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Infra-red and Fluorescence—Excitation Spectroscopic studies on Synthetic Humic Acids

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The condensation of amino acids L (−) asparatic acid, glycine and L (+) Lycine with phenolic component (vanillic acid) under alkaline oxidation conditions resulted in the formation of three different humic acids. One nitrogen free compound and their hydrolysed products were characterised by infra-red and fluorescence excitation spectroscopic studies. The structure of the humic acid was infra-red from the studies.

Key Words: Syn. Humic Acids, Spec. studies

Introduction

Oxidative degradation products of humic acids show fluorescence under U V light and the wave length of fluorescence maxima of these aromatic degradable compounds are much shorter than observed in case of natural humic acids. It is likely that synthetic humic acids or their degradable compounds may exhibit fluorescence. Current view (Seal at al. 1964, Visser 1983) is that humic fluorophore are aromatic moiety with electron donating functional groups similar to phenols, carbazoles etc. and not a nitrogen bearing group. Visser (1983) observed a positive correlation between the aromatic OH group content of humic matter and the wave length of the main excitation peak. Mataga et al. (1956 a,b) observed a shift in the fluorescence maximum identical with that of absorption one on changing solvent from hydrocarbon to ether to alcohol and considered as due to the gradual increase in solute-solvent hydrogen bond formation. This effect of solute-solvent hydrogen bonding on the electronic spectra of organic molecules has already been discussed by Nagakura and Baba (1952) in terms of FRANCK-CONDON principle by Pimental (1957). The present study attempts to investigate some of these facts from spectral analyses of synthetic humic acids.

Attempt has hitherto been made to understand the structure of natural and synthetic humic compounds, by several researchers (Stevenson & Goh 1971, Adhikari et al. 1978) for the assignment of I R spectral bands of natural and synthetic products. In the present paper, I R spectral of some synthetic humic acids were studied with reference to their respective reactants with an objective to get an insight into the assignment of spectral band and also to get an idea about the nature of amino acid involved in humification.

Materials and Methods

The preparation of synthetic humic acids was done as reported earlier (Adhikari & Roy Choudhury 1988). These were synthesized in alkaline media using $K_2S_2O_8$ as an oxidant (Adhikari et al. 1978) and hydrolysed with 6 N HCl for 6 hr as stated by Ozebk (1977).

Starting materials	Humic compound	Hydrolysed product
Vanillic acid and L (-) asperatic acid (acidic)	HA_1	HA_1 (HY)
Vanillic acid and Glycine (neutral)	HA_2	HA_2 (Hy)
Vanillic acid and L(+) Lysine (basic)	HA_3	HA_3 (Hy)
Vanillic acid (self condensation product)	HA_4	—

The I R spectra of humic compounds are taken following KBr disc technique in PERKIN ELMER-782 I R spectrophotometer.

Fluorescence spectra are recorded with the help of PERKIN ELMER MPF-44B. Dry sodium salts of the humic acids were dissolved in the solvent immediately before recording the spectra. Their solubility in non-aqueous solvents are very low. However, both the excitation and fluorescence monochromators are varied until the detector shows a fluorescence peak. Then the excitation monochromator is set at this wave length (λ_{ex}) and the fluorescence monochromator is allowed to scan the sample to find out wave length of maximum radiation $(\lambda_{em})_{max}$. The fluorescence monochromator is next set at this wave length $(\lambda_{em})_{max}$ and the excitation monochromator is thus allowed to scan the sample to record the fluorescence excitation specturm.

Results and Discussion

Comparative assignment of I R spectal bands of synthetic humic acids and their hydrolysed products were carried out with reference to their respective reactions as shown in figure 1,2, and 3 respectively. It is observed that the broad band observed near 3500 cm^{-1} region in HA_4 becomes more flatter towards higher wave length for the other three compounds due to N-H stretching vibration.

The next characteristic difference in spectral band is observed in 1500-1700 cm^{-1} region. Stevenson and Goh (1971) distinguished humic acid (HA) and fulvic acid (FA) into three types depending upon the absorption in 1600-1700 cm^{-1} region of I R spectral band. The first type (I) showed equal absorption band at 1720 cm^{-1} and 1600 cm^{-1}. The second type (II) represented by some FA, had a strong 1720 cm^{-1} band with only a shoulder at 1650 cm^{-1}. The third type (III) have had a broad band centered at 1620-1650 cm^{-1} with only a weak shoulder at 1720 cm^{-1}. The latter contained

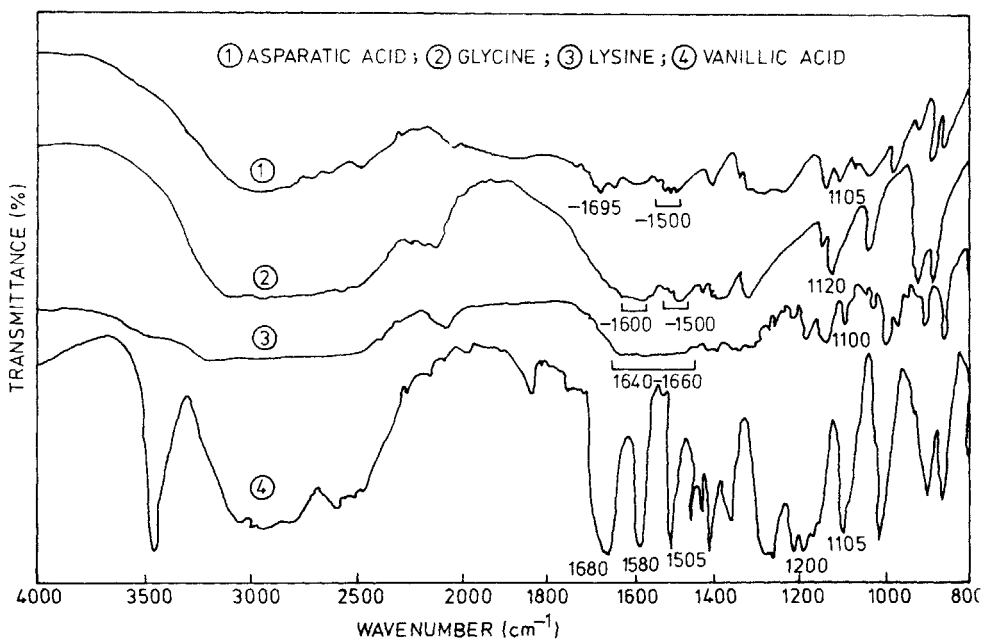

Figure 1.

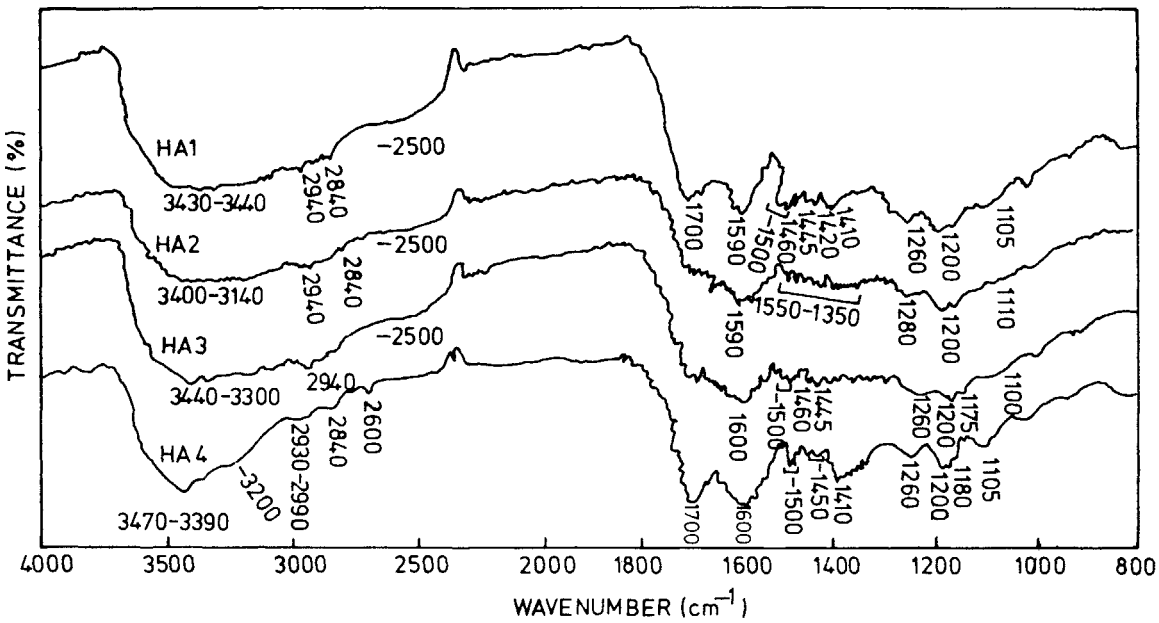

Figure 2

more peptide linkage as indicated by a strong shoulder in the 1540-1520 cm^{-1} region. Again Ballamy (1975) assigned 1600 cm^{-1} and 1510 cm^{-1} as for C=C aromatic ring, recently called ring breathing hands. In the present study, HA_1, HA_1(Hy) and HA_4 resemble type I, whereas HA_2 & HA_3 are like type III. The presence of ß-carboxyl group is HA_1 accounts for C=O Str. band at 1700 cm^{-1} which suggests the non-involvement of second carboxyl group in humification to HA_1. This is further evidenced by the presence of 1410 cm^{-1} band for NH_4 and salt of carboxylic acid. The band at 1700 cm^{-1} is present in nitrogen free synthetic product HA_4. The band for C.....O str. (anti) of carboxylate ion at ~1600 cm^{-1} region is present both in HA_1 and HA_4. The strong shoulder at 1650-1700 cm^{-1} in HA_2 and HA_3 is due to polypeptide group (amide I band), upon hydrolysis (Filip et al. 1974), this band splits into 1600 cm^{-1} and 1700 cm^{-1} band in HA_3 resembling type I (figure 3). In case of HA_2 due to hydrolysis 1600 cm^{-1} band becomes more intense but surprisingly 1700 cm^{-1} band does not appear. Instead, the shoulder at 1650-1700 cm^{-1} region appears but is less intense. Such a possibility for product has earlier been ascribed to either lower no. of carboxylic group (Stevenson & Goh 1971, Martin-Martinez 1967, Saiz-Jimenez & Martin-Martinez 1972) or to the presence of carboxylate ion (Salfeld 1964). Since the cation exchange value for HA_2 is high (table 5), the latter possibility may exist which is further evidenced by 1420 cm^{-1} band for C..... Str. (Sym.) of carboxylate ion.

The amide II band for polypeptide linkage in the 1500 cm^{-1} region is found along with C ..... C Str. band of aromatic nucleus (figure 2). Upon hydrolysis, complexity in this region disappear leaving a fairly residual narrow band similar to HA_4 for aromatic C=C Str. (figure 3).

It is noted that band at 1100 cm^{-1} region, on hydrolysis becomes more intense particularly for HA_1. Flaig (1964) assigned this band to arylalkyl ether linkage like syringyl component. Because of the appearance of bands at this region in their reacting components, the proper assignments is difficult. The probability of acid catalysed ether formation (like syringyl component) during hydrolysis is evidenced from the fact that the band at 1200 cm^{-1} is more intense for HA_2 and HA_3 while in case of HA_1, this is a weak broad band peak centered at 1100 cm^{-1} (figure 3).

This study reveals that on the basis of absorption at 1600-1700 cm^{-1}region, these synthetic compounds

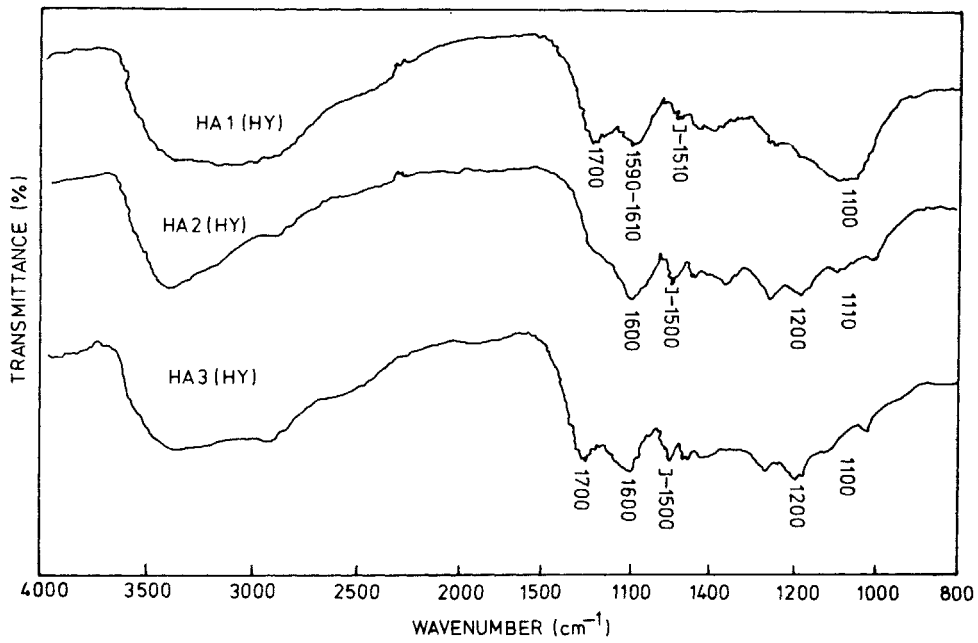

Figure 3.

Table 1 *Data on Fluorescence* spectral *study and acidity content of synthetic compounds*

	$(\lambda_{em})_{max}$ in ethanol	$(\lambda_{ex})_{max}$ in			Acidity (in m.eq/g)	
		Cyclohexane	Ethanol	Water	Phenolic	Carboxyl
HA_1	When (λ_{ex}) = 320 nm sig. −10 353 nm.—	When $(\lambda_{em})_{max}$ = 353 nm sig. −3 300 nm.—	When $(\lambda_{em})_{max}$ = 353 nm sig. −10 295 nm.—	When $(\lambda_{em})_{max}$ = 353 nm sig. −100 317 nm.—	0.78	2.46
HA_2	When (λ_{ex}) = 320 nm. sig. −10 355 nm. 415 nm	When $(\lambda_{em})_{max}$ = 355 nm = 415 nm sig. −3 Sig. −30 297 nm. 260 nm	When $(\lambda em)_{max}$ = 355 nm = 415 nm Sig. −3 Sig. −10 300 nm. 317 nm	When $(\lambda_{em})_{max}$ = 355 nm = 415 nm Sig. −100 Sig. −30 311.75 nm. 324 nm	1.08	2.94
HA_3	When (λ_{ex}) = 320 nm. Sig. −10 328 nm. 335 nm	When $(\lambda_{ex})_{max}$ = 354 nm = 413 nm Sig. −10 Sig. −10 294 nm. 258 nm.	When $(\lambda_{em})_{max}$ = 354 nm. = 413 nm. Sig. −10 Sig. −10 297 nm. 317 nm.	When $(\lambda_{em})_{max}$ = 354 nm. = 413 nm. Sig. −100 Sig. −10 318.5 nm. 316 nm	0.58	1.25
HA_4	When (λ_{ex}) = 293 nm Sig. −10 328 nm. 335 nm	When $(\lambda_{em})_{max}$ = 328 nm = 335 nm Sig. −3 Sig. −3 288 nm. 296 nm	When $(\lambda_{em})_{max}$ = 328 nm. = 335 nm Sig. −10 Sig. −3 286 nm 292 nm	When $(\lambda_{em})_{max}$ = 328 nm. = 335 nm. Sig. −100 Sig. −100 295 nm 302 nm	2.52	3.08

Abbreviations: λ_{ex} excitation wave length, λ_{em} emission wave length, λ_{max} wave length peak

can be classified into two types e.g. HA_1 and HA_4 as one type, where both bands at 1600 cm^{-1} and 1700 cm^{-1} are present and HA_2 and HA_3 is another type. Again, identification between HA_1 and HA_4 can be made on the basis of 1500 cm^{-1} region for the absence of polypeptide group in HA_4, whereas for HA_2 and HA_3 from their hydrolysed products where absence of band at 1700 cm^{-1}in HA_2 is noted. Fluorescence excitation spectra revealed that (table 1) excitation wave length, peak (λ_{max}) increases with increase in polarity of the solvent for HA_1, HA_2 and HA_3 compounds resulting into red shift. This suggests that both ethanol and water act as proton acceptors to π-electron system as a result of which $\pi - \pi^*$ transition occurs. But with HA_4, changing to ethanol from cyclohexane as solvent, blue shift occurs suggesting that $n - \pi^*$ transition occurs due to the proton donation property of ethanol. Both the excitation λ_{max} and emission λ_{max}, are slightly different in HA_1, HA_2 and HA_3 due to the difference in extent of electron donation ability of peptide group substituted to the aromatic ring. The absence of such group in HA_4 results very lower λ_{max}, which accounts for blue shift in ethanol medium.

With increase in polarity of solvent (e.g. Cyclohexane-ethanol — water), the instrumental signal gain is made high to take the spectra due to the decrease in fluorescence intensity (table 1) with increase in polarity of solvent, solubility increases and hence flexible nature of the compounds are more pronounced as a result low frequency vibrational motion on the parts of the molecule occurs with respect to each other causing internal quenching and hence reduced fluorescence.

The present study does not show correlation between the –OH content of humic acids and the wave length of the main excitation peak (λ_{max}).

It appears from the present study that I R spectral bands at 1500 cm^{-1} and 1600-1700 cm^{-1} regions of these synthetic humic acids or hydrolysed product help to differentiate humic acids with respect to their respective nitrogen involvement in the structure during humification process and the presence of polypeptide group as identified by 1500 cm^{-1} band plays very important role in determining the excitation and emission wave length peak (λ_{max}). The absence of this polypeptide group in HA_4 accounts for wide difference in λ_{max} for HA_1 from the rest of numic acids.

Acknowledgements

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