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Analysis of potentiometric titrations of heterogeneous natural polyelectrolytes in terms of counterion condensation theory: application to humic acid

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Abstract

A model, developed within the framework of the counterion condensation theory of linear polyelectrolytes, is presented in this paper to describe the acid–base properties of linear polyelectrolytes, consisting of several types of functional ionizable groups. This formalism has been successfully applied to Fluka humic acid under salt-free conditions, as well as in the presence of supporting simple 1:1 salt (KNO_3) at three different concentrations. As part of this approach, the charge density of the humic acid is obtained from the activity coefficient measurements of potassium counterions at different humic acid concentrations at a constant degree of dissociation of the polyelectrolyte. The humic acid average charge density was found to be 0.80 ± 0.05. Using the present model, we are able to satisfactorily describe the experimental data obtained from acid–base potentiometric titrations. Four main functional groups making up the polymer are determined through their fractional abundances (X_i) and intrinsic pK (pK_i^0) values. The fractional abundances remained constant and independent of the ionic strength, indicating that the humic acid constitution does not depend on the concentration of excess salts. The pK_i^0 values show a small change with ionic strength, which can be explained by the polyelectrolytic behavior of the solution. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Humic acid; Polyelectrolytes; Ionic strength; Counterion condensation

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1. Introduction

Many aspects of the polyelectrolytic behavior of monoprotic linear charged polymers in solution have been successfully described by the Counterion Condensation (CC) theory of linear polyelectrolytes [1–4]. This theory has provided rather simple functional relationships to describe several solution properties in the dilute regime, including specific interactions between the different types of counterions in solution and the polyelectrolyte [5–7]. Nevertheless, little work has been reported under this theoretical framework on the more complicated case of polyelectrolytes with different functional groups. Paoletti et al. [8] have analyzed, through the analysis of potentiometric titrations, acid–base properties of co-polyelectrolytes. They have shown that ‘anomalous’ titration curves, which could ambiguously be taken as indicative of polymeric conformational transitions, correspond in some cases to linear polyelectrolytes with two different types of functional groups.

Motivated by experimental work and modeling analysis (other than the CC theory) that indicate that some important (bio)polyelectrolytes consist of more than one type of functional group [9–14], a generalization of the CC theoretical treatment is presented here. A direct advantage of the polyelectrolytic approach is the straightforward determination of the relevant thermodynamic variables, as well as their dependence on the charge density of the polyelectrolyte and physicochemical solution variables.

The objective of this paper was, therefore, to present a theoretical treatment for the analysis of acid–base properties of natural polyelectrolytes with different types of ionizable groups. The theoretical approach is then applied to the analysis of acid–base potentiometric titrations performed on purified Fluka humic acid (HA) at different concentrations of supporting salt (KNO_3).

Humic acids are known to be heterogeneous polyacids with a polymeric structure constituted by different types of functional groups. Formed by the random condensation of the degradation products of plants and animals, composition of humic material strongly depends on its origin, extraction procedure and purification treatment [15]. A direct consequence of this complexity and variability is that humic material has not been defined in terms of precise and unique structural parameters. Hence, an analysis within the framework of the CC theory must face the additional problem concerning the determination of the charge density of the humic acid sample. In the polyelectrolyte theory [1,16], the charge density of the polyelectrolyte is a central magnitude determining the different physico-chemical properties of the polyelectrolytic solution. Its value has usually been determined from the conformation of the polymer in the crystalline state or, more often, in the fibrous one, and thereafter used in the study of solution properties with satisfactory results. In the present work the polyelectrolyte charge density was determined on the basis of activity coefficient measurements of monovalent counterions at different HA concentrations. It was shown that this experimental/theoretical approach results in a successful characterization of the HA, including the determination of its charge density and fractional abundances (X_i) and intrinsic pK (pK_i^0) values of the functional groups making up the polymer.

2. Theory

Working in the framework of the counterion condensation (CC) theory [1], the polyelectrolyte is modeled as a uniform linear array of charges separated (along the polymer axis) by an intercharge distance, b, which determines the polyelectrolyte charge density, ξ:

$$\xi = \frac{l_B}{b} = \frac{e^2}{\varepsilon kTb} \tag{1}$$

where l_B is the Bjerrum length; e is the elementary charge; ε is the bulk dielectric constant; k is Boltzmann’s constant; and T is the absolute temperature.

If the value of ξ is greater than a certain critical value, ξ_{crit}, the theory predicts that a fraction, r, of the counterions will condense per unit of polymeric charge. The fraction of condensed counterions r is determined by minimization of the ionic free energy of the system, made up of the sum of the electrostatic and entropic (mixing) contributions [17]:

$$G^{\mathrm{ion}} = G^{\mathrm{elec}} + G^{\mathrm{mix}} \tag{2}$$

Manning [1] has shown that for the simple case of only monovalent species, the electrostatic contribution can be written (for the case of $\xi < \xi_{\mathrm{crit}}$) as:

$$\mathrm{G}^{\mathrm{elec}} = -n_{\mathrm{p}} RT \xi \ln[1 - \exp(-\kappa b)] \tag{3}$$

where R is the gas constant and n_{p} the number of moles of equivalent polymeric charged groups. The Debye–Hückel parameter κ is given by [18]:

$$\kappa^2 = \frac{8\pi e^2 N_A}{\varepsilon kT 1000} I \tag{4}$$

N_A is Avogadro's number and I is the ionic strength of the solution.

If counterion condensation occurs ($\xi > \xi_{\mathrm{crit}}$), the entropy of the mixing contributions to the free energy should be included, according to the concentration and valences of the small ions in solution. The interested reader is referred to Paoletti et al. [4] for full details.

2.1. Determination of the charge density

The CC theory provides a simple functional form for the activity coefficients that can be used in the thermodynamic determination of the polyelectrolytic charge density. If the ionic free energy of the system is known, the single ion activity coefficient can be obtained by using [1]:

$$\ln \gamma_{\mathrm{i}} = \left(\frac{\partial G^{\mathrm{ion}}}{n_{\mathrm{p}} RT \partial C_i} \right)_{C_{\mathrm{P}}, T, C_{j \neq i}} \tag{5}$$

where C_i is the concentration of ions of type i.

Manning [1] has shown that for the particular case of monovalent polyelectrolyte groups and counterions (with no simple salt added), $\kappa b \ll 1$ and assuming a random distribution of functional groups, the mean activity coefficient is a function of the equivalent concentration of ionizable groups, C_{p}:

$$\ln \gamma_{+P} = -\frac{1}{2} \alpha \xi \ln(\alpha C_{\mathrm{p}}) + \mathrm{const} \quad \alpha \xi \leq \xi_{crit} \tag{6a}$$

$$\ln \gamma_{+P} = -\left(1 - \frac{1}{2}(\alpha \xi)^{-1}\right) \ln(\alpha C_{\mathrm{p}}) + \mathrm{const} \quad \alpha \xi \geq \xi_{crit} \tag{6b}$$

where, for generality's sake, it has been considered as a case of a partially ionized linear weak polyelectrolyte at degree of ionization α.

As usual the mean activity coefficient, γ_{+P}, is defined by: $\ln \gamma_{+P} = \frac{1}{2}(\ln \gamma_{+} + \ln \gamma_{\mathrm{P}})$. The subscript '+' refers to the counterion and 'P' to the equivalent fixed charge on the polyion (polyanion in this case). According to Eqs. (6a) and (6b), for a constant value of α, a plot $\ln(\gamma_{+P})$ against $\ln(\alpha \mathrm{C}_{\mathrm{p}})$ shows a linear dependence with a negative slope related to the polyelectrolyte charge density, ξ.

2.2. The apparent dissociation constant, pK_a, of multifunctional weak polyelectrolyte

Once the charge density and relevant solution variables are known, we can use the polyelectrolyte theory to analyze potentiometric titration data. For the case of interest here, and in order to interpret the potentiometric titrations of HA, an expression is derived for the dependence of the apparent pK, p$K_{\mathrm{a}}(\alpha)$, with the degree of ionization, α, for the case of a multifunctional polyelectrolyte. In general, for the common case of a homopolymer one can write:

$$\mathrm{p}K_{\mathrm{a}}(\alpha) = \mathrm{p}K^0 + \Delta \mathrm{p}K_{\mathrm{a}}(\alpha) \tag{7}$$

where pK^0 is the intrinsic pK of the ionizing group constituting the polyelectrolyte. If the ionic free energy, $G^{\mathrm{ion}}(\alpha)$, is known, polyelectrolyte theory provides an analytical expression for the change in the apparent pK upon ionization:

$$\Delta \mathrm{p}K_{\mathrm{a}}(\alpha) = \frac{1}{n_{\mathrm{p}}\, 2.303\, RT} \frac{\partial G^{\mathrm{ion}}}{\partial \alpha} \tag{8}$$

For the simple case of a linear polyelectrolyte with only one type of functional group and monovalent species, the CC theory gives (in the non-condensation region, $\alpha\xi \leq \xi_{\mathrm{crit}}$ [3]):

$$\Delta \mathrm{p}K_{\mathrm{a}}(\alpha) = -\frac{\alpha\xi}{2.303}\left\{2\ln(1-e^{-\kappa\alpha b}) + \frac{\kappa\alpha b}{e^{\kappa\alpha b}-1}\left[\frac{1}{2}\frac{\alpha}{2R_{\mathrm{S}}+\alpha}-1\right]\right\} \tag{9}$$

where $R_{\mathrm{s}} = C_{\mathrm{s}}/C_{\mathrm{p}}$ is the ratio between the analytical concentration of simple 1:1 salt added, C_{s}, and the equivalent concentration of ionizable groups on the polymer, C_{p}.

For the complete description of the theory, including the cases of mixing of counterions of difference valences, the interested reader is referred to references [3,19].

If the polyelectrolyte has more than one type of functional groups one can be faced with 'anomalous' titration curves of an exceedingly large pK_{a} change, and the above theory does not apply anymore. Paoletti et al. [8] have extended the CC theory in order to consider co-poly(monoprotic) acids containing two different acidic groups in the polymeric chain, each with different intrinsic pK_{i}^{0}'s and fractional abundances.

Since important natural polyelectrolytes, like humic acids, contain more than two functional groups, we extend, in the present work, this formalism in order to deal with an arbitrary number of functional groups making up the polyelectrolyte.

Following the formalism of Paoletti et al. [8], let the polymer be constituted by N different monoprotic acids: $\mathrm{H}_1, \mathrm{H}_2, \ldots, \mathrm{H}_N$ with dissociation constants $K_1, K_2, \ldots, K_N$ and concentrations $C_1, C_2, \ldots, C_N$. Each of these groups will show, at a given pH of the solution, different degrees of ionization: $\beta_1, \beta_2, \ldots, \beta_N$. Therefore, the overall polyelectrolytic degree of ionization, α, can be written as:

$$\alpha = \sum_{i=1}^{N} X_i \beta_i \tag{10}$$

where X_i denotes the fractional abundance of species i, defined as:

$$X_i = \frac{C_i}{\sum_{j=1}^{N} C_j} \quad \left(\sum_{j=1}^{N} C_j = C_{\mathrm{p}}\right) \text{ for } i = 1, \ldots, N \tag{11}$$

For the ionization of each functional group we can write a Henderson–Hasselbalch equation:

$$\mathrm{p}K_i(\beta_i) = \mathrm{pH} + \log\left[\frac{1-\beta_i}{\beta_i}\right] \text{ for } i = 1, \ldots, N \tag{12}$$

defining:

$$\Delta \mathrm{p}K_i = \mathrm{p}K_1 - \mathrm{p}K_i = \log\left[\frac{\beta_i(1-\beta_1)}{\beta_1(1-\beta_i)}\right] \text{ for } i = 2, \ldots, N \tag{13}$$

and:

$$q_i = 10^{\Delta pK_i} = \frac{\beta_i(1-\beta_1)}{\beta_1(1-\beta_i)} \text{ for } i = 2, \ldots, N \tag{14}$$

from which it is possible to obtain each β_{i} as a function of β_1:

$$\beta_i = \frac{q_i \beta_1}{1 + t_i \beta_1} \text{ for } i = 2, \ldots, N \tag{15}$$

where:

$$t_i = q_i - 1 \text{ for } i = 2, \ldots, N \tag{16}$$

Eqs. (10)–(16) provide a complete set of N independent equations for the unknown ionization degrees, β_i. With the above definitions, the pH of the solution can be written as:

$$\mathrm{pH}(\alpha) = \mathrm{p}K_i + \log\left[\frac{\beta_i}{1\text{-}\beta_i}\right] \text{ for any } i = 1,\ldots, N \tag{17}$$

and the intrinsic $\mathrm{p}K^0(\alpha)$, associated with the removal of a proton from the mixture of different functional groups, takes the form:

$$\mathrm{p}K^0(\alpha) = \mathrm{p}K_i^0 + \log\left[\frac{\beta_i}{(1-\beta_i)}\frac{(1-\alpha)}{\alpha}\right] \text{ for any } i = 1,\ldots, N \tag{18}$$

Eq. (9) from Paoletti et al. [8] is a particular case of the extension presented here, obtained by just setting $N = 2$. Therefore, in order to analyze HA potentiometric titration data (and remembering that its charge density is smaller than critical), we use Eq. (7) for the theoretical calculation of $\mathrm{p}K_a(\alpha)$, with $\Delta\mathrm{p}K_a(\alpha)$ given by Eq. (9) and $\mathrm{p}K^0(\alpha)$ by Eq. (18).

3. Experimental

3.1. Materials

The humic acid used was a commercial sample from Fluka (lot/product number 19582067/53680). To prepare a stock solution, 10 g of the HA was added to 1 l of water. By the addition of aliquots of a NaOH solution (approx. 3 mol l^{-1}), a constant pH of 9 was reached. After shaking the mixture for 24 h, the pH was slowly reduced to pH 3 by adding a $\mathrm{HNO_3}$ solution (approx. 3 mol l^{-1}) under continuous stirring of the mixture. After leaving the mixture standing for 24 h, the precipitate was centrifuged at 5000 rev.min^{-1} for approxmately an hour. The pH of the decanted supernatant was brought to pH 7, to prevent further coagulation. The solution obtained was dialyzed against Millipore water, using a Spectrapore tubing with a molecular mass cut-off of 3500. Dialysis was continued until the conductivity of the refreshed dialyzate remained constant at approximately 7 μS cm^{-1}. The dialyzed solution was subsequently treated with an ion exchanger of the type AG 50W-X4, to transfer the material into the acid form. The final stock solution had a DOC content of 80×10^{-3} mol l^{-1} C. The pH and conductance of this solution was 2.87 and 590 μS cm^{-1}, respectively. The concentration of chargeable groups in the final stock solution was determined by conductometric titration and was found to be $16.2 \pm 0.3 \times 10^{-3}$ mol l^{-1}.

The polyacrylic acid (PAA) solution was obtained from Acros (lot/product number A010694201/18501-2500) and was used without further treatment. According to the manufacturers, the PAA concentration was 25% weight units. Stock solutions of approximately 0.1 mol l^{-1} (in monomers) were prepared by dilution with water. As for HA, the concentration of chargeable groups was determined by conductometric titration.

All stock solutions were stored in the dark at approximately 7°C until usage. Potassium nitrate of an analytical reagent grade was obtained from Merck. Titrisol potassium hydroxide solutions (Merck), at a concentration of 0.100 mol l^{-1}, were used as titrating solutions. Titrisol pH buffer solutions (Merck) were used for calibrating the potentiometer. Water was obtained from a Millipore reverse-osmosis system and was de-gassed with nitrogen before use.

3.2. Methods

Two different methods were employed to obtain appropriate experimental data: (1) potassium activity measurements of solutions of the polyacid in the potassium salt form at different total polyelectrolyte concentrations; and (2) potentiometric titrations of polyacid solutions in the presence of different concentrations of potassium nitrate (0, 1×10^{-3}, 10×10^{-3}, and 100×10^{-3} mol l^{-1}). In all cases, a digital mV meter from Radiometer (model PHM 95) was used.

The potassium activity measurements were carried out with a potassium ion selective electrode (K-ISE: 597-13-096) in combination with a double junction reference electrode (REF 251), both obtained from Radiometer. From calibration curves it was found that the K-ISE exhibited Nernstian behavior with a slope equal to 58 mV. The quality of the electrode was checked daily using standard potassium nitrate solutions at two different concentrations. Different total polyelectrolyte concentrations (in terms of chargeable groups) at constant degree of dissociation ($PAA/\alpha = 0.2$; $HA/\alpha = 0.5$), and thus at a constant K/C_p ratio, were obtained by appropriate dilutions of the stock solution and potassium hydroxide solution to a total volume of 30 ml. Note that in case of HA, the range of performance is limited to the relatively low concentration of chargeable groups of the stock solution as a consequence of the origin of the HA and the pre-treatment procedure. Activity measurements of the potassium nitrate solutions without the polyelectrolyte were performed at equal ionic concentrations as the polymeric solution (the so-called iso-ionic condition). The potential was determined after a stabilization period of 15 min.

For the measurement of pH, a combined glass/calomel electrode was used (GK2401C, obtained from Radiometer). The electrode system was calibrated daily using two buffers (pH 4 and 7) and controlled with a standard solution of pH equal to 6.88. The potentiometric titrations were performed with potassium hydroxide charging the poly(acrylic acid) and humic acid at a fixed concentration of 2.5×10^{-3} mol l^{-1} of chargeable groups. The pH readings were carried out after 6 min of stabilization.

All experiments were performed in duplicate in a glass vessel thermostated at 25°C (±0.3°C) under a nitrogen atmosphere to avoid introduction of CO_2 in the solution.

3.3. Data treatment

The mean (relative) activity coefficient, γ_{+P}, was calculated on the basis of the difference of the potentials of the counterion/polymer solution, E_{pol}, and of the simple salt solution, E_{salt}, at equal ionic concentrations, by:

$$\ln\gamma_{+P} = \frac{F}{2RT}(E_{pol} - E_{salt}) \tag{19}$$

obtained from the corresponding Nernst equations [20]. Here F is the Faraday constant.

The apparent dissociation constant, $pK_a(\alpha)$ is obtained, as usual, from the experimental measurements of pH and the Henderson–Hasselbalch equation:

$$pK_a(\alpha) = pH + \log\left[\frac{1-\alpha}{\alpha}\right] \tag{20}$$

where for α we take the experimental value of the degree of dissociation, given by:

$$\alpha = \frac{[KOH]_a + [H^+] + [OH^-]}{C_p} \tag{21}$$

$[KOH]_a$ is the added concentration of potassium hydroxide.

4. Results and discussion

4.1. Determination of polyelectrolytic charge density

In order to determine the polyelectrolytic charge density of the studied polymers, measurements of the mean activity coefficient of K^+/polyelectrolyte systems at different polymer concentrations were performed under salt-free conditions. In Fig. 1, experimental values of $\ln(\gamma_{+P})$ are plotted as a function of $\ln(\alpha C_p)$ for the K^+/PAA ($\alpha = 0.2$) and K^+/HA ($\alpha = 0.5$) systems. The data points show a linear dependence with a negative slope in agreement with the theoretically predicted polyelectrolyte behavior. From the linear fitting we obtain a value of the slope equal to -0.282 ± 0.016 for the K^+/PAA system. Under these experimental conditions the charge density is certainly below the critical value for monovalent counterions. Hence, Eq. (6a) should be used, resulting in $\xi_{PAA} = 2.82 \pm 0.16$, which is in an excellent agreement with the usually accepted value of $\xi = 2.85$ [21].

For HA, a linear decrease of $\ln(\gamma_{+P})$ with increasing values of $\ln(\alpha C_p)$ was also observed (see Fig. 1). The slope for this case is -0.200 ± 0.014. This analysis indicates that under the present experimental conditions, at least in the local

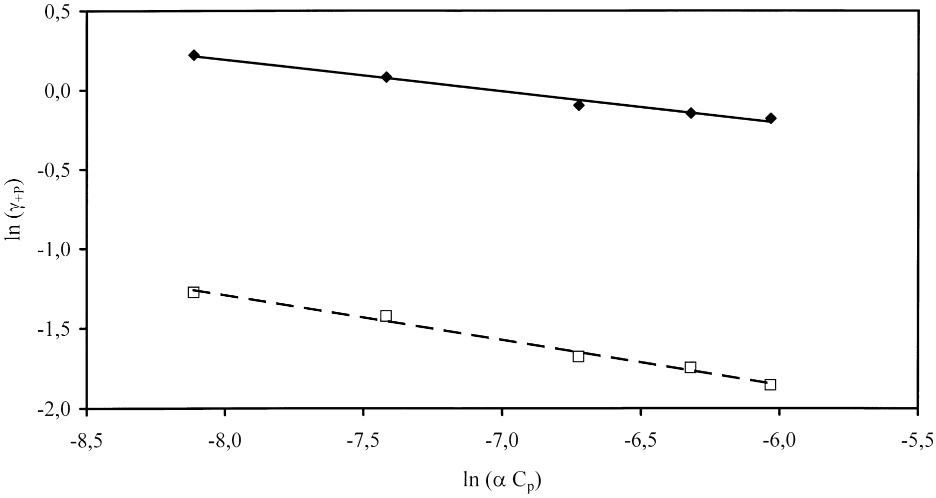

Fig. 1. Experimental data for $\ln(\gamma_{+P})$ against $\ln(\alpha C_p)$ for the K^+/PAA (□) and the K^+/HA (◆) systems under salt-free conditions. $\alpha = 0.2$ for PAA and $\alpha = 0.5$ for HA. The lines correspond to linear least square fits to the data.

conformation, the HA behaves as a linear polyelectrolyte. On the basis of conductometric experiments, Van den Hoop et al. [22] have shown that there is no association of monovalent counterions with the HA under these conditions ($\alpha = 0.5$), indicating that the charge density of the HA is below its critical value. Hence, analysis through Eq. (6a) is performed, resulting in a calculated value of the HA charge density of $\xi_{HA} = 0.80 \pm 0.05$.

4.2. Analysis of acid–base properties

The pH measurements corresponding to the potentiometric titrations of HA, at a concentration of 2.5×10^{-3} mol l^{-1} of chargeable groups at four different concentrations of KNO_3 (0, 1×10^{-3}, 10×10^{-3}, and 100×10^{-3} mol l^{-1}), are reported in Fig. 2. The four sets of data show similar features and a general agreement with previously reported data for other types of humic material [12–14,23].

In Fig. 3, a Henderson–Hasselbalch plot is shown for PAA and HA under salt-free conditions. For PAA a linear decrease in pH is observed, whereas the decrease in pH for HA is non-linear. This suggests that the HA consists of more than one functional ionizable group [24,25], which is in line with the previous findings of other authors [10–13].

In Fig. 4a, pK_a values are presented corresponding to the potentiometric titration of HA in water without additional salt [curve (d)]. A remarkable feature is the very large change in pK_a over the range of α values presented ($\Delta pK_a \approx 5.2$ pK units). It was noted that a poly (monoprotic acid) of equal charge density ($\xi = 0.8$) typically shows a total change in pK_a of approximately 1 pK unit in the same range of ionization. The theoretical calculation for this latter case is also represented in the same figure [curve (a)] for comparison. The other notable feature is the appearance of three 'S' shaped regions at low, medium and high α values. These 'S' shaped regions have been traditionally interpreted as being indicative of polymeric conformational transitions upon ionization [26]. No such evidence has so far been reported for humic acids. Moreover, there are two additional indications that no conformational transition takes place. The first one derives from the shape of the pK_a vs. α curve, which does show inflection points, but a monotonously increasing mode, which is at variance with what was found for, e.g. poly (glutamic acid) [26]. The later evidence is a very large pK_a change, which is rather unusual in such instances.

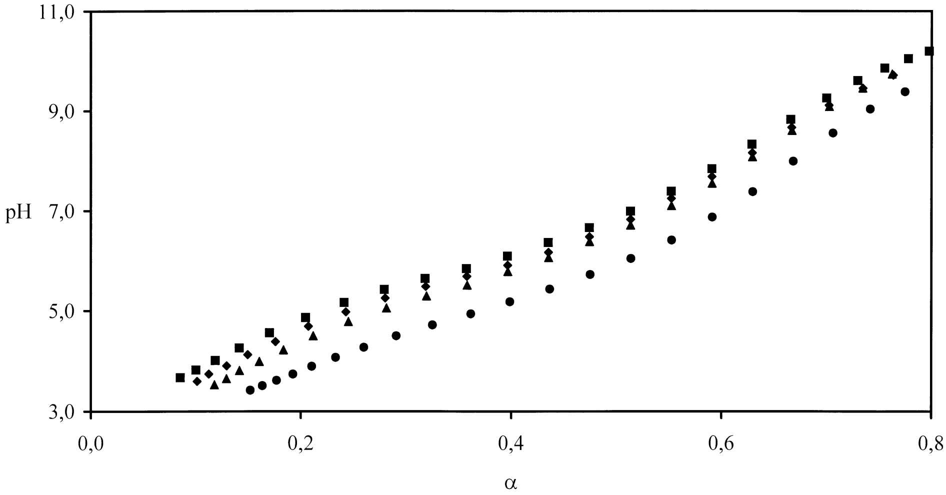

Fig. 2. Acid–base potentiometric titrations of HA at four concentrations of KNO_3: 0 (■), 1×10^{-3} (◆), 10×10^{-3} (▲) and 100×10^{-3} (●) mol l^{-1}. $C_p = 2.5 \times 10^{-3}$ mol l^{-1}.

Therefore, following the results of previous experiments and data analysis [10–13], we have applied the theory described above with the assumption that the HA is made up of more than two functional groups.

To determine the number of different functional groups, the data analysis started by assuming two different functional groups [see curve (b) in Fig. 4a]. The quality of the agreement between the experimentally determined and theoretically calculated pK_a values is expressed in terms of the sum of the square of the differences, and appears to be 19.56 in the case of two different functional groups. This number was subsequently increased to three and four functional groups for which the sum of the square of the differences were found to be 0.43 and 0.14, respectively [see curves (c) and (d), respectively]. No significant improvement

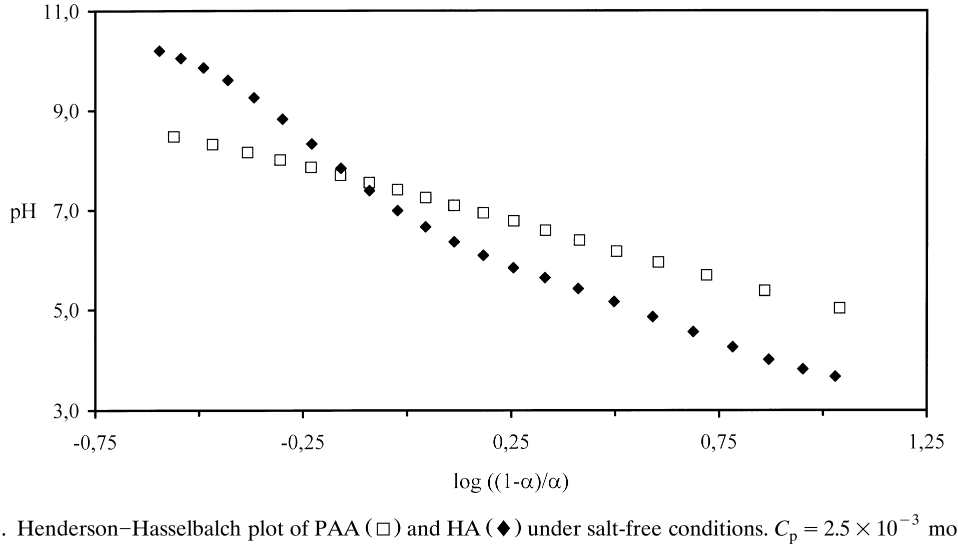

Fig. 3. Henderson–Hasselbalch plot of PAA (□) and HA (◆) under salt-free conditions. $C_p = 2.5 \times 10^{-3}$ mol l^{-1}.

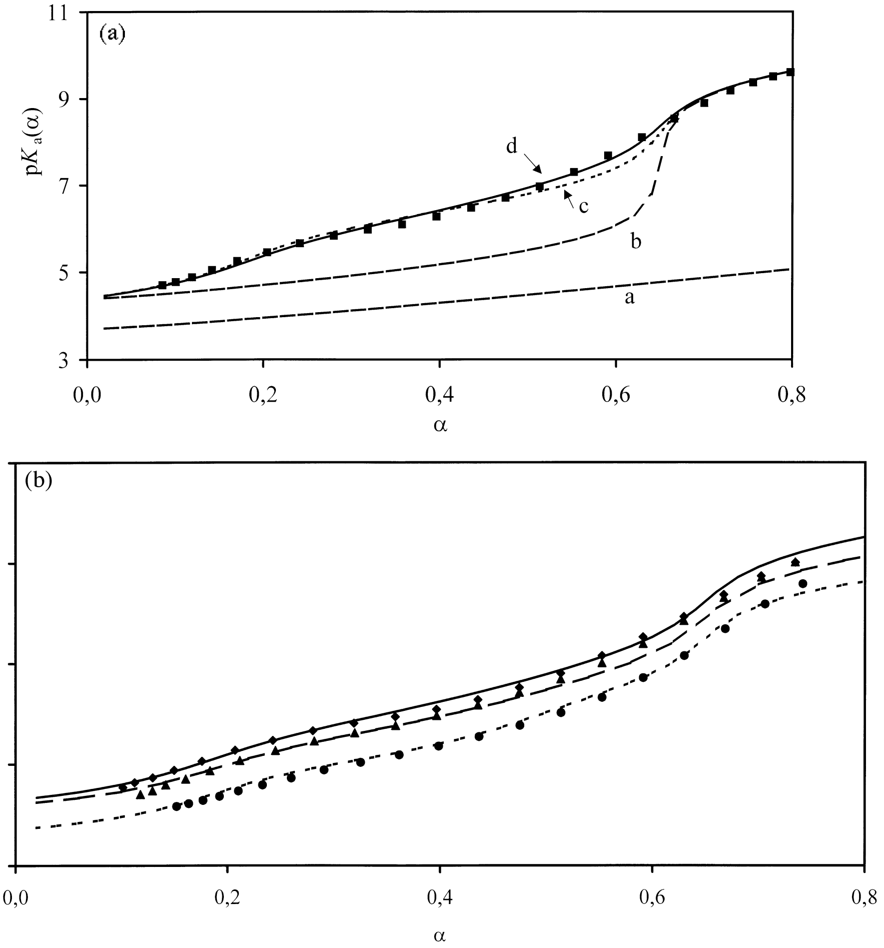

Fig. 4. (a) Experimental (■) and theoretical (curves a, b, c and d) pK_a vs. α for HA under salt-free conditions. Curves: model calculation for a monoprotic polyelectrolyte (a); for two functional groups (b); for three functional groups (c); for four functional groups (d). $C_p = 2.5 \times 10^{-3}$ mol l^{-1} and $\xi = 0.8$ in all cases. Further explanation in the text. (b) Experimental (symbols as in Fig. 2) and theoretical (lines) pK_a vs. α for the K^+/HA system at three KNO_3 concentrations. Lines represent the theoretical simulation using $\xi = 0.8$ and parameters of Table 1. $C_p = 2.5 \times 10^{-3}$ mol l^{-1}.

Table 1
Values of intrinsic pK_i^0 and fractional abundances X_i of functional groups determined from acid–base potentiometric titrations of HA at different ionic strengths[a]

[KNO_3] 10^{-3} mol l^{-1}	pK_1^0	X_1	pK_2^0	X_2	pK_3^0	X_3	pK_4^0	X_4
0	3.7	0.19	5.4	0.29	6.4	0.17	9.0	0.35
1	3.6	0.19	5.3	0.29	6.4	0.17	9.0	0.35
10	3.5	0.19	5.2	0.29	6.4	0.17	9.0	0.35
100	3.0	0.19	4.8	0.29	6.4	0.17	9.0	0.35

[a] $C_p = 2.5 \times 10^{-3}$ mol l^{-1}; $\xi = 0.8$

in the fitting process was reached by assuming an additional fifth functional group (the sum of the square of the differences $= 0.11$). Following this procedure, four main functional groups have been consistently determined with all main features of the titration data correctly reproduced. The corresponding fractional abundances X_i and intrinsic pK_i^0 of these groups are reported in the first row of Table 1. It is important to note that in this process we kept the number of functional groups as small as possible, i.e. our analysis indicates that four functional groups is the minimum number of such groups that are compatible with our data. In this thermodynamic approach, one should keep in mind that other functional groups may be present, but at much smaller fractional abundances and complying with the condition $\sum_{i=1}^{N} X_i = 1$.

Ionic strength effects on acid–base properties of HA were studied by adding a simple 1:1 salt (KNO_3) to the titrated solutions. The results (pK_a vs. α) at three different concentrations of KNO_3 (1×10^{-3}, 10×10^{-3} and 100×10^{-3} mol l^{-1}), are shown in Fig. 4b. In principle, all of the curves present patterns similar to the titration data under salt-free conditions. The salt effect is apparent in two features of the potentiometric titration data. The first is a systematic decrease of pK_a for increasing ionic strengths at corresponding degrees of dissociation. It reflects the fact that the more effective electrostatic shielding of the charged species at higher salt concentrations decreases the free energy of ionic origin. The polyelectrolyte theory properly takes into account this screening effect of the fixed charges (which, in general, tend to compensate for the effect of the increasing charge density upon titration) through the ionic strength dependence of ΔpK_a in Eq. (7).

The other effect is the small lowering of the initial pK of the titration curves, when the ionic strength is increased. This lowering of less than 1 unit of pK was pointed out previously by other authors for different types of polyelectrolytes [26,27]. This has been attributed to a lowering of the intrinsic free energy of ionization (pK^0) when the simple salt concentration is increased.

Through an analysis similar to the one performed for the salt-free condition, the intrinsic pK_i^0 and the fractional abundances of the four functional groups of the HA are obtained and have been also reported in Table 1. We note that the fractional abundances remain constant, independent of the ionic strength, indicating that the HA constitution does not depend on the concentration of excess salts as might be expected [14]. The pK_i^0 values of the first two groups show a small decrease with increasing ionic strength, fully in line with the discussion above.

5. Conclusions

An extension of the Counterion Condensation Theory of linear polyelectrolytes was successfully developed and applied to the analysis of potentiometric titration data obtained for the K^+/HA system in water solution under salt-free conditions as well as in the presence of supporting simple 1:1 salt. Four main functional groups have been consistently identified for the HA sample used, with their intrinsic pK_i^0 values and fractional abundances determined, both in a water solution and at three different concentrations of KNO_3, covering a salt concentration range of two orders of magnitude. The pK_i^0 values obtained from the fitting procedure cover a range of approximately 6 units, from 3.0 to 9.0. These values are reasonable for humic acids, encompassing values from the most common ones of carboxylic groups to those of (substituted) phenolic-type acidic functionalities. However, further structural work is necessary to reach a sound conclusion on their chemical identity. The polyelectrolytic approach also allows for the thermodynamic determination of the HA charge density, a parameter that is essential for a proper description of the experimental data based on first principles.

This consistent picture reinforces our assumption that HA can be considered, at least in the local conformation, as a linear polyelectrolyte. The good results obtained from the present modeling encourages the analysis of further experimental work aimed at the study of structural and environmental aspects of humic acids. For instance, recent theoretical work on the binding of divalent counterions to linear polyelectrolytes [6,19] can be used to analyze the interactions of heavy metals with humic material. The proposed method for the determination of pK^0 and the fractional abundance of poly (multifunctional acids) [8] has proven to be effective and reliable. Moreover, also the CC theory of linear polyelectrolytes has once more been demonstrated to be a very handy and efficient tool which lends itself to simple modifications able to answer practical needs of the experimental biophysical chemist.

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