
PART 3

SHAPE OF HUMIC SUBSTANCES ISOLATED FROM
SOIL FROM VISCOMETRY

3.1 INTRODUCTION

In this part, a further extension of the additivity principle of the viscosity B coefficient to a more complex heterogeneous system has been made to work out the shape (folded, or linear) of a polymer of natural origin, namely humic acid (HA), a component of soil organic matter, humus. Viscometry can be applied with reasonably satisfactory results to determine the state of conformation of such large macromolecules. Soil humus is doubtless one of the most complex materials present in nature. The term refers more specifically to the non-living components of heterogeneous character, resulting from microbial and chemical transformations of organic debris in soil¹.

The fraction HA is further known to be polyfunctional in nature, and contains, among important groups, the carboxylic, phenolic (OH), and amino groups. In the present case, the single species B coefficients of the simple constituents of HA have been determined by independent experiments (as already elaborated in section 1.3) as well as those of HA suspension measured. The carboxylic, phenolic (OH) and amino group contents of HA are also determined by standard analytical techniques (pH-metric titration etc.), and then the principle of additivity of viscosity B coefficient is invoked to decide whether or not the HA molecules consist of long elongated (uncoiled) chains, or folded ones.

A semi-quantitative model has been proposed to correlate the findings to the surface area of the polymer chain exposed to water by using the concept of changes in microviscosity of the solvent brought about by the polymer.

3.2 REVIEW OF LITERATURE

The chemistry of humic acid (HA) and fulvic acid (FA) has been extensively reviewed by Hayes and Swift² fairly recently, and a vast amount of works are reported in the literature³ on the characterisation of HA and FA. In the following, a brief review of past works, employing viscometry as a tool for the purpose, is given.

Reports³ have appeared demonstrating the shape and polyelectrolyte behaviour of humic substances by viscosity measurements. For distinguishing the polyelectrolyte behaviour of such substances as distinct from neutral salts, the dependence of the reduced viscosity (η_{sp}/C) on concentration has been considered. Mukherjee et al⁴. observed that the reduced viscosity of a coal-HA sample at various concentrations corresponded to the polyelectrolyte behaviour. This observation was supported by other workers^{5,6,7} as well.

Piret et al⁵ demonstrated for a sample of peat-HA the non-spherical polyelectrolytic as well as the polydispersion behaviour which contradicted the observations of Flaig and Beutelspacher⁸ that both natural and synthetic HA's are spherical.

Chen and Schnitzer^{9a} have reported that humic acid/fulvic acid are rod-shaped which was in agreement with the scanning electron microscopic investigations^{9b}.

From the determination of viscosity numbers (η_{sp}/C), Visser¹⁰ suggested that HA's were spherocolloids while other workers^{11,12} reported that HA's were within the range of spherocolloids and also linear colloids.

Adhikari et al¹³ compared the viscometric behaviour of naturally occurring humic substances with those prepared by chemical and microbial processes. They compared the acids with the corresponding nitro- and sulphonated derivatives, and observed a flexible polyelectrolytic nature for all the samples.

In the following, we report our work on such shape of humic acid by utilising, as said above, the viscosity B coefficient of the Jones-Dole's equation¹⁴, and its additive characteristics.

3.3. THEORETICAL RELATIONS

A rearrangement of the Jones-Dole's equation¹⁴ leads to

$$\left(\frac{\eta}{\eta_0} - 1\right) / \sqrt{C} = A + B \sqrt{C} + \dots \quad (3.3.1)$$

so that a plot of left-hand side of the eq.3.3.1 against \sqrt{C} should yield a straight line of slope B and intercept A. The B coefficient thus obtained obviously has the unit of (concentration)⁻¹.

There have been attempts in the past also to use eq.3.3.1, or its modified forms, for colloidal suspensions, even though the attention has been primarily concentrated upon the molecular weight determinations from what has been termed the intrinsic viscosity¹⁵.

For the present purpose, the viscosities of a number of solutions of different molar concentrations of various simple constituents of HA are determined, and then recourse is made to the above graphical technique to evaluate the corresponding B's.

The B coefficients of the given HA suspension is next determined in the same way. Because the molecular weight of the polymeric acid is uncertain, B coefficient for HA is obtained in percent concentration unit, and then suitable conversion factors

are worked out for changing B lit.mol⁻¹ values to B (percent)⁻¹ units for the simple constituents of the acid. For instance, the following relation holds,

$$\frac{B \text{ in (percent)}^{-1} \text{ unit}}{B \text{ in lit.mol}^{-1} \text{ unit}} = \frac{1}{0.1 M} \quad (3.3.2)$$

where M is the molecular weight of the compound (or, functional group) in question. The latter is necessary for the purpose of correlations (see latter).

The B coefficient of HA, obtained by direct experiment, is finally compared with the "additive value" computed by summing up the B value contributions due to contents of various hydrophilic groups in HA. Lack of concordance, if any, and especially if the latter is wide (i.e., $B_{\text{expt}} < B_{\text{Calc}}$), is taken to indicate that HA chain is coiled, and not unfolded, so that the whole of its surface area is not exposed to water to affect its "local structure" through the interactions with the polar head groups (such as -COOH, phenolic -OH, -NH₂) of the former.

3.4 EXPERIMENTAL DETAILS

(A) APPARATUS

The temperature of the study was maintained at 35°C ± 0.05°C by the use of a water thermostat (SICO), provided with a solid state relay unit and an electrical contact thermometer (JUMO-B.D.P).

An Ubbelohde type (three necked) all-glass viscometer was used for the determination of viscosities. The flow time was recorded by means of a stopwatch (ROCAR, antimagnetic), reading up to 0.1 of a second. The average flow time for water (10 ml)

through the capillary of the viscometer used was 350.0 secs (at 35°C).

The pH meter used was a Pye Cambridge model capable of reading pH upto 0.1 unit.

(B) CHEMICALS

The chemicals used, namely anilinium chloride, caustic soda, barium hydroxide, calcium acetate, perchloric acid, glacial acetic acid, potassium hydrogen phthalate were of AnalaR grade.

The solutions were prepared in distilled water of conductivity less than $3 \mu \text{ mho.cm}^{-1}$. An electrical balance, reading upto 10 μg , was used for recording weights in the preparation of solutions and measurements of density (by using a specific gravity bottle).

The HA used in the present study was isolated, by following the standard procedure¹, from the humus suspension from Bigra (Burdwan, West Bengal) Pond Sediment.

(C) FUNCTIONAL GROUP ANALYSIS

In order to correlate the B coefficient due to -COOH group content in HA to pure components, it is necessary to convert these -COOH groups to $\text{COO}^- \text{Na}^+$ form while leaving the phenolic (OH) groups unchanged, in view of the fact that the B values of the fully dissociated salts of the simple carboxylic acids such as sodium formate are measured, and on subtraction of Na^+ value, that for the $-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{O} \end{array}$ group may be obtained, while B for almost fully undissociated phenolic compounds can readily be determined. For the purpose, aqueous suspension of HA (0.6%, 10 ml) was titrated pH-metrically against standard NaOH solution. Two inflexion points for HA were obtained and the alkali equivalent corresponding to

the first inflexion point (which gives a measure of the amount of -COOH groups) was added to the bulk of the suspension to be used for viscosity measurements.

(i) -COOH GROUP DETERMINATION

The original suspension of HA was subjected to the standard pH-potentiometric procedure¹⁶ to determine the carboxylic acid and phenolic (OH) group contents of the polymer. A brief outline of the procedures are given below :

To 25 mg of HA in a 125 ml glass-stoppered Erlenmeyer flask, 10 ml of 1(N) calcium acetate solution and 40 ml of CO₂-free distilled water were added. A blank consisting of 10 ml of 1(N) calcium acetate solution plus 40 ml of CO₂-free distilled water only was simultaneously set up. Both were shaken for 24 hrs. at room temperature, then filtered, washed with distilled water and the combined filtrate and washings for both the cases were titrated potentiometrically (using a glass-calomel electrode) with 0.099(N) NaOH solution to pH 9.8 (vide. figures 3.4.1a and 3.4.1b).

The calculations are as follows :

$$\frac{(\text{titre for sample} - \text{titre for blank}) \times (N) \text{ base} \times 1000}{\text{weight of the sample, mg}}$$
$$= \text{meq. of CO}_2\text{H groups / g of HA (vide. table 3.5.1).}$$

(ii) PHENOLIC (OH) GROUP DETERMINATION¹⁶

The amount of phenolic hydroxyl group can be calculated in the following manner :

$$\text{meq. of total acidity / g of HA} - \text{meq. of COOH groups / g of HA}$$
$$= \text{meq. of phenolic (OH) / g of HA (vide. table 3.5.2).}$$

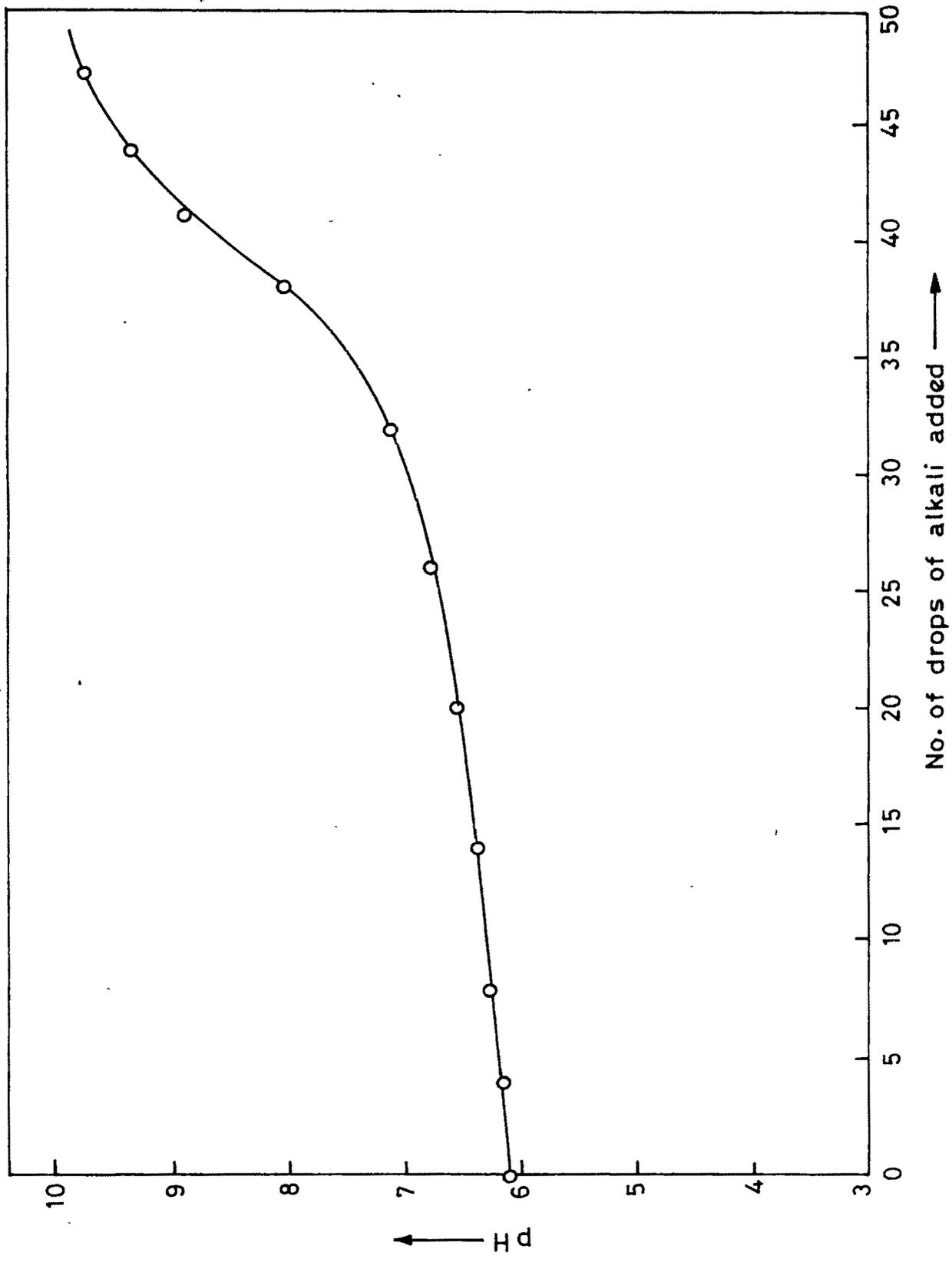


Fig. 3-4.1a

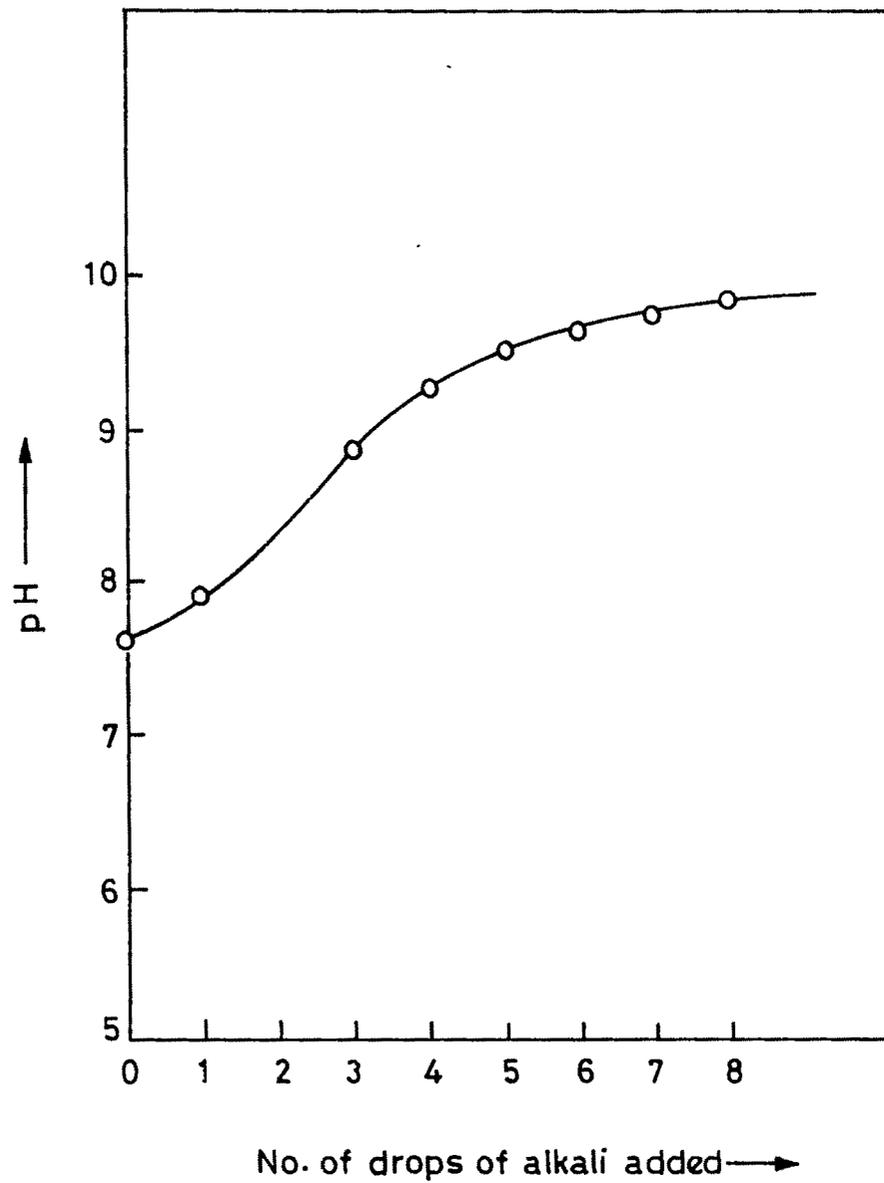


Fig. 3.4.1b

DETERMINATION OF TOTAL ACIDITY

To 25 mg of HA taken in a 125 ml glass-stoppered Erlenmeyer flask, 10 ml of 0.2 (N) Ba(OH)₂ solution were added. A blank containing only 10 ml of 0.2 (N) Ba(OH)₂ solution was also set up. Both were shaken at room temperature for 24 hrs., filtered, washed with distilled water and the combination of filtrate and washings was titrated potentiometrically (glass-calomel electrode) with 0.56(N) HCl solution to pH 8.4 (vide. figures 3.4.2a. and 3.4.2b).

The calculations are as follows :

$$\frac{(\text{titre for blank} - \text{titre for sample}) \times (N) \text{ acid} \times 1000}{\text{weight of sample, mg}}$$

$$= \text{meq. of total acidity / g of HA (vide. table 3.5.2).}$$

(iii) -NH₂ GROUP DETERMINATION

The amino group content of HA was estimated by following the analytical technique of Ficken and Lane¹⁷. A short outline of the method is given below :

An aqueous suspension containing 134 mg of HA in a 250 ml conical flask was boiled gently with 20 ml of glacial acetic acid for about one minute. Then it was allowed to cool, one drop of crystal violet indicator was added and titrated with 0.1(N) perchloric acid in glacial acetic acid (perchloric acid was standardised against standard (0.092N) potassium hydrogen phthalate) until the indicator changed colour to blue through the intermediate stage of purplish to pink. The flask was then placed in an air oven at approximately 100°C. When the original colour returned, a further addition of perchloric acid was made, the process being repeated until a permanent end-point colour was obtained. As a precautionary

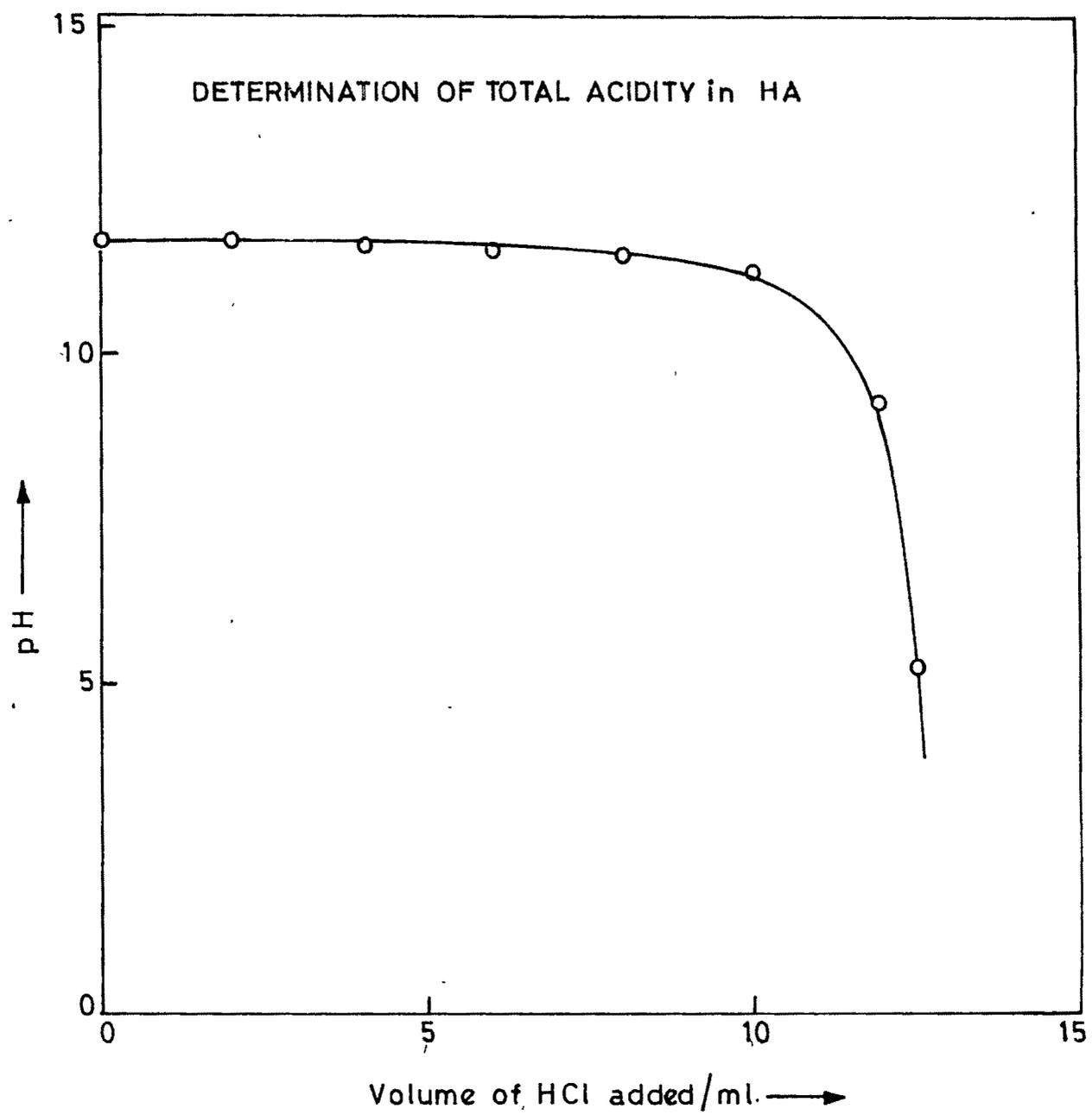


Fig.3.4.2a

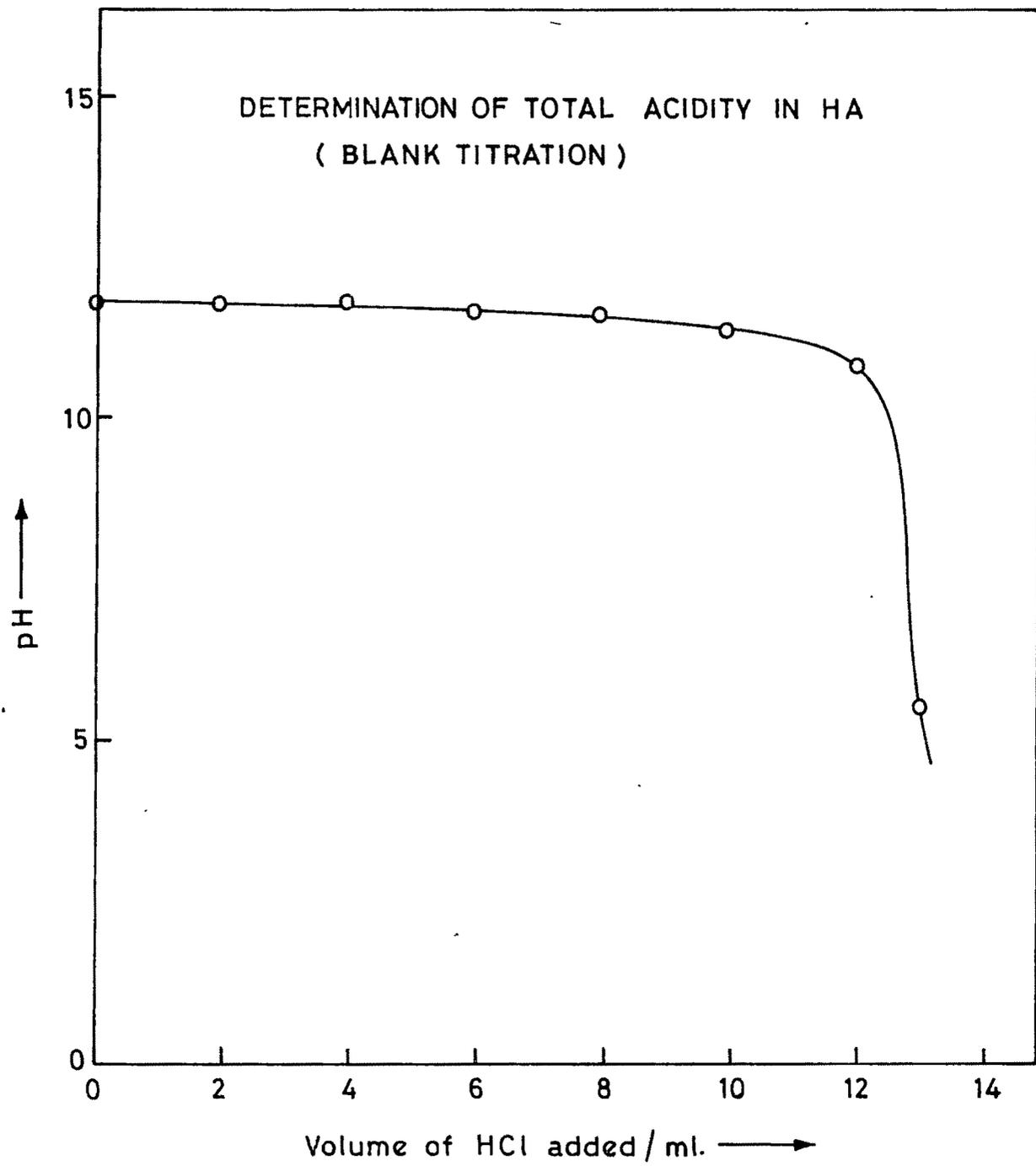


Fig.3.4.2b

check, the flask was left overnight at 40°C when no further colour change should be observed.

The calculation is as follows :

$$\frac{\text{volume of perchloric acid} \times (\text{N})_{\text{acid}} \times 1000}{\text{weight of HA, mg}}$$

= meq. of $-(\text{NH}_2)$ group/g of HA (vide. table 3.5.3).

The amino group content of the polymer thus obtained was compared with the total nitrogen content of HA obtained by Duma's method; the latter turned out nearly equal to the amino group content (in equivalent terms) of the polymer indicating that nitrogen was present almost exclusively in the amino form.

(D) DETERMINATION OF VISCOSITIES

(i) The viscosity B coefficients of simple constituents of HA as reported earlier in part 1^{18,19}, have been used in the present study.

(ii) Next, the viscosity B coefficient of HA suspension (Na-salt) was measured in the way as indicated in the section on theory. The B value of the humate anion was next obtained on subtraction of the Na^+ value (on percent⁻¹ scale, as obtained by using the literature value²⁰, and eq.3.3.2).

(iii) The B coefficients of simple compounds were converted to percent unit values by taking help of eq.3.3.2.

(iv) The "Composite (Calculated) B", B_{theor} , for HA computed for contributions made by various hydrophilic groups, was next obtained by a method as elaborated shortly in the section 3.6 on "Discussion".

(v) B_{theor} value was then compared with the experimentally

obtained value of B for HA, and the extent of coiling in HA was inferred therefrom.

3.5 RESULTS

The results for the estimation of different hydrophilic functional groups, namely -COOH, phenolic (OH) and -NH₂ groups, present in the given HA are recorded in tables 3.5.1 to 3.5.3.

Table 3.5.4 records the B coefficients of simple constituents at 35°C and table 3.5.5 the estimated (by a method as elaborated below) B coefficient of the functional groups of HA at 35°C, as well as that of the HA worked out from these estimated (group) B values and the contents of the functional groups of HA, as given in tables 3.5.1 to 3.5.3.

Table 3.5.6 records the experimental B coefficient of the sodium salt of HA, and also that of the humate anion (B_{expt}) at 35°C.

TABLE 3.5.1

Percent concentration of HA for titration	Amount of HA titrated W / mg	Volume of NaOH consumed V' / ml	Volume of NaOH for blank V'' / ml	Strength of alkali / N	Amount of -COOH group of HA / meq.g ⁻¹ $Q_1 = \frac{(V' - V'') \times N(\text{base}) \times 10^3}{W}$
0.6	25.0	1.875	0.125	0.099	7.42

TABLE 3.5.2

Percent concentration of HA for titration	Amount of HA titrated W / mg	Volume of HCl consumed V' / ml	Volume of HCl for blank V'' / ml	Strength of HCl / N	Total acidity of the HA / meq.g ⁻¹ $Q_2 = \frac{(V'' - V') \times N(\text{acid}) \times 10^3}{W}$	Amount of phenolic (OH) group of HA / meq.g ⁻¹ $= Q_2 - Q_1$
0.6	25.0	12.1	12.6	0.56	11.25	3.83

TABLE 3.5.3

Amount of HA taken W/mg	Volume of perchloric acid required V / ml	Strength of perchloric acid / N	Amount of -NH ₂ group of HA/ meq. g ⁻¹ $= \frac{V \times N(\text{acid}) \times 10^3}{W}$
134.0	4.80	0.092	3.29

TABLE 3.5.4

Temperature 35°C

Constituents	B coefficients / lit. mol ⁻¹	Reference
Phenol	0.213	19
Resorcinol	0.216	19
Aniline hydrochloride	0.293	-
Sodium formate	0.199	18
Na ⁺	0.0859	20
Cl ⁻	0.0048	20

TABLE 3.5.5

Temperature 35°C

Functional group/ Substance	B coefficient/ lit.mol ⁻¹	B coefficient/ percent ⁻¹
-COO ⁻	0.113	0.025
Phenolic (OH)	0.003	0.0017
⁺ -NH ₃	0.078	0.040
Humic acid	-	0.069 (B _{theor})

TABLE 3.5.6

η_0 at 35°C = 0.7194 cp; ρ_0 at 35°C = 0.9940 g.ml⁻¹

Suspension	Percent concentration C	Relative viscosity η/η_0	B coefficient/ percent ⁻¹ (from the slope of the graph) B	B _{expt} of humate anion/ percent ⁻¹ B _{expt} = B - B _{Na⁺}
	0.010	1.0009		
	0.025	1.0025		
HA (Na-salt)	0.050	1.0045	0.071	0.034
	0.075	1.0055		
	0.100	1.0073		

3.6 DISCUSSION

(A) Methods of Calculation :

(i) Group contribution towards B values

The principle is first to evaluate the B coefficient contributions of -COOH (as carboxylate anion), phenolic (OH) and amino groups (in -NH_3^+ form; the pH of HA used for viscometry was in the acidic range) from those of simple constituents as entered in table 3.5.4. Thus a subtraction of Na^+ value from sodium formate B would result in that for HCOO^- ion, which may very nearly be taken as equal to that for the $\text{-C}\begin{smallmatrix} \text{O} \\ \text{O} \end{smallmatrix}\text{-}$ ion in view of the supposition that the formate H can readily be accommodated in the hydrogen-bonded water structure without having to modify the latter to any great extent, and hence "local" changes in viscosity can be ignored, i.e., the B contribution of the formate H may be disregarded.

For the phenolic (OH)-contribution, one may subtract the phenol B value from that of resorcinol, while the -NH_3^+ group contribution was obtained as follows :

$$B_{\text{NH}_3^+} = B_{\text{C}_6\text{H}_5\text{NH}_3^+\text{Cl}^-} - B_{\text{Cl}^-} - (B_{\text{C}_6\text{H}_5\text{OH}} - B_{\text{phenolic(OH)}})$$

These B values were obtained in lit.mol^{-1} units and converted to $(\text{percent})^{-1}$ unit by using eq.3.3.2, where M is the group weight of the various functional groups concerned. The table 3.5.5 summarises these B coefficients.

(ii) Correlation between experimental B and that computed on the basis of additivity

On the assumption that the density of HA suspension (0.6% aqueous suspension) used for the present study was nearly 1.0 g.ml^{-1}

(it was, in reality, 1.003 g.ml^{-1} , at 35°C), it follows from table 3.5.1 that the percent concentration of $-(\text{COO})^-$, i.e., $-(\text{COOH})$ group in HA used

$$= 7.42 \times 44 \times 10^{-3} \times 0.6 \text{ g of } -(\text{COO})^- \text{ group per 100 ml of HA suspension } (C_1)$$

Similarly, percent concentration of phenolic (OH) group in HA (vide. table 3.5.2)

$$= 3.83 \times 17 \times 10^{-3} \times 0.6 \text{ g of phenolic (OH) group per 100 ml of HA suspension } (C_2),$$

while that of $-(\text{NH}_3^+)$ group (vide. table 3.5.3)

$$= 3.29 \times 17 \times 10^{-3} \times 0.6 \text{ g of } -(\text{NH}_3^+) \text{ group per 100 ml of HA suspension } (C_3).$$

Now, if B_{theor} be the computed overall B coefficients, assuming additive contributions of various (important) hydrophilic groups of the polymer, in question, and C_1, C_2, C_3 be the respective percent concentrations of these groups as indicated above, then

$$B_{\text{theor}} \times C_{\text{av}} = \left[(B_1 C_1)_{-\text{COO}^-} + (B_2 C_2)_{\text{phenolic(OH)}} + (B_3 C_3)_{-\text{NH}_3^+} \right] \dots \quad (3.6.1)$$

where C_{av} is the average percent concentration of the various functional groups considered of the polymer suspension as employed in such analysis, and is given as,

$$C_{\text{av}} = \frac{1}{3} (C_1 + C_2 + C_3)$$

$$\begin{aligned} \text{i.e., } C_{\text{av}} &= \frac{1}{3} [7.42 \times 44 \times 10^{-3} \times 0.6 + 3.83 \times 17 \times 10^{-3} \times 0.6 + 3.29 \times 17 \times 10^{-3} \times 0.6] \\ &= \frac{1}{3} [0.195 + 0.039 + 0.033] \\ &= 0.089\% \end{aligned}$$

The eq.3.6.1 obviously neglects the B contribution of various aromatic rings (which are largely hydrophobic, and may lead to significant increase in "local" viscosity of water through hydrophobic hydration²¹), and this may constitute an important source of error, even though the corresponding additional terms (on the right-hand side of eq.3.6.1) may possibly be deduced, in an approximate manner though, from the molar volumes of the polymer. However, the associated error (of such neglects) is expected to be small for predominantly "aromatic" humic acids owing to a possible even cancellation in the latter of the hydrophobic hydration effects of (C-H) bonds by the inability of water molecules to accommodate themselves, for spatial restrictions, inside the benzene rings. Furthermore, the "aromaticity" i.e., the π -electron cloud, above and below the plane of a benzene ring, may also facilitate the break-down of "water-clusters", adjacent to the rings, by transmitting, through cooperative hydrogen-bonding, the cluster-disruptive influences from water molecules lying outside the cluster region²¹. The latter leads to a lowering of "local" viscosity, and hence the B coefficients. The aliphatic chains of HA, as well as, to some extent, the alicyclic rings also (having no π -electron clouds), are expected, on the other hand, to make a greater contribution to the B coefficient of HA, and as such, the error of neglecting the hydrophobic surface in computation of B_{theor} , as being discussed here, is also expected to be greater, the higher the "aliphatic" character of the HA chains. In fact, such a partial "loss" of the structure-making influence of the methylene groups on water (i.e., leading to a decrease of B), induced by "aromaticity", is in line with the general higher water-solubilities of aromatic compounds (exerting

a lower "negentropic" effect on water-structure) than those of the saturated hydrocarbons (as already mentioned earlier in Part 1), and is further supported by the reported larger "entropy loss" for the transfer of open chain amines and ethers from the gaseous state (at 1 atmosphere pressure) to dilute aqueous solutions, as compared to that for the corresponding cyclic analogues²².

Returning to eq.3.6.1, it follows, on substitutions of various concentration terms, as noted from tables 3.5.1 to 3.5.3, and of the B contribution of the hydrophilic groups (noted from table 3.5.5),

$$B_{\text{theor}} \times C_{\text{av}} = \left[0.025 \times 7.42 \times 44 \times 10^{-3} \times 0.6 + 0.0017 \times 3.83 \times 17 \times 10^{-3} \times 0.6 \right. \\ \left. + 0.04 \times 3.29 \times 17 \times 10^{-3} \times 0.6 \right]$$

$$\text{or, } B_{\text{theor}} \times C_{\text{av}} = \left[0.025 \times 0.195 + 0.0017 \times 0.039 + 0.04 \times 0.033 \right]$$

$$\text{or, } B_{\text{theor}} \times 0.089 = 0.0062$$

$$\text{whence, } B_{\text{theor}} = 0.069$$

A comparison of this B_{theor} value with the experimental value of B coefficient for HA (vide. table 3.5.6) reveals that the latter is much less than the former. In view of the approximate nature of the treatment, such a finding may be taken to indicate, with reasonable justification, that HA chain is coiled (folded); as a consequence, the hydrophilic groups (such as, -COO^- , phenolic (OH) , -NH_3^+), as well as the hydrophobic surface area of HA chain, are not fully exposed to the neighbouring water so as to enforce "ordering" in the latter. This cuts down the experimental value of the B coefficient which gives a direct measure of the solute-solvent interactions, causing the local viscosity of the solvent

to change.

In the next section, an attempt has been made to assess again such correlations between the experimental B (B_{expt}) and B_{theor} of the polymer (anion) in a semi-quantitative manner, on the basis of a simplified model²³ as outlined below.

(B) SEMI-QUANTITATIVE TREATMENT

Let us suppose the polymer, humic acid (HA), to be cylindrical (say P_0), arranged in a linear chain, and having a diameter D and length L ; the chain being very long, it is evident that $L \gg D$ (vide. figure 3.6.1).

The total surface area (S_0) of the cylinder is given by,

$$\begin{aligned} S_0 &= 2 \pi \frac{D}{2} \times L + 2 \times \frac{\pi D^2}{4} \\ &= \pi DL + \frac{\pi D^2}{2} \end{aligned} \quad (3.6.2)$$

On the other hand, if we further suppose that the chain is coiled on a cylinder of diameter d in a closely packed fashion ($d <$ diameter of a water molecule; the implied assumption is that the polymer chain, on coiling, does not actually touch face to face; also, $d \ll L$), the outer diameter of the new cylindrical system (say P') becomes equal to $(d + 2D)$ (vide. figure 3.6.1). The external rings, encircling the inner cylinder, are, in reality, very closely packed (unlike what is depicted in figure 3.6.1) so as to touch each other laterally.

The length (L_{eff}) of the new cylinder (P') is obtained as,

$$L_{\text{eff}} = \text{Length of each coil along the axis of the cylinder } P' \times \text{Number of such coils forming the cylinder } P'.$$

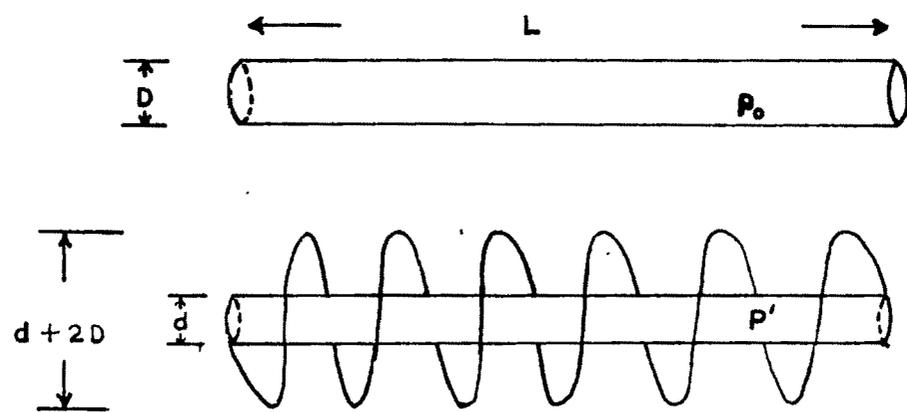


Fig. 3·6·1

$$= D \times \frac{L}{\frac{2\pi(d+2D)}{2}} \quad (3.6.3)$$

In evaluating the number of coils in eq.3.6.3, the circumference of each ring was arrived at by considering that of the outer surface of each ring forming P', i.e., a value, $2\pi(d+2D)/2$.

Therefore, the exposed surface (S') of the cylinder P' is given by,

$$S' = 2\pi \frac{(d+2D)}{2} \times L_{\text{eff}} + 2 \times \frac{\pi \left(\frac{d+2D}{2}\right)^2}{4} \quad (3.6.4)$$

Using the value of L_{eff} , as given by eq.3.6.3, we obtain,

$$S' = LD + \frac{\pi(d+2D)^2}{8} \quad (3.6.5)$$

Taking the ratio of the exposed surface areas of the coiled system to that of the linear chain, it follows from eqs.3.6.2 and 3.6.5,

$$\begin{aligned} \frac{S'}{S^0} &= \frac{LD + \frac{\pi}{8}(d+2D)^2}{\pi LD + \frac{\pi D^2}{2}} \\ &= \frac{1 + \frac{\pi}{8LD}(d+2D)^2}{\pi \left(1 + \frac{D}{2L}\right)} \\ &\approx \frac{1}{\pi}, \dots \quad (3.6.6), \end{aligned}$$

remembering that $L \gg D$ or d .

Because B coefficient relates to the exposed surface area (to water) of the solute (or, the disperse phase), the ratio, $B_{\text{expt}}/B_{\text{theor}}$, of the polymer chain is expected to approximate to the value of the corresponding ratio, $\frac{S'}{S^0}$, as given by eq.3.6.6, in case of a coiled polymer. However, the former for HA turns out to be 0.034/0.069, i.e., 0.49, whereas the latter, even though of

the same order of magnitude, is much lower, being $\frac{1}{\lambda}$, i.e., about 0.31. In view of the fact that the contribution of the hydrophobic surfaces of HA to enforce water - structure in the neighbourhood, through hydrophobic hydration, has been neglected in the present calculations while computing B_{theor} (which, if included, would have the effect of bringing down the ratio, $B_{\text{expt}}/B_{\text{theor}}$), and also the various simplifying assumptions made in the above semi-quantitative picture of the situation, coupled with a degree of uncertainty in the actual structure and state of conformation of the "natural" humic acid, as well as its "purity", the correlations obtained between the experimental results, and those predicted for the model, as presented, are considered satisfactory.

Further to testing the semi-quantitative model as above with a natural humic acid sample, attempt has been made to examine its suitability to describe the extent of coiling in a number of "synthetic" low molecular weight humic acids of much more well defined composition than that of the natural sample.

A scrutiny of literature²⁴ reveals that the given model has already been used for this purpose. Thus, a number of synthetic and microbial humic acids were prepared starting from :

- (i) hydroquinone and glycine (sample HA₁)
- (ii) hydroquinone and β -alanine (sample HA₂)
- (iii) glucose subjected to acid-refluxing (sample HA₃)
- (iv) catechol and glycine (sample HA₄)
- (v) catechol and β -alanine (sample HA₅).

The "chemical" humic acids were prepared by oxidation and polycondensation of the simple constituents, as above, in the

presence of $K_2S_2O_8$ ²⁵. In addition, a sample (HA₆) of the microbially synthesised humic acid (in presence of cultures of streptomyces sp.) is also used. The details of the methodology followed for preparation of these samples, HA₁ to HA₆, have already been given²⁵.

The data are recorded in table 3.6.1, which gives the estimated (theoretical) B_{theor} , as well as B_{expt} of these synthetic samples (as humates) of HA (HA₁ to HA₆) at 30°C, and also the ratio, B_{expt}/B_{theor} , obtained by the same methods as elaborated here.

The agreement between the postulated value of 0.31 (from the model presented earlier) of the ratio, S'/S_0 (vide. eq.3.6.6) and (B_{expt}/B_{theor}) ratio of the HA samples (vide. last column of table 3.6.1), is seen to be excellent, except for HA₃ which exhibits a rather large positive deviation. The interpretation of such "error" for the sample HA₃ may be sought from the corresponding aliphatic character which is also expected to be the highest in HA₃ among the various HA samples. This gets a qualitative support from the reported highest value of the ratio, E_4/E_6 , for HA₃ (E_4/E_6 , which is the ratio of optical densities of the HA suspension at 465 and 665 nm, is taken to denote the content of aliphatic chains in HA) among the present samples²⁴, and thus corroborates further what has been said earlier (in the section dealing with "method of calculation") regarding the error of neglect of B contribution of the hydrophobic surface of HA in computation of B_{theor} by eq.3.6.1. Such a "hydrophobic" term, if included to raise B_{theor} (vide. eq.3.6.1), would have the effect of bringing down the ratio, B_{expt}/B_{theor} , for HA₃ (from the observed value of 0.44), and hence have the desired effect in so far as concordance between S'/S_0

TABLE 3.6.1

Temperature = 30°C

Humic acid sample	B_{expt} of sodium humate/percent ⁻¹ B	B_{expt} of humate anion/percent ⁻¹ $B_{\text{expt}} = B - B_{\text{Na}^+}$	$B_{\text{expt}} / B_{\text{theor.}}$
HA ₁	0.098	0.091	0.30
HA ₂	0.097	0.089	0.27
HA ₃	0.120	0.110	0.44
HA ₄	0.096	0.089	0.28
HA ₅	0.089	0.082	0.28
HA ₆	0.106	0.096	0.29

and $B_{\text{expt}}/B_{\text{theor}}$ is concerned. As mentioned, such hydrophobic terms may possibly be deduced from the partial molal volumes of HA in order for the purpose that one may wish to have a greater degree of "quantitative" insight into the conformation of these large macromolecules in aqueous medium²⁶.

Similar experiments have been conducted with the fulvic acid (FA) fraction also, isolated from the Bigra Pond Sediment described earlier, and the results are recorded in table 3.6.2.

TABLE 3.6.2

Temperature 35°C

Percent concentration of FA for estimation of functional group	Amount of -COOH group of FA/1 meq.g	Amount of phenolic (OH) group of FA/1 meq.g	Amount of -NH ₂ group of FA/1 meq.g	Bcoefficient of Na-fulvate/1 percent (B _{expt})	B _{expt} of fulvate anion/1 percent (=B-B _{Na+})
0.4	38.4	10.56	2.39	0.450	0.413

The data indicate that the contents of -COOH and phenolic (OH) groups are much greater in FA than those in HA, while -NH₂ group content in FA is slightly less. Indeed FA, being an earlier condensation product of soil humus than HA, is expected to contain larger amount of various hydrophilic (e.g., -COOH group) functional groups (than HA); the latter get involved in the elimination of water and carbon dioxide in course of progressive gradual condensation. Furthermore, the fulvic acid chain at low pH being largely uncoiled^{9b} such (more numerous) carboxylic groups with their positive contributions to B, will increase the experimental B, in agreement with what is observed actually (B_{expt} for FA anion is 0.413, much more than that for HA).

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