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Acid-Base Properties of Humic Acids Isolated from Different Sources

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POTENTIOMETRIC titration was used for obtaining detailed information on the acid-base properties of humic acids. The studied humic acids were isolated from different four sources, *i.e*., alluvial soil (HAs), farmyard manure (HAf), compost (HAc) and poudrette (HAp). The isolated humic acids were purified and performed. Back titration using 0.1M HCl as the titrant and direct titration using 0.1M NaOH as the titrant of humic acid solutions (2 mg/ml) at the ionic strength of 0.1 were executed. Moreover, total acidity and functional groups of humic acids were estimated.

The studied humic acids were similar in their acid-base properties: i) the data of direct and back titration curves which limited the position and composition of dissociation or ionization of functional groups of the studied humic acids were more similar, ii) total functional groups of HA determined from back and direct potentiometric titration was ranged between 5.40-6.54 and 4.20-5.00 mmolc/g HA, respectively, iii) total functional groups of humic acids determined using potentiometric titration were varied from those determined using normal titration methods, where total functional groups at normal titration methods ranged between 11.32 and 19.58 mmolc/g HA and iv) humic acid extracted from poudrette has the highest value of total acidity followed by compost, farmyard manure, while the soil came late.

Keywords: Potentiometric titration, Acid-base properties, Humic acids, Functional groups

Numerous publications are concerned with the study of acid-base equilibrium in solution of humic acids (Aleixo *et al*., 1992, Masini, 1994 and Fukushima *et al*., 1995). However, until now, there is no unified approach to the interpretation of obtained data, which can be explained by several reasons.

Perdue *et al*. (1984) showed that, the humic substances (HS) are amorphous, polydisperse colloids. It is only possible to determine an average molecular weight, which depends on the measurement method applied. HS show a strong cation exchange capacity, generally from 5 to 12 mmolc/g, which is due to the presence of functional groups such as carboxylic and phenolic acids. Because of the huge variety and number of acidic groups, HS acid-base properties cannot be described by discrete values of pK_a.

First, humus acids contain various carboxyl, phenolic, alcohol, amino and other functional groups heterogeneous in their properties: groups of the same chemical nature occupying different positions in a molecule can differ in acidity (Stevenson, 1994 and Orlov, 1990). Titration curves generally have no pronounced inflections typical for individual acids, which complicates the exact determination of equivalence points and titration end points.

Second, humus acids belong to polyelectrolytes, they have important pH dependent negative charges due to different ionized groups. The presence of these charges results in inter- and intermolecular electrostatic interactions between the functional groups of humus acids, which affect their dissociation constants.

Third, the intensity of electrostatic interactions depends on the molecular weight of the macromolecule (Tipping *et al*., 1990 and Masini, 1993). Humic acids are polydispersed substances; therefore, a nonfractionated preparation contains an important set of fractions differing in molecular weight, which complicates calculation.

Tipping *et al*. (1990) showed that, if all functional groups of HA of the same nature in a macromolecule are similar in affinity to hydrogen ions, the proton dissociation can be described by means of (a) the intrinsic pK_a (pK_0) that depends only on the chemical nature of the groups (this is the dissociation constant, in the absence of electrostatic interactions, when the molecule is in the hypothetical uncharged state) and (b) the apparent pK_a (pK_{app}) that depends on the electrostatic interactions due to the negative charge accumulated on the macromolecule surface. In the absence of electrostatic interactions, as is typical for electrolytes with low accumulated charge, the pK_{app} value is constant and close to pK_0 .For significant accumulated charge, when electrostatic effects take place, pK_{app} increases and can vary by more than 2 pK units. Also, Zavarzina and Demin (1999) showed that, the potentiometric titration was used for obtaining detailed information on the acid-base properties of HAs where they found that HA preparations isolated from different sources were found to be similar in acid-base properties.

This investigation was carried out to study the relation of humic acids origins to their acid-base properties, the general shapes of their potentiometric titration curves, the content of functional groups and their elemental analysis.

Material and Methods

The used humic acids in this study were isolated from four sources, *i.e*., HAs from surface layer (0-20 cm) of alluvial soil at Experimental Farm, Faculty of Agriculture, Minufiya University; HAf from farmyard manure which was taken from Animal Farm, Faculty of Agriculture, Minufiya University; HAc from compost produced from a mixture of maize stalks and cow faeces and HAp from poudrette of Elgabal Elasfar station, Cairo Governorate.

Isolation and purification of humic acids was performed as described by Posner (1966). Elementary composition or the content of C, N, H and O % were determined. Carbon (C) was estimated by Walkley and Black method (Page *et al*., 1982). Nitrogen (N) was determined by using micro-steam Kjeldahl distillation apparatus under alkaline conditions (Page *et al*., 1982). Determination of Hydrogen (H) was executed according to Mann and Sounders (1960). Oxygen (O) content was calculated by subtracting the content % of C, N and H from the total of 100. Ash content of humic acids was estimated by burning the oven-dry humic acid at 750 ºC for 24 hr (Holder and Griffith, 1983).

Total acidity and functional groups as mmolc/g acid (COOH, total-OH, phenolic-OH and alcoholic-OH) were carried out according to Dragunova (1958). Optical density (E4/E6) which meaning the ratio of optical densities at 465 and 665 nm, was determined by dissolving 1.0 mg of each HA in 5 ml of sodium bicarbonate ($NaHCO_3$) and the pH was adjusted to 8.3 with NaOH. The absorbance of the prepared humic acids solutions at 465 and 665nm was measured spectrophotometrically (Rivero *et al*., 1998).

Potentiometric titration

Potentiometric titration was used for obtaining detailed information on the acid–base properties of the humic acids of the running study, *i.e*., HAs, HAf, HAc and HAp. Titration conditions or potentiometric titration of HA was conducted at 25 ± 0.1 ºC. The initial solution volume of HA was 25 ml. The ionic strength was kept constant (0.1 mole/l) by means of 0.1 M KCl. Both 0.1M HCl and 0.1M NaOH was used as the titrant. The humic acid concentration was selected experimentally, it was chosen equals to 2 mg/ml, because this concentration corresponded to well-pronounced inflexion points on the titration curve and the sample dilution at the second equivalence point did not exceed 10% (Zavarzina and Demin, 1999).

Titration procedure

Humic acid (50 mg) was dissolved in 20 ml of 0.05 M NaOH (pH about 12). The mixture was left overnight in a covered plastic beaker to complete the dissolution. About 1-1.5 h before titration, the HA solution and the titrant solutions in covered beakers were placed into a thermostat (T = 25ºC). Immediately before titration, the HA solution was transferred into a glass beaker of 50 ml and 2.5 ml of 1M KCl were added using an automated dosimeter, as well as about 1 ml of 0.5M HCl in order to bring the pH to 11.5. The solution volume was brought to 25 ml with distilled water. The back titration (from pH 11.5 to pH 2.5) using 0.1M HCl as the titrant was performed first and was immediately followed by the direct titration (from pH 2.5 to pH11.5) using 0.1M NaOH as the titrant. The titrant was added by 0.1 ml aliquot portions. Instrument readings were taken when the pH value remained stable for 1 min. At the back titration in the pH range from 8.5-9.0 to 6.0 from 10 to 40 min were required to establish equilibrium after the addition of each titrant portion. Therefore, the back and direct potentiometric titration of each preparation took about 7 h, as has been described by Zavarzina and Demin (1999). Determination of the initial and final titration points and the equivalence points has been described by Takamatsu and Yoshida (1978). Also, determination of the content of functional groups titrated has been described by Takamatsu and Yoshida (1978) and Zavarzina & Demin (1999).

All analyses of humic acids were carried out in triplicate, and these data were statistically analyzed according to the method described by Snedecor and Cochran (1989). The least significant difference (LSD) range test was used to compare different categories means.

Results and Discussion

Elementary composition of humic acids

The elemental composition, atomic ratio, degree of internal oxidation and ash content of humic acids are shown in Table 1. Data show that carbon and oxygen are the predominant elements in the studied humic acids. The carbon content of the examined humic acids ranges from 46.40 to 52.55%, lower than that of Aldrich HA (55.17%). There were high significant differences among the studied humic acids in their content of C, with the highest values (52.25%) recorded to HAc and lowest C content (46.40%) appeared in HAp. The hydrogen content of the studied humic acids ranges from 4.30 to 5.40%, higher than that of Aldrich HA (4.29%). Humic acid isolated from poudrette achieved the highest H content (5.40%) with significant difference between Hap and other humic acids and no significant differences between the other tested humic acids (HAs, HAf and HAc) in their contents of hydrogen. As can be seen in Table 1, the humic acids were characterized by a varied content of nitrogen ranging from 2.70 to 3.76%, much higher than 1.71% of Aldrich HA. There were high significant differences among the studied humic acids in their content of N. On the other hand, oxygen content (%) in the isolated humic acids ranged between 41.70 and 44.44%, higher than that of Aldrich HA (36.26%). The tendency of lower C and higher H, N and O contents of the studied humic acids in comparison with Aldrich HA has been reported by other works (Schnitzer *et al*., 1988 and Stevenson, 1994). Also, these results may be attributed to the chemical composition of humic acids sources and humification degree of the humic substances in these sources (Sokołowska *et al*., 2012).

Changes in elemental composition are accompanied by changes of atomic ratios (H/C and C/N) as shown in Table 1. On the basis of C/H ratio one can make an approximate estimation of the degree of aromatization of humus substances. The value of C/H atomic ratio is inversely proportional to the aromaticity of humus compounds (Kononova, 1966). Humic acid extracted from soil (HAs), farmyard manure (HAf) and from compost (HAc) had different values of C/H ratio. Taking the value of C/H ratio as the basis, we can arrange the acids extracted from different sources in accordance with increasing share of aromatic structures (HAs > HAc > HAf > HAp). The values of C/H ratio of the studied humic acids were ranged between 8.59 and 11.93, lower than the value

(12.86) of Aldrich HA. Also, this result suggests that the isolated humic acids have more aromatic and less aliphatic character than Aldrich HA.

TABLE 1. Elementary composition, atomic ratio, internal oxidation degree (ω) and ash content (%) of the studied humic acids and Aldrich HA .

Humic acids	Elementary content (%)				Atomic ratio		ω	Ash content (%)
	C	H	N	O	C/H	C/N		
HAs	51.30 b	4.30 b	2.70 d	41.70 c	11.93	19.00	1.70	1.80 a
HAf	50.75 c	4.35 b	2.95 c	41.95 b	11.67	19.20	1.74	1.65 ab
HAc	52.55 a	4.42 b	3.28 b	42.11 b	11.89	16.02	1.71	1.57 bc
HAp	46.40 d	5.40 a	3.76 a	44.44 a	8.59	12.34	2.04	1.40 c
LSD (0.05)	0.55	0.13	0.09	0.19	-	-	-	0.18
Aldrich HA	55.17	4.29	1.71	36.26	12.86	32.26	1.41	4.53

Notes. Means in the same category followed by different letters are significantly different at the 0.05 level of probability. HAs = soil humic acid, HAf = farmyard manure humic acid, HAc = compost humic acid and HAp = poudrette humic acid.

The C/N ratio was more diversified. The values of that ratio fell within the range from about 12.34 to 19.20 (Table 1). The widest C/N values were found in humic acids extracted from the farmyard manure (HAf) and the narrowest one was from poudrette (HAp). The lowest C/N ratio of HA isolated from poudrette indicates that it was the highest mature compared to the other humic acids (Sokołowska *et al*., 2012).

On the basis of the elemental composition, the values of the degree of internal oxidation (ω) according to Sokołowska *et al*. (2012) were calculated from the equation:

$\omega = [(2O + 3N) - H]/C$

The degree of internal oxidation (ω) is a good indicator for the quality of humus compounds. The value of ω of the tested humic acids extracted from different sources was within the range from 1.70 to 2.04 (Table 1), which indicates to the variation of the degree of internal oxidation. The lowest value of ω was found for the humic acids extracted from soil (HAs) and the highest one was from poudrette (HAp). It is supposed that the degradation process involves the loss of CH_3 groups and partial oxidation, thus decreasing the number and length of aliphatic chains in the humic substance. High positive values of ω indicate a high degree of internal oxidation (Sokołowska *et al*., 2012).

Also, data in Table 1 reveal that the humic acid isolated from alluvial soil (HAs) had an ash content (%) greater than that extracted from the other sources. Generally the ash content of the four studied humic acids was low. Such low values may be due to their treatment with Khan's mixture (Schnitzer and Riffaldi, 1972) which removed inorganic impurities.

Total acidity and functional groups of humic acids

Total acidity (sum of carboxyl and phenolic hydroxyl) explains an extremely important properties of humic acids namely, their participation to exchange reactions (Kononova, 1966). Data in Table 2 show that, the highest value of total acidity was that of humic acid extracted from poudrette (HAp), where the lowest value was found in the humic acid isolated from farmyard manure (HAf). Thus according to the content of total acidity, the examined HA take the following order: HAp > HAc > HAs > HAf. Total acidity values of HAp and HAc acids are higher than that of Aldrich HA (7.97mmolc/g), while the other two (HAs and HAf) were less. The data of Table 1 and 2 appeared that, the total acidity of all the humic acids increases with increasing oxygen content. Similar results were found by Yanagi *et al*. (2003) and Sokołowska *et al*. (2012).

Data in Table 2 denote that, the COOH groups of humic acids isolated from different studied sources were the predominant functional group as compared with the other functional groups. The highest content (7.36 mmolc/g) of COOH groups was that of humic acid extracted from poudrette (HAp) while the lowest content (4.02 mmolc/g) was that of HAf isolated from farmyard manure. There are highly significant differences between different studied humic acids in their content of COOH groups, wherever two humic acids (HAp and HAc) were higher and the other two were lower than that (4.86 mmolc/g) of Aldrich HA. These characteristics are related to the original composition of the residues and the nature of humification processes (Stevenson, 1994). The phenolic-OH group contents of the studied humic acids appeared with highest significant values (5.14 mmolc/g) for HAp isolated from poudrette and lowest value (3.18 mmolc/g) of HAf extracted from farmyard manure. The same trend holds true for the content of alcoholic-OH groups. The humic acid isolated from farmyard manure (HAf) had the lowest content (0.47 mmolc/g) of alcoholic-OH compared with the other humic acids. Aldrich HA recorded 3.11 and 0.37 mmolc/g of phenolic and alcoholic-OH groups respectively, which were lower that of any studied humic acids. These results may be attributed to more oxidation processes for the different organic residues under the conditions of poudrette. The data in Table 2 elucidate that the content of total-OH groups is markedly higher in the humic acid extracted from poudrette (HAp) followed by that one of humic acid isolated from compost (HAc), from alluvial soil (HAs) and finally that of humic acid extracted from farmyard manure (HAf). Generally, the differences in the content of total-OH groups could be related to the chemical composition of the organic residues as well as the effect of humification processes (Stevenson, 1994, Rivero *et al*., 1998 and Sokołowska *et al*., 2012).

TABLE 2. Total acidity, functional groups and optical density (E_4/E_6) of the studied humic acids and Aldrich HA .

Humic acids	**Total acidity mmolc/g HA**	**Functional groups mmolc/g HA**				**E_4/E_6**
		COOH	**Phenolic - OH**	**Alcoholic- OH**	**Total-OH**	
HAs	7.50 c	4.24 d	3.26 c	0.52 c	3.78 c	2.14 d
HAf	7.20 c	4.02 c	3.18 c	0.47 c	3.65 c	2.30 c
HAc	9.11 b	5.36 b	3.75 b	0.68 b	4.43 b	2.41 b
HAp	12.50 a	7.36 a	5.14 a	0.97 a	6.11 a	2.55 a
LSD (0.05)	0.44	0.14	0.21	0.05	0.23	0.04
Aldrich HA	7.97	4.86	3.11	0.37	3.48	7.20

Notes. Means in the same category followed by different letters are significantly different at the 0.05 level of probability. HAs = soil humic acid, HAf = farmyard manure humic acid, HAc = compost humic acid and HAp = poudrette humic acid.

The ratio of optical densities at 465 and 665 nm, known as E_4/E_6 is widely used for detecting the characterization of humic substances. It usually decreases with increasing molecular weight and condensation and is believed to serve as an index for degree of humification (Schnitzer and Riffaldi, 1972 and Stevenson, 1994). Data presented in Table 2 declare that the ratios of the optical densities of humic acids isolated from different studied sources, E_4/E_6 ratios ranged between 2.14 and 2.55, lower than that of Aldrich HA (7.2). According to the ratio of E_4/E_6, the tested humic acids taking the following descending order HAp > HAc > HAf > HAs. This order indicates that, the extracted humic acid from poudrette had a relatively lower molecular weight, more aggregated humic macromolecule and a greater aliphatic character. This trend may be ascribed to that the formation of humic acids is controlled by many factors of extreme complexity, such as dissolved oxygen and organic matter, temperature, light, inorganic constituents associated with organic matter, their origin,… *etc*. (Stevenson, 1994, Rivero *et al*., 1998 and Sokołowska *et al*., 2012).

Back and direct potentiometric titration curves

The obtained titration curves of the tested humic acids are illustrated in Fig 1A and 1B. The data represented by these figures were similar. The figures demonstrate that, at the same volume of NaOH or HCl the pH values of the direct titration were higher than the pH of back titration. The titration curves shown in Figure (1A and 1B) can be broken down into three zones (I, II and III). The first zone (I), *i.e*., the lowest of the acid region where COOH groups are known to dissociate, the third zone (III) probably represents dissociation of phenolic-OH groups and the second zone or the zone marked (II) is an intermediate area where ionization of weak COOH and very weak acid groups (Posner, 1964 and Zavarzina & Demin, 1999).

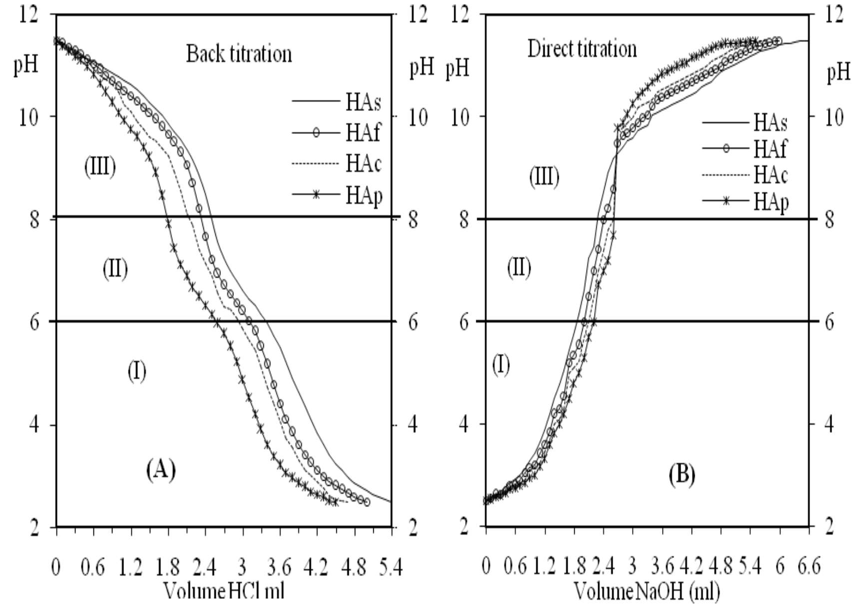

Fig. 1. Back (A) and direct (B) potentiometric titration curves of humic acids isolated from alluvial soil (HAs), farmyard manure (HAf), compost (HAc) and poudrette (HAp).

Data of the back potentiometric titration of the humic acids isolated from the alluvial soil (HAs), farmyard manure (HAf), compost (HAc) and poudrette (HAp) are given in Figure (1A). Data illustrate that, the volumes of HCl are required to change humic acid solutions from pH 11.5 to pH 2.5 were varied. The studied humic acids could be arranged according to the highest volumes of HCl required to convert humic acid solution pH from 11.5 to pH 2.5 in the following descending order: HAs (5.4ml) > HAf (5.0ml) > HAc (4.7ml) > HAp (4.5ml). The high values of HCl required in titration may refer to the excess of unionized functional groups. These results pointed out that, the content of unionized functional groups in HAs > HAf > HAc > HAp. The gradual decrease in pH with added acid is an evidence for the high buffering capacity of the studied humic acids especially that isolated from the alluvial soil. This conclusion is in accordance with the concept that the HA behave as weak acid polyelectrolytes. The wide range of pH illustrates the high buffering capacity of the HA solutions and the possibility of occurrence of configurational changes as electrostatic effects on high-molecular-weight polyacids by the decrease in pH. The potentiometric titration properties of HA isolated from different sources were deviate somewhat from those typical weak acid polyelectrolytes because of the heterogeneous nature of the high molecular weight acids. The difference in the volumes of HCl required for changing the pH of the humic acids solutions from 11.5-2.5 may be attributed to the variation in the acidic groups of the different isolated humic acids, and to the difference in the regular space appearance of molecules (Stevenson, 1994 and Zavarzina & Demin, 1999).

Also data in Fig. 1A reveal that, the volumes of HCl required to decrease the alluvial humic acid (HAs) solution pH from 11.5 to 8.0 (3.5 pH unit), from 8.0 to 6.0 (2 pH unit) and from 6 to 2.5 (3.5 unit) were 2.2, 1.2 and 2.0 ml, respectively. This results illustrate that, the least volume of HCl was that required to decrease the pH from 8.0 – 6.0. On the other hand, the slope of the titration curve in the second area (II) was of the highest value. While the slopes of the first (I) and third areas (III) were lower than that of obtained in the second one. The similar results were obtained for HAf, HAc and HAp. The rapid equilibrium during titration by HCl for humic acids may be due to the ionization of weak COOH and phenolic-OH groups at the pH range from 8.0 to 6.0 (Posner, 1964 and Stevenson, 1994).

Results of the direct potentiometric titration of humic acid isolated from the alluvial soil (HAs), farmyard manure (HAf), compost (HAc) and poudrette (HAp) are presented in Figure 1B. Data elucidate that, the tested humic acids were differed in volumes of NaOH required to change pH of humic acids solutions from 2.5 to 11.5, where the studied acids could be arranged according to these volumes of NaOH following order: HAs > HAf > HAc > HAp. These results refer to the highest content of functional groups of HAp which can be ionized when the pH values of the media increased from 2.5 to 11.5. The gradual rise in pH with added NaOH attests to the high buffering capacity of HA. Buffering effect is shown over a wide pH range because of electrostatic effects on high-molecular-weight polyacids and the likelihood of the configurational changes occurs with the increase in pH. Direct titration of humic acids would be expected to have a more “smeared out” appearance than those of monomeric acids. The volumes of NaOH which were required to increase the pH of isolated humic acids from 2.5 to 11.5 were 6.6, 6.0, 5.7 and 5.5ml for HAs, HAf, HAc and HAp respectively. These results mean that the acidic groups of humic acids were differed in their types and cannot be considered to be regularly spaced shape of the molecules (Stevenson, 1994 and Zavarzina & Demin 1999). Also, data in Fig. 1B reveals that, the least volume of NaOH was that required to increase the pH from 6.0 – 8.0. These results show existence of intense slope in pH values, due to ionization of both COOH and weak COOH groups (Posner, 1964 and Stevenson, 1994).

Regarding to the data illustrated in Fig. 1A and 1B it can be noticed that, the volumes of NaOH which were used in the direct titration are higher than the volumes of HCl which were used in the back titration. These results may be due to the strong acidity which was obtained previously in back titration. This may be also, due to the volumes of NaOH consumed through autoxidation of organic matter in the presence of even trace amounts of oxygen (Stevenson, 1994).

Differential curves of back and direct potentiometric titration

The differential curves of back and direct potentiometric titration of the selected humic acids were illustrated in Fig. 2. The figures show that, there are two apparent equivalence points (EPs) which can be calculated by the highest values of ΔpH/ΔV from Fig. (1A and 1B), distinguished at pH 4.90 and 8.01 for HAs, 4.78 and 7.23 for HAf, 5.65 and 9.25 for HAc and pH 4.54 and 7.93 for HAp on the differential curves of back titration (Fig. 2A). It can noticed that, the studied humic acids presents two broad peaks, the first corresponds to strong acid sites (carboxylic groups) and the second to weak acidic sites (phenolic groups). The examined humic acids declared also, a small or developing peaks at more acidic pH values (≤ 4), indicating, probably, a presence of stronger acidic sites. On the other side, only a single point was readily apparent on the direct titration curves at pH 8.11, 7.43, 7.00 and 6.73 for HAs, HAf, HAc and HAp, respectively (Fig. 2B). Moreover, as observed in Fig. 2 the greater widths of peaks indicating a large numbers of acid sites while the narrow widths refers to small numbers of acid sites. Also, the differences in height of the peaks reflect the concentration of each acidic site and therefore, their buffer capacity contribution. On this basis, the back titration curves can be divided into three intervals and the direct titration curves into only two intervals corresponding to the titration of specific groups with inherent dissociation constants. It can be suggested by analogy with proteins that, the predominant weak-acid carboxyl groups of different acidities dissociate at pH 3-7, extremely weak-acid phenol groups dissociate at pH higher than 9-10, and the intermediate pH range about 7-8.5 corresponds to the simultaneous titration of the both groups and amino groups. These results are in agreement with those obtained by Takamatsu and Yoshida (1978), Stevenson (1994) and Zavarzina & Demin (1999).

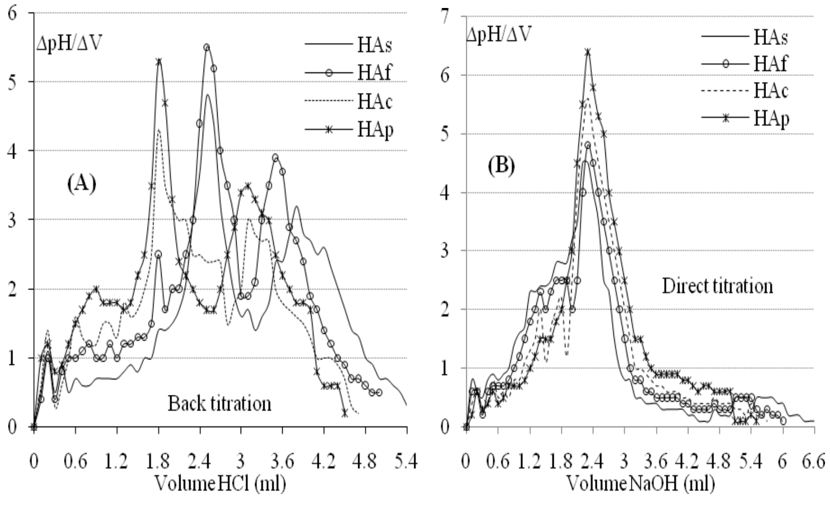

Fig. 2. Differential curves of back (A) and direct (B) potentiometric titration of humic acids isolated from alluvial soil (HAs), farmyard manure (HAf), compost (HAc) and poudrette (HAp).

Functional groups of humic acids

The content of functional groups of the tested humic acids isolated from the different sources as determined from the data of the potentiometric titration curves (back and direct titrations) as mmolc/g HA (Fig. 1) are given in Table 3. The data show the content of groups titrated within the allocated pH intervals. The total content of functional groups in the tested humic acids have a little variation where it ranged from 5.40 - 6.54 and from 4.20 - 5.00 mmolc /g HA for back and direct titrations, respectively. Also, the obtained data reveal that, the total content of functional groups determined from back titration curves was higher than that determined by direct titration curves. This trend was found with the four tested humic acids. These results may be attributed to the formation of inter-and intramolecular hydrogen bonds affecting the reactivity of functional groups (Zavarzina and Demin, 1999). According to the content of total functional groups (mmolc/g HA) determined at different pH intervals from both back and direct titration curves, the tested humic acids took the following descending order HAs > HAf > HAc > HAp. These results reveal to hysteresis probably due to the conformational changes in the polyelectrolyte molecules was high for HAs. The total content of acidic groups as determined by the potentiometric titration (Table 3) was lower than the corresponding values obtained by volumetric titration which were recorded in Table 2. This can be attributed to the fact that the determination was performed at higher pH values in the latter case. These results are in agreement with those obtained by Orlov (1990), Falzoni *et al*. (1998) and Zavarzina & Demin (1999).

TABLE 3. Functional groups of the tested humic acids as determined from the data of potentiometric titration as mmolc/g HA.

Tested HA*	**Back titration**				**Direct titration**		
	pH interval			**Σ**	**pH interval**		**Σ**
	3.5-5.5	**5.5-8.5**	**8.5-10.5**		**3.5-8.5**	**8.5-10.5**	
HAs	1.40	2.60	2.54	6.54	2.60	2.40	5.00
HAf	1.20	2.20	2.40	5.80	2.40	1.80	4.20
HAc	1.10	2.40	2.10	5.60	2.50	1.70	4.20
HAp	1.00	2.60	1.80	5.40	2.40	1.80	4.20

[*] Note: Σ denotes the total content of functional groups, it is supposed that the pH ranges of 3.5-5.5, 5.5-8.5 and 8.5-10.5 correspond to the titration of carboxyl, amino and phenol groups, respectively.

Conclusion

1. The data of direct and back titration curves which limited the position of dissociation or ionization of functional groups show that there are more similar in the composition of the studied humic acids.
2. Total functional groups of humic acids determined from back and direct potentiometric titration was ranged between 5.40-6.54 and 4.20-5.00 mmolc/g HA, respectively.
3. Total functional groups of humic acids determined using potentiometric titration was varied from that determined using normal titration methods.

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خواص الحموضة والقلوية لأحماض الهيوميك المستخلصة من مصادر مختلفة

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استخدمت طريقة المعايرة الرجعية أو التنشيومترية لإظهار وإعطاء معلومات أكثر وضوحاً عن خواص الحموضة والقلوية لأحماض الهيوميك. أحماض الهيوميك المدروسة تم إستخلاصها من أربع مصادر مختلفة وهى الأراضى الرسوبية (HAs)، السماد البلدى (HAf)، الكمبوست (HAc) و البودريت (HAp). وتم تنقية أحماض الهيوميك المستخلصة من هذه المصادر. أجريت المعايرة الرجعية لمحاليل أحماض الهيوميك المختلفة بإستخدام حامض الهيدروكلوريك 0,1 مولر كمحلول معايرة كما أجرى لها معايرة مباشرة بإستخدام هيدروكسيد الصوديوم 0,1 مولر كمحلول معايرة، حيث كان تركيز محاليل أحماض الهيوميك (2 ملليجرام/مل) وقوتها الأيونية مقدارها 0,1. علاوة على ذلك تم تقدير الحموضة الكلية والمجاميع الوظيفية لأحماض الهيوميك المختلفة.

أوضحت النتائج المتحصل عليها أن هناك تشابهاً فى خواص الحموضة والقلوية لأحماض الهيوميك المختلفة تحت الدراسة حيث: 1- أوضحت نتائج منحنيات كل من المعايرة الرجعية و المباشرة والمحددة لمواقع وتركيب المجموعات الفعالة التى يحدث لها تأين و تفكك أن هذه المجموعات متشابهه فى أحماض الهيوميك المدروسة، 2- أن المحتوى من المجاميع الفعالة والمقدرة من المعايرة الرجعية والمباشرة لأحماض الهيوميك تحت الدراسة تراوحت بين 5,40 إلى 6,54 و4,20 الى 5,00 ملليمول/جرام حامض هيوميك على الترتيب، 3- إختلف المحتوى المقدر من المجموعات الفعالة الكلية فى أحماض الهيوميك بإستخدام طريقة المعايرة التنشيومترية عن ذلك المحتوى المقدر بطريقة المعايرة العادية، حيث تراوحت المجموعات الفعالة بطريقة المعايرة العادية بين 11,32 و 19,58 ملليمول/جرام حامض هيوميك، 4- أعلى قيمة للحموضة الكلية سجلت لصالح حامض الهيوميك المستخلص من البودريت ثم الكمبوست ثم السماد البلدى بينما جاءت التربة فى المرتبة الأخيرة.