

## Characterization of humic acids isolated from soils under mulberry vegetation

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Two different samples of humic acids were isolated from soils under mulberry vegetation. The isolated acids have been characterized by electrometric (potentiometry) titrations and visible spectroscopy with a view to probing their physicochemical properties. Attempts have also been made to correlate the results obtained from these experiments with coiling-decoiling behaviour as well as aliphatic-aromatic balance of the acids.

HUMIC acids are nothing but the natural polymeric acids having complex molecular nature<sup>1</sup>. Characterization of the same is necessary, not only for having some information about their structural arrangements, but also for the effective use of these substances in mulberry cultivation<sup>2</sup>. The principal features of humic acids—the polyelectrolytic behaviour and coiling-decoiling phenomenon—are probed in detail in the present paper by looking at different properties of two humic acid samples isolated from soils under mulberry vegetation located at two different agroclimatic conditions.

For isolation of humic acids, soil samples were treated with sodium carbonate, followed by acidification of the alkali-extracted material with dilute HCl to pH 2 (ref. 3). The purified humic acids were denoted as HA<sub>b</sub> (humic acid sample isolated from the soil under mulberry vegetation of Central Sericultural Research and Training Institute, Berhampore, Gangetic alluvium of West Bengal, 24°4' N–88°9' E) and HA<sub>k</sub> (humic acid sample isolated from the soil under mulberry vegetation of hill nursery, Kalimpong, West Bengal, 27°2' N–88°17' E).

Potentiometric titrations were carried out by using standard NaOH solution. Carboxylic and phenolic –OH group contents were reckoned from the acidity equivalents of the initial and final inflexion points of the titration curves<sup>4</sup>. The amount of fresh alkali required to attain a stable higher pH, following a fall in the final pH (on standing after initial titration), as well as the time elapsed for the same were also recorded for each sample.

The optical densities at 465 nm and 665 nm (ref. 1) for the humic acid solution (Na form) were noted with the help of a systronics spectrophotometer at two different pH values 7.0 and 9.0.

Table 1 records the carboxylic and phenolic –OH group contents and a pH difference namely,  $\Delta\text{pH} = \text{pH}_{3/4} - \text{pH}_{1/4}$ , where  $\text{pH}_{1/4}$  and  $\text{pH}_{3/4}$  refer to the values corresponding to one-fourth and three-fourths of the final inflexion points. The  $\Delta\text{pH}$  values of the humic acid samples are greater than 0.954, which signifies the polyprotic nature of both the acid samples<sup>5</sup>. Again, the approximate charge density (number of charges per unit mass) of humic acid molecule is computed from the cumulative contribution of carboxylic and phenolic –OH contents. This, in turn, governs the 'adsorptive capacity' of humic acid molecules, which enables them to combine with metal ions, clays, pesticides, fertilizers and different organic compounds<sup>6,7</sup>. HA<sub>k</sub> is found to be superior to HA<sub>b</sub> in this aspect.

The amount of fresh alkali needed to attain a stable higher pH as well as the time required for the same have been reported to be correlated with the coiled nature of humic acid molecule<sup>8-10</sup>. Addition of fresh alkali for stabilizing the higher pH (when HA molecules were completely unfolded) indicates an amount of remainder hydrogen ions which are not accessible to alkali initially. This, in turn, is governed by the steric hindrance associated with a particular conformation of the molecules due to their coiled shape. Thus, for higher degree of coiling, greater amount of fresh alkali is

Table 1. Potentiometric information about HA samples

HA sample	Amount of COOH (meq · g <sup>-1</sup> )	Amount of phenolic –OH (meq g <sup>-1</sup> )	Approximate charge density (Number of charges g <sup>-1</sup> ) × 10 <sup>-20</sup>	pH at first inflexion point	pH at final inflexion point	$\Delta\text{pH}$	Conclusion
HA <sub>b</sub>	1.63	4.14	34.73	8.72	9.48	4.74	Polyprotic
HA <sub>k</sub>	4.03	6.05	60.68	7.96	8.66	4.33	Polyprotic

Table 2. Potentiometric stability and spectrophotometric analysis of HA samples

HA sample	Amount of fresh alkali required to attain stable pH (meq g <sup>-1</sup> )	Time required to attain stable pH (h)	$E_d/F_0$ at pH		$\Delta E_d/F_0$
			7.0	9.0	
HA <sub>b</sub>	0.87	23.00	3.32	2.75	0.57
HA <sub>k</sub>	13.12	69.50	4.88	4.07	0.81



required. Again, the time required to attain a stable higher pH depends on the ease of decoiling, which is inversely related to the intramolecular hydrogen bonding. Formation of such bonds is often interrupted by the alkyl groups available in the humic acid molecule; this, in turn, governs the 'flexibility' of the molecule. Thus, poorer flexibility exhibits longer time requirement. From Table 2 it may be concluded that  $HA_k$  is more coiled but less flexible than  $HA_b$ .

The spectrophotometric analysis data have been recorded in Table 2. The  $E_4/E_6$  values, being greater than unity at both pH values, establish that the absorbance by the aliphatic part (i.e. absorbance at 465 nm) is greater than that by the aromatic part (i.e. absorbance at 665 nm) due to a particular conformational arrangement of the aliphatic and the aromatic moiety<sup>1,9</sup>.

Table 2 also shows the data on  $\Delta(E_4/E_6)$  ratio, namely,  $\Delta(E_4/E_6) = (E_4/E_6)_{pH-7} - (E_4/E_6)_{pH-9}$ . The latter may be taken as an 'index of coiling' of the humic acid molecules owing to the fact that addition of alkali (NaOH) rendered the acid molecules 'unfolded under thermodynamic compulsion'<sup>11</sup>. This leads to the destruction of the tertiary structures (i.e. three-dimensional configuration) of the molecules following the release of the hidden aromatic

parts, which thereby leads to a lowering of  $E_4/E_6$  value, giving a positive value of  $\Delta(E_4/E_6)$ . The higher  $\Delta(E_4/E_6)$  value of  $HA_k$  signifies that the latter is more coiled than  $HA_b$ . This has got close resemblance with potentiometric study discussed earlier. It may further be concluded that  $HA_k$  is more aromatized than  $HA_b$ .

1. Sarmah, A. C. and Bordoloi, P. K., *J Indian Soc. Soil Sci.*, 1993, 41, 642-648.
2. Inamatsu, K., *Bull. Seric. Expt. Stn.*, 1977, 27(1), 1-96.
3. Adhikari, M. and Hazra, G. C., *Indian J. Appl. Chem.*, 1970, 33, 322-328.
4. Schnitzer, M. and Khan, S. U., *Humic Substances in the Environment*, Marcel Dekker, New York, 1972, pp 2-3.
5. Sturrock, P. E., *J Chem Educat.*, 1968, 45, 258-259.
6. Kononova, M. M., *Soil Organic Matter*, Pergamon, Oxford, 1966, pp. 190-199.
7. Stevenson, F. J., *Humus Chemistry*, Wiley, New York, 1982, pp. 349.
8. Davis, H. and Mott, C. J. B., *J. Soil Sci.*, 1981, 32, 393-397.
9. Saha, P. B. and Sanyal, S. K., *J Indian Soc. Soil Sci.*, 1988, 36, 35-42.
10. Kar, R. and Sanyal, S. K., *J. Indian Chem. Soc.*, 1988, 65, 834-837.
11. Sanyal, S. K., *Chem Edn.*, 1984, 1, 14-18.

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