
CHAPTER - IV

CHARACTERISATION OF THE HUMIC SUBSTANCES

SECTION - A
ELEMENTAL ANALYSIS

Nowadays comprehensive information is available on the elemental composition of the humic substances. Gillam¹ found no essential differences between the chemical composition of humic acids isolated from different soil. However, Kononova², with the humic acids, demonstrated on the basis of % C, % O, % H that some humic acids in some soil are complex in structure and some are simpler. Fulvic acids in general contain a lower % C and higher % O than the corresponding humic acids which indicate the simpler structure of the former³. The elemental composition of the synthetic humic acid too lie in the ranges of those of the natural ones⁴.

Experimental

The ash-free samples were analysed by the well known Duma method.

Results and Discussion

The scrutiny of the results suggest, in the case of soil, the percentage of carbon in humic acid is greater than that in the corresponding humatmelanic acid and such is the case in respect of nitrogen content also.

It apparently indicates that the hymatomelanic acid is simpler in form compared to the humic acid of the same origin. Again the fulvic acid contains a lower percentage of carbon than the corresponding humic and hymatomelanic acid in soil pointing to the simpler structure of the former³. Another point of interest is that the percentage of nitrogen is maximum in the synthetic humic acid. The percentage of carbon, hydrogen, nitrogen, oxygen and ash content of different humic materials are presented in table - 1. The results are mostly in good harmony with the inferences drawn by Kononova³.

TABLE - 1

Elemental composition of humic substances

Sample	% C	% H	% N	% O	% Ash content
SHA	58.46	3.80	3.97	33.77	1.7
PHA	56.91	4.00	0.91	38.18	0.9
SFA	37.11	3.28	1.95	57.66	3.2
PFA	38.17	3.30	2.20	56.33	3.8
SHYA	54.56	5.51	2.04	37.89	2.2
SYNHA	57.54	3.08	4.44	34.94	-

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SECTION - B
POTENTIOMETRY

The polybasic character of humic acids owes its' existence mainly to the presence of carboxyl and phenolic hydroxyl groups. Potentiometric titrations are usually employed in order to identify the functional groups and to determine their amount and relative orientation in a polyfunctional macromolecule .

The acid nature of humic acids and its titratability were the subjects of controversy for many years. Oden¹ suggested that the humic acids should be treated as true acids, whereas Baumann and Gully² were of opinion that they were colloidal complexes with high power of adsorption because the acidity appeared only on the addition of salts. These two contradictory hypotheses were bridged by Rindell³ .

Eichhorn⁴ explained that the 'increased acidity', caused by the interaction of either peats or humates with the neutral salts, had been due to a 'Cation exchange phenomenon', on the other hand Daikuhara⁵ assumed that adsorbed iron and aluminium salts were replaced and their hydrolysis in solution could be responsible for the observed acidity. Later workers⁶ inferred that both the processes might operate simultaneously.

Anderson et al.⁷ potentiometrically titrated an electro-dialysed humic acid sol as well as a synthetic humic acid prepared by the action of sulphuric acid on sucrose. They noted that CEC of soil humic acid was greater than that of the synthetic one, but similar to the results obtained by

Thiele and Kettner⁸, the titration curves showed no inflexion points but were somewhat similar to the adsorption isotherms. Later Feustel⁹ found definite breaks in the curves in very dilute solutions, possibly due to some secondary reactions, e.g. hydrolysis of complex esters, attack on the phenolic groups, etc., breaks were absent in the concentrated solutions. Gillam¹⁰ reported two distinct inflexion points in the potentiometric titration curves. Some investigators are of the opinion that the carboxyl groups are neutralised below pH 7.0, provided there are only two acid groups involved. A new calculation of the results obtained from the titrations made by Thiele and Kettner⁸ led Pommer and Breger¹¹ to establish that the humic acids behaved like weak acids and the curves were not similar to the isotherms as observed earlier.

Gillam¹⁰ also attempted to have a quantitative evaluation of the contribution individually made by -COOH and phenolic -OH groups with the help of electrometric titrations of acetylated and methylated humic acids. Investigations on fulvic acids by Gamble¹² suggested that the carboxyl groups present are of two types of which one is ortho to the phenolic hydroxyl. Electrometric titration of humic acids by Zadnarski¹³ with alkali and alkaline earth hydroxides displayed a greater rise of pH value with the former and the exchange capacity of the base in the process at pH 7 followed the order, $\text{Ba(OH)}_2 \rangle \text{Ca(OH)}_2 \rangle \text{KOH} \rangle \text{LiOH} \rangle \text{NaOH}$. However, this order of reactivity was contradicted

by Chatterjee et al.¹⁴, who found the sequence as Ca(OH)_2 }
 Ba(OH)_2 } NaOH. Posner¹⁵ carried out the potentiometric
 titration of humic acids to study the effect of ionic
 strength on their titration properties and thereby to
 prove their polyelectrolytic behaviour. Martin and Reeve¹⁶,
 Khanna and Stevenson¹⁷ also made similar investigation in
 this direction. They all established the polyelectrolytic
 character of humic acids by their observations. A detailed
 study in this field by Mukherjee et al.^{18,19} and others²⁰
 also suggested that the humic acids were weak polyelectrolytic
 acids with exchange capacities varying from 200-800 me/100 gm .

Experimental

The potentiometric titrations were carried out with the
 aid of a digital pH meter with combined electrode (E.C. -
 Toshniwal uóller, 0 to 14 pH, 0°C to 17°C). The titrations have
 been carried out continuously with NaOH .

The titration curves are shown in fig. 1, and electro-
 chemical data calculated from them are presented in Table 2.

Results and Discussion

The electrochemical behaviours of all the samples studied
 showed close resemblance to each other. Moreover, each of them
 exhibited weak polyelectrolyte acid character¹¹.

The electrometric titration curves with the bases alone
 always showed two breaks, one for carboxyl and the other for

phenolic hydroxyl groups, with other features akin to those of weak acids. However the plot of $\frac{dpH}{dv}$ vs. v . (not shown) reveals a few other inflexion points, none-too-prominent and not detectable on superficial scrutiny of the curves, indicative of the polyprotic nature of the acids.

The shapes of the titration curves with NaOH, displaying a steeper rise of pH values, as was also observed by Zadnarski¹³. The high rate of pH increase for NaOH may, perhaps, be attributed to the hydrolysis of sodium salt of the humic substances formed in course of titration. This potentiometric titration curves in presence of salts (which we have not considered) in each case yielded a stronger acid character and a suppression of initial pH. This manifested the polyelectrolyte character of the samples. On the addition of salts, the groups which were shielded from neutralization due to the macromolecular conformation become available to the added alkali, and the CEC values increased appreciably. Moreover instead of two inflexions, a single inflexion was observed.

With regard to the variable concentration of the humic acids, a scrupulous examination was made not only of the nature of the titration curves but also of the calculated electrochemical parameters, viz. CEC, first and second inflexions, initial pH and at the equivalence point.

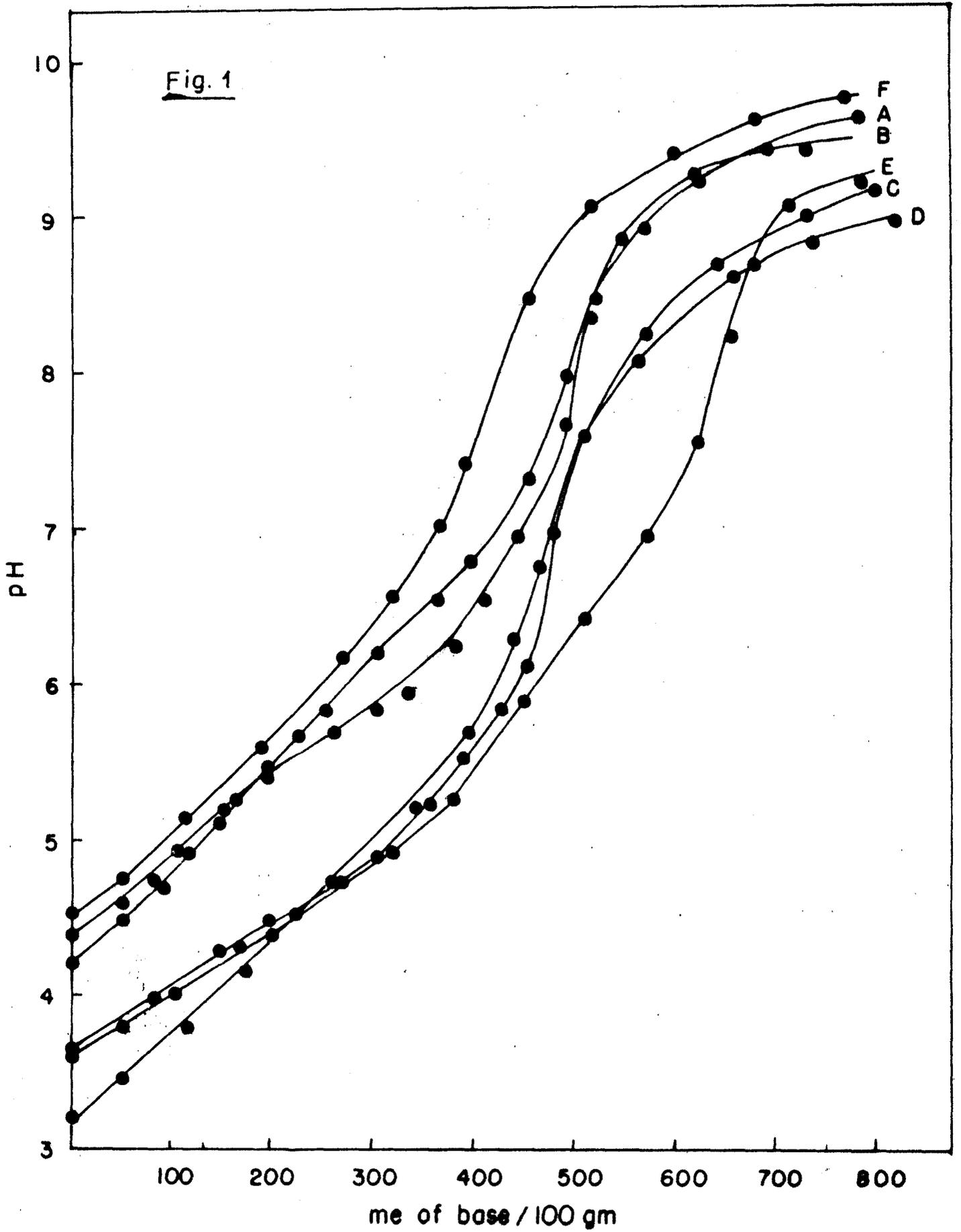
The results are seen to be in good agreement with those reported by the previous workers^{13,14,20}. The CEC value of synthetic humic acid is found to be less than that of soil humic

acid. This is understandable in view of the Lesser number of -COOH groups present in the former acid.

T A B L E - 2

Electro chemical parameters of humic acid fractions from potentiometric titration with NaOH

Sample	Potentiometric inflexion		Total acidity me/100 gm	Initial pH	pH at inflexion point
	First	Second			
Soil Humic acid (A)	250.0	462.0	462.0	4.2	7.70
Peat Humic acid (B)	300.0	496.0	496.0	4.4	7.50
Soil Fulvic acid (C)	270.0	500.0	500.0	3.6	7.20
Peat Fulvic acid (D)	250.0	470.0	470.0	3.2	7.50
Soil Hymatomeianic acid (E)	350.0	522.0	522.0	3.6	7.30
Synthetic Humic acid (F)	225.0	400.0	400.0	4.5	7.20



POTENTIOMETRIC TITRATION CURVES OF
HUMIC SUBSTANCES

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SECTION - C

VISCOSITY

The polyelectrolytic behaviour, particle size and weight of macromolecules can be determined by viscometric investigations. Several workers^{1,2} have shown the polyelectrolytic behaviour of coal humic acids with the help of such studies. Piret³ also reported the polyelectrolytic character of peat humic acids by their viscosity and sedimentation behaviours, which are in direct contradiction with the work made by Flaig et al., who showed the spherical shape of humic acids by viscometric⁴, ultracentrifugation⁵ and electron microscopic⁶ measurements. Recently the polyelectrolytic behaviour of natural and synthetic humic acids have been confirmed by Mukherjee et al.^{7,8} with the aid of viscometric measurements.

However, Wershaw et al.⁹ attempted to bridge between the aforesaid two contradictory theories by assuming the particles not only variable in size but also in shape a proposition which was further supported by Kahn¹⁰ who considered that humic acids might contain a mixture of both linear and spherocolloids. The presence of charged groups along the chain makes polyelectrolytes remarkably different from all other polymers, and all of their differences with respect to neutral polymers are a consequence of these charges which are an integral part of the polyionic molecule .

According to Fuoss and Strauss¹¹, reduced viscosity of a non-electrolytic polymer is practically independent of concentration. But, for polyelectrolytic macromolecules reduced viscosity usually rapidly rises with diminishing concentration, an observation made by several investigators^{2,8} in case of humic substances. However,

Chen and Schnitzer¹² reported that the reduced viscosity of humic and fulvic acids increased with diminishing concentration particularly at lower concentration region. The same observation was also made by Terayama and Wall¹³.

The present viscometric investigation is confined to the studies of reduced viscosity in respect of concentration for all the systems. No attempt has, however, been made to determine their particle size or particle weight .

Experimental

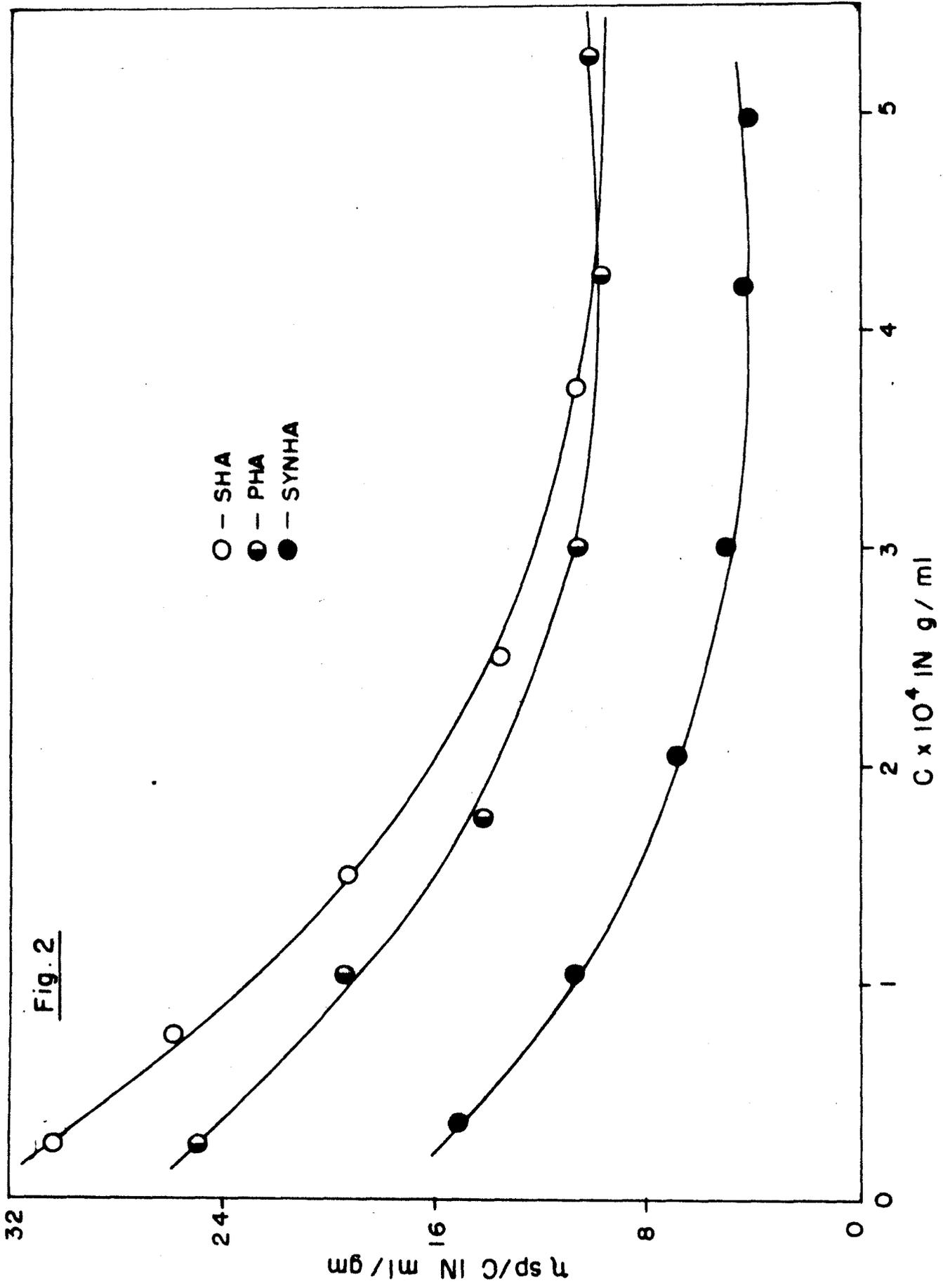
Viscometric measurements of sodium salts of humic, hymato-melanic and fulvic acids at pH 7.5 in aqueous solutions were carried out with the help of Ubbelohde viscometer (capacity - 15 ml) having a flow time 300 sec. kept in water thermostat at $30^{\circ}\text{C} \pm 1$.

The solutions, being very dilute, were assumed to have the same density as that of the solvent.

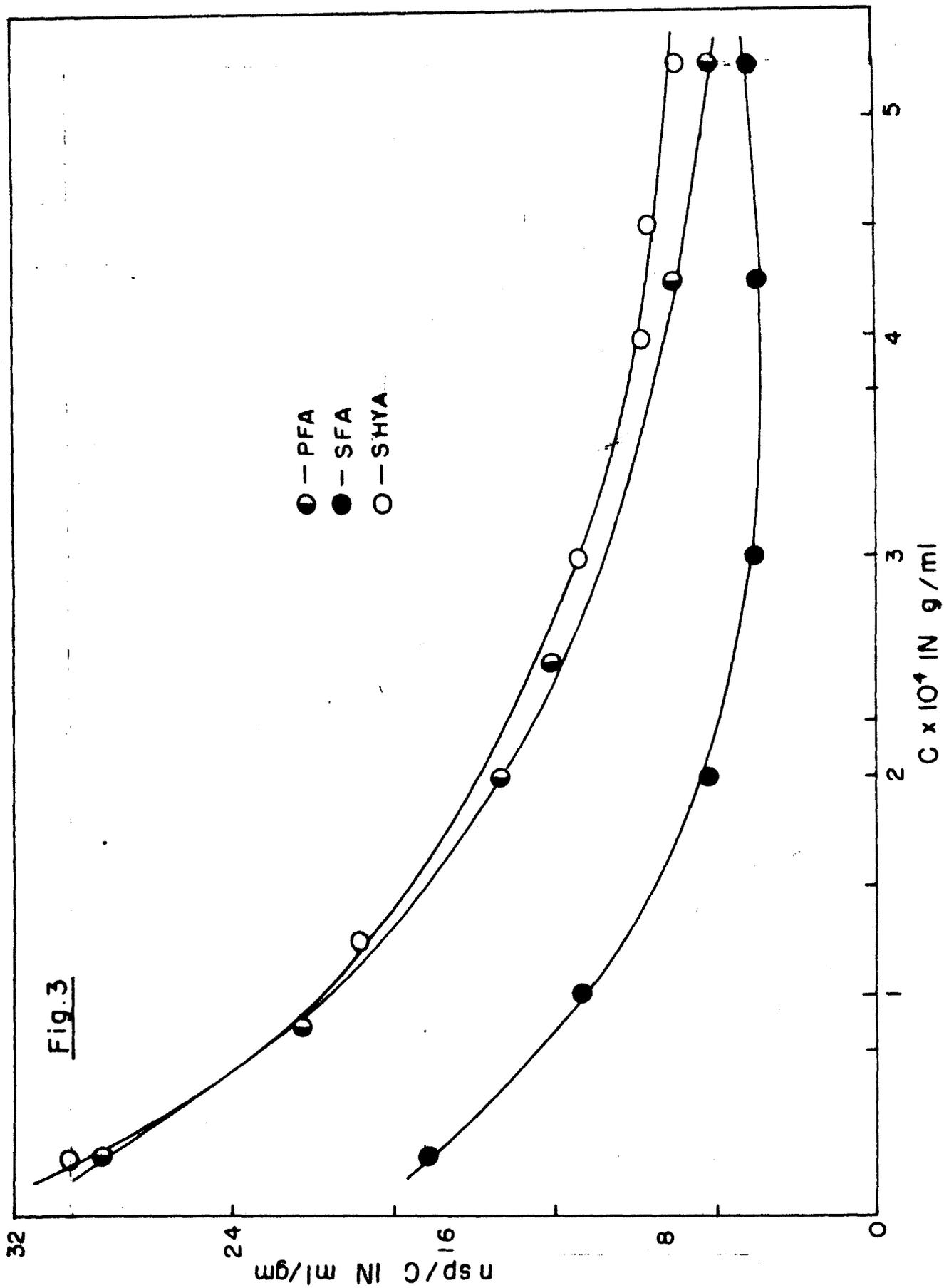
Discussion

The typical behaviour of polyelectrolytes has been displayed by all the reduced viscosity - versus - concentration curves (Figs. 2 & 3). From the graph it is found that the reduced viscosity increases with diminishing concentration. As the concentration is gradually decreased, counterions

diffuse away from the polymer backbone and the repulsion between similarly charged centres on the polymer chain causes the latter to expand. The chain is coiled up in solution, the coiling does not vary appreciably with concentration at least in the dilute region in a good solvent, the extent of coiling being dependent on the kinetic energy and internal Brownian motion. In case of polyelectrolytes, at infinite dilution, all the ionizable groups are dissociated and the molecule assumes an extended configuration accompanied with the maximum possible charge separation¹⁴. The upward rise of the reduced viscosity against concentration, in the dilute region is thus caused by intra-molecular repulsion. In addition there is the electroviscous effect and the long range electrostatic coupling between the macromolecules¹⁵. Viscometry offers an almost unique opportunity to study a number of important characteristics of humic substances, such as particle weights, particle volumes, particle shapes and dimensions but here we have examined only the polyelectrolytic behaviour of humic substances .



VISCOSITY BEHAVIOUR OF HUMIC SUBSTANCES



VISCOSITY BEHAVIOUR OF HUMIC SUBSTANCES

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SECTION - D

VISIBLE ABSORPTION SPECTROSCOPY

The visible absorption spectra of humic substances are featureless. The absorption decreases monotonically with increasing wave length exhibiting no maxima or minima and gives little structural information¹.

The chromophoric groups formed by oxidation are likely to be responsible for absorptions in the visible region. The typical chromophores, known to occur in humic compounds, are C=C, C=O etc., and the auxochromes which are supposed to be present are C-O, C-NH₂ etc.^{2,3}.

Several attempts have been made^{2,4}, to obtain some information regarding the structure and properties of humic substances from the absorption spectroscopic measurements. The most widely accepted absorption spectroscopic information is the ratio of optical densities at the wave lengths 465 nm and 665 nm. The ratio referred to as E_4/E_6 ratio, appears to be independent of the concentration of humic substances, but varies with differences in the genesis of the soil organic matter in diverse soil environments⁵.

Some workers⁶ have suggested that light absorption of aqueous humic acid and fulvic acid solutions in the visible region of the spectrum increases with the ratio of carbon in aromatic nuclei to carbon in aliphatic side chains, the total carbon and the molecular weight. In general, the progressive humification and increased condensation are indicated by a decrease in E_4/E_6 ratio, so that this ratio could serve as an index of humification. These ratios for humic acids from

different soil types have been reported to be in the range 2.2-5.0⁷. Fulvic acids, however, show higher values of this ratio ranging between 6.0-8.5⁵.

Later on, Chen et al.⁸ studied thoroughly in this field. According to them, though the E_4/E_6 ratio was independent of the sample concentration, they were unable to obtain evidence that the ratio was directly related to the concentration of condensed aromatic structures. They also concluded that the E_4/E_6 ratio was governed by particle size, affected by pH and related to free radical concentrations, contents of O, C, COOH and total acidity .

Experimental

The spectra of aqueous solutions of all the humic materials at pH 7.5 (raised by the addition of NaOH solution) were recorded with the help of a Beckman Spectrophotometer (Model - DU-2). The spectral ranges examined were 350 nm to 700 nm. The measurements were carried out at room temperature, and the concentration of the solution was 0.01% in each case. Optical density (O.D.) against wavelength (λ) plots were obtained for each experiment in the wavelength ranges mentioned above. E_4/E_6 ratios, i.e. the ratio of the optical densities at 465 nm and 665 nm, were evaluated from the above mentioned plots and are presented in table - 3 . The absorption curves (O.D vs λ) are depicted in Figs. 4 & 5 .

Results and Discussion

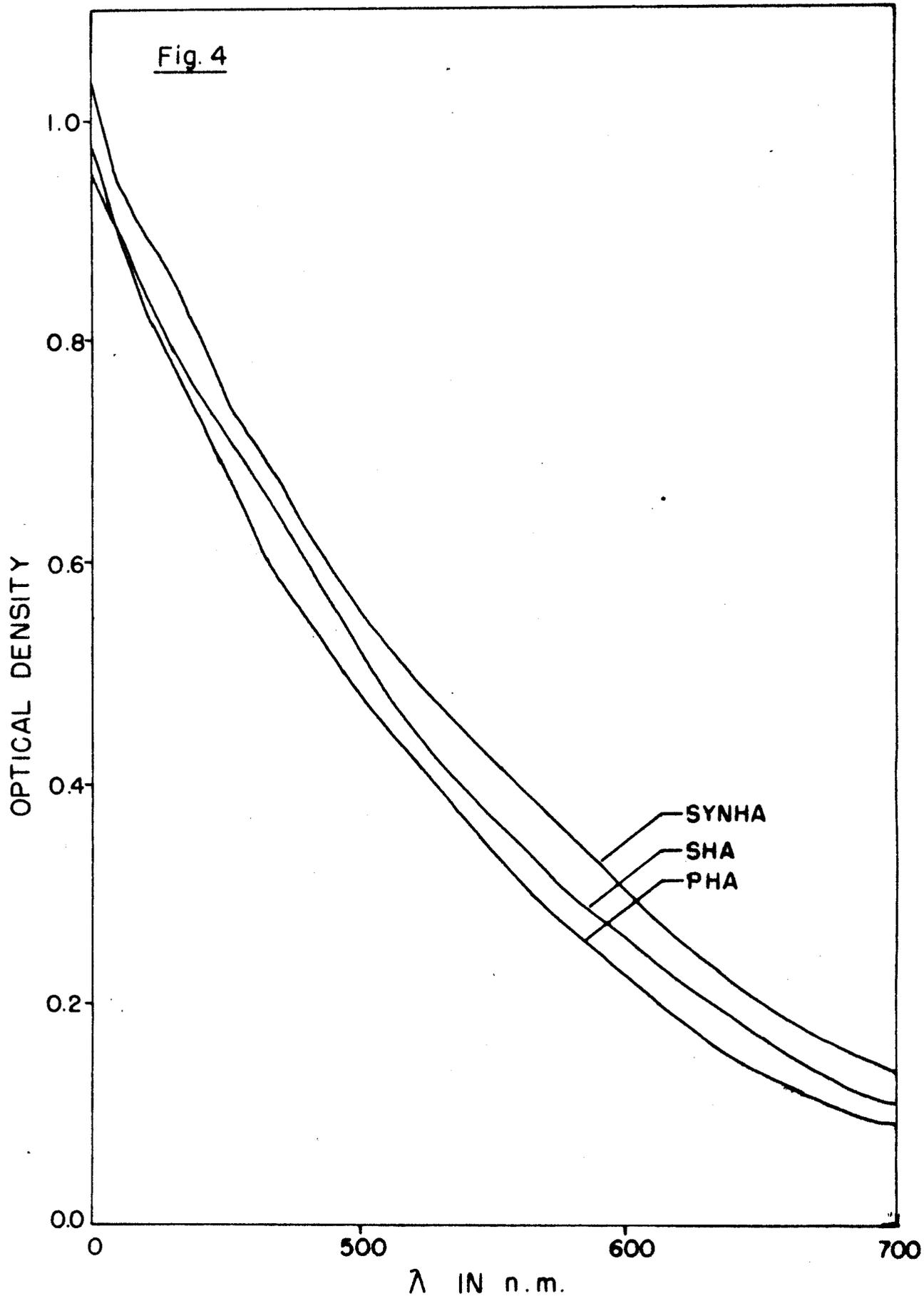
All the recorded spectra (Figs. 4 & 5) are featureless and clearly resemble to each other, which suggest that all the humic materials have similar chemical structures with overlapping chromophoric absorptions. The non-specific nature of the spectra points to the fact that the molecules contain similar types of π -electron distribution. High optical density at shorter wave lengths may be attributed to the increased mobilities of π -electrons over aromatic carbon 'nuclei' and unsaturated structures conjugated with these 'nuclei'.

The optical densities, from the curves, of humic and hymatomelanic acids are always greater than those of corresponding fulvic acids.

The table, where E_4/E_6 values were recorded, indicates that this ratio for humic and hymatomelanic acid is lower than that of the fulvic acid from the same origin, followed by the lesser condensation of the aromatic net of carbon atoms in the latter. Again these values for humic and hymatomelanic acids from the same source seem to suggest that the former is a little more condensed than the latter⁵.

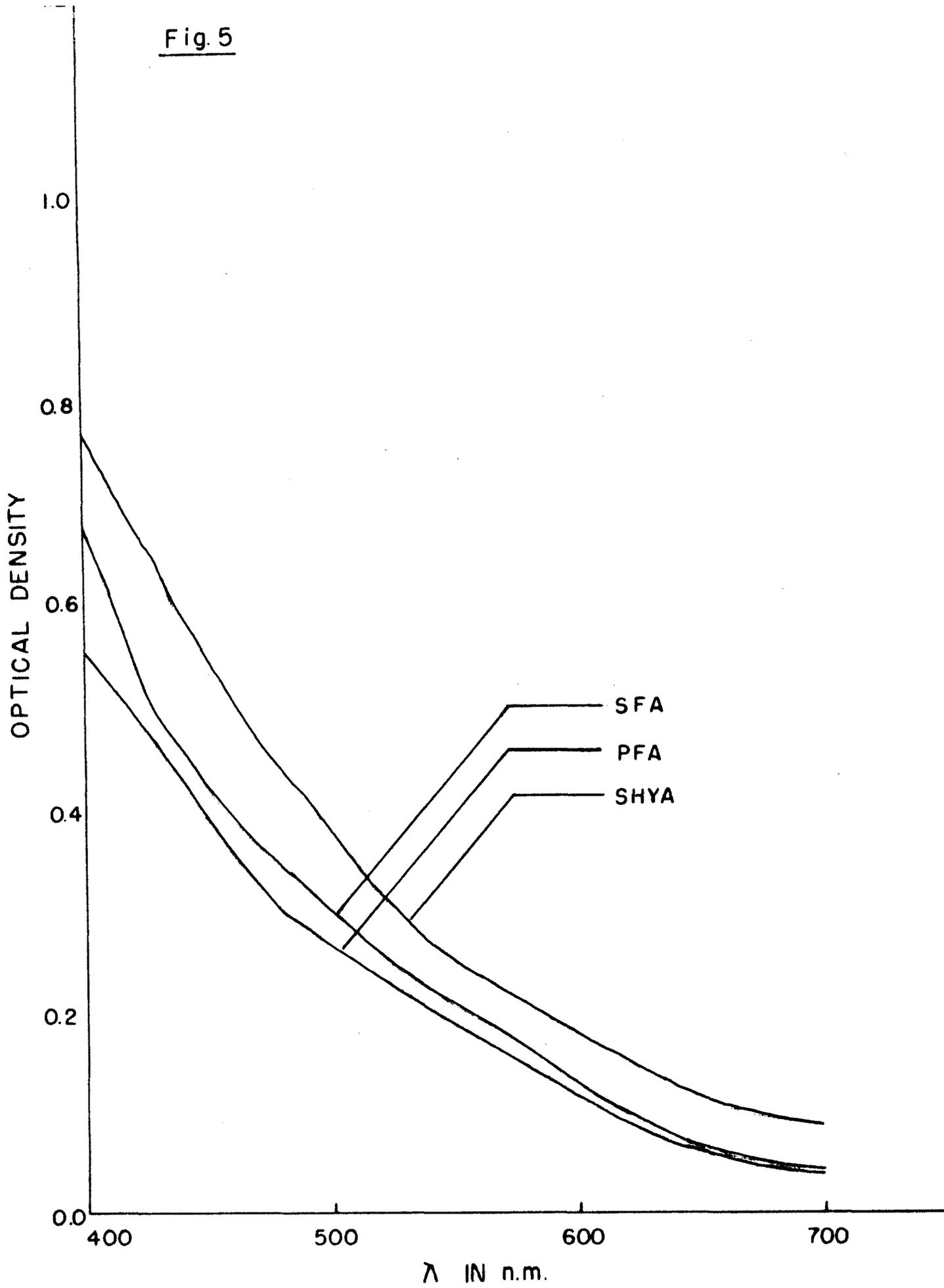
TABLE - 3Visible absorption spectral data of humic
substances

Sample	E_4/E_6
SHA	4.52
PHA	5.00
SFA	7.40
PFA	7.20
SHYA	4.79
SYNHA	3.96



VISIBLE ABSORPTION SPECTRA OF THE HUMIC SUBSTANCES

Fig. 5



VISIBLE ABSORPTION SPECTRA OF THE HUMIC SUBSTANCES

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SECTION - E

I-R SPECTROSCOPY

Attempts have been made by several investigators to characterise humic substances, both natural and synthetic with the help of I.R. Spectroscopy^{1-7,9}. As the organic matters are, in most instances, the conglomeration of more or less complex molecules with different types of linkages and functional groups leading to the overlapping of the absorption bands, the assignment of specific absorbances in their cases suffer from limitations. The I.R. Spectra of humic matter in fact, display some absorbances, characteristic of the chemical nature of the molecules, probably originating from the similar groups in different molecular surroundings, and not from the identical structural features².

The present investigation includes I.R. - Spectroscopic studies on different samples of natural humic, humatomelanic and fulvic acids as well as of synthetic model humic acid .

Experimental

All the humic materials (both natural and synthetic) prepared according to methods presented in Chapter-III. The compounds were dried at 60^oc in an air oven and then kept in vacuum for 48 hours. The infrared spectra were recorded by Beckmann Model IR-20 infrared spectrophotometer. The samples were prepared in the form of discs using KBr - pellet technique⁸.

2.0 mg of thoroughly dried material was ground manually with 200 mg of oven-dry KBr in a agate mortar for about 5 minutes

at room temperature. Then an additional 200 mg of KBr was added and the grinding was continued for another 5 minutes. The mixture was transferred to a KBr - discs press (Beckmann, U.S.A.) which was evacuated. The pellets were pressed at 10 tons total pressure for 15 minutes. The KBr disc with the sample was placed in one beam of the spectrophotometer and the blank consisting 400 mg of another KBr disc treated in the same manner as the samples was placed in the comparison beam .

Results and Discussion

The I.R. spectra of different samples recorded are displayed in the figures 6 and 7 and the main absorption bands observed in each case are listed in the table 4.

The spectra are qualitatively similar to those obtained by earlier workers^{3,5,7,9,10} .

The absorbance near 3300 cm^{-1} and 3400 cm^{-1} observed in the humic substances may be attributed to the hydrogen bonded OH - groups. The C = O of COOH group and C = O stretch of carbonyl groups are responsible for the intense bands at $1720\text{-}1740\text{ cm}^{-1}$. But in case of synthetic humic acid the band 1720 cm^{-1} is not pronounced (or weak) because of the lower number of -COOH group present in the molecule. A number of groups e.g. hydrogen bonded C = O of carbonyl, aromatic C=C, double bond conjugated with carbonyl and COO - group may have

given rise to another intense band found in the region 1600-1680 cm^{-1} . It has been found that for SFA and PFA the absorption bands near 1725 cm^{-1} region are quite intense while those near 1600 cm^{-1} region are not much prominent. This is perhaps, due to much more COOH groups than COO^- groups present in these acids. The bands near at 1200 cm^{-1} , intensify with the synthetic sample but weaken for the natural ones; this may be assigned to C - O stretch or OH- deformation of COOH groups. The absorption band in the range of 1050-1150 cm^{-1} , found with SHA, SHYA, SFA and PFA, are probably attributable to Si-O-Si and Si-O-C valences and deformation vibrations. The band in this region at 1080 cm^{-1} is very prominent for SFA and PFA. The band at 1720-1730 cm^{-1} is found to be intense for the samples from natural sources while it is nearly a shoulder in the case of those of synthetic origin, which suggests the presence of considerably more COOH groups in the former than in the latter. Another noticeable point of difference between the samples of natural origin and the synthetic ones is the appearance of the intense band at 820 cm^{-1} in the spectra of the latter. This band originates from the out of plane vibration of C - H in the aromatic ring⁷.

However, no remarkable difference in the spectral pattern of natural humic, hymatomelanic and fulvic acids, which may precisely help to distinguish between them, is discernible .

TABLE - 4

I.R. spectral data of humic substances

Sample	Main absorbance band frequencies (cm ⁻¹)					
SHA	3300,	1720,	1630,	1230,	1100,	1030
PHA	3300,	1720,	1600,	1200,	1100,	
SFA	3300,	1730,	1625,	1360,	1230,	1080, 970
PFA	3300,	1720,	1620,	1400,	1220,	1080
SHYA	3400,	1720,	1620,	1250,	1120,	1030
SYNHA	3300,	1600,	1200,	1100,	820	

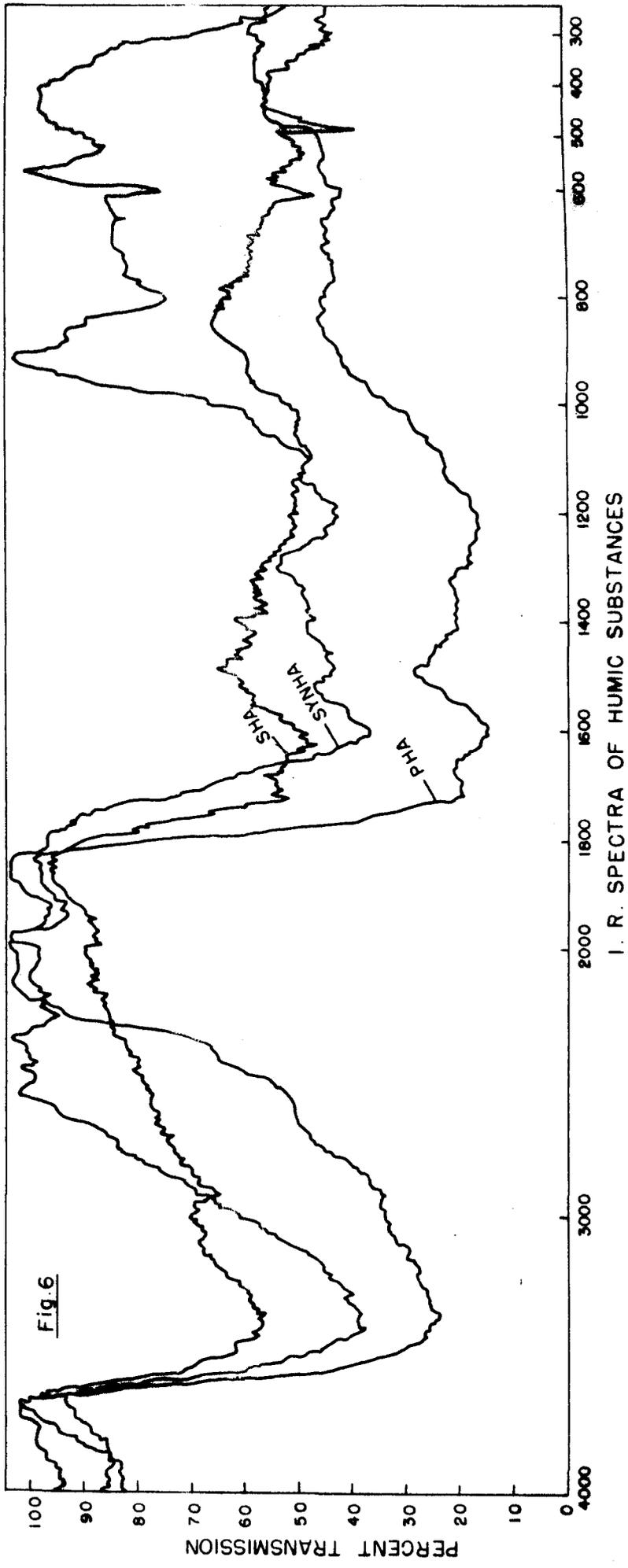


Fig. 6

I. R. SPECTRA OF HUMIC SUBSTANCES

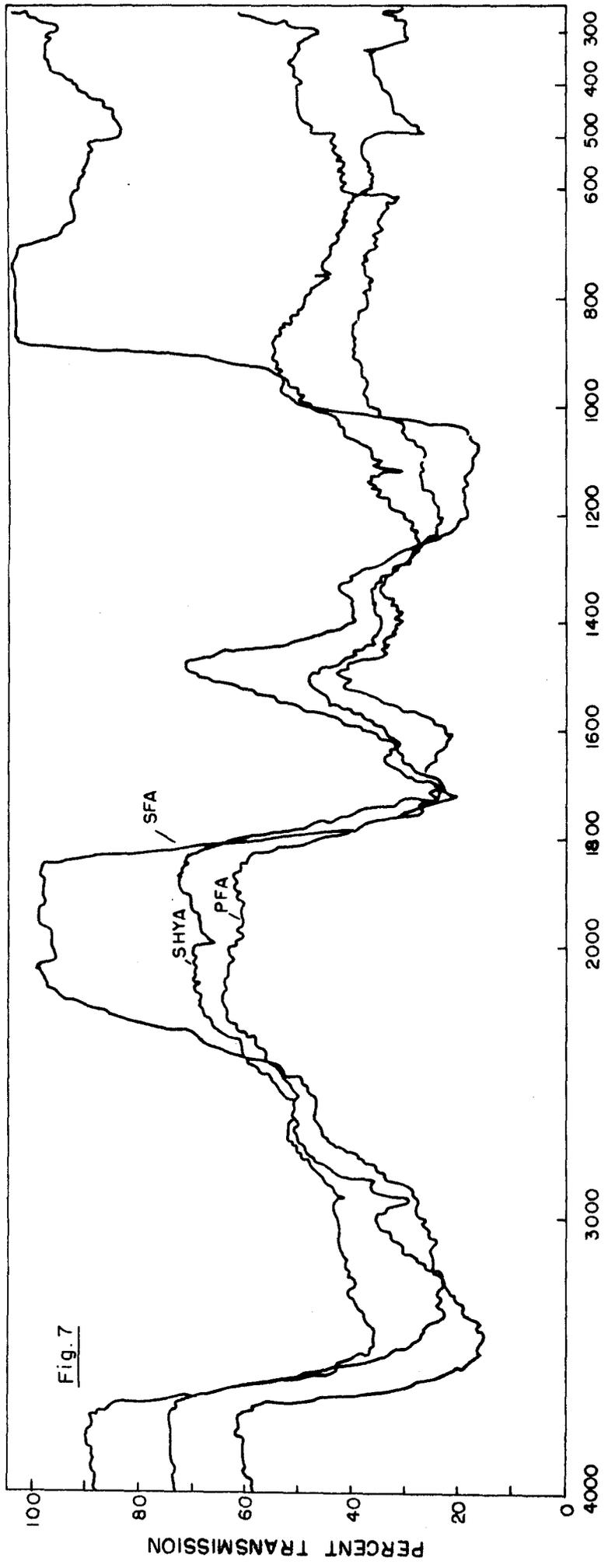


Fig. 7

I. R. SPECTRA OF HUMIC SUBSTANCES

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SECTION - F

E.S.R. SPECTROSCOPY

Electron spin resonance is based on the fact that atoms, ions, molecules and molecular fragments that have an odd number of electrons or unpaired spin exhibit characteristic magnetic properties.

An electron may be in any of the two possible and distinct spin states. The electro magnetic moment for an electron in such a state is generally expressed as $g\mu_B$ where μ_B is the Bohr magneton, a true constant and 'g' is the spectroscopic splitting factor which is variable. For a completely free electron, the value of 'g' is 2.0023 MC/gauss, but when strong coupling occurs, the value differs appreciably. This method of study is a relatively new technique in humic acid field and has been applied by a number of workers¹⁻⁸, and almost all of them concluded that humic compounds contain relatively high amounts of stable free radicals of the semiquinone type.

Apparently they are stable over periods of years under air and can survive the geochemical processes of humification and coalification, and may have wide ranging effects on many reactions that occur in soil. It is likely that the free radicals participate actively as intermediates in oxidation reduction reactions in terrestrial and aquatic environments.

Thus these compounds play an important role as electron donors and acceptors in nature and so are capable of governing many biogeochemical reactions that occur in these systems.

It is well known that flavin electron transport involves the formation of semiquinone intermediates. Similarly, humic substances rich in phenolic and quinonoid groups^{9,6,10} can mimic flavoproteins by disproportionating as metastable semiquinones¹¹. Although it appears that semiquinone intermediates are involved in oxidation-reduction process of humic substances.

Experimental

Thoroughly dried, finely ground humic materials were taken in standard ESR sample tubes which were tightly stoppered. ESR spectra were recorded in all cases at room temperature on a varian associates E-3 spectrometer, employing 100 KHZ modulation and a nominal operating frequency of 9.5 GHZ with a Scan range 400 G. The spin concentrations were estimated by comparison with standard values of DPPH. The peak to peak separation of the derivative signal was taken as line width. The magnetic field at the sample was calibrated with DPPH (diphenyl-picrylhydrazyl). 'g' values were calculated by using the equation

$$\frac{H_2}{H_1} = \frac{g_u}{g_k} \quad \text{where } H_2 \text{ is the magnetic field at which resonance}$$

occurred for the DPPH of known g (g_k) value and H₁ for the sample under study of unknown g-value (g_u) .

Results and Discussion

ESR spectra, representative of all humic substances that we have examined, consist of a single symmetrical line devoid

of any hyper fine splitting, which indicates that the free radicals are extremely complex in structure.

Rex¹ first observed that humic acid contain free radicals, produced as a result of dehydrogenation and oxidation in which H-atoms were removed from such groups as aromatic $-OH$, $-NH_2$ or $-SH$ present in humic acids. Thus wide range of compounds can form semiquinone type of structures which appear to be trapped in humic acid molecule. But Tollin and Steelink² have rejected the existence of trapped free radicals but believe that humic acid itself is a stable free radical or a mixture of free radicals of semiquinone type.

In the present study, all the humic substances have got 'g' values which correspond to the existence of free radicals in them. Soil and synthetic humic acid have got nearly similar 'g' values and line width indicating almost similar type of free radicals in them. In case of soil and peat fulvic acid, the 'g' values and line width are almost similar which also indicates the similar type of free radicals in them.

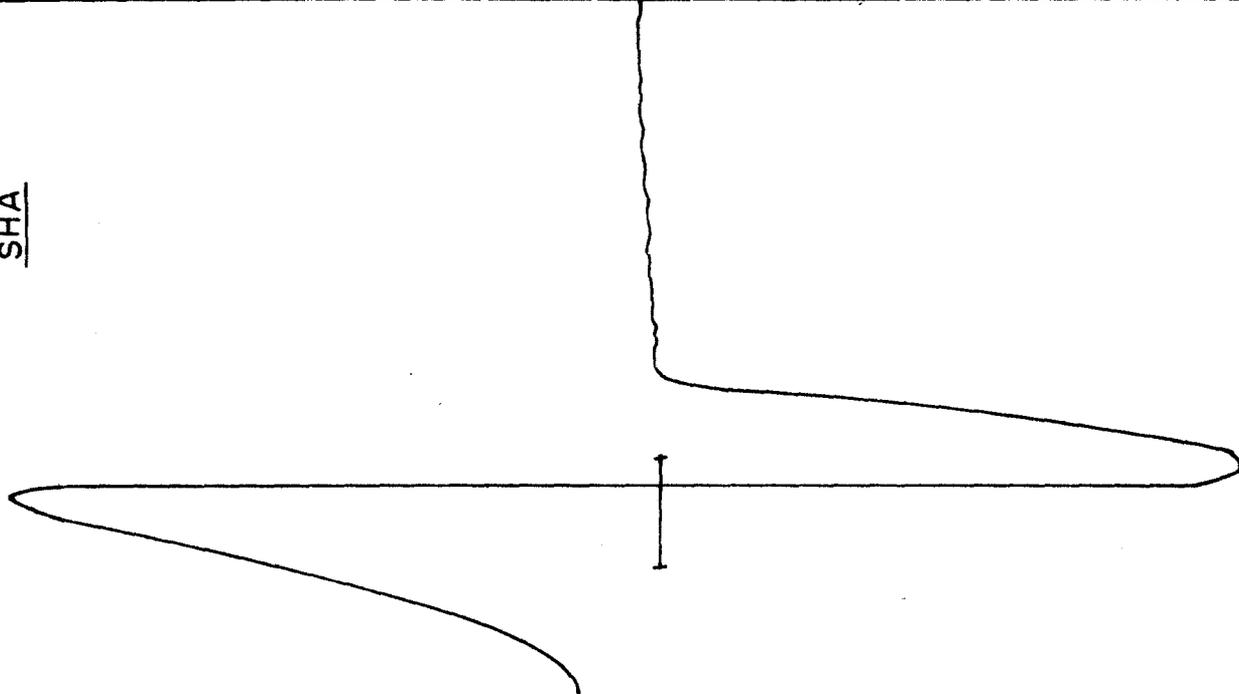
The line width in case of fulvic acid of both soil and peat origin are greater than that of the humic substances which indicates the lower number of free radicals present in fulvic acid molecule than humic acid molecule. The parameters calculated from ESR study of humic materials were listed in table - 5 and the figures were placed in figs. 8,9 & 10.

T A B L E - 5
ESR parameter of humic substances

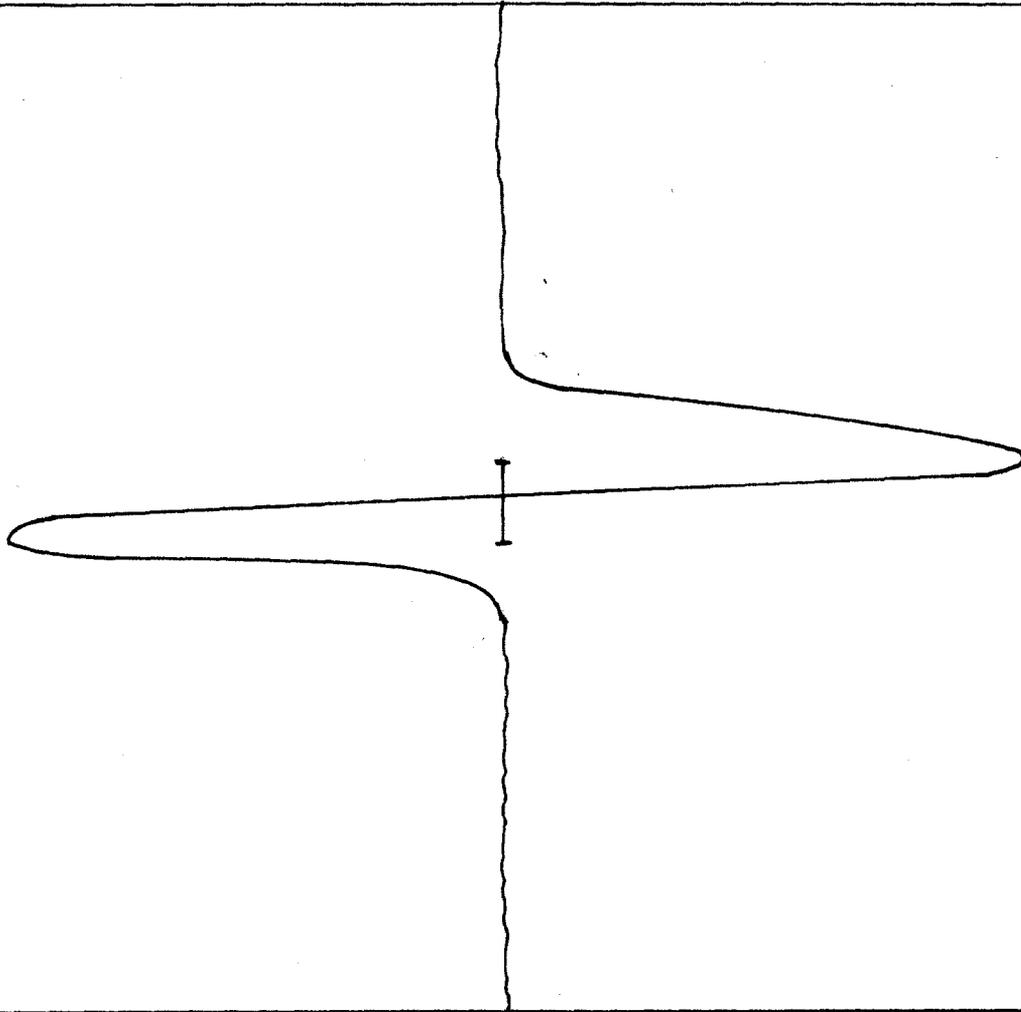
Sample	Line width in gauss	"g"	Spins/gm
Soil humic acid (SHA)	10	2.0035	73.25×10^{16}
Peat humic acid (PHA)	6	2.0034	68.52×10^{16}
Synthetic humic acid (SYNHA)	9	2.0038	89.57×10^{16}
Soil Hymatomelanic acid (SHYA)	8	2.0036	80.64×10^{16}
Soil fulvic acid (SFA)	12	2.0040	14.79×10^{16}
Peat fulvic acid (PFA)	12	2.0042	16.47×10^{16}

Fig. 8

SHA



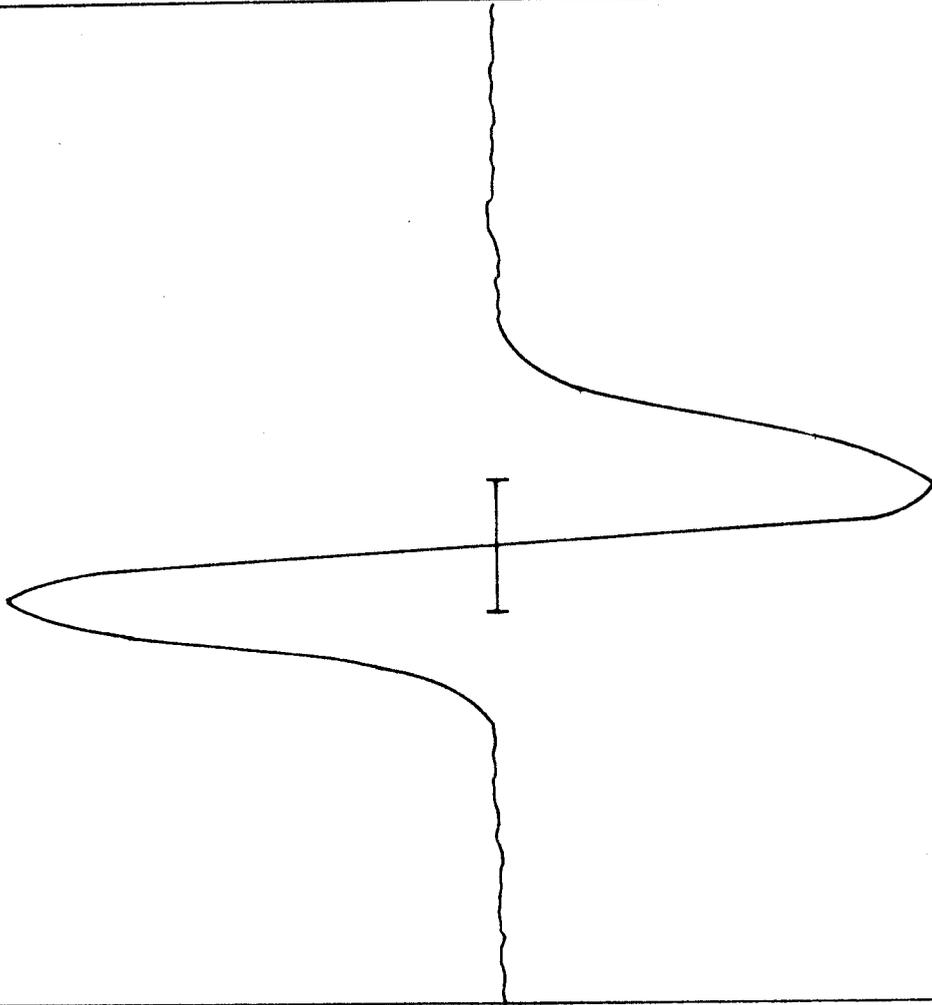
PHA



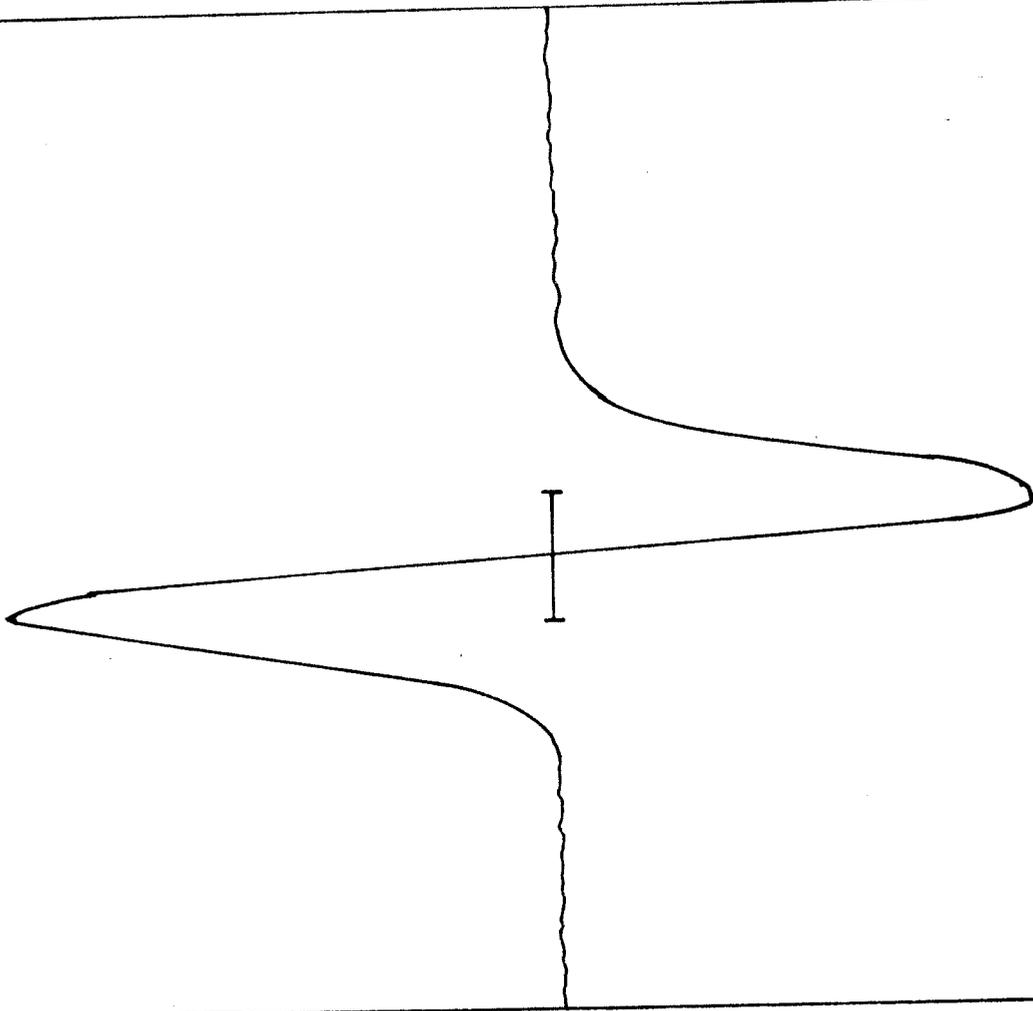
E. S. R. SPECTRA OF HUMIC SUBSTANCES

Fig. 9

SFA



PFA

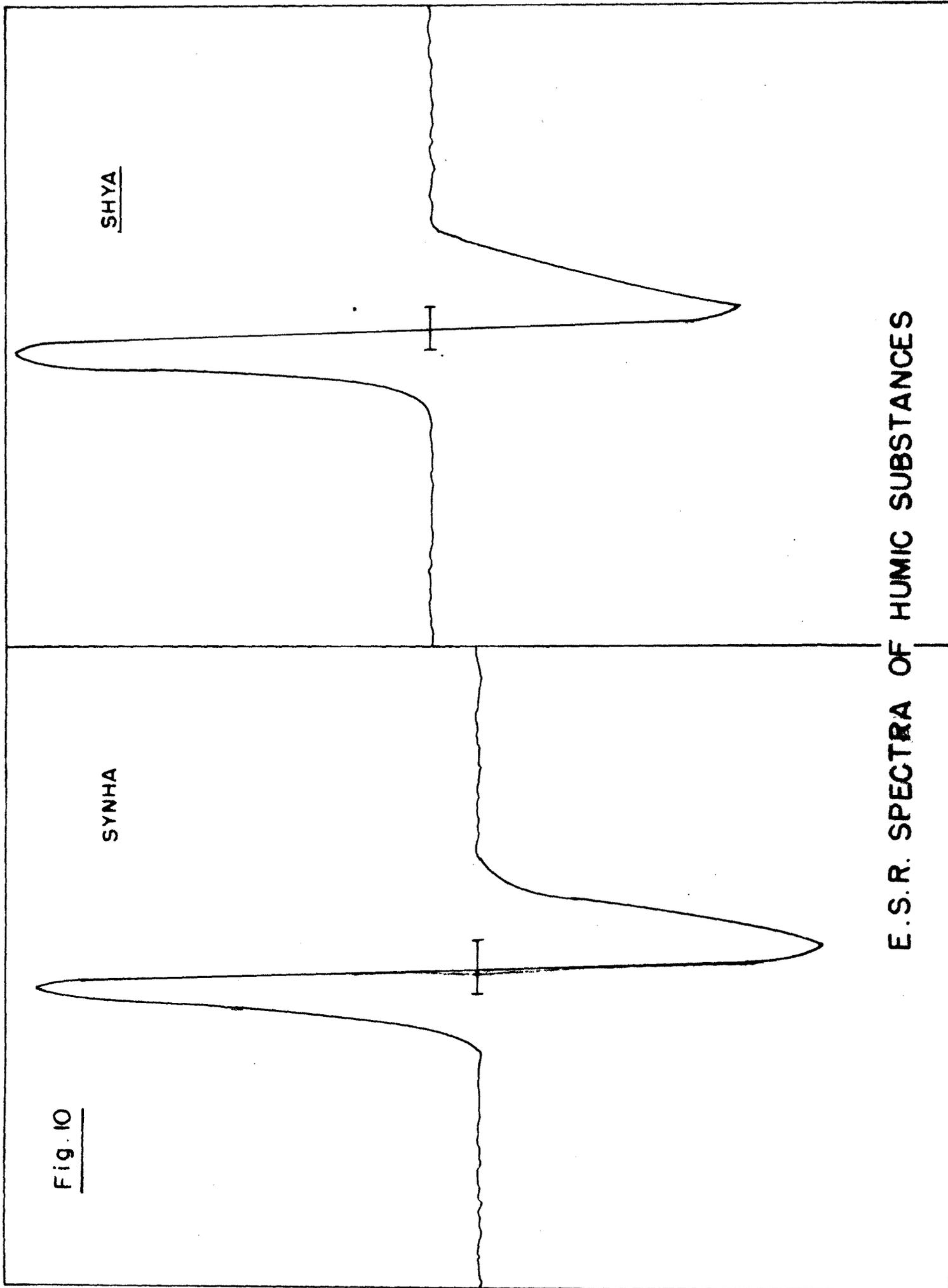


E.S.R. SPECTRA OF HUMIC SUBSTANCES

Fig. 10

SYNHA

SHYA



E.S.R. SPECTRA OF HUMIC SUBSTANCES

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SECTION - G

FLUORESCENCE EXCITATION SPECTROSCOPY

Many investigators^{1,2,3} showed while fractionating humic acids by chromatographic method, the fluorescence characteristics of humic acids. Humic acids are heterogeneous in nature containing a number of fluorescing groups. During their study of fluorescence characteristic of humic and fulvic acids, some workers^{4,5,5a} noted that all the fractions of humic acids exhibit fluorescence with emission maxima at 470 m μ .

The present study aims to obtain information about the molecular structure of different humic acids in terms of fluorescence excitation spectra.

Experimental

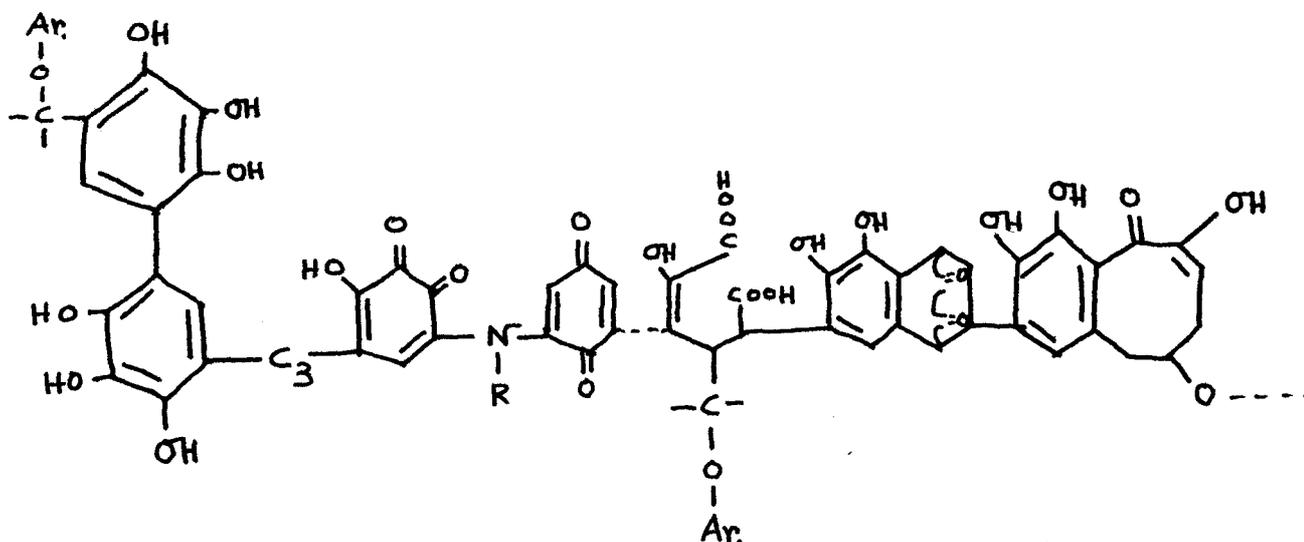
The fluorescence measurement was made in a Farrand Spectrofluorometer. The fluorescence attachment consists of a high pressure Xenon arc lamp which serves as the source of ultraviolet and visible energy imaged on to the entrance slit (1 mm) of an exciting monochromator. Energy of any selected wavelength is directed from this monochromator on to a sample. The resulting fluorescence of the sample at right angle to the exciting beam enters an "analysing" monochromator which feeds into a photomultiplier producing a signal that might be read on a microammeter. The samples in water were taken in a fused quartz cell of 1 cm thickness. The fluorescent excitation at different wavelength were recorded in terms of microammeter reading. The fluorescence spectra of sodium salts of natural and synthetic humic substances were recorded.

Results and Discussion

Humic acids are weakly fluorescent⁵ and required the use of large slits (1 mm). It has been found that in addition to Rayleigh scattering, there exists to a smaller extent a form of scattering at the wavelength of about 450 m μ called Raman Scattering. This Raman effect has been found to be independent of the frequency of incident light. All solutions were kept sufficiently dilute so that Lambert-Beer's law may obey. Dilution of solution is also necessary to lesson quenching effect⁶. The fluorescent excitation spectra which are recorded in terms of microameter reading have however been drawn in terms of excitation of radiation taking it as equivalent to microameter reading.

The fluorescence excitation spectra of all the humic substances are more or less similar in shape, indicating the presence of nearly identical nature of groups of fluorophor in them. The intensity difference in the fluorescence of different humic acid fractions may be due to the concentration variation of fluorescent groups present in them. The exact nature of fluorescing groups could not be identified in the present investigation but the fluorescing property itself suggests the presence of either (a) aromatic nucleus substituted by at least one electrodonating group such as $-\text{NH}_2$, $-\text{OH}$ or (b) congugated unsaturated system capable of high degree of resonance in humic acids. The former view is supported by a number of workers^{7,8} who isolated a number of aromatic compounds

from oxidation degradation products of humic acids. On the other hand presence of stable free radicals supports the conjugated structure⁹. According to Rex¹⁰ humic acid are large sized organic free radicals but steelink and Tollin¹¹ are of opinion that two stable free radicals, one of a semiquinone - catechol - resorcinon type copolymers and the other electron transfer complex of quinhydrone type co-exist in humic acids. But still no definite structure of humic acids can be assigned, yet Flaig¹² tried to give some idea of the structure of humic acid by a number of tedious experiments. According to him humic acid has got the structure like this :



The structure indicates a number of electron donating (e.g. -OH, -OCH₃), electron withdrawing (-COOH, C=O, -N-R) groups

R

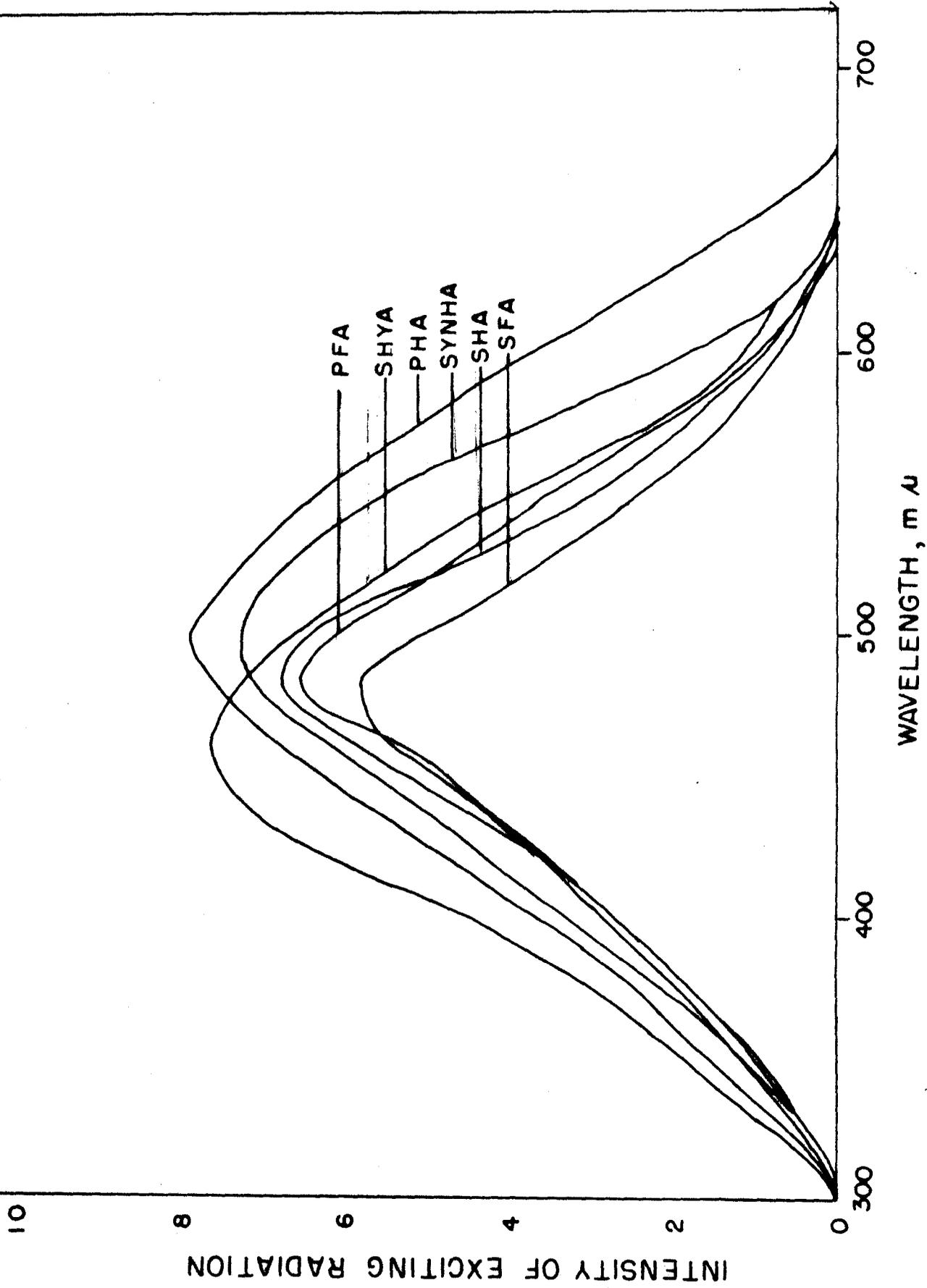
and resonating structure which are essentials for the fluorescence of the whole. The fluorescence with excitation maxima curves of humic materials are depicted in Fig. 11 and the results are in Table 6.

TABLE - 6

Fluorescence excitation spectral data of humic substances

Sample	Wavelength of maximum excitation in m μ
SHA (Sodium salt)	480
PHA (" ")	492
SFA (" ")	472
PFA (" ")	480
SHYA (" ")	470
SYNHA (" ")	500

Fig. II



FLUORESCENCE EXCITATION SPECTRA OF HUMIC SUBSTANCES

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SECTION - H

POLAROGRAPHY

The polarographic technique was applied to humic materials by Tokuoka and Ruzica¹, who found no reduction in case of humic acids but with hymatomelanic acids, they obtained definite polarographic wave at $-1.44V$ against standard calomel electrode where lithium hydroxide was used as a supporting electrolyte.

It has been found that the half-wave potential is dependent on concentration and shifted towards more positive values with increasing concentration. Further, with the concentration of hymatomelanic acid less than 0.085%, the diffusion current was observed to be proportional to concentration.

Lucena-Conde and Gonzalex-Crespo² reported that the three fractions from soil organic matter, when subjected to polarographic studies with the addition of $HClO_4$, $LiClO_4$ and $LiOH$ as supporting electrolyte, were found not reducible at the dropping mercury electrode. A small reduction wave at 0.45 V against Hg-electrode in dimethyl sulphoxide solution of natural humic acids was observed by Lindbeck and Young³. This was attributed to easily reducible functional groups e.g. a quinone or some other system of highly conjugated functional groups. Another attempt by Kothoff and Lingane⁴, to estimate the redox potential of soil humic acid fractions by polarography proved ineffective because these organic matters were not electro-reducible at the dropping mercury electrode.

Experimental

The polarograms of natural humic, hymatomelanic and fulvic acids including synthetic model humic acids were recorded in 0.1 M

KCl as the supporting electrolyte at different pH values in the potential range 0 to 2.0 V with the help of a recording d.c. polarograph (Radelkis-Type OH-102) having sensitivity up to 2×10^{-2} amp./div. The dropping mercury electrode with capillary characteristics, $m = 1.87$ mg/sec and $t = 4.2$ sec measured in an open circuit, served as a cathode and the saturated calomel electrode as an anode. In the suspensions pure nitrogen gas was passed for 10 minutes in each polarographic measurement and pH of the sample was varied by the addition of alkali (NaOH).

Results and Discussion

The polarograms of humic materials are shown in Fig. 12-17. It is found that at low pH, well defined waves are obtained for each sample, however with increasing pH, the wave appears virtually to disappear. The same observation has been noted by earlier workers^{1,5,6}. In case of fulvic acid, the wave height is seen to be reduced to maximum extent with the increase of pH. It may also be noted that the half-wave potentials ($E_{1/2}$) obtained in case of synthetic humic acid approaches that of natural system. The pH value and the corresponding $E_{1/2}$ value have been listed in Table 7. Although no definite inference can hardly be made from these data, the nature of the polarogram indicates that all the compounds appear to possess identical chemical structure .

TABLE - 7
Polarographic results of humic substances

Sample	pH	$E_{1/2}$ (Volt)
SHA	3.63	-1.30
PHA	3.61	-1.30
SFA	2.80	-1.50
PFA	2.80	-1.45
SHYA	3.58	-1.25
SYNHA	3.50	-1.60

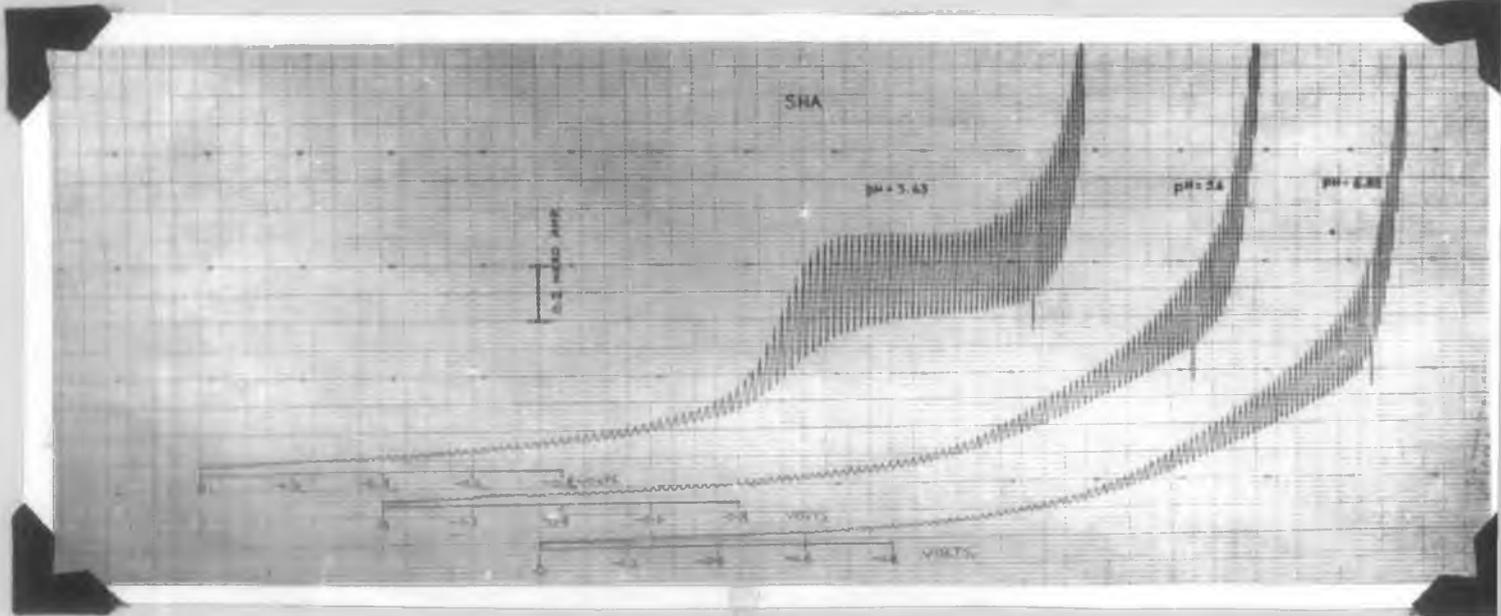


Fig. 12 The Polarogram of Soil Humic acid

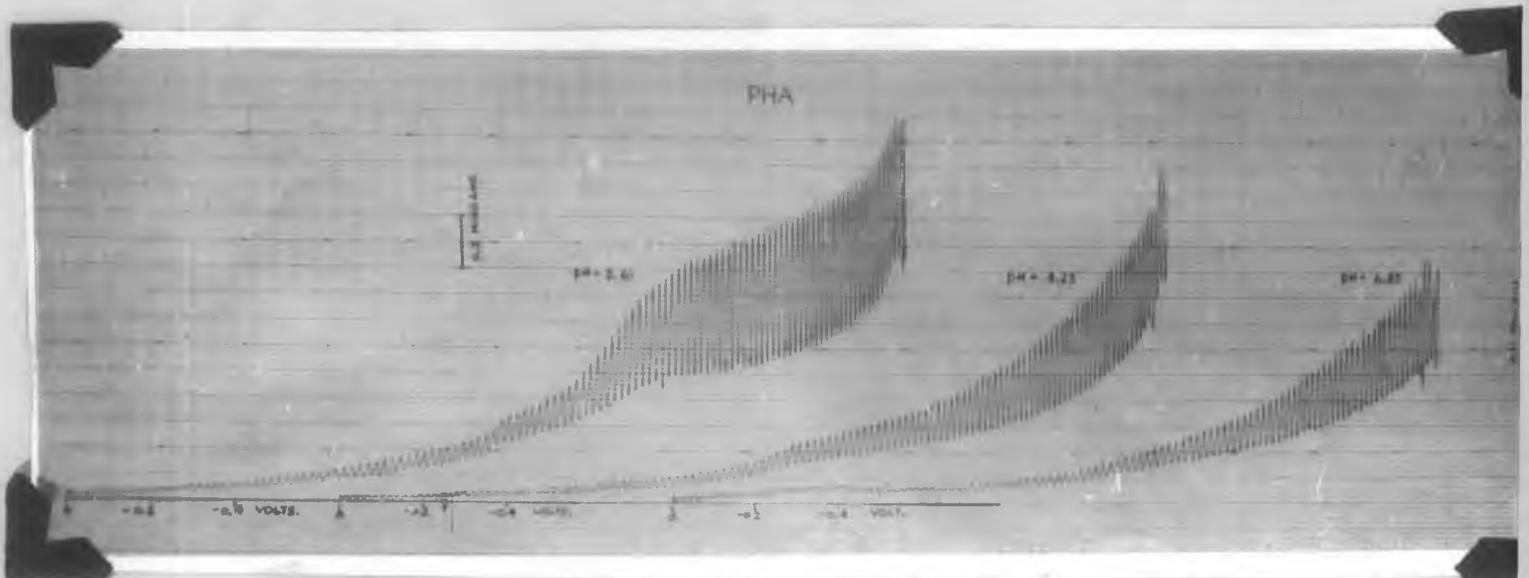


Fig. 13 The Polarogram of Peat Humic acid

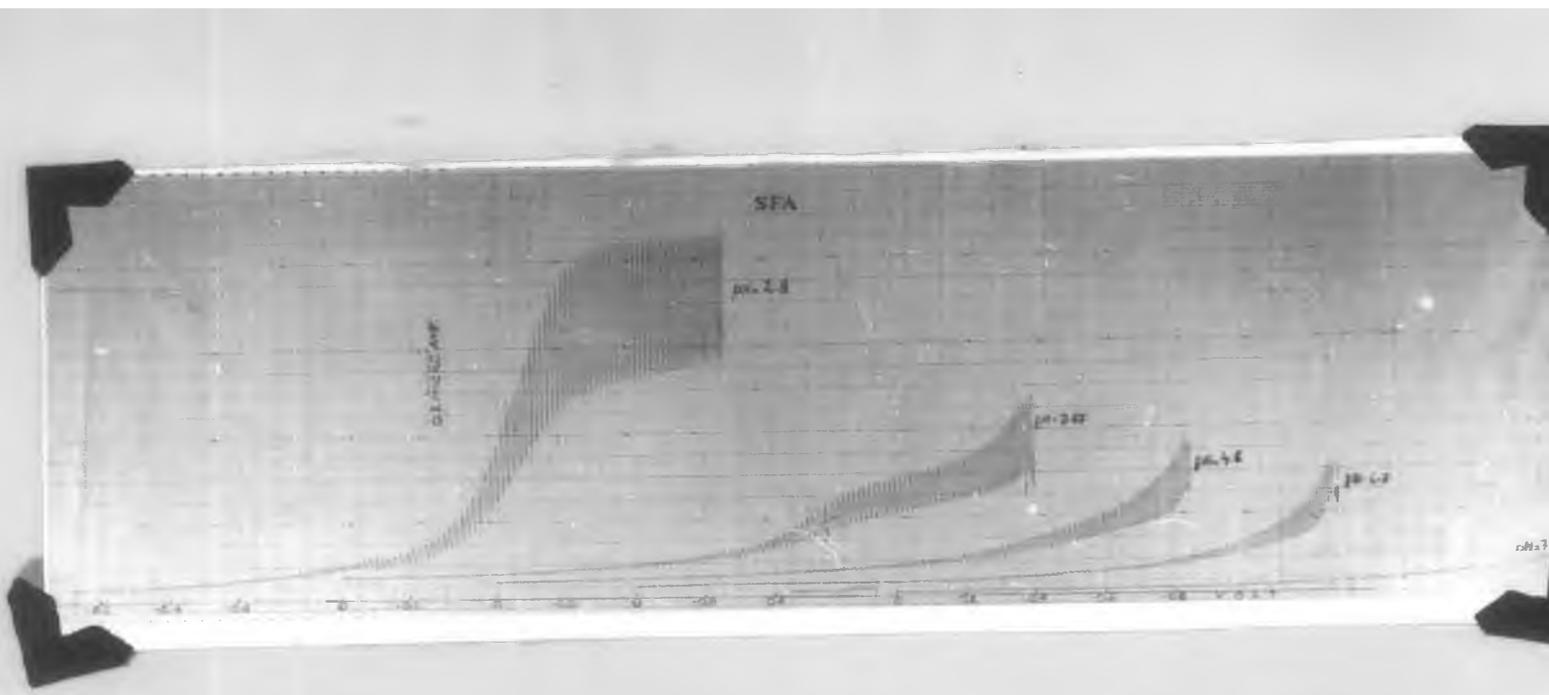


Fig. 14 The Polarogram of Soil Fulvic acid

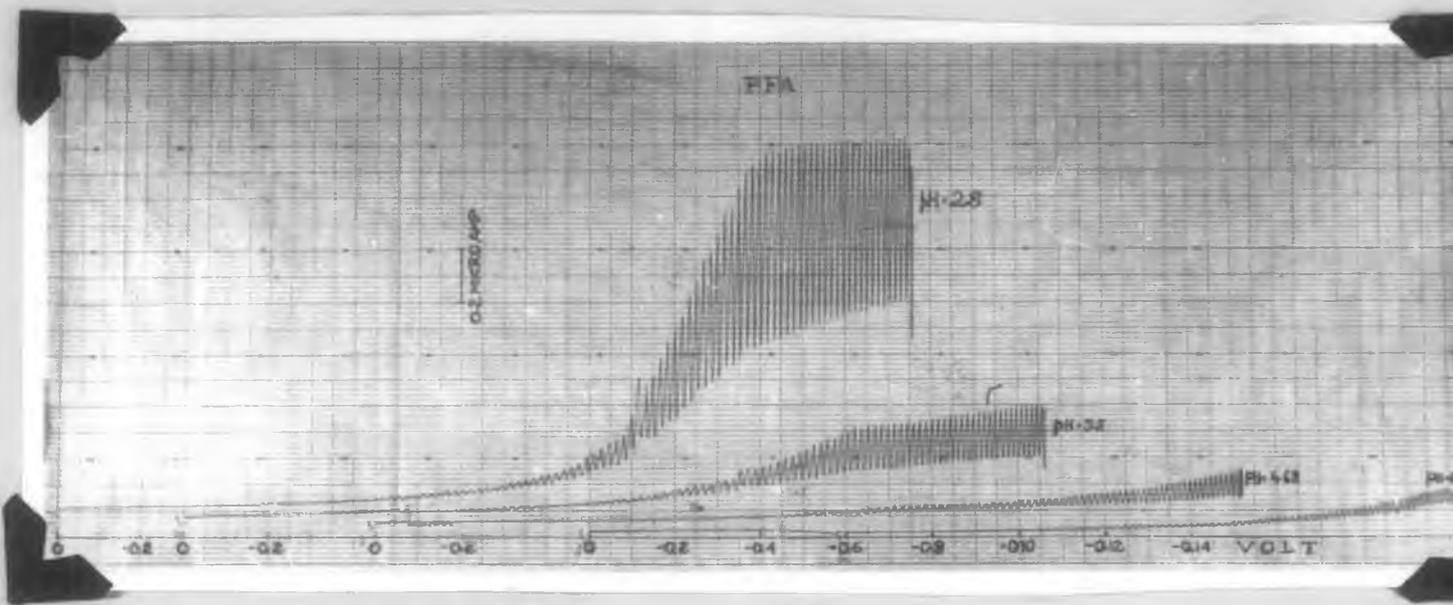


Fig. 15 The Polarogram of Peat Fulvic acid

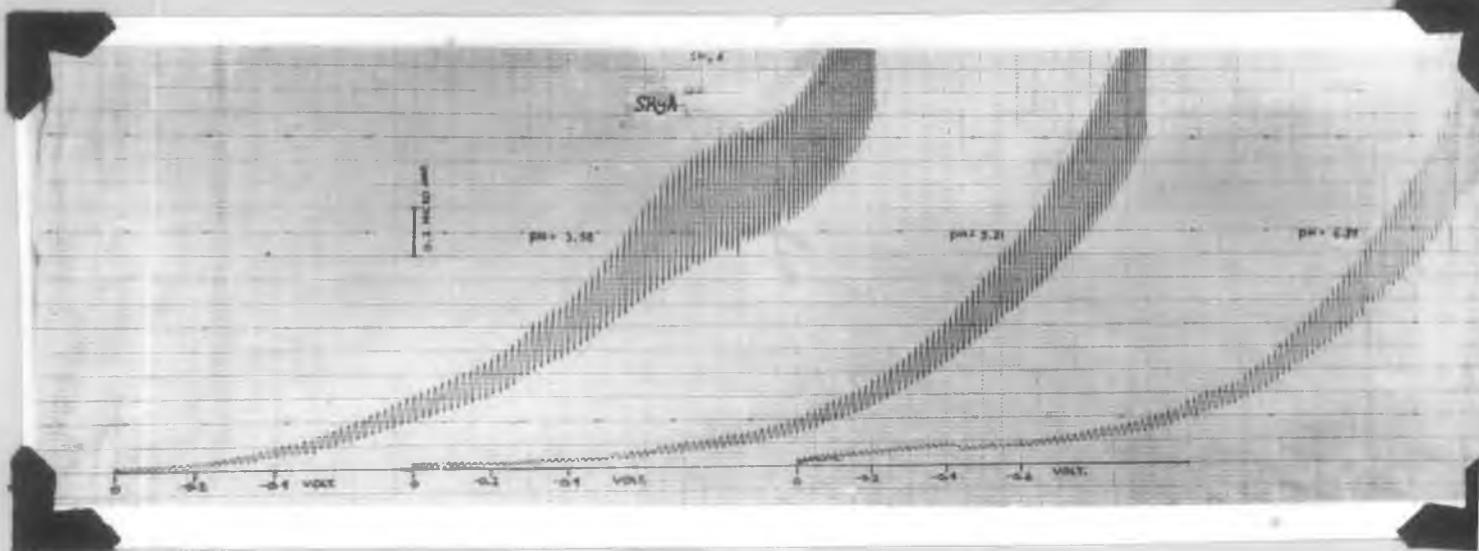


Fig. 16 The Polarogram of Soil Hymatomelanic acid

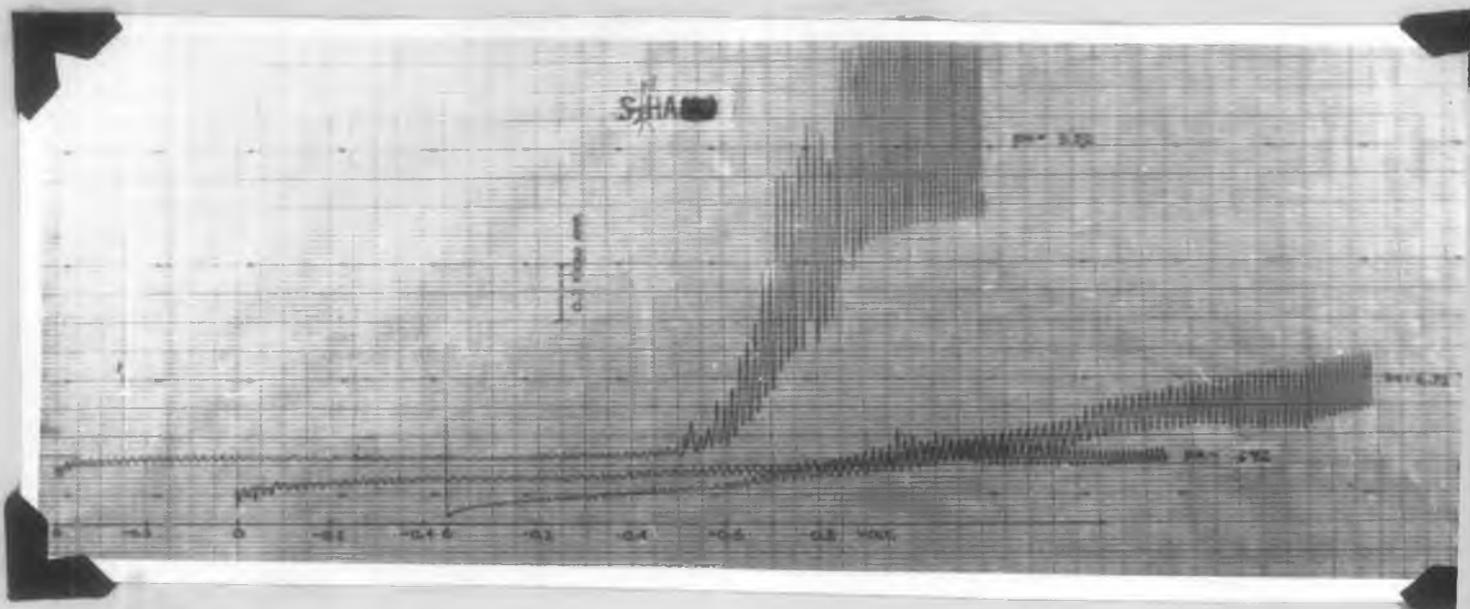


Fig. 17 The Polarogram of Synthetic humic acid

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