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ORIGINAL ARTICLE

Solution equilibrium of metal ions-binary complexes with 3-(2-ethylamino)-1-hydroxyethyl] phenol (Effortil)



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Abstract Formation of binary complexes of Al(III), Cr(III), Fe(III), Th(IV), UO₂(II), Ce(III), La(III), and Gd(III) with 3-(2-ethylamino)-1-hydroxyethyl] phenol (Effortil) were studied potentiometrically at 37.0 °C and $I = 0.16 \text{ mol dm}^{-3} \text{ NaNO}_3$ in aqueous solution. The acid–base properties of Effortil were investigated and discussed. The order of stability of the complexes was investigated and is discussed in terms of the metal ion. The experimental pH titration data were analyzed in order to evaluate the formation constants of various intermediate species formed. The concentration distribution of various species formed in solution was evaluated.

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1. Introduction

During the last two decades we have found that many drugs used in the treatment of hyper- and hypotension form stable chelate complexes with some metal ions (see e.g., Bontchev and Pantcheva, 2002; Bontchev et al., 1989, 2000, 2001). Such investigations in this field lead to the hypothesis that the complexation can modify the biological activity of the drugs by changing the homeostasis of the metalloelements and/or changing the reactivity of the medication when it is bound in the form of a metal complex (Getova et al., 2006). The essen-

tial role of transition metal ions in biological systems is well known (Eichhorn, 1975; Eichhorn and Marzilli, 1979; Martell, 1980; Sigel, 1974; Sigel and Sigel, 1990, 1996; Bertini, et al., 1992). The interest in rare earth elements results from their unique physical chemical characteristics. The entire group exists exclusively as trivalent ions in natural waters, and their ionic radius decreases by less than 20% across the fifteen member series. Group III cations exhibit an essentially similar chemical behavior in aqueous solution. Under physiological conditions these cations exist as metal complexes. They are known to bind tightly to human serum transferrin in the blood. Numerous published studies on the interactions of group III metals with transferrin are reviewed, with particular attention being given to the comparative analysis of the binding constants and to the kinetics and mechanisms of metal ion uptake and release (Harris and Messori, 2002).

In fact, little studies showing the interactions between metal ions and Effortil have been appeared in the literature (Bontchev et al., 2000). We observed that no report is concerned about the suitability of Effortil and Al(III), Cr(III), Fe(III),

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Th(IV), UO₂(II), Ce(III), La(III), and Gd(III) metal ions. In this context, we believed that the study of the interactions of some metal ions with Effortil may have implications on biochemical studies.

2. Experimental

2.1. Materials and solutions

3-(2-Ethylamino)-1-hydroxyethyl] phenol (Effortil) was from Sopharma, Bulgaria. The metal salts were provided by BDH (England) analytical-grade products as nitrates. Stock solutions of the metal salts were prepared in bidistilled water; the metal ion concentration purity was checked by a standard method (Welcher, 1965). Carbonate-free sodium hydroxide (titrant, prepared in 0.10 mol dm⁻³ NaNO₃ solution) was standardized potentiometrically with KH phthalate (Merck AG). A nitric acid solution (0.04 mol dm⁻³) was prepared and used after standardization. Sodium hydroxide, nitric acid, and sodium nitrate were from Merck p.a.

2.2. Apparatus

The titrimetric data were obtained using a 702 titroprocessor equipped with a 665 dosimat (Switzerland) made by Metrohm (coupled with a Metrohm combined glass electrode Ag/AgCl). The glass electrode was calibrated before each titration with two Merck standard buffer solutions in nitrate medium: first with the pH 7.0 solutions (the same as in the bulb) and then with a pH 4.0 solution. All titrations were performed at 37.0 ± 0.10 °C by coupling the titration cell with a thermostatic bath.

2.3. Procedure

The ligand concentration was varied in the range 0.001–0.006 mol dm⁻³. Three to six different metal-to-ligand ratios, ranging from 1:1 to 1:6 for binary systems were investigated (metal:ligand). The investigated solutions were prepared (total volume 50 cm³) and titrated potentiometrically against standard CO₂-free NaOH (0.10 mol dm⁻³) solution. A stream of nitrogen was passed throughout the course of the experiment in order to exclude the adverse effect of atmospheric carbon dioxide. Each of the investigated solutions was thermostated at the required temperature with an accuracy of ±0.10 °C and the solutions were left to stand at this temperature for about 15 min before titration. Magnetic stirring was used during all titrations. About 100–140 experimental data points were available for evaluation in each run. Titrations were performed up to pH ≈ 11. Each set of titrations was performed at least four times and the reproducibility of the titration curves was within 0.01-pH unit throughout the whole pH range. The pH titrations were terminated when either of the pH readings became unstable, showing a downward drift. In all cases, no calculations have been performed beyond the precipitation point; hence, the hydroxyl species likely to be formed after this point could not be studied. Also, because of the rather sluggish ligand-exchange kinetics of Al(III) and the precipitation reactions when equilibration could not be reached within 10 min, the corresponding titration points were omitted from the calculations (Kiss et al.,

1997). The stability constants of the binary complexes were calculated by means of computer program based on unweighted linear least squares fit (Irving et al., 1953). Standard deviations were also evaluated for the corresponding equilibrium constants. The concentration distribution of various complex species existing in solution as a function of pH was obtained using the SPECIES program (Gans and Sabatini, 1996). All solutions used throughout the experiments were prepared freshly in ultra pure water obtained from a NANO pure-ultrapure water system with resistivity of 18.30 MΩ cm. All of the aqueous solution samples were prepared gravimetrically.

2.4. Calibration of glass electrode cell

A computer program (GLEE, glass-electrode evaluation) (Gans et al., 2000) was used for the calibration of glass electrode by means of a strong acid-strong base titration. This program provided an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential, slope of the electrode, optionally, the concentration of the base and pK_w. It used a non-linear, least-squares refinement to fit a modified Nernst Eq. (1),

$$E = E^0 + s \log[\text{H}^+] \quad (1)$$

Where E is the measured electrode potential, E^0 and s are parameters of the refinement and represent the standard electrode potential and slope, and $[\text{H}^+]$ represents the hydrogen ion concentration.

In acidic solutions the hydrogen ion concentration is obtained from the mineral acid concentration, T_H , as calculated from Eq. (2), that is, $\log[\text{H}^+] = \log(T_H)$

$$T_H = \frac{(a_H v_0 + \gamma b_H v)}{(v_0 + v_1 + v)} \quad (2)$$

a_H is the concentration, mol dm⁻³, of acid of which v_0 cm³ was added to the titration vessel; b_H is the concentration, mol dm⁻³, of base in the buret (by convention given a negative sign), v_1 is the volume, cm³, of background electrolyte solution added to the titration vessel; and v , cm³, is the volume of base added from the buret; and γ is a correction factor for the base concentration, where γ is refined and the calculated base concentration is γb_H .

In the alkaline solutions the effective concentration of the base is usually reduced by the presence of a small amount (preferably < 1%) of carbonate contamination. The extent of this contamination can be estimated by means of a gran plot (Gans et al., 2000). Initially E^0 is estimated from the acidic region and s is taken as the ideal Nernstian slope (T/5.0399 mV). Then Eqs. (3) and (4) are fitted by linear least-squares regression. Acidic region:

$$(v_1 + v_1 + v) 10^{\frac{E-E^0}{s}} = m^a v + c^a \quad (3)$$

Alkaline region:

$$(v_1 + v_1 + v) 10^{\frac{E-E^0}{s} - pK_w} = m^b v + c^b \quad (4)$$

A typical Gran plot was constructed. From the slopes and intercepts of the fitted lines two estimates are obtained of the volume of base consumed at the equivalence point: $v_e^a = -c^a/m^a$ for the acidic region and $v_e^b = -c^b/m^b$ for the alkaline region. Assuming that the difference is due to

carbonate, the effective base concentration is reduced by the factor v_e^a/v_e^b in the alkaline region. The mineral acid concentration in the alkaline region is then given by Eq. (5).

$$T_H = \frac{(a_H v_0 + \gamma \left(\frac{v_e^a}{v_e^b}\right) b_H v)}{(v_0 + v_1 + v)} \quad (5)$$

where T_H is negative and $\log[H^+] = -pK_W - \log(T_H)$. Stability constants β_n^l are defined in terms of the equilibrium between a metal complex and its components, except that the free ligand concentration is replaced by total concentration of all ligand species not actually complexed to the metal, and the free metal ion term includes hydrolyzed metal ion and metal ion bound to other complexing species. Calculation of β_n^l can done by

$$\beta_n^l = \frac{\beta_n}{\alpha_M \cdot (\alpha_L)^n} \quad (6)$$

where

$$\alpha_M = ([M] + [MOH] + [M(OH)_2] + \dots) / [M] \quad (7)$$

Many metal ions hydrolyze to form polynuclear species so that α_M would be concentration-dependent, but in the presence of excess strong ligand it is usually sufficient to consider only the formation of mononuclear species. Under these conditions, α_M reduced to

$$\alpha_M = 1 + 10^{(pH-pK_1)} + 10^{(2pH-pK_1-pK_2)} + \dots \quad (8)$$

where pK_1, pK_2, \dots are the successive pK_a values for the loss of a proton from a hydrated metal ion. The metal ion hydrolysis constants published in the IUPAC stability constants data base and other sources have been used (Database Royal Society of Chemistry IUPAC, 2004; Cornelis et al., 2003). All the possible hydrolytic species resulting from the formation of the different hydroxyl complexes including different metal ions have been taken into consideration during the calculations. Initial estimates of the stability constants of different normal and protonated binary and ternary complexes formed in solution have been refined with the computer program. The quality of the fit during this refinement was judged by the values of the sample standard deviations.

3. Results and discussion

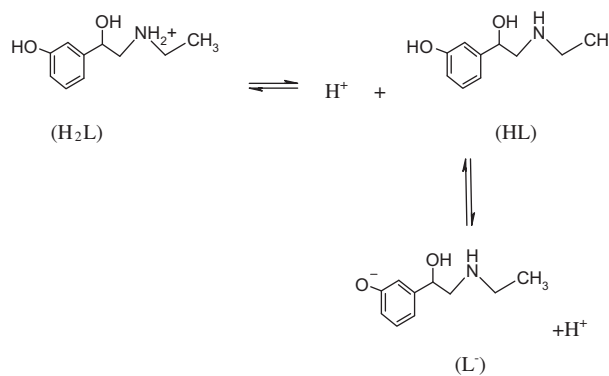
The accurate evaluation of the dissociation constants of phenylalkanolamines is important to understand their association and chelation reactions, their stability, and their comparative biological activity. Equilibrium studies of Effortil and its complex formation were carried out in aqueous solution because the stoichiometric protonation constants of the investigated ligand were determined at 37.0 °C and $I = 0.16 \text{ mol dm}^{-3}$ NaNO₃ potentiometrically using a computer program based *Irving and Rossotti* (Irving et al., 1953) and these constants are tabulated in Table 1. Upon the addition of NaOH deprotonation of ligand occurs, representation of the acid dissociation constants (K_a) in stability study is expressed in terms of proton-ligand formation constant or protonation constant (Eqs. (9), (10)), and also used in the present investigation. Analysis of the potentiometric titration curve using the computer program gave best fit for two protonation constants (Table 1). The protonation steps are described as follows (charges for simplicity):

Table 1 Dissociation constant of Effortil and stability constants of 1:1, 1:2 and 1:3 binary complexes at 37 °C and $I = 0.16 \text{ mol dm}^{-3}$ (NaNO₃).

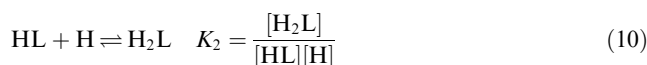
Species	p	q	r	$\log \beta(\pm\sigma)^a$
HL	0	1	1	9.24 (0.01)
H ₂ L	0	1	2	16.63(0.01)
[ThLH]	1	1	1	18.45 (0.04)
[ThL]	1	1	0	14.14 (0.02)
[ThL ₂]	1	2	0	25.48 (0.03)
[ThLH ₋₁]	1	1	-1	11.15 (0.01)
[ThLH ₋₂]	1	1	-2	18.08 (0.01)
[UO ₂ L]	1	1	0	11.66 (0.01)
[UO ₂ L ₂]	1	2	0	19.17 (0.02)
[UO ₂ L ₃]	1	3	0	24.37 (0.03)
[UO ₂ LH ₋₁]	1	1	-1	6.25 (0.05)
[UO ₂ (OH) ⁺]	1	0	-1	5.96 ^b
[GdLH]	1	1	1	17.29 (0.04)
[GdL]	1	1	0	11.33 (0.02)
[GdL ₂]	1	2	0	18.32 (0.01)
[LaLH]	1	1	1	14.30 (0.01)
[LaL]	1	1	0	8.56 (0.01)
[LaL ₂]	1	2	0	15.30 (0.04)
[CeL]	1	1	0	6.45 (0.05)
[CeL ₂]	1	2	0	6.35 (0.03)
[AlLH]	1	1	1	15.65 (0.03)
[AlL]	1	1	0	10.48 (0.01)
[AlL ₂]	1	2	0	20.11 (0.01)
[AlLH ₋₂]	1	1	-2	12.30 (0.05)
[CrLH]	1	1	1	15.66 (0.03)
[CrL]	1	1	0	10.54 (0.01)
[CrL ₂]	1	2	0	16.99 (0.02)
[FeL]	1	1	0	13.38 (0.03)
[FeL ₂]	1	2	0	26.89 (0.02)
[FeL ₃]	1	3	0	37.62 (0.02)
[FeLH ₋₁]	1	1	-1	9.83 (0.01)
[FeL ₂ H ₋₁]	1	1	-1	21.61 (0.01)

^a Standard deviation.

^b Ref. (Cornelis et al., 2003).



Scheme 1 Protonation of Effortil.



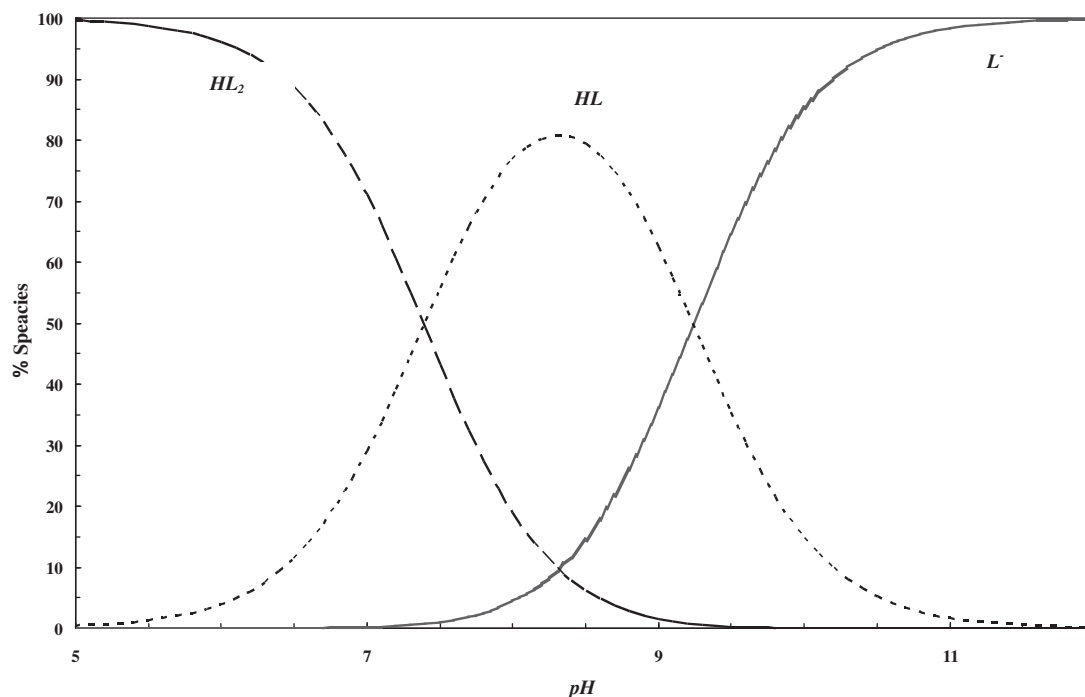


Figure 1 Representative concentration distribution curves as a function of pH calculated for Effortil system at 37 °C, $I = 0.16 \text{ mol dm}^{-3}$ NaNO_3 and $C_{\text{ligand}} = 0.001 \text{ mol dm}^{-3}$.

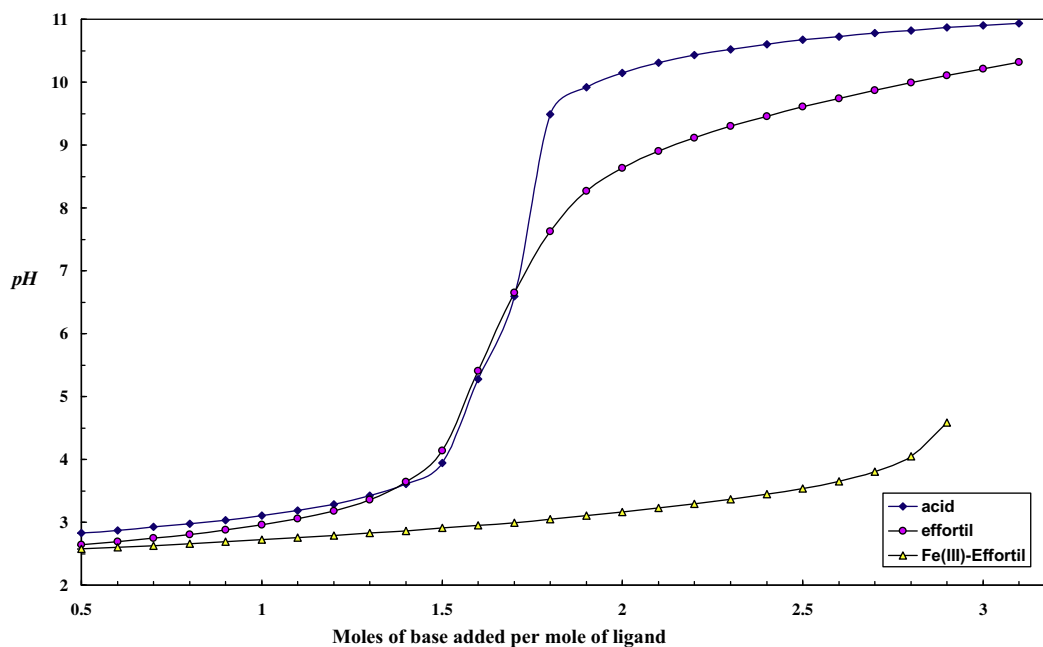


Figure 2 Potentiometric pH -titration curves for the Fe(III)-Effortil system at 37 °C, $I = 0.16 \text{ mol dm}^{-3}$ NaNO_3 and at 1:1 metal–ligand ratios; $C_{\text{Effortil}} = 0.001 \text{ mol dm}^{-3}$.

These equilibria are described as protonation reactions to give protonated ligand and accordingly the equilibrium constants are stability constants. As is apparent from these equations, K_1 , and K_2 are equal to the reciprocals of the respective acid dissociation constants (K_a). Since the two protonation constants are related to the protonated amine nitrogen and

phenolate, respectively as shown in Scheme 1. This is also illustrated in the species distribution of the Effortil ligand in Figure 1 as a function of pH which indicates that in acidic solution effortil initially exists 100% in the fully protonated form as H_2L below $pH < 5$. By rising of pH, the ligand (H_2L) loses the first proton forming HL, which is the

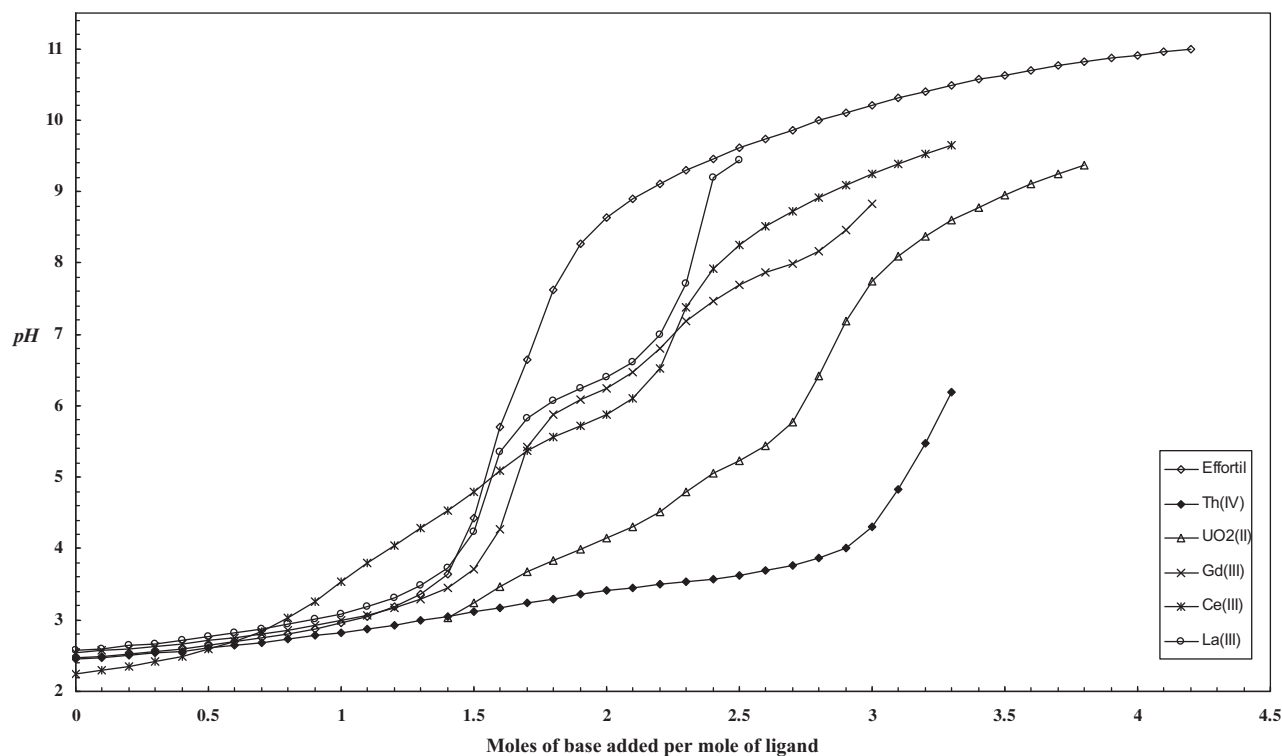


Figure 3 Potentiometric pH -titration curves for the Effortil and for the M-Effortil systems at $37\text{ }^{\circ}\text{C}$, $I = 0.16\text{ mol dm}^{-3}\text{ NaNO}_3$ and at 1:1 metal-ligand ratios; $C_{\text{Effortil}} = 0.001\text{ mol dm}^{-3}$.

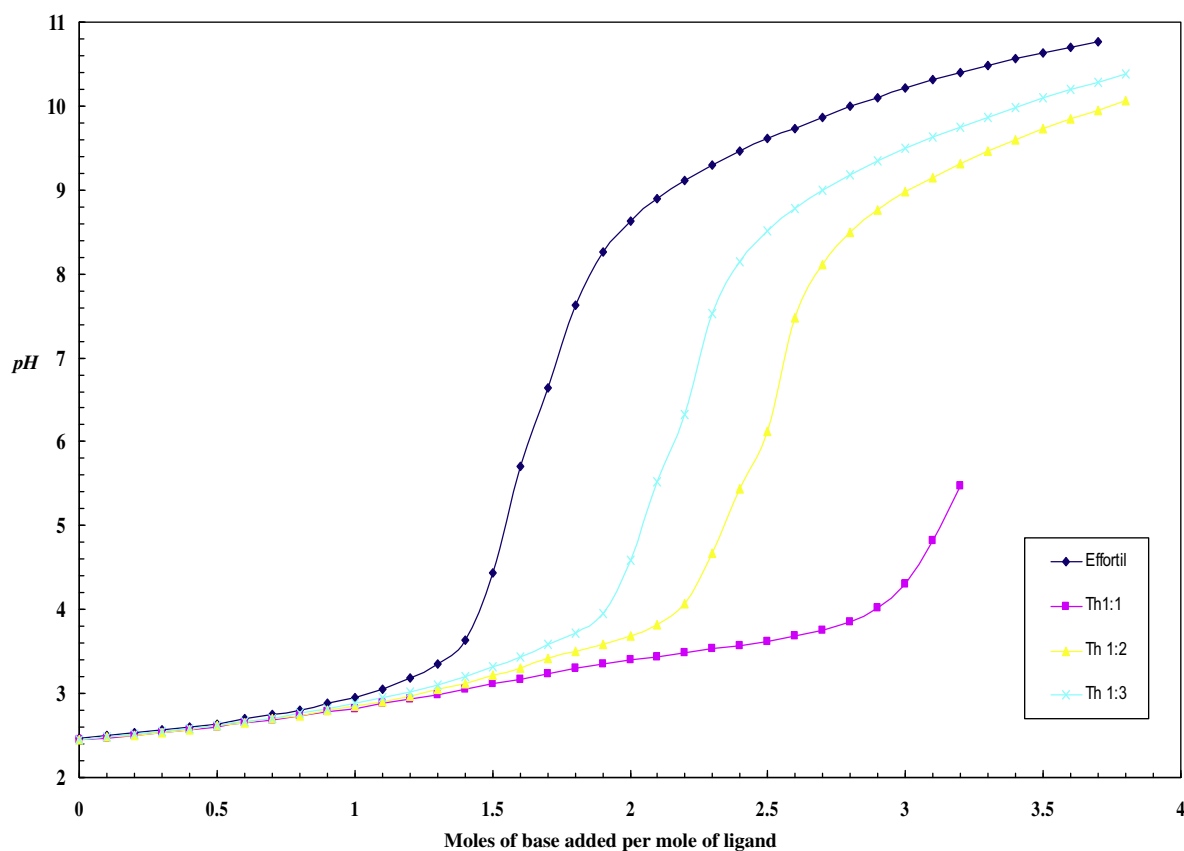


Figure 4 Potentiometric pH -titration curves for the Effortil and for the Th(IV)-Effortil system at $37\text{ }^{\circ}\text{C}$, $I = 0.16\text{ mol dm}^{-3}\text{ NaNO}_3$ and at 1:1; 1:2 and 1:3 metal-ligand ratios; $C_{\text{Effortil}} = 0.001\text{ mol dm}^{-3}$.

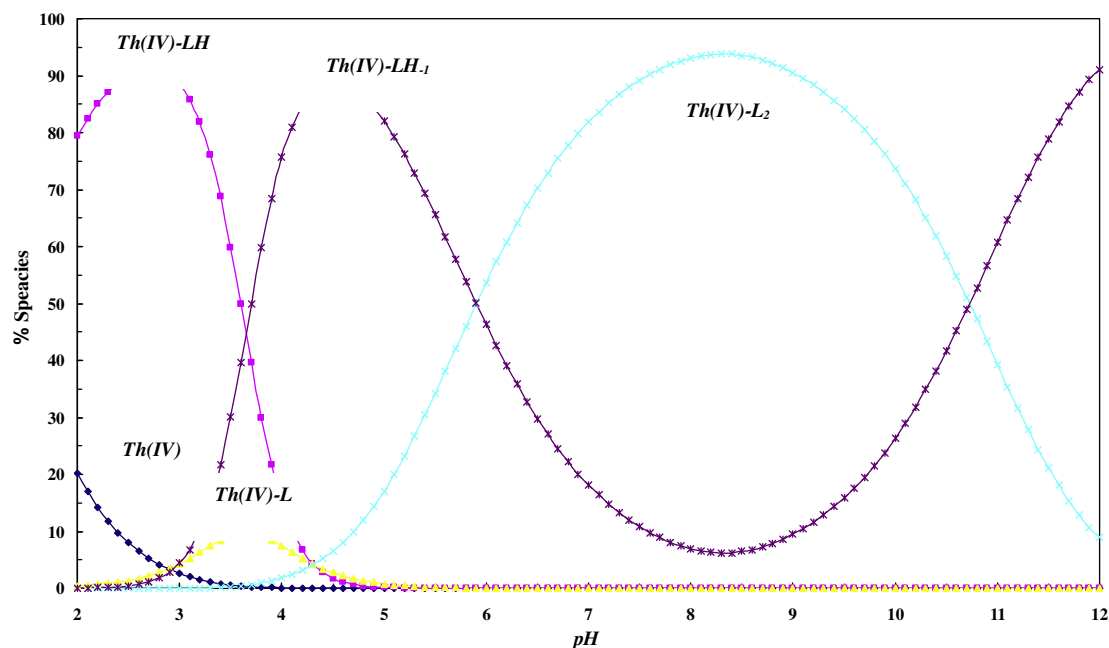


Figure 5 Representative concentration distribution curves as a function of pH calculated for Th(IV)-Effortil system in the ratio 1:2 at $37\text{ }^{\circ}\text{C}$, $I = 0.16\text{ mol dm}^{-3}\text{ NaNO}_3$ and $C_{\text{ligand}} = 0.001\text{ mol dm}^{-3}$.

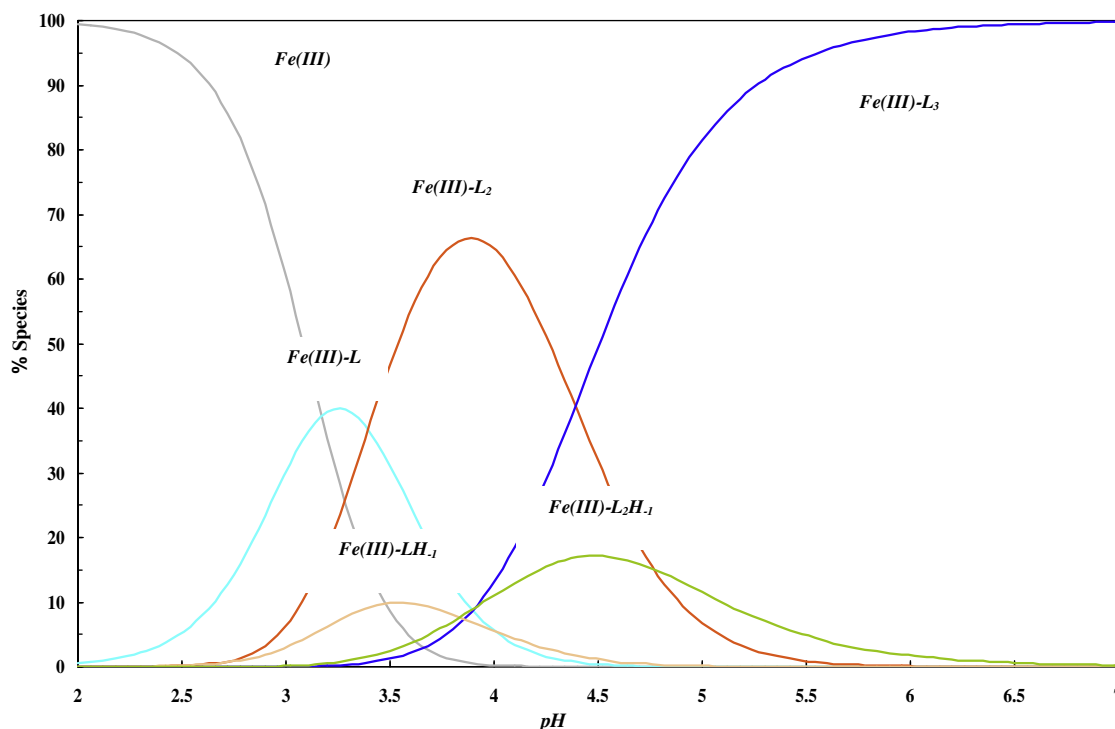
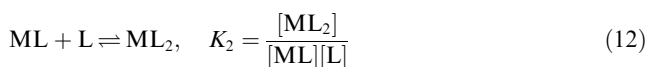
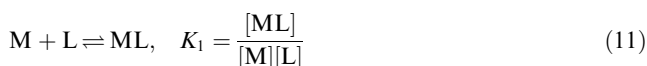


Figure 6 Representative concentration distribution curves as a function of pH calculated for Fe(III)-Effortil system in the ratio 1:4 at $37\text{ }^{\circ}\text{C}$, $I = 0.16\text{ mol dm}^{-3}\text{ NaNO}_3$ and $C_{\text{ligand}} = 0.001\text{ mol dm}^{-3}$.

predominant species in pH 8.30 with maximum concentration of 80.79%. As the pH increases, the second proton begins deprotonation of the free ligand L^- anion reaching a maximum percent of 99.70% at pH 11.70.

Effortil was titrated in the presence and absence of metal ion. The titration curve of the complex is lowered from that

of the free Effortil curve (Figure 2). This indicates a complex formation associated with release of hydrogen ions. The formation constants were determined by fitting potentiometric data on the basis of possible composition models. The equilibria involved in the formation of the metal-Effortil binary complexes may be represented as follows:



Examination of the stability constants of the binary complexes investigated (Table 1) reveals the following remarks:

- In general, the values of $\log K_1$ reflect the affinities of the used ligand for chelation with the metal ions under investigations. This affinity may be attributed to the simple structure of the used ligands and consequently no steric hindrance in the formed 1:1 complexes.
- The complex stability of the binary complexes with respect to the metal ion present follows the order: Th(IV) > UO₂(II) > Gd > Ce(III) > La(III). This behavior can be attributed to the fact that the actinides have much higher densities and greater tendency to form complexes compared with the lanthanides (see Figure 3). Thus, in actinides the 5f orbitals extend into space beyond the 6s and 6p ones and participate in bonding. In contrast, for lanthanides, the 4f orbitals are buried deep inside the atom, totally shielded by outer orbitals and unable to take part in bonding. Stabilities of binary complexes involving UO₂(II) ion are lower than those containing Th(IV), which can be attributed to the smaller charge of the linear dioxouranium. With respect to lanthanides binary complexes, one can deduce that the values of stability constants of Ce(III) systems are higher than those of La(III) complexes. This is due to the smaller ionic size of Ce(III) (1.020 Å) than that of La(III) (1.032 Å) and also the lower basicity of Ce(III) than La(III) ion (Cornelis et al., 2003).
- Also, it can be observed that the stability constants of different 1:3 or 1:2 metal–ligand and complexes are lower than those of the corresponding 1:1 complex species (See Figure 4), as expected from statistical consideration. The $\Delta \log K = (\log K_3 - \log K_2 \text{ or } \log K_2 - \log K_1)$ values are negative (Table 1). The reduction in the values of stepwise constants is principally due to the fact that the entropy contribution to the free energy change becomes less favorable from one step to the next (Eqs. (11), (12)).
- The high stability of Fe(III) with the ligand under study can best be viewed in light of the observation that Fe(III) is a hard Lewis acid with a high charge. The presence of negatively charged O, N donor atoms in both amino and phenol groups play a major role in producing thermodynamically stable complexes with Fe(III) (Fazary et al., 2011).
- The acid dissociation constant of the protonated complex given by ($pK_{MLH}^H = \log K_{MHA}^M - \log K_{MA}^M$) amounts to 4.31 for Th(IV), 5.96 for Gd(III), 5.74 for La(III), 5.17 for Al(III), and 5.12 for Cr(III)–Effortil binary systems. The lowering of this difference can be ascribed to coordination of Effortil with the metal ion.
- In our investigation, measurements were carried out in a wide range of reagent concentrations and ligand/metal molar ratios in order to reach high pH values (up to pH ≈ 8.5) at 37 °C, $I = 0.16 \text{ mol dm}^{-3} \text{ NaNO}_3$. It is worth mentioning that the pH values do not change during the titration time, ruling out any hydrolysis occurring under these experimental conditions and thus the formation of hydroxo species (extensive hydrolysis is unlikely).

- Studies of pH-metric titration, as a “hard acid”, Al can effectively coordinate with the amino group of effortil in aqueous solution owing to the high affinity of Al toward nitrogen. The 1:1 chelating complexes are major forms in acidic aqueous solutions.
- Good linear relationships exist for these data which demonstrate that metal ion affinity for a particular chelate increases with increasing metal ion charge (Raymond et al., 1984; Angkawijaya et al., 2011; Cam et al., 2011).

The species distribution diagram of Th(IV)–Effortil, taken as a representative and given in Figure 5, shows that protonated complex prevails with formation degree (91%) at pH ca 2.60. The deprotonated species predominates in the physiological pH range. And the concentration distribution diagram of the Fe(Effortil) system is shown in Figure 6. The hydrolyzed species FeLH–1 has been formed above pH 3.00 and has 9.82% relative concentration at pH 3.5 and FeL2H–1 shows the predominance at 4.40 pH with 16.92%.

4. Conclusion

Potentiometric data were employed to calculate formation constants of Al(III), Cr(III), Fe(III), Th(IV), UO₂(II), Ce(III), La(III), and Gd(III) with 3-(2-ethylamino)-1-hydroxyethyl] phenol (Effortil). The complex formation equilibria were investigated to ascertain the composition and stability constants of the complexes.

The concentration distribution diagrams of the complexes were evaluated. The effect of metal ion on the formation of the binary complex, the size of the central metal cations and the steric requirements of the ligands are important. These results can help understand biological interactions among the ligand under study and the metal ions.

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