from the solution of the ligand (b) as shown in Fig. (1). Therefore, the replacement of the hydrogen ion is a result of the construction of complexes. Therefore, the stability constants of the metal-ligand and the proton were gained as shown in Table 3. The extreme number of protons that can be free from folic acid is three protons, and when titrated with the base (0.2 M) in the range of pH 2.9-12.1. FA behavior as a triprotinic acid [H_3-FA], and the proton release centers are the initial carboxylic group, the second carboxylic set, and the imino set of glutamic acid. The acid-base property of FA in 0.2 M acid at ionic power (I=0.2 M $NaClO_4$) shows that one proton from the first carboxylic set is deprotonated in the pH variety 3.65-4.96. The other H^+ from the second carboxylic group in the pH variety 5.2-8.5, and the third H^+ from the imine group in the pH range 8.50-9.30. The values of log K_1^H, log K_2^H, and log K_3^H (the primary, secondary, and third proton dissociation constants of folic acid, respectively) are the pH values equivalent to ńA = 0.5, 1.5, and 2.5, one-to-one. The values of log K_1^H (8.5), log K_2^H (4.9), and log K_3^H (3.5) are shown in Table 3.

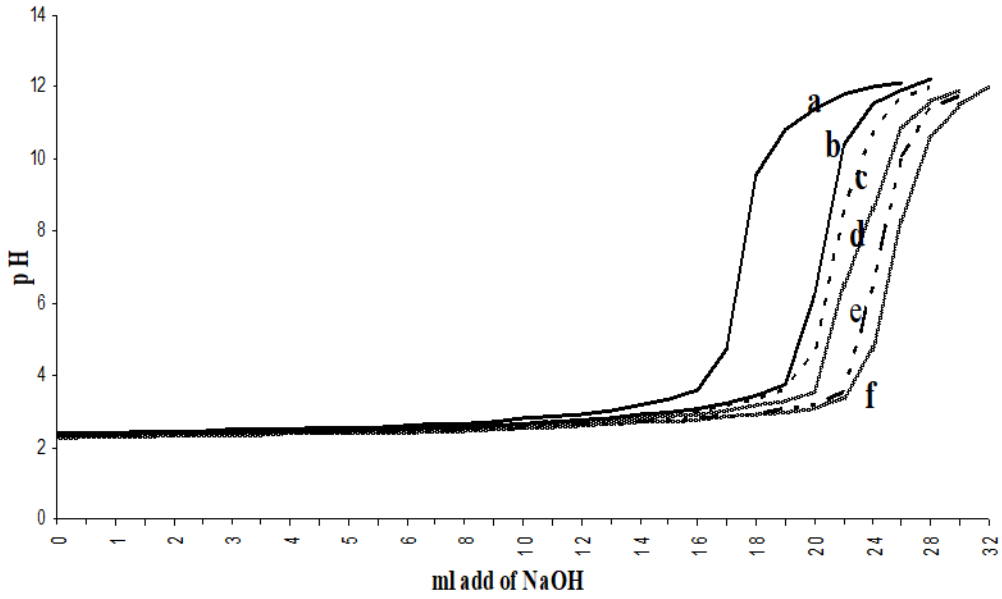


Fig.1. Typical pH-metric measurements curvatures of folic acid (FA), at 0.1M sodium perchlorate and 25 ± 1ºC: a = 0.01 M $HClO_4$, b = a + 0.001 M FA, c = b + 0.001 M Cu(II), d = b + 0.001M Pb (II), e = b + 0.001 M Fe(III), and f = b + 0.001M Co(II)

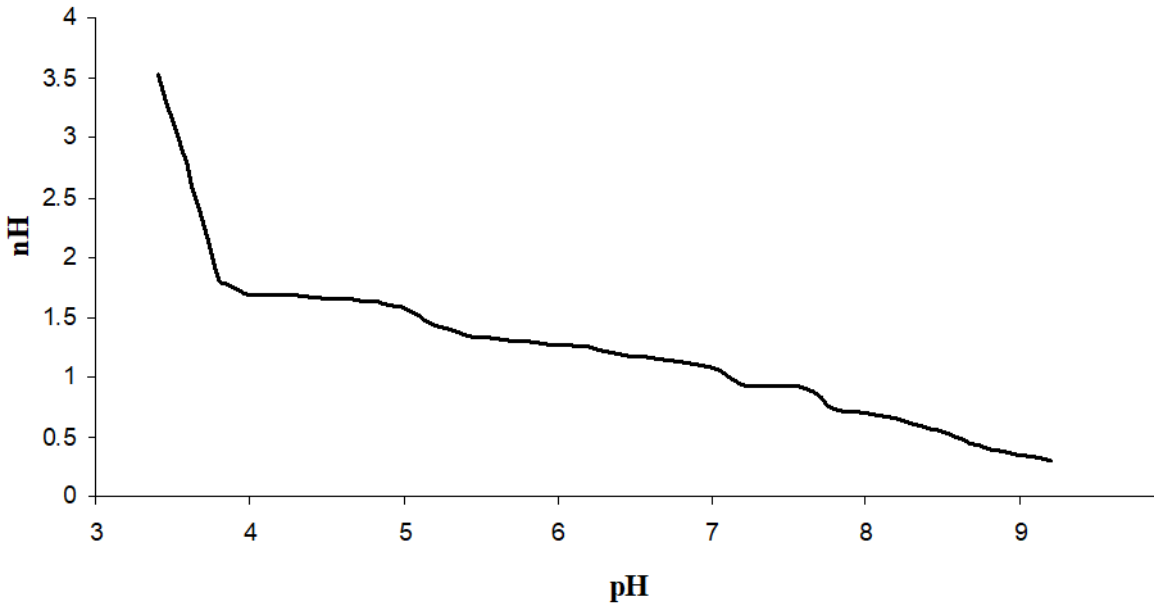


Fig. 2. Association constant curve of folic acid at 0.2 M $NaClO_4$ and 25±1ºC

3.A.2. Metal-folic acid complexes

. As for these titration curve, $\bar{n}$ (the mean number of FA particles attached per metallic ion) and pL (free FA exponent) values were computed by Irving and Rossoti equation[27].

$$\bar{n} = \frac{(V_3 - V_2)(N^0 + E^0)}{(V_0 + V_2)\,n\,HTcM^o} \longrightarrow (2)$$

$$pL = Log\left[\frac{(1 + K_1^{H}[H^+] + K_2^{H}[H^+]^2 + K_3^{H}[H^+]^3 + ----)}{(Tcl^0 - \bar{n}TcM^0)} \times \frac{V_0 + V_3}{V_0}\right] \longrightarrow (3)$$

Wherever V_1, V_2, V_3 are the volumes of alkaline to achieve the equal pH in free FA, free acid + FA and free acid + FA + metal, one-to-one. TcM^0 refers to the entire concentration of metallic ions existent in the solution. The values were plotted versus the equivalent pL values to obtain the creation curves of the metal complex equilibria. The association curves are shown in Fig. (3). From these association curves, the values of the stability constants at ionic strength of 0.2 M $NaClO_4$ recorded in Table (1) were determined by the half-integration technique [27].
The balances recognized from this investigation can be displayed in the subsequent formula eqs (4, 5 and 6):

$$H_3{-}FA \rightleftharpoons H_2{-}FA^{-} + H^{\oplus} \qquad pH = 3.63 - 4.95 \ldots\ldots 4$$

$$H_2{-}FA^{-} \rightleftharpoons H{-}FA^{2-} + H^{\oplus} \qquad pH = 5.1 - 8.4 \ldots\ldots 5$$

$$H{-}FA^{2-} \rightleftharpoons FA^{3-} + H^{\oplus} \qquad pH = 8.6 - 9.2 \ldots\ldots 6$$

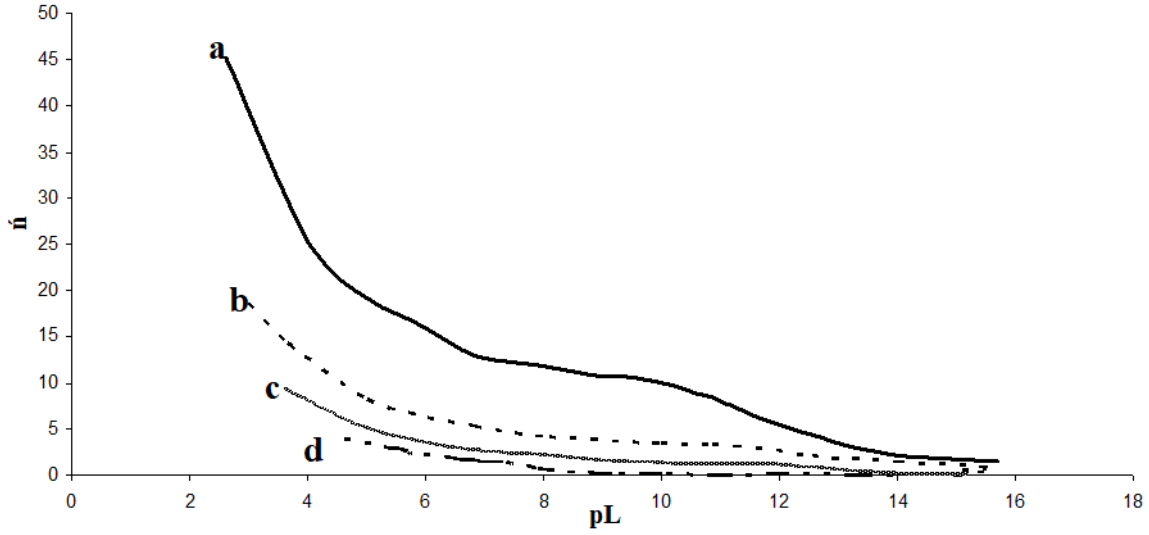


Fig. 3. typical Demonstrative association plots of twofold metallic ion interaction with folic acid at I = 0.1M: a) Co(II), b) Pb(II), c) Cr(III) and d) Th (IV).

In Table 1 we can see that metallic ions similar to iron(III), Thorium(IV), Cupper (II), Magnesium (II), Cobalt (II) and Nickel (II), form (1:1), (1:2) and (1:3) metal-ligand interactions, furthermore, Ca(II) and Cr(III) form only one (1:1) metallic-ligand compound, but in the case of lead (II) ions which form (1:2) and (1:3) metallic-ligand complexes, the association constants of the primary complex were obtained only because the association of the next complex is distorted by hydrolysis and precipitation of the metallic ion. This owing to the nature of the metallic ion, folic acid activity and ionic strength. Thus, the experimental information in this pH range will not be beneficial for mathematical calculations. Furthermore, these data cannot be in equilibrium, so the pH values at this step exhibit unstable deviation. However, compared our data obtained with the previous research we found that are in very agreement to the data from reference 19 as shown in table 1..

Table 1: Association constants of FA and stability constants of metallic ion complexes at 0.2 M $NaClO_4$ and at room temperature.

Metallic ion	Log K_1 (Metal : ligand)*	Log k_2 (Metal : ligand)*	Log K_3 (Metal : ligand)*
H+	**8.5**	4.9	**3.5**
Pb (II)	-------	**13.73(14.83)** **1:2**	**12.21(13.22)** **1:3**
Ca (II)	**7.5(7.1)** **(1:1)**	------	-----
Co (II)	**14.6 (14.81)** **1:1**	**12.93 (13.93)** **1:2**	**11.24 (12.24)** **1:3**
Fe (III)	**13.70 (14.7)** **1:1**	**12.54 (13.54)** **1:2**	**11.95 (12.04)** **1:3**
Mg (II)	**13.15 (14.05)** **1:1**	**11.56 (12.76)** **1:2**	**9.85 (10.01)** **1:3**
Ni (II)	**13.27 (14.37)** **1:1**	**12.5 (13.6)** **1:2**	**10.85 (11.85)** **1:3**
Cr (III)	**8.5 (8.12)** **1:1**	**……**	**……**
Th (IV)	**10.8 (12.92)** **1:1**	**9.02 (9.53)** **1:2**	**7.14 (7.14)** **1:3**
Cu (II)	**14.06 (14.06)** **1:1**	**11.59 (11.59)** **1:2**	**8.45 ([8.62)** **1:3**

The data between brackets ( ) are from reference number 19.

Stability constants of the complexes formed between folic acid and the elements under investigation recorded in Table 1are in the same direction of stability of the different two complexes formed between folic acid and metallic ions investigation in this work and follow the predictable Irving-Williams order [32].

Co(II) > Fe(III) > Ni(II) > Mg(II) > Cu(II) > Th(IV) > Cr(III) > Ca(II)

The behavior of folic acid may depend on the three dentate nature coordinated through the first and second carboxylic groups of glutamic acid and the imino group of glutamic acid, making established six-member chelating ring.

The space between curves c and b corresponds to the stepwise association ion of complex types ML, ML_2 and ML_3. The outcomes gained for the construction of the binuclear complexes examined are recorded in Table 1.

3. A.3. Conductometric method for titration of folic acid (FA):

The conductivity investigation method is widely used to trace the formation of complexes. This method is very useful as a delicate method to test the fraction differences in the ionic diameters of the examined transitional metallic elements as presented in Figure 4. The conductivity examination method is built on the variation in the electrolytic conductance results of solutions as a resulting of complexing association. Which in turn depends on the variations in the number of ions existent and their movements. The results of conductance measurements are also used to trace the diverse forms of chelates that form between metallic ions and the folic acid.

The conductivity titration curve of the double ligand system having Mg(II), Ca (II), Co(II), Ni(II), Cu(II), pb(II), Cr (III), Fe(III) and Th(IV) metallic elements displays primary reduction in conductance and a least at 1:1. This might be owing to the neutralizations of hydrogen ions resultant from the association of the [Metal -FA] interaction as shown in Figure 4.

Furthermore, the conductivity increases a little among 1:1, 1:2 and/or 1:3, perhaps owing to the association of the double Complexes and the releasing of hydrogen ions from folic acid. However, both of suggested potentiometric and conductometric procedures were utilized to confirm the stoichiometry of M-FA formed binary complexes.

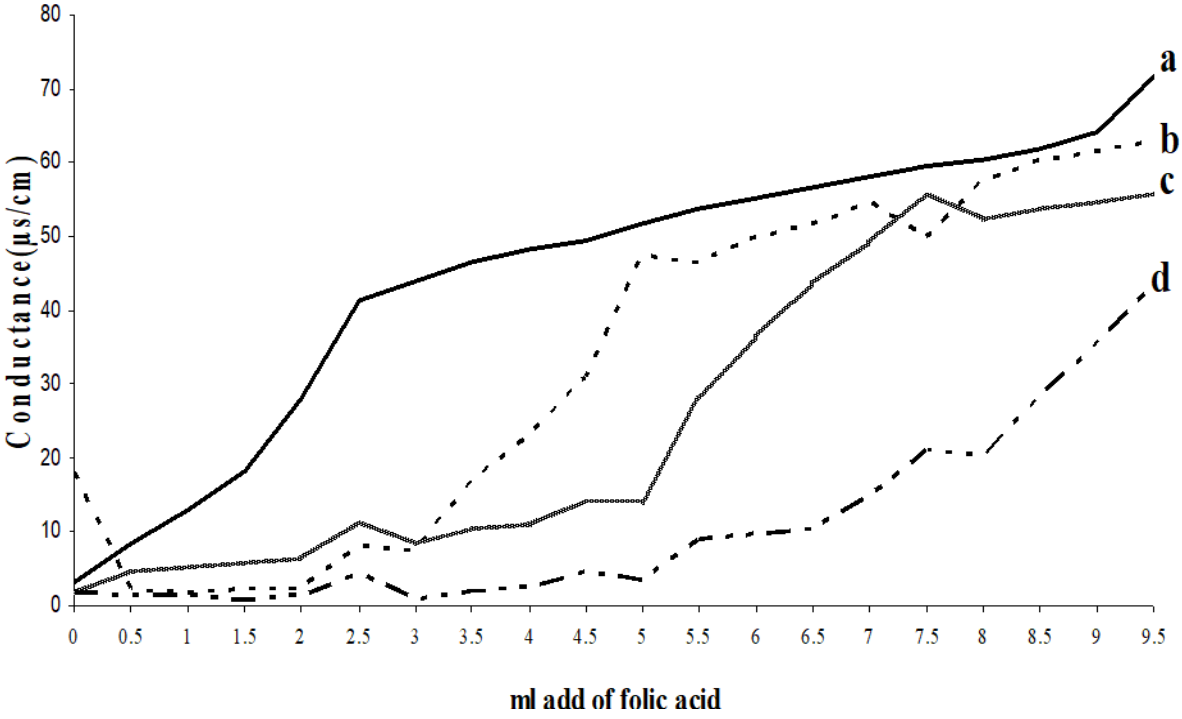


Fig. 4: Typical conductometric titration at 25 ml 0.001 M metallic ions with 0.01 M of folic acid: a) Co(II), b) Cu(II), c) Ca(II) and d) Fe(III)

3.A.4. Ionic distribution diagrams of folic acid (FA):

From the dispersal curves of folic acid revealed in Figure 5 at ionic strength I = 0.01M $NaClO_4$, we find that in the pH range (2.7 - 6.6) the main types is αo (H_3-FA), but in the pH range (3.2 - 6.7) the main species is α1 (H_2-Folic acid), however the absence of α2 ($H\text{-}FA^{-2}$) was found, while in the pH range (4.95 - 11.8) the main types is α3 (FA-3).

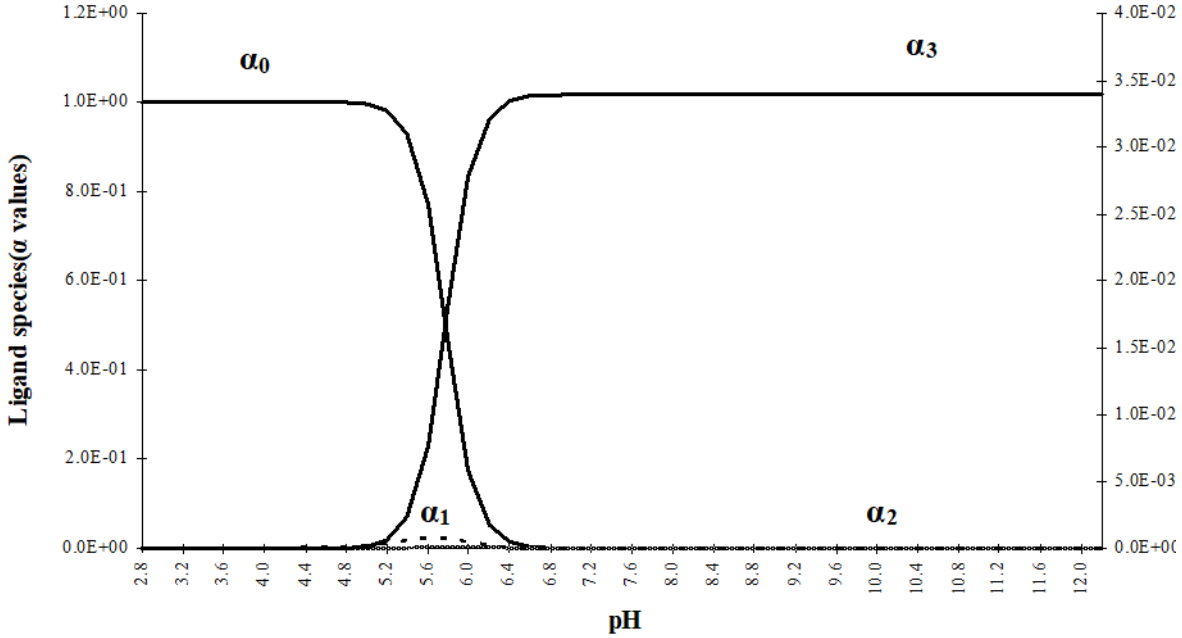


Fig. 5: Ionic equilibria of FA in different pH range.

When plotting metallic ion types and pH, ionic distribution curves can be attained. As revealed in Figure 5, by analyzing the resulting charts, we find that at little pH values, most of the metallic elements are in the form of free metallic ions, indicating that no complexation occurs in the acidic solution. With growing FA concentration, which is produced by growing the pH of the medium during titration, the mole fraction of the free metallic ion tends to fall, while the mole fraction of the ML types begins to grow in the reasonably acid medium. But, the value of log K_1>log K_2 has a significant concentration of the ML types in this area of pH. As the pH of the medium increases, the main variation becomes growth in the contents of the ML_2 species and a fall in the ML types. Beyond this area, the metallic elements are almost entirely in the form of ML or ML_2 species when the pH of the medium growths. The intersection of some types of fractions with the extreme pH can be denoted by complexes. So that values of –log [H] at the joining points (αM, αML) and (αML, αML_2) are consistent with values of the stability constant for every metallic-FA interaction in study as logged in Table 1.

3.B Potentiometric determination of Folic acid:

The dissociation constants of folic acid (FA) at ionic strength of 0.1 M sodium perchlorate at room temperature were previously estimated by potentiometric titration [19]. Folic acid is a triprotic acid [H_3-FA], with the first and second carboxyl groups as the first and second proton centers, while the imino group of glutamic acid represents the third proton. The acidic and basic properties of FA in acidic medium of 0.2 M at ionic strength (I = 0.2 M $NaClO_4$) show that one H^+ of the first carboxylic group dissociates in the pH range of 3.65-4.96. The second H^+ from the second

carboxyl group dissociates in the pH range of 5.2-8.5, while the triproton dissociates from the imino group in the pH range of 8.50-9.30. The pH constants corresponding to the first carboxylic group are PK_1 8.5. Therefore, folic acid can be detected by direct potentiometric titration of folic acid with NaOH, folic acid act as a single basic acid with an ionization constant of $PK_1 = 8.5$ (first carboxylic group). Observing this value for folic acid, the titration curve gives a clear deviation from the first equivalence point $PK_1 = 8.5$. Figure 6 shows the curves (a) of the pH – metric titration curve with only one variety point. In the suggested technique, the variation at the end point of the titration was large enough to obtain a potentiometric titration curve with a clear and acceptable shape for precise and reproducible end point detection. Curve (b) displays the first derivative of the potentiometric titration plot by a common glass electrode for pH, while curve (c) represents the second derivative.

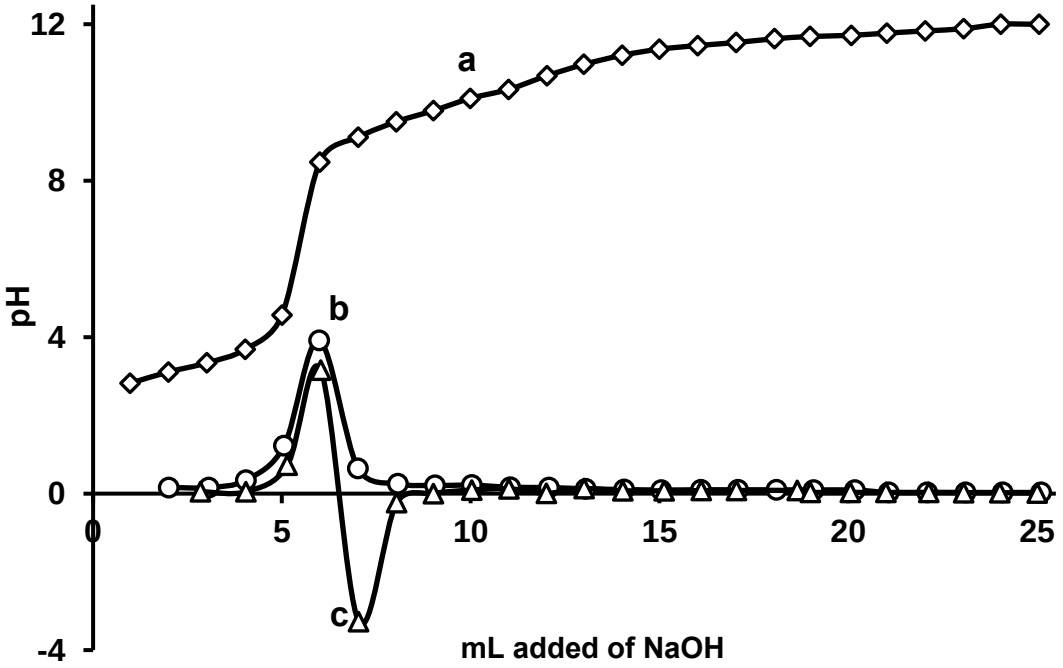


Fig 6: potentiometric titration curve of folic acid (pure), a) blank titration curve, b) first derivative curve, c) a second derivative curve, $\mu = 0.5$ M $NaClO_4$ and at 25 ±1.0 °C

3.B.1 Influence of ionic strength on detection of folic acid:

The influence of ionic strength in the variety of 0.04 M to 1.5 M $NaClO_4$ was studied by potentiometric method. The best ionic strength, which gave a recovery of 99.1±0.21 (100% closed), was found at 0.5 M $NaClO_4$. Therefore, the ionic power of 0.5 M was chosen as the best value and was used in the detection of pure FA and its dosage forms. As seen in Table 2.

Table (2): influence of ionic strength on the detection of folic acid (FA) by Potentiometric technique at room temperature.

Ionic Strength (M)	Adding from pure (mg)	Founding (mg)	Percentage recovery ± SD (%)	Relative standard deviation (RSD) n=5
0.04	10	10.6	106± 0.16	0.15
0.1	10	10.3	103 ±0.25	0.23
0.5	10	10.2	102 ±0.11	0.10
1.0	10	10.5	105±0.17	0.16
1.5	10	10.8	108 ± 0.15	0.14

3.B.2 Folic acid determination in pure form:

Looking at Table (3), we find that the values of the recovery percentage of folic acid in the pure form by the potentiometric method linear range from 95.2 - 100.5%, and we obtained them under the best ideal conditions (I = 0.5 M $NaClO_4$). It was found that the recovery percentages are very close to 100%; within a range of standard deviations ranging from SD (n = 5) 0.1-0.4 and the RSD(n=5) 0.14 – 0.37, as well as the confidence range from 0.07 - 0.35 at a confidence level of 95%. These values indicate the accuracy and clarity of the method used.

Table (3): Estimation of folic acid (pure) by the potentiometric method at 0.5 M $NaClO_4$ and at room temperature.

pure Added (mg)	Founding (mg)	Recovery (%)	Standard deviation (n=5)	Relative standard deviation (RSD) n=5	Confidence (n=5) α =0.05
0.1	0.11	110	0.31	0.37	0.29
0.2	0.23	115	0.18	0.22	0.11
0.3	0.28	93.3	0.25	0.30	0.1
0.5	0.51	102	0.15	0.18	0.26
0.8	0.78	78	0.23	0.28	0.22
1.0	1.08	108	0.31	0.37	0.27
2.5	2.4	96	0.112	0.14	0.08
3.5	3.5	100	0.190	0.23	0.16
5.9	6.3	1.06	0.28	0.34	0.33

The detection limits can be calculated by the equation 3σ/b and the quantitative limits can be computed also from the relationship 10σ/b [29]. wherever b is the slope and σ = SD. The detection limits for folic acid were found to be 0.004 mg/ml, with a correlation coefficient R = 0.9853, (n = 5) and with a standard deviation (SD = 0.001). A linear relationship was successfully gained over a variety of concentrations from 0.004 to 0.065 mg/ml as presented in Fig. 7.

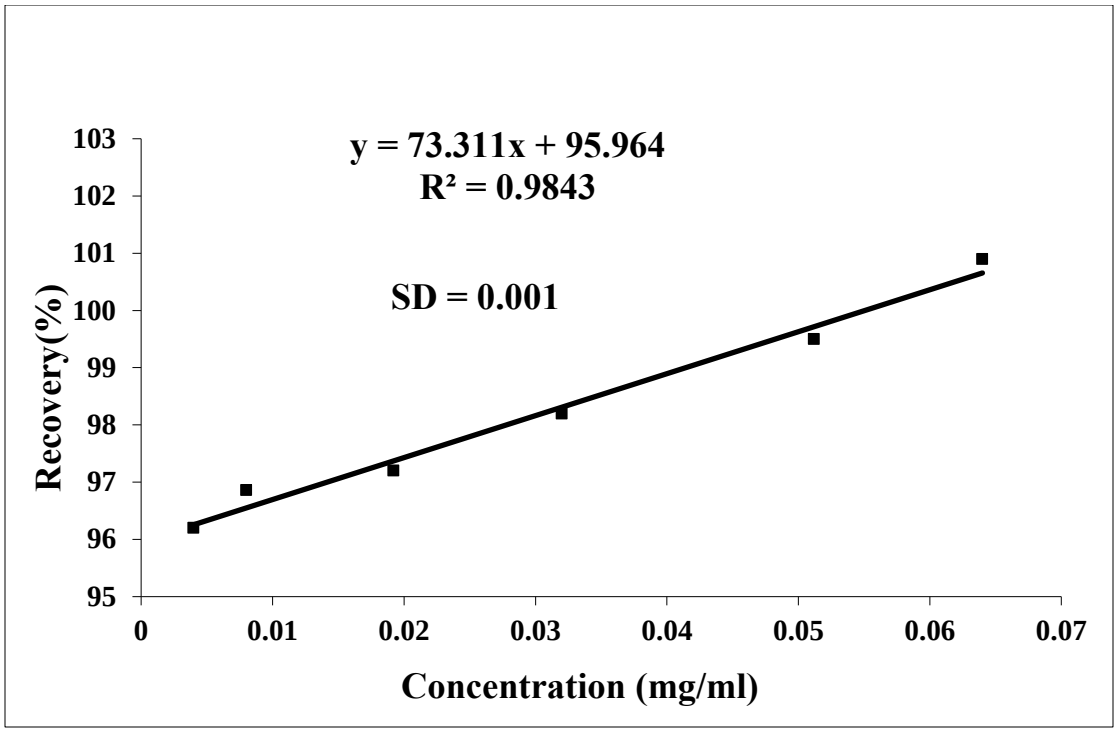


Fig 7: Linearity range of folic acid (pure) at 0.5 M $NaClO_4$ and 25±1.0°C.

3.B.3. Influence of interferons:

To evaluate the utility of the technique used, the influence of common constituents (extracts and auxiliaries) that often accompanying the determination of folic acid in its pure form, such as sodium chloride and sodium acetate, in addition to (D(+) lactose monohydrate, was studied in a concentration range of at smallest 100 times greater than folic acid. No interferences were detected in the concentration range used.

3.B.4. Analytical application:

3.B.4.1 Dosage Forms:

The suggested technique was effectively useful to the detection of FA in pharmacological tablets (Folvite tablet: (400 µg, , 800 µg,1000 µg and injectable solution (5 mg/ml)) with different doses. In Fig. 4, curves a, b, c is: representative pH- metric calibration plot with only one reflection point, first derived of potentiometric curve and second derived, one-to-one.

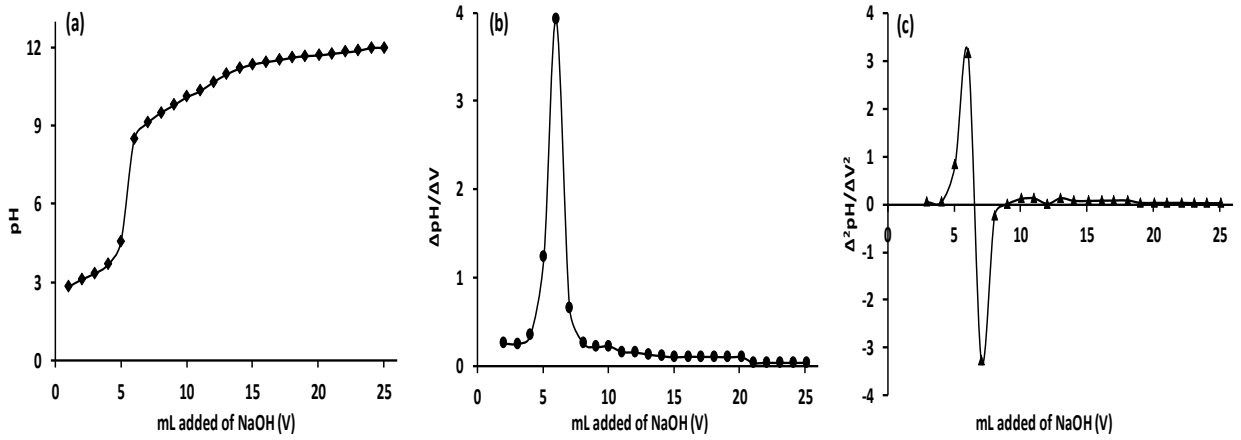


Fig 8: Typical pH - metric titration plot of folic acid (tablet) 400 µg, a) blank titration curve, b) first derivative curve, c) a second derivative curve, µ = 0.5 M $NaClO_4$ and at room temperature

Table (4): -Potentiometric detection of folic acid in dosage forms at µ=0.5$NaClO_4$ and 25±1.0°C.

Sample	Manufacture	Labeled to content/ µg		Proposed procedures		
			Founding (mg)	Recovery (%)	Standard deviation (%) n=5	Realative standard deviation (RSD) n= 5
Folvite tablets (400 µg)	(CO.E.I.p.I.CO-EGYPT)	400	390	97.5	0.35	0.37
Folvite tablets (800 µg)	(UNIMED- TUNISIE)	800	780	97.5	0.17	0.18
Folvite tablets (1000 µg)	(CO.E.I.p.I.CO-EGYPT)	1000	987	98.7	0.36	0.38
Injectable solution (5 mg/ml)	CO.E.I.p.I.CO-EGYPT)	5mg/ml	4.5	90	0.26	0.27

Looking at Table (4), we find that the recovery variety of the dosage forms (labeled to the content of 400, 800 and 1000 µg folvite) by the potentiometric method is linear in the variety of 90 - 98.7%, which was gained under ideal conditions (I = 0.5 M $NaClO_4$). The recovery ratios were establishing to be very near to 100%; with a standard deviation error, SD (n = 5) of 0.17-0.36 and the RSD(n=5) 0.18 – 0.37, as These outcomes indicate the precision and accuracy of the technique.

4. CONCLUSION:

In this research, a complex interaction between FA and several metallic elements, namely; Magnesium (II), Calcium (II), Cobalt (II), Nickel (II), Cupper (II), lead (II), Chromium (III), iron (III) and Thorium(IV), was conducted by potentiometric and conductometric methods. By these methods, the complexes formed and the Log K and species distribution of FA and their metallic interactions at diverse pH values were determined. Moreover, as far as it's known, we stayed the first to introduce potentiometric and conductometric techniques for the determination of FA in pharmaceutical tablets, and obtained recovery values ranging from 90 to 97.7%. Compared with many existing procedures for the estimation of folic acid, which require special instruments, substances and expertise, our technique was characterized by simplicity, rapid response, low cost and enough accuracy in the detection of folic acid in pure form and pharmacological tablets. The recovery of folic acid for different vial dosage forms ranged from 96.67% to 101.3%, without observing any interferences.

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