

STUDIES ON PHYSICO-CHEMICAL PROPERTIES OF NATURAL, SYNTHETIC AND MICROBIAL HUMIC ACIDS

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A comparative study has been made on the physico-chemical properties of naturally occurring humic acid with that prepared from synthetic processes (chemical and microbial), along with their nitro- and sulphonated products from IR spectroscopy, electrometric titration and E_4/E_6 values. A flexible polyelectrolytic nature with a three dimensional structure for humic acid is reported. Natural humic acid is considered to have more complex structure with condensed aromatic rings in comparison to other products.

INTRODUCTION

HUMIC SUBSTANCES, the naturally occurring "biopolymer", have a great ability to combine with metal ions, clay and a variety of organic agricultural chemicals due to the large number of functional groups present in the molecule. For the characterization of 'Humic Acids', infrared absorption spectroscopy (Adhikari & Chakraborti 1973 ; and Theng *et al.* 1966) and electrometric titrations (Adhikari & Hazra 1970; Chatterjee & Bose 1952; and Roy *et al.* 1973) have been widely used. The aromatic nature and the presence of major groups like $-\text{COOH}$, $-\text{C}=\text{O}$, $-\text{OH}$ (Phenolic), $-\text{NH}$, $-\text{COOR}$ etc., are indicated from IR spectroscopy while the electrometric and high frequency titrations show the polyelectrolytic behaviour and the presence of two types of acidic functional groups viz., carboxylic $-\text{COOH}$ and phenolic $-\text{OH}$. The E_4/E_6 ratio, the ratio of the optical densities of dilute aqueous solutions at 465 nm and 665 nm serves as an index of humification and the degree of condensation of aromatic rings in humic substances (Kononova 1966; and Schnitzer & Khan 1972).

In the present paper a comparative study from IR spectroscopy, electrometric titrations and E_4/E_6 ratios, has been made on humic acids from natural source with that from synthetically prepared by chemical and microbial processes as well as with those of their respective nitro- and sulphonated products. Since controversies exist regarding the process of formation of humus, the main source for its formation is Nature and the presence of nitrogen in the molecule whether it is a part of the structural unit or an accidental contaminant; different types of synthetic humic acids are prepared chemically and microbially and their properties are compared. The object of nitration and sulphonation is to detect any change in chemical behaviour and the structural modifications, if any, by these processes.

MATERIALS AND METHODS

Pond sediments, being an enriched source for humus in Nature, was used as a natural source for humus. Isolation of humus was done from pond sediments (0–15 cm depth, Bighra, Burdwan) by 0.5 (N) Na_2CO_3 solution and was fractionated and purified adopting usual selective solubility procedures (Adhikari & Hazra 1970). The synthetic humic acids were prepared through the oxidation and polycondensation of an aromatic compound viz., hydroquinone and pyrocatechol with an amino acid viz., glycine and alanine in the presence of $\text{K}_2\text{S}_2\text{O}_8$ (Roy *et al.* 1973). Equal proportions of an amino acid and hydroquinone/pyrocatechol were dissolved in 0.1 (N) NaOH and heated on waterbath for an hour with the gradual addition of $\text{K}_2\text{S}_2\text{O}_8$ solution. Humus from carbohydrate material was synthesized by dissolving 35 g of glucose in 9–10% HCl and then refluxing the solution on oiling waterbath for 30 hours (Roy *et al.* 1973). For the production of microbially synthesized humic acid, the cultures of *Strachybotrys atra* and *Streptomyces* sp were grown in separate sterile medium containing 4.5 g dextrose, 0.015 g yeast extract 0.30 g K_2HPO_4 , 0.0375 g KCl, 0.075 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.125 g asparagine dissolved in 150 ml of tap water in one litre prescription bottle and kept for an incubation period of 5 weeks at 28°C following the method of Martin *et al.* (1967). The usual procedures of fractionation and purification were followed in all the cases.

The different humic acids thus obtained were treated with 15% HNO_3 with five times its weight of crude humic acid at 80°C for two hours with constant shaking for nitration (Higuchi *et al.* 1970) and for sulphonation (Sasaki *et al.* 1969) with a $\text{BF}_3/\text{H}_2\text{SO}_4$ mol. ratio of 0.134 at 100°C for 90 minutes. The products were thoroughly dialysed and purified as before.

IR Spectroscopy

Thoroughly dried samples (1.5 mg each) were dispersed in KBr (200 mg) by mechanical grinding for 5 minutes and pressed discs were prepared (Theng *et al.* 1966; Schnitzer and Khan 1972). The spectra were recorded at room temperature with the aid of a Beckmann model 20 infrared spectrophotometer fitted with a NaCl prism.

Electrometric Studies

Conductometric titration—Both continuous and discontinuous conductometric titrations of the different samples were carried out with standard solutions of NaOH (SM, GR) and $\text{Ba}(\text{OH})_2$ (BDH, AR) separately, using systronic conductivity bridge at a constant temperature of $28^\circ \pm 0.1^\circ\text{C}$.

Potentiometric titration—Both continuous and discontinuous potentiometric titrations were performed with the aid of glass electrode pH meter using standard solutions of NaOH and $\text{Ba}(\text{OH})_2$ and also in the presence of 1.0 M NaCl (BDH, AR).

 E_4/E_6 Values

The O.D. values corresponding to 465 nm and 665 nm for the samples at a concentration of 0.024 g/100 ml were noted with the aid of a Beckmann DU-2 spectro-

photometer. The pH of the solutions were adjusted to 8.0 by 0.05 (N) NaHCO_3 solution (Schnitzer & Khan 1972).

RESULTS AND DISCUSSION

The different humic acids are symbolized as follows :

- | | | |
|-----|--|----------------|
| (1) | Pond sediment humic acid | —H. A. (P. S.) |
| (2) | Synthetic humic acid from hydroquinone and glycine | —H. A. (A) |
| (3) | -Do- from hydroquinone and alanine | —H. A. (B) |
| (4) | -Do- from glucose | —H. A. (C) |
| (5) | -Do- from pyrocatechol and glycine | —H. A. (D) |
| (6) | -Do- from pyrocatechol and alanine | —H. A. (E) |
| (7) | Microbial humic acid from strachybotrys atra | —H. A. (Fa) |
| (8) | -Do- from Streptomyces SP | —H. A. (Fb) |

IR spectroscopy data as obtained from the frequency vs. absorbance plots (Fig. not shown) and the tentative arrangements as done (Adhikari & Chakraborti 1973; Schnitzer & Khan 1972; and Theng *et al.* 1966) are recorded in Table I. From the table, it is seen that all the natural, synthetic and microbial humic acids and their derivatives have, in general, some common absorption bands viz., 3400 cm^{-1} (H bonded OH, NH stretch), 2900 cm^{-1} (aliphatic —CH stretch), 2300 cm^{-1} (zwitter ions of $> \text{NH}^+$ and $-\text{NH}_2^+$ etc), 1700 cm^{-1} (C=O stretching of —COOH and Ketones), 1600 cm^{-1} (aromatic C = C), 1060 cm^{-1} (C—O in cyclic and aliphatic esters and alcohols), 880 cm^{-1} (H bonded OH, deformation in carbonyl group). The band at 1510 cm^{-1} due to NO_2 vibration overlaps with aromatic C = C but the peaks are more sharper and stronger in case of nitro- compounds than in the original and sulphonated products. Again the absorption band in the region 1360 cm^{-1} due to S = O in sulphone group is overlapping with aliphatic CH, NO_2 and N-O stretching modes. But this band is obtained for nitro and sulphonated compounds and not for the original humic acids. This band thus corresponds to S = O in sulphone group for sulphonated products and NO_2 and NO stretching modes in the nitro- products. The band in the region $1200 - 1270\text{ cm}^{-1}$ due to C = O stretching and OH deformation modes of CO_2H exists for the samples except for natural humic acid. Since SO_3H group has absorption band at 1260 cm^{-1} region, the presence of this band is due to this group of sulphonated natural humic acid and also the other sulphonated products.

Though the clear picture of the macromolecular structure can hardly be obtained from IR spectra, yet from the relative sharpness and intensity of the different absorption bands tentative conclusion can be drawn for the comparison of natural humic acid with synthetic and microbial humic acid and for the nitro and sulphonated humic acids. A broad similarity is found among the natural and the different synthetic and microbial humic acids except that the peaks are more in number and sharper in the latter cases than in the former. This indicates more complex nature of natural humic acid with more polymeric condensation of aromatic rings which is also reflected in the E_4/E_6 ratio (discussed later). Regarding the nitro and sulphonated products these respective groups are added in the molecule without any major change in the

TABLE I
IR Spectra
Frequency in cm⁻¹

H.A. (P.S.)	H.A. (A)	H.A. (B)	H.A. (C)	H.A. (D)	H.A. (E)	H.A. (F _a)	H.A. (F _b)	Nitro H.A. (P.S.)	Nitro (A)	Nitro (B)	Nitro (C)	Nitro (D)	Nitro (E)	Nitro (F _a)	Nitro (F _b)	Sulpho H.A. (P.S.)	Sulpho (A)	Sulpho (B)	Sulpho (C)	Sulpho (D)	Sulpho (E)	Sulpho (F _a)	Sulpho (F _b)	Tentative assignments	
3400 (b, st)	3300 (b, st)	3400 (b, st)	3300 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3300 (sp, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (sp, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3400 (b, st)	3300 (sp, st)	H-bonded OH and NH stretch	
2940 (sp, w)	2900 (w)	2900 (w)	2940 (s _{sp} p, w)	2900 (w, b)	2900 (m, w)	2900 (w)	2900 (sp, m)	2920 (sp, w)	2940 (w)	2920 (sp, m)	2900 (b, w)	2920 (w)	2920 (w)	2920 (w)	2920 (sp, w)	2920 (sp, w)	2920 (w)	2920 (w)	2900 (sp, st)	2900 (b, w)	2900 (w)	2940 (w, b)	2900 (sp, m)	Aliphatic CH stretch	
2380 (sp, w)	2320 (sp, w)	2320 (w)	2320 (m, w)	2340 (b, w)	2300 (b, w)	2320 (w)	2320 (sp, w)	2320 (w)	2300 (b, w)	2300 (b, w)	2360 (b, w)	2340 (b, w)	2300 (w)	2300 (w)	2320 (sp, w)	2380 (w)	2400 (w)		2400 (w)	2300 (w)	2300 (w)	2320 (sp, w)		Zwitter ions of NH ⁻ and NH ₂ ⁺ etc	
1700 (sp, m)	1700 (sp, w)	1700 (sp, w)	1700 (sp, w)	1710 (b, w)	1710 (sp, w)	1720 (sp, w)	1700 (sp, w)	1700 (sp, b)	1700 (sp, b)	1700 (sp, w)	1700 (sp, b)	1700 (sp, b)	1700 (b, w)	1710 (sp, b)	1710 (sp, w)	1710 (sp, m)	1700 (b, m)	1700 (sp, m)	1700 (sp, st)	1710 (sp, st)	1700 (sp, m)	1720 (sp, st)	1710 (sp, st)	C=O of CO ₂ H and ketones	
1600 (m, st)	1600 (m, st)	1600 (w, b)	1600 (sp, b)	1590 (sp, b)	1600 (sp, b)	1600 (sp, m)	1630 (sp, st)	1600 (sp, st)	1620 (sp, b)	1600 (b, m)	1600 (b, m)	1600 (sp, b)	1600 (sp, st)	1600 (sp, st)	1600 (sp, st)	1620 (b, m)	1630 (sp, m)	1620 (sp, b)	1620 (sp, b)	1610 (sp, b)	1600 (sp, b)	1610 (sp, st)	1620 (sp, st)	Aromatic C=C	
	1400 (b, w)	1500 (m, w)	1480 (m, w)	1510 (sp, w)	1525 (b, w)	1510 (b, w)	1530 (sp, w)	1510 (sp, w)	1510 (sp, st)	1530 (b, w)	1500 (sp, st)	1500 (w)	1500 (b, w)	1510 (sp, st)	1510 (sp, st)	1500 (b, w)	1510 (sp, st)		1510 (b, m)		1510 (b, w)	1520 (b, w)	1500 (sp, w)		Aromatic C=C and NO vibration
								1370 (b, m)	1370 (b, w)	1390 (sp, w)	1340 (sp, w)	1360 (w)	1360 (b, w)	1360 (sp, w)	1390 (sp, w)	1360 (b, w)	1340 (b, m)	1360 (w)	1380 (m, m)	1340 (b, w)	1340 (b, w)	1370 (b, w)	1360 (b, w)		S=O in sulphone group and aliphatic CH or NO ₂ and N-O Stretching
	1180 (sp, st)	1250 (sp, w)	1180 (sp, m)	1270 (b, w)	1270 (b, w)	1230 (b, w)	1230 (b, w)	1250 (b, w)	1200 (b, st)	1220 (b, w)	1200 (sp, st)	1270 (b, w)	1270 (b, m)	1200 (b, w)	1250 (sp, w)	1230 (sp, b)	1200 (sp, b)	1200 (sp, b)	1200 (sp, b)	1200 (m, b)	1270 (sp, b)	1220 (sp, b)	1220 (sp, w)		C=O stretch and OH OH deformation of -CO ₂ H
1060 (m, st)	1080 (sp, w)	1080 (sp, w)	1090 (b, m)	1060 (b, w)	1120 (b, w)	1070 (sp, m)	1070 (b, m)	1080 (b, m)	1100 (b, w)	1100 (sp, b)	1100 (sp, w)	1090 (b, m)	1110 (sp, w)	1090 (sp, m)	1090 (sp, m)	1110 (sp, w)	1050 (b, w)	1060 (sp, w)	1110 (w)	1050 (m, b)	1030 (m, b)	1060 (sp, m)	1060 (b, m)		C=O in cyclic and aliphatic esters and alcohols
	810 (sp, m)	800 (b, w)	800 (sp, m)	870 (m, w)	870 (b, m)	870 (sp, w)		980 (m, w)	830 (b, m)	800 (b, m)	820 (sp, b)	980 (sp, b)	880 (b, w)	870 (sp, b)	890 (sp, w)	980 (sp, w)	820 (sp, b)	800 (sp, b)	800 (sp, m)	870 (b, w)	870 (sp, m)	890 (sp, m)	820 (sp, w)		Aromatic CH, H- bonded OH, or de- formation in carbo- xyl group

sp—sharp, m—medium, b—broad, st—strong, w—weak

other functional groups in the molecule during the process of nitration and sulphonation. However, such treatments have resulted in smaller molecules which show sharp peaks and this is also reflected in the E_4/E_6 values and electrometric titrations (discussed later) with an increase in acidity due to the presence of NO_2 and SO_3H groups in the molecule respectively. The data for $p\text{H}$ metric and conductometric titrations (figures not shown) are tabulated in Table II. The c.e.c. values were calculated from the final inflexion/break of both $p\text{H}$ metric and conductometric titrations. The pK av. values are taken to be the $p\text{H}$ corresponding to 50% neutralization. From the table, which shows the initial $p\text{H}$ and specific conductance values, pK av, pK int, c.e.c. calculated for both NaOH and $\text{Ba}(\text{OH})_2$ titrations as well as titrations in presence of neutral salt solution, it can be inferred that the synthetic and microbial humic acid resemble natural humic acid having more or less similar titration figures with poly-electrolytic character. A comparatively stronger acidic character is observed in the cases of nitro and sulphonated products than their respective parent counterparts. This is due to the presence of $-\text{NO}_2$ group, having electron withdrawing effect, which increases the acidity of the phenolic— OH and other weakly acidic groups present in the molecule SO_3H group being itself acidic, increase in acidity in sulphonated products is obvious.

The peculiar behaviour of colloidal acids as distinct from simple acids in true solution is noted in $p\text{H}$ metric titrations with a suppression of initial $p\text{H}$ in presence of neutral salt solution and a comparatively strong acid feature of the titration curve. In $p\text{H}$ metric titration for humic acids, in general, two inflexion points—first one due to COOH group and second one due to phenolic OH group, are noted. But a single inflexion point in presence of neutral salt is obtained. This is due to the replacement of H^+ ions by the cations of the added electrolyte and the relative power of replacement is $\text{Ba} > \text{Ca} > \text{Na}$. The c.e.c. values whether calculated at $p\text{H}$ 7.0 or higher, follow the order $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{KOH} > \text{NaOH}$. The above observations are in agreement with that reported by previous workers (Adhikari & Hazra 1970; Chatterjee & Bose 1952; and Roy *et al.* 1973). The pK int. values are determined (Tanford 1961) from the intercept at $\alpha = 0$ of the plot $p\text{H} - \log \frac{\alpha}{1 - \alpha}$ vs. α , where $\alpha \left(\frac{r}{n} \right)$ is the degree of dissociation, r being the average number of protons dissociated per molecule and n the average total number of such dissociable protons per molecule. These plots for different humic acids (Figs. for natural and one synthetic humic acid both in absence and in presence of NaCl are shown in Fig. 1) are not linear which suggest that the size of the molecule changes with the ionization of the molecule (Tanford 1961). In presence of salt, the deviation from linearity is less pronounced which is due to the molecule becoming rigid in presence of neutral salt. The pK int. value for natural humic acid viz., 5.5 is a little higher than that for synthetic and microbial humic acid and the latter lie between 5.0 to 5.4. The values are somewhat close to the value of pK int. for side chain $-\text{COOH}$ viz., 4.7 (Tanford 1961). The addition of NaCl causes a lowering in the pK int. values of 0.4 to 0.8 units for the different humic acids; lowering being maximum for the case of natural humic acid. Nitration and sulphonation also cause the lowering of pK int. values; the value being 0.10 to 0.55 units for nitro products and 0.10 to 0.80 units for sulphonated products; maximum for synthetic nitro

TABLE II
Electrometric titrations and E_4/E_6 Ratio
 (Concentration used for all the samples was 0.024g/ml)

Sample	Initial pH		pKav (at $\alpha=0.5$)		pKint		C.E.C. at last inflexion point (Potentiometric) in meq/100 g		Initial specific conductance ($\alpha=0$) in mhos $\times \text{cm}^{-1} \times 10^4$	C.E.C. at final break ($\alpha=1$) (Conductometric)		E_4/E_6 at pH 8.0		
	NaOH/ Ba(OH) ₂ titration.	NaCl/ NaOH	NaOH	Ba(OH) ₂	in absence of salt	in NaCl	NaOH	Ba(OH) ₂		NaOH	Ba(OH) ₂		(13)	(14)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
H.A. (P.S.)	4.15	3.60	6.95	7.25	6.4	5.5	4.7	4.00	425	432	3.575	408	436	3.1
H.A. (A)	3.8	3.4	6.3	6.65	5.9	5.0	4.45	342	353	356	6.2	350	354	4.5
H.A. (B)	3.7	3.45	6.5	6.7	5.85	5.2	4.8	326	358	360	4.05	334	352	4.8
H.A. (C)	3.75	3.35	6.6	6.85	6.15	5.3	4.9	388	408	416	3.83	396	418	5.1
H.A. (D)	3.8	3.4	6.4	6.7	5.95	5.1	4.6	308	336	338	6.8	312	322	4.48
H.A. (E)	3.7	3.35	6.25	6.5	5.8	5.4	5.0	314	332	340	4.34	318	336	4.3
H.A. (F _a)	3.9	3.4	6.45	6.75	6.1	5.1	4.7	372	386	394	5.46	376	388	4.1
H.A. (F _b)	4.0	3.6	6.55	6.9	6.2	5.0	4.6	356	368	372	5.82	362	374	4.4

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Nitro H.A. (P.S.)	4.1	3.6	6.7	6.85	6.4	5.3	5.0	4.22	436	442	12.36	414	446	3.8
Nitro (A)	3.7	3.35	5.9	6.2	5.75	4.9	4.6	350	348	354	22.7	368	359	4.8
Nitro (B)	3.6	3.2	6.25	6.50	6.00	5.0	4.7	328	342	336	15.2	352	366	5.2
Nitro (C)	3.7	3.3	6.2	6.05	5.95	4.9	4.7	392	412	416	20.4	412	428	5.8
Nitro (D)	3.75	3.35	6.15	6.46	6.0	5.0	4.8	325	332	338	23.5	346	336	5.15
Nitro (E)	3.7	3.3	5.95	6.2	5.6	4.85	4.6	312	324	322	18.6	334	345	4.6
Nitro (F _a)	3.25	3.45	6.0	6.35	5.8	4.9	4.75	368	374	376	21.8	382	376	5.25
Nitro (F _b)	3.9	3.5	6.4	6.5	6.1	4.8	4.6	370	382	378	17.4	368	378	4.95
Sulpho H.A. (P.S.)	4.05	3.6	6.25	6.1	5.7	4.8	4.4	432	446	454	14.7	438	448	4.28
Sulpho (A)	3.7	3.3	5.8	5.6	5.4	4.7	4.5	354	358	362	20.3	402	412	5.9
Sulpho (B)	3.65	3.2	6.1	5.9	5.55	4.8	4.6	322	340	334	14.6	370	384	6.1
Sulpho (C)	3.65	3.25	6.0	6.3	5.8	4.5	4.4	408	410	414	23.8	431	436	5.6
Sulpho (D)	3.7	3.3	5.9	6.1	5.45	5.0	4.7	332	336	344	27.4	385	378	4.9
Sulpho (E)	3.65	3.25	5.7	5.95	5.5	4.7	4.6	320	328	326	24.5	352	364	5.3
Sulpho (F _a)	3.8	3.3	4.85	5.9	5.45	4.8	4.5	372	380	382	18.6	404	428	5.6
Sulpho (F _b)	3.85	3.6	6.2	6.4	6.0	4.6	4.4	376	392	388	14.8	396	410	5.4

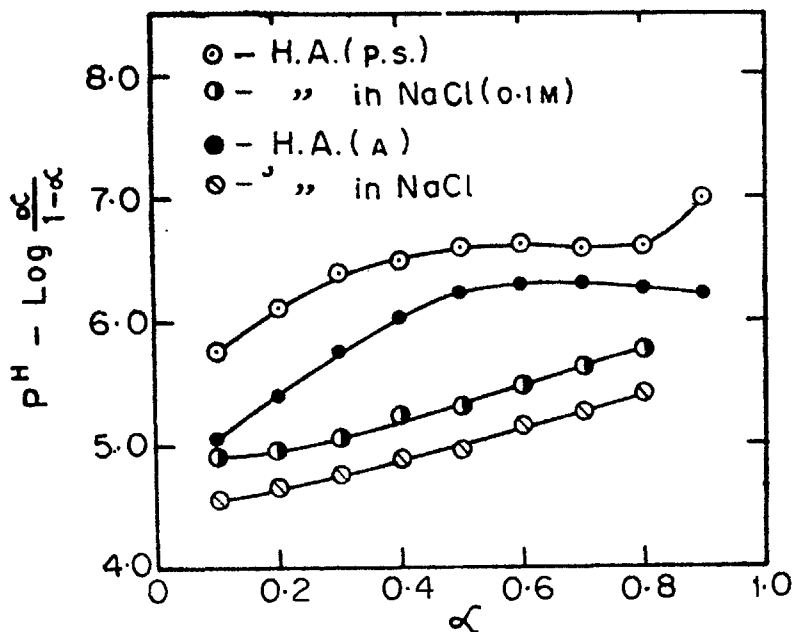


FIG. 1. Plots for different humic acids and one synthetic humic acid.

(E) and sulpho (C). The lower values for nitro- and sulphonated products further establish their stronger acidic and less polymeric nature than their parent humic acids.

The nature of the curves viz., $pH - \log \frac{\alpha}{1-\alpha}$ vs. α may be explained in the

following way. Near the start of the titration, when α is small, the chain is effectively uncharged and hence PK_0 value may be expected to be close to that for a small simple molecule. As the titration proceeds the chain accumulates more of negative charges; thus it becomes difficult to remove each of successive protons and hence the effective PK_0 value steadily rises. Therefore, the measured pH values during the titration no longer conform to the conventional Henderson-Hasselbach equation for ordinary electrolyte.

$$pH = PK_0 + \log \frac{\alpha}{1-\alpha}$$

Taking this into account, the equation can be written in a little modified form to be applicable for a macromolecule with interacting charged groups (Birshtein & Ptitsyn 1966).

$$pH = PK_0 + \log \frac{\alpha}{1-\alpha} \Delta PK_{(\alpha)},$$

where $\Delta PK_{(\alpha)} = PK_{(\alpha)} - PK_0$; and PK_0 is the value of PK at $\alpha=0$. The plot

$pH - PK_0 - \log \frac{\alpha}{1-\alpha}$ against α , which describes directly the effect of interaction of

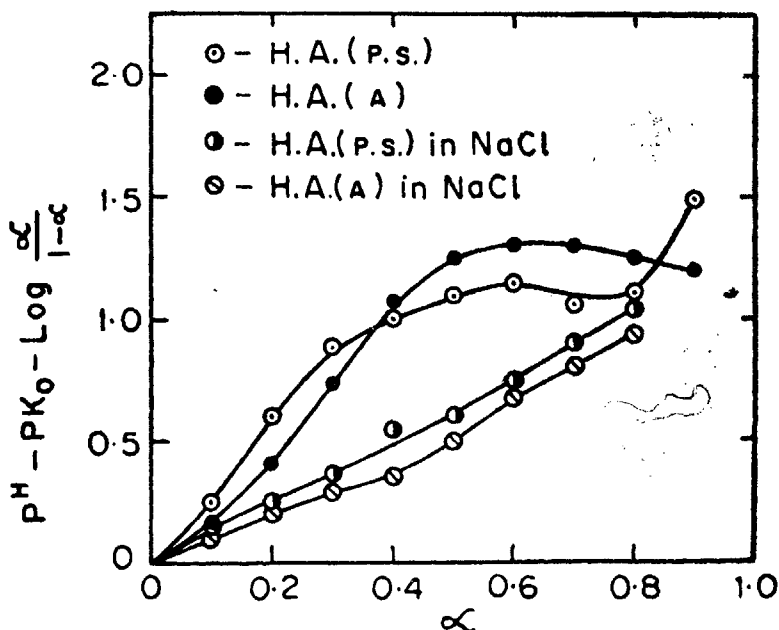


FIG. 2. The effect of interaction of the charged groups on the titration curve.

the charged groups on the titration curve is shown in Fig. 2. These figures show the parameter $pH - PK_0 - \log \frac{\alpha}{1-\alpha}$ increases with increase in the value of α .

The curve undergoes an inflexion. This indicates a conformational transition (Birshstein & Ptitsyn 1966), which may be occurred from the loose open fibrous structure at low pH to a more sheet like one at higher pH . These types of transitions with changes in pH for humic acids were observed earlier from scanning electron microscopic study (Chen & Schnitzer 1976). In presence of NaCl, the nature of curve is changed and no

sharp inflexion is obtained and the value of the parameter $pH - PK_0 - \log \frac{\alpha}{1-\alpha}$

for each value of α is less than for the case of humic acid along. This is due to the less electrostatic interaction between the groups and a more rigid structure in the presence of salt. From the above discussion it can be concluded that humic acid has the flexible poly-electrolytic character and its geometrical configuration is affected by pH and salt solution.

The E_4/E_6 values for the different samples are recorded in Table II. The values for the different humic acids lie in the range of 3.10 to 5.10; the lowest value is for natural humic acid and highest for synthetic humic acid (C). Comparatively greater values are obtained for nitro- and sulphonated products than their respective parent molecules; the increase being 0.30 to 1.25 units for nitro- and 0.40 to 1.50 units for the sulphonated products. The sulphonated products, in general, have the greatest values. The increase in E_4/E_6 values is maximum for microbial humic

acid on nitration and sulphonated. This ratio is an indication of humification and the degree of condensation of aromatic rings in humic substances. Lower is the value, greater is the humification with higher degree of condensation of aromatic rings (Kononova 1966). In the present study, natural humic acid having lowest value is most humified with highest degree of aromatization and obviously it has highest aromatic —C to aliphatic —C ratio. On the other hand sulphonated acid (B) having the highest value viz., 6.10 has the lowest aromatic —C to aliphatic —C ratio. The greater values for nitro and sulphonated products than the parent humic acid are considered as due to the smaller size of these products with less aromatization. Between nitro- and sulphonated products, comparatively lower value for nitro products can further be corroborated to the contribution of nitrogen and spin contents which have positive influence on light absorption (Schnitzer & Khan 1972).

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