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Influence of Amino Acids on Humification Process

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The condensation of amino acids L (−) Asparatic acid, Glycine and L (+) Lysine with phenolic compound vanillic acid was carried out through alkaline oxidation resulting into formation/synthesis of humic acid like substances. One nitrogen-free humus was also synthesised by self condensation of vanillic acid. The synthetic products were characterized by physicochemical methods. Asparatic acid was found to be more helpful in humification.

Key Word: Humic acids, Amino acids, Humification

Introduction

Acid hydrolysis of humic substances yields valuable information regarding building blocks of humus substances (Sowden & Schintzer 1967). It had been reported that the amount of amino acids and hence the nitrogen content differs depending upon the origin of the natural humic acids (Flaig et al. 1975). Molecular weight fractionation of low molecular weight fraction of humic acids, e.g. fulvic acids, showed that the nitrogen content declined with the decreasing molecular weight (Piper & Posner 1968). Further, Chattopadhyay et al. (1985) observed that nitrogen content was directly related to molecular weight. Sowden et al. (1976) observed that humic acids not only contained most of the soil nitrogen, but also relatively higher amount of basic amino acids. In the present study, attempts have been made to investigate the role of three different types of amino acids viz., acidic, neutral and basic in the humification process.

Materials and Methods

Synthesis of the Humic Compounds

The humic compounds were synthesized according to (Adhikari et al., 1978) in alkaline media, using equimolar amounts of phenolic component (e.g. vanillic acids) and an amino acid in the presence of an oxidant, $K_2S_2O_8$. One nitrogen-free polymer was synthesized under similar conditions by self-condensation of phenolic component. The products thus obtained are denoted as follows:

Starting materials	Humic compounds
Vanillic acid and L(−) Asparatic acid (acidic)	HA_1
Vanillic acid and Glysine (neutral)	HA_2
Vanillic acid and L(+) Lysine (basic)	HA_3
Vanillic acid (self condensation)	HA_4

The products formed characterized by different physio cochemical methods to understand the difference in chemical nature of these compounds.

(i) Total nitrogen content of the products was determined by Micro Kjeldhal method as described by Bremner (1960).

(ii) Electrometric Titration was carried out to determine the total acidity of the compound by titration with 0.01N NaOH solution (Schintzer & Gupta 1964) and carboxyl acidity was determined by Ca-acetate method (Schintzer & Gupta 1964). The phenolic OH content was determined from the difference between the total acidity and carboxyl

acidity. The "used up" carboxyl acidity was calculated from the difference between that of the product and theoretical value for the initial mixture.

The $p_{K_{int}}$ values were calculated as Tanford 1961

$$log\frac{a}{1-a} - pH = p_{K_{int}} - 0.868\ WZ$$

where a is the degree of neutralisation W, the electrostatic interaction factor, and Z, the net average charge. $p_{K_{int}}$ was determined from the plots of $pH - \log a/1-a$ vs the degree of neutralisation (a), extrapolated at zero neutralisation. Further, the change in p_K value (Δp_K) of carboxyl group was calculated from the difference between the $p_{K_{int}}$ value of the synthetic product and p_K value for α-carboxyl of amino acid.

(iii) Viscosity study was carried out to determine weight average molecular weight (Tager 1978) and β — coefficient value (Mondal & Sanyal 1984) of the synthetic products. The viscosity was measured at pH corresponding to the first inflexion point of the pH metric titration curve of each sample, at 25 ± 0.1°C. Ostwald Viscometer was used for this purpose.

According to the modified empirical relation of Staudinger (1930) known as Mark — Kuhn — Houwink equation (Tager 1978)

$$(\eta sp/c)_{c \to 0} = [\eta] = kM\alpha$$

where ηsp/c is the reduced viscosity;
[η], the intrinsic viscosity; and
M, the molecular weight
and K and α are characteristic constants dependent on the nature of polymer and solvent. For flexible polyelectrolytes, Gaberman and Litovkee (1968) determined the value of α and K as 0.65 and 7.3 × 10^{-4} respectively.

Again, to determine degree of solvation from viscosity measurement Jones-Donee equation (Jones & Dole 1929) was used.

$$\frac{\eta s_p}{c} = A + Bc$$

where A and B are empirical constants. A is related to electrostatic interaction of the solute ions with one another, while constant B corresponds to the interaction between the solvent and the solute ions. The knowledge of B is useful to understand the degree of solvation of polyelectrolytes.

(iv) *Spectrophotometric studies:* The E_4/E_6 value was determined by taking the absorbances at 465 and 665 nm at pH corresponding to the first inflexion point of electrometric titration curve in each sample as suggested by Schnitzer (loc cit).

(v) *Surface property studies:* The measurement of interfacial tension (γ) (dynes/cm) was carried out at different concentrations C (g/100 ml) of the synthetic product employing Dunöuy's torsion balance.

The maximum surface absorption Γ_{max} is related by the equation (Gibbs 1931)

$$\Gamma_{max} = -\frac{1}{RT} \cdot \frac{d\gamma}{d\ln c} \text{ mole/cm}^2$$

Further, the minimum cross-sectional area, A_{min} (Å^2/molecule), occupied by a single molecule at the surface is related by

$$A_{min} = \frac{1}{\Gamma_{max} N} \text{ cm}^2\text{/molecule}$$

where N is the Avogadro's number. A_{min} was calcuated from Γ_{max}.

Results and Discussion

It was observed (table 1) that the percentage of yield of the synthetic products followed the order $HA_3 < HA_2 < HA_1$. Further, it was observed (table 2) that the self condensation product HA_4 had the lowest molecular weight. Moreover, with the increase in acidity of amino acids, there was rise in the degree of condensation resulting in higher molecular weight and acidity of the product.

Exchange capacity of these products followed the order $HA_3 < HA_1 < HA_2 < HA_4$, so also their contents of carboxyl and phenolic acidity (table 1). It was noted

Table 1 *Data on yield and electrometric titration*

Synthetic products	% of yield	Acidity in m. eq./g					Used up carboxyl
		Reaction mixture		Synthetic product			
		Carboxyl	Phenolic	Total	Carboxyl	Phenolic	
HA_1	11.96	10.48	3.31	3.14	2.36	0.78	8.12
HA_2	5.13	9.63	4.11	4.02	2.94	1.08	6.69
HA_3	3.35	6.36	3.18	1.83	1.25	0.58	5.14
HA_4	—	5.94	5.94	5.60	3.08	2.52	2.86

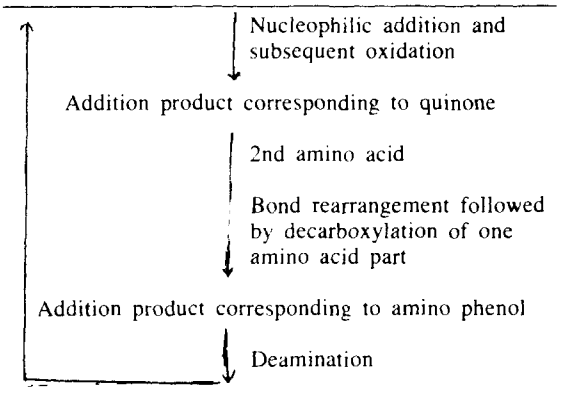
Table 2 *Data on molecular weight, molar acidity and degree of condensation*

Synthetic products	(η)	Molecular weight $\times 10^{-4}$	Molar acidity $\times 10^{-4}$ (m.eq./mole)			E_4/E_6
			Total	Carboxyl	Phenolic	
HA_1	0.7874	4.634	14.55	10.93	3.61	2.39
HA_2	0.5847	2.933	11.79	8.62	3.16	3.65
HA_3	0.4807	2.170	3.97	2.71	1.25	4.42
HA_4	0.2347	0.7204	4.03	2.21	1.81	6.45

that during condensation, the "used up" carboxyl acidity increased from HA_4 to HA_1.

The principal feature of reaction mechanism may be stated with reference to the work of Trautner and Roberts (1950)

Phenolic components + Amino acid



Therefore, the order of "used up" carboxyl acidity (i.e. loss of caboxyl acidity through decarboxylation process) may reflect the order of "nucleophilic addition" (Haider et al. 1965) to the substituted quinone. The reverse trend was followed by "used up" nitrogen (i.e., loss of nitrogen through deamination process) from HA_1 to HA_2 (table 3). It may be inferred that higher the degree of nucleophilic addition of amino acids, lesser would be the degree of their deamination during condensation. This view gains further support from p_k change (table 4). During condensation "loss of Zwitter ion character" and hence the "increase in strength of acidity" is found to be highest for HA_2. However, the break in the trend at HA_3 with respect to both p_k value and "used up" nitrogen indicated that the role of t-amino group in lysine was of little importance in the deamination process. It may thus be inferred that with the progressive decrease in basic character of amino acid (Lysince → glycine → asparatic acid),

Table 3 *Nitrogen content of reaction mixture and synthetic products*

Synthetic products	Percentage of nitrogen		"used up" nitrogen (%)
	Reaction mixture	Synthetic product	
HA_1	4.64	1.83	2.81
HA_2	8.90	2.30	6.60
HA_3	5.75	4.03	1.72
HA_4	0.0	0.0	0.0

Table 4 p_k *values of reactants and products*

Amino acids	p_k for α-carboxyl	$p_{k_{im}}$ for synthetic compounds		Δp_k
Asparatic acid	2.0	HA_1	5.9	3.9
Glycine	2.3	HA_2	6.8	4.5
Lysine	2.18	HA_2	5.05	2.87
—	—	HA_4	6.1	—

i.e. with the increase in carboxyl to amino group, ratio of amino acid molecule, percentage of yield, degree of condensation and molecular weight increased.

In case of natural humic acids, Chen et al. (1977) observed that the molecular size increased with the decrease in carboxylic acidity. The present study showed that the 'used up' acidity varied directly with the molecular weight.

The result of the surface property measurement (table 5) showed that HA_1 and HA_2 were more surface active than HA_2 and HA_1, surface activity being dependent both on the length of the nonpolar part as well as on the hydrophilic part. The length of nonpolar part becomes longer for HA_1 and HA_3 (both asparatic acid

Table 5 *Surface property measurement and computation of β-coefficient values*

Synthetic products	dγ Inc		Γ_{max} (moles/cm^2) × 10^{10}		A_{min} (Å^2/molecule)		β-coefficient in	
	Air/water	Oil/water	Air/water	Oil/water	Air/water	Oil/water	(Per cent)$^{-1}$	1(Mole)$^{-1}$ × 10^{-4}
HA_1	6.18	5.3	2.49	2.13	66.49	77.59	0.19	0.09
HA_2	1.22	1.0	0.49	0.40	338.98	411.28	0.03	0.01
HA_3	5.20	4.8	2.09	1.93	79.49	85.72	0.03	0.005
HA_4	1.25	1.2	0.50	0.48	329.05	343.05	0.06	0.004

and lysine have longer carbon chain). Therefore, the shooting up of nonpolar part of adsorbent molecules from the respective interfaces is more prominent in case of HA_1 and HA_3. However, under comparable length of nonpolar parts, charge density (−COOH group/gm) is a dominating factor, thus HA_1 was more surface active than HA_3. On the other hand, under comparable charge density, the length of the nonpolar part is a dominating factor for which HA_2 was more surface active than HA_4. The higher A_{min} value at the oil/water interface than at the air/water interface, could be attributed to the fact that in the oil layer, the cohesive forces are minimised and the dissolution of the polymer film is more than in water.

The extent of solvation as indicated by the β-coefficient value (lit. mole^{-1}) of macromolecules followed the same order as molar acidity content, i.e. $HA_1 > HA_2 > HA_3 > HA_4$. This is in agreement with the extended principle of additivity (Trautner & Roberts 1950) in respect of acidic (−COOH) group contribution towards β-coefficient values. The extent of solvation did not follow the same sequence of surface active behaviour, a fact which supported indirectly the role of nonpolar part to the surface activity of these compounds.

In conclusion, it might be stated that the basic amino acid served as better nitrogen source for its higher nitrogen content of humic material. But acidic one was more helpful for humification which was probably due to greater nucleophilic addition of amino acid to the substituted quinone. This view was supported by the fact that the synthetic product HA_1 had higher yield, greater degree of condensation higher molar weight and acidity. The extent of solvation of the synthetic product was dependent on the degree of hydrophilic part of amino acid, while its surface acitivity was dependent on the length of the hydrophobic part and charge density.

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