

Charge Determination of Humic Acid and Humic Extract by Spectrophotometry

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(Manuscript received on 11 November 1998)

The charge densities of humic acid and humic extract have been determined by back titration using spectrophotometry as the method of end point detection and several cationic polymers of different charge density (copolymers of acylamide and dimethylaminoethylacrylate and polydiallyldimethylammonium chloride, PDDA), and a cationic indicator o-toluidine blue (o-Tb). The charge densities of humic acid and humic extract obtained depend on the charge densities of cationic polymers used. When the polymers of lower charge density are used, lower apparent charge density values are obtained, but for polymers of high charge density (above 2.5 meq/g), the measured values are almost identical, but also are nearly the same as that obtained for PDDA which has a high charge density (about 6 meq/g). For polymers of lower charge density, it is considered that the optimum dosage does not correspond to 1:1 charge neutralization between anionic and cationic groups. Polymers of high charge density should be used in order to achieve a complete charge neutralization. As humic acid and humic extract are dissociable acids, their ionization is heavily pH dependent and so higher charge density values are obtained with increasing pH.

Key words : charge density, humic acid, humic extract, spectrophotometry, back titration, cationic polymer

1. Introduction

Natural organic matter (NOM) in surface and ground waters mainly consists of partially decomposed plant material and most is found as dissolved organic matter such as humic substances, hydrophilic acids and single organic compounds. Humic substances make up 50-80% of the dissolved organic matter in natural waters (Thurman, 1985), and ground waters in NOM can be termed humic ground waters. The presence of humic substances in water causes a yellow to brown color, may result in the formation of trihalomethanes (THMs) during chlorination (Chadik, 1983; Prakash, 1983), can result in microbial regrowth in distribution systems, and impedes the removal of iron and

manganese from the water (Prakash and McGregor, 1983). Humic substances include a wide variety of compounds with similar constitution and properties. Oden (1919) classified them in terms of four fractions—humic acid, fulvic acid, humin and humatomelanic acid. In natural waters, the predominant fractions present are humic and fulvic acids (Smith, 1986).

Several techniques are used to remove organic materials in drinking water treatment plants and a very common method is coagulation/flocculation, followed by sedimentation or flotation. Most dissolved organic materials (DOM) in natural waters is anionic in character and can be effectively removed by cationic additives, such as hydrolyzing metal salts and cationic poly-electrolytes. In these cases there is a close

stoichiometric relationship between the anionic charge carried by the DOM and the cationic charge by the additive and optimum dosages can be predicted if the appropriate charge information is available (Bernhardt, 1993). For these reasons, the charge density of natural and synthetic polyelectrolytes is of importance and convenient experimental methods are needed.

As applied to the determination of charge carried by dissolved macromolecules, the most widely used is the technique of colloid titration introduced by Terrayama (1952). The method is based on the stoichiometric reaction between oppositely charged polyelectrolytes. For end point detection, Terrayama used the interaction of the chromotropic cationic indicator, *o*-toluidine blue [*o*-Tb, 3-amino-7-dimethylamino-2-methylphenothiazin-5-ium] with the anionic polyelectrolyte poly(potassium vinyl sulfate) (PPVS), which leads to a hypsochromic shift in the absorption spectrum of *o*-Tb and thus to a visible color change. As the color change in this visual titrimetry is not evident to the titration of polyelectrolytes of low charge density, the accurate equivalence point can not be measured (Kam, 1998). Alternatives to visual titrimetry include conductimetry (Toei and Kohara, 1976), spectrophotometry (Kawamura, 1966; Kawamura, 1967), phototitrimetry (Horn, 1978, 1980; Wasser, 1991) and streaming current detector method (Fischer, 1988; Bernhardt, 1993). When the reaction between oppositely charged polyelectrolytes leads to the formation of a precipitate, end point detection by turbidimetry (Josephs, 1963) or by other particle detection methods (Gregory, 1984; Gregory, 1985, 1990) can also be used. In the present paper, the charge densities of humic acid that is a major component of humic substances and humic extract obtained from natural waters have been determined using spectrophotometry as the method of end point detection.

2. Principles

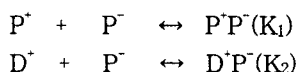
Colloid or polyelectrolyte titration is based on the reaction between oppositely charged polyelectrolytes, or between polyelectrolytes and charged surfaces. When aqueous solutions of cationic and anionic polyelectrolytes are mixed, a neutralization reaction will occur and in many cases this reaction is stoichiometric in the sense of 1:1 charge neutralization (Terrayama, 1952; Horn, 1980). Thus, if a polyelectrolyte of known charge density (or equivalent weight) is available, it may be used as a titrant in determining the charge density of an oppositely charged polyelectrolyte, provided that a method for detecting the equivalence point is available and that the assumption of 1:1 stoichiometry is valid. The mutual neutralization of polyelectrolyte charges usually leads to a reduction in solubility and eventual precipitation.

In the determination of the charge density by colloid titration, the counterions are displaced by the added polymers in the course of titration, so that the total charge of the polymer is determined. The charge density in this way often correlates well with the properties of the polymers investigated in practical applications, for instance adsorption and coagulation, since a displacement of counterions also takes place in such cases. In other techniques, such as electrokinetic or conductance methods, part of the counterion charge is carried with the polyelectrolyte and so the charge determined is rather less than the total charge determined by titration.

Colloid titration can in principle be carried out in two ways: as a direct titration (Terrayama, 1952; Horn, 1978, 1980) with an oppositely charged polymer or as a back titration in which a defined amount of an oppositely charged polymer is added to the solution to the investigated and the excess is then titrated (Terrayama, 1952; Horn,

1978; Horn, 1983). The latter method is suitable for the determination of anionic polymers with o-Tb as the indicator and has been employed successfully, e.g., for the charge determination of proteins (Horn, 1983). The charge densities found agreed well with those calculated from the compositions of the proteins and such results confirm the stoichiometric 1:1 reaction between the oppositely charged polymer molecules. Owing to the easily detectable color change and well-defined interactions with PPVS, which are shown in the formation of an isobestic point in the absorption spectrum, the cationic indicator o-Tb is usually employed. This shows a distinct color change, from blue to red-violet, on binding to anionic polymers. Using the system o-Tb/PPVS, cationic polymers can be determined directly and anionic polymers by back titration.

The 1:1 reaction between cationic polymer (P^+) and anionic polymer (P^-) and the indicator reaction are shown schematically in Fig. 1. At the end point, the anionic titrant reacts with a cationic dye (D^+) by direct or back titration. The metachromatic band shift occurring at the same time can be detected. The overall reaction can be described by the two following equilibria:



Where K_1 and K_2 are the equilibrium constants. The equilibrium constants differ according to the binding affinity of the polymer/polymer vs polymer/dye interaction (Horn, 1980). With a sufficiently large parameter ($K_1/K_2 \geq 100$), the anionic polyelectrolyte-dye binding only takes place after the polymer complex formation is virtually complete. This results in a sharp break point at the equivalence point. If $K_1/K_2 < 100$, the equivalence point is less clearly-defined and colloid titration based on an indicator color change

becomes less useful.

Both the polymer complex formation and the indicator reaction are based on electrostatic and co-operative interactions. The binding constant between a polyelectrolyte and an oppositely charged ligand increases steeply with the number of binding sites or charges of the ligand (Horn, 1978, 1980). This manifests itself in a molecular weight dependence of the binding constants between the oppositely charged polymer molecules (Horn, 1980).

The color change is ascribed to an interaction of dye molecules with each other, which occurs when the distances between the molecules are small (Gummow and Roberts, 1985). A high structural charge density, and thus a small distance (<1 nm) between the charge centers, is a necessary condition for the chromotropic properties of a polyelectrolyte. Formation of dye aggregates is induced with polymer of high charge density and electrostatic binding of dye ion to the polymer matrix. However, these aggregates are not formed in too large an excess of the polyelectrolyte, as the binding of the dye ions to the polymer then takes place randomly at larger distances from each other (Ortona, 1984; Gummow, 1985).

3. Experimental

3.1. Reagents and Apparatus

Analar grade reagents were used and the solutions were prepared with deionized water from an Elga 'Spectrum' unit. Humic acid was prepared as described earlier (Kam, 1997). Humic extract from aqueous environment were donated by Prof. N. J. D. Graham (Dept. of Civil Engineering, Imperial College, UK). This was extracted from

the Valve House at the Chellow Heights Water Treatment Plant between August 15th and September 12th, 1991, concentrated by reverse osmosis, freeze-dried to solid, and subsequently stored in a desiccator. Humic extract was prepared as a 2 g/L aqueous solution by adding 1 g of the solid to 500 mL of water, shaking manually for 90 sec, and exposed to ultra-sound for a further 90 sec. Similar short exposes to ultra-sound(2 min) have been determined not to affect the molecular weight distribution of organic molecules in reconstituted solutions(Whittle, 1988). The humic solution was then filtered under vacuum through a prewashed 0.45 μm cellulose nitrate membrane and adjusted to pH 7 with 0.01 N HNO_3/NaOH .

Cationic polymers used were supplied by Allied Colloids Ltd., UK. These were copolymers of acrylamide and dimethylaminoethyl acrylate (DMAEA), the latter being quaternized, so that these polymers are positively charged over a wide

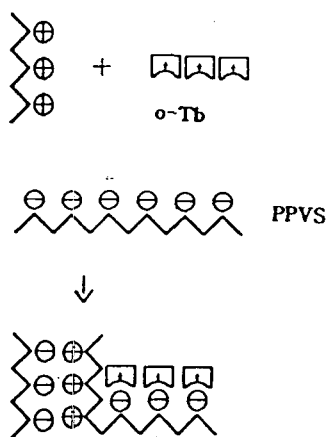
pH range. The properties of the polymers, designated A-F, are given in Table 1. The molar portion of DMAEA determines the charge density. Polymers A-E were supplied as solids and were prepared as 0.1% aqueous solutions by wetting 0.1 g of the solid with 2 mL methanol, adding 98 mL of water and agitating for 12 hours. Polymer F was supplied as an aqueous solution with 20% of solid concentration and was diluted as 0.1%.

As an example of a much lower molecular mass cationic polymer, a commercial coagulant, polydiallyldimethylammonium chloride(PDDA, Allied Colloid Ltd., UK) was used. This has a molecular mass of $3-4 \times 10^4$ and was prepared as a 0.1% aqueous solution by diluting the 40% product supplied.

All of the 0.1% solutions were used within one week of preparation.

PPVS was obtained from Aldrich Chemical Co., UK. The solution was prepared by dissolving 180 mg to 1 L with water. The charge density of this

a) Cationic polymer



b) Anionic polymer

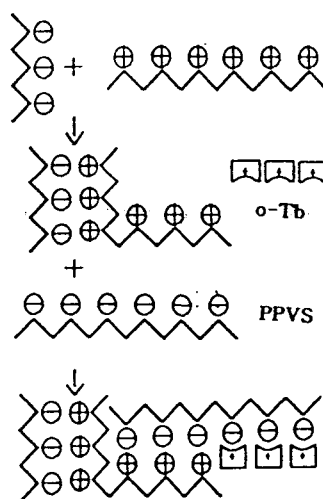


Fig. 1. Schematic illustration for charge determination of a cationic polymer by direct titration(a) and an anionic polymer by back titration(b) using anionic polyelectrolyte(PPVS) and a cationic dye(o-Tb).

Table 1. Properties of polymers used.

Polymer	Supplied		Measured	
	Approx. Charge Density (meq/g)	Approx. Molecular Mass/10 ⁶	Charge Density (meq/g)* in water (pH 5)	Charge Density (meq/g)* at pH 7
A	0.5-1.5	11-16	0.838	0.695
B	1.5-2.5	11-16	1.570	1.295
C	2.5-4.0	11-16	2.870	2.610
D	2.5-4.0	7-10	2.975	2.690
E	2.5-4.0	4-6	3.021	2.727
F	2.5-4.0	1-3	3.255	2.940
PDDA	about 6	0.03-0.04	5.730	5.730

* From Kam(1998)

solution was standardized with a cationic surfactant, cetyltrimethylammonium bromide(CTAB), obtained from BDH Co., UK and prepared as a 0.5 or 1 meq/L solution.

o-Tb was obtained from BDH Co., UK and prepared as a 0.01% aqueous solution.

Buffer solutions(1 mmol/L) were used in investigations of pH effects. These were acetic acid/sodium acetate for pH 3, 4 and 5, NaH₂PO₄/Na₂HPO₄ for 6 and 7, and NH₄OH/NH₄Cl for pH 8, 9 and 10.

A CamSpec UV/Vis spectrophotometer and a Kontron 922 scanning spectrophotometer were used for all absorbance measurements and absorption spectra. Continuous titration of samples in a 10 mm quartz cuvette was carried, using a syringe pump(Cole-Palmer Instrument Co., USA) and a magnetic cuvette stirrer(Rank Brothers Ltd., UK). A Cranwell Universal pH 950 pH meter was used for pH measurements.

3.2. Procedure

The charge densities of humic acid and humic extract were determined by back titration and the

procedure was as follows: 1.65-1.70 mL(for humic acid) or 1.7-1.8 mL of water or buffer(3-10), 0-0.05 mL of humic acid(1 g/L) or 0-0.2 mL of humic extract(2 g/L) and 0.1 mL of each of the cationic polymers and magnetic stirrer bar were placed in a 10 mm quartz cuvette which was inserted into spectrophotometer, on the cuvette stirrer unit. The sample was then fully in the light beam of the spectrophotometer. After the cuvette contents were stirred for 1 min, 0.2 mL of o-Tb was added and then PPVS solution was introduced from the syringe pump at a flow rate of 0.06 mL/min. The spectrophotometer output was connected to a chart recorder, so that the absorbance at 635 nm could be monitored. The syringe pump and chart recorder were stopped when the graph showed little change in absorbance. The experimental set-up is shown schematically in Fig. 2.

4. Results and Discussion

In order to select the suitable wavelength for the charge determination of humic acid and humic extract against PPVS in the presence of o-Tb spectrophotometrically, the absorption spectra of o-Tb at different stages of complex formation with PPVS were depicted. As shown in Fig. 3, there is a distinct metachromatic band shift with increasing PPVS concentrations. A hypsochromic shift occurs on binding o-Tb to PPVS, i.e., the absorption maximum of o-Tb is shifted from 635 nm(uncomplexed, blue form of o-Tb) to 530 nm(complexed, red-violet form of o-Tb and PPVS) with increasing PPVS concentration, exhibiting a well-defined isobestic point. For measurements at a single wavelength, it is more sensitive to determine at 635 nm than 530 nm, which decreases as o-Tb binds to PPVS. Therefore absorbance measurements were made

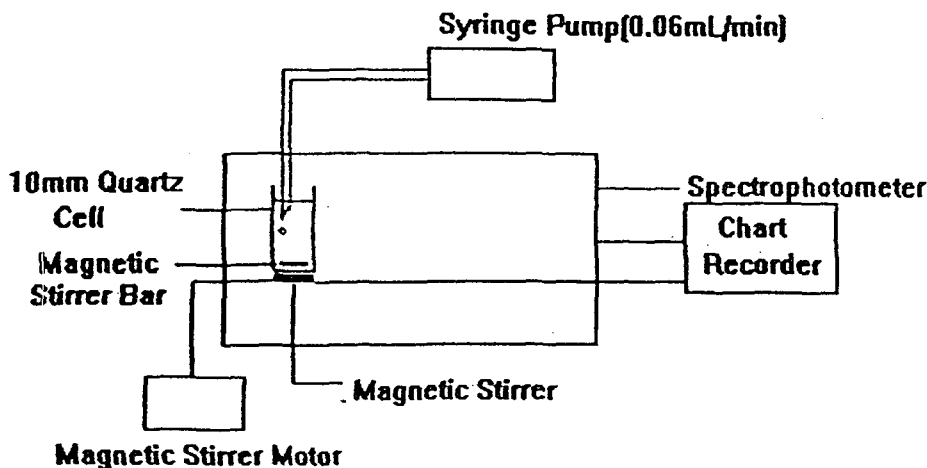


Fig. 2. Schematic diagram of experimental set-up by spectrophotometry.

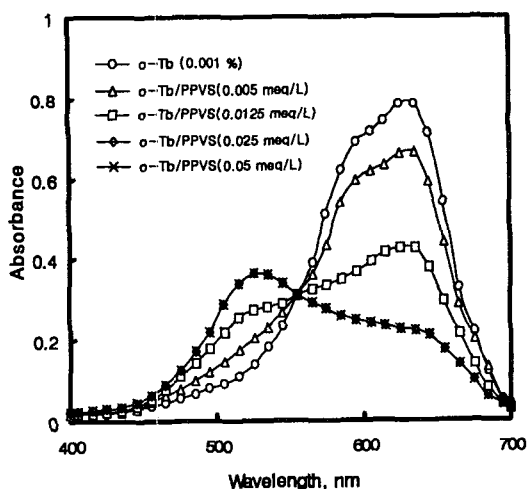


Fig. 3. Absorption spectra of *o*-Tb/PPVS complex in water.

at 635 nm in order to determine the charge densities of cationic polymers and humic extract. It is also possible to use a dual wavelength system in an automated procedure (Wasser et al., 1991).

As humic substances are weakly dissociable

acids, their ionization is heavily pH dependent and can be described as follows (Zhou, 1994):



Increasing pH increases the ionization of humic substances and hence the concentration of negatively charged anion A^- . As the charge density of humic substances is pH dependent and the pH of those from natural waters is close to neutral pH, the pH of humic acid and humic extract is adjusted to pH 7 using 0.01 N HNO_3/NaOH . As suitable metachromatic anionic indicator dyes are present by now, the charge densities of humic acid and humic extract were measured by back titration, i.e., a defined amount of each of the cationic polymers was added to the solution of humic acid or humic extract and the excess of the former was then titrated with PPVS in the presence of *o*-Tb.

Fig. 4 and 5 show the spectrophotometric titration curves of polymer A (of the lowest charge density used in this study) with the added time of *o*-Tb against PPVS in the presence of humic acid (25 mg/L) and humic extract (200 mg/L),

respectively. It can be known that the reaction of polymer A and humic acid or humic extract occurs very rapidly (less than 30 seconds). In this study, each of the cationic polymers was added to the solution of humic acid or humic extract and 1 min later, o-Tb was added.

Titration curves at different concentrations of humic acid (0–25 mg/L) or humic extract (0–200 mg/L) were carried out in order to determine the charge densities of those. The titration curves of polymer C in buffer solutions (1 mM) at pH 7 are shown in Fig. 6 and 7. The decrease in absorbance in the indicator reaction is plotted against the volume of PPVS metered in. The curves are displaced to lower x values with increasing humic acid or humic extract concentration. The titration curve at 0 mg/L is the blank reading curve, i.e., the curve which polymer C is titrated against PPVS in the absence of humic acid or humic extract. The PPVS equivalence volume results from the break point in the two straight lines drawn from blank curve minus the values of different humic acid or humic extract concentrations

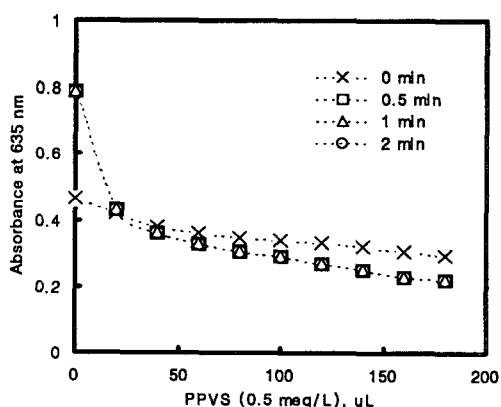


Fig. 4. Spectrophotometric titration curves of polymer A with the added time of o-Tb against PPVS in the presence of humic acid at pH 7 ($C_{\text{polymer A}} = 50$ mg/L, $C_{\text{Humic acid}} = 25$ mg/L, $C_{\text{o-Tb}} = 0.001\%$).

determined in a corresponding manner. This is illustrated in Fig. 6. It was reported that three end points (upper and lower break points, and inflection point) can be derived from the titration curves and the charge densities obtained from those were almost identical, in a previous paper (Kam, 1998). In this study, the upper break point was used as a equivalence volume of the titrant. The charge densities of humic acid and humic extract were obtained from the calibration graphs using the values for cationic polymers at pH 7 in Table 1 and the equivalence volume of PPVS, and the results are shown in Table 2. As shown in Table 2, it can be known that the high correlation coefficients show that there is a very good linear relationship, but the charge densities of humic acid and humic extract obtained depend on the charge densities of the polymers used for the titration. When lower charge density polymers are used, lower charge density values of humic acid and humic extract are obtained, but for polymers of high charge density (above 2.5 meq/g), the measured charge density values of

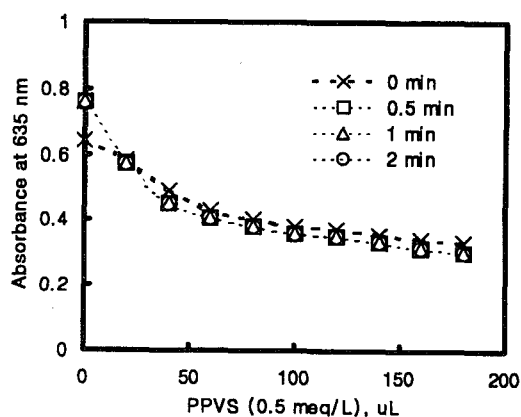


Fig. 5. Spectrophotometric titration curves of polymer A with the added time of o-Tb against PPVS in the presence of humic extract at pH 7 ($C_{\text{polymer A}} = 50$ mg/L, $C_{\text{Humic extract}} = 200$ mg/L, $C_{\text{o-Tb}} = 0.001\%$).

Table 2. The charge density values of humic acid and humic extract obtained for each of the cationic polymers at pH 7.

Polymer	Charge density(meq/g)	
	Humic acid(r^*)	Humic extract(r^*)
A	1.133(0.982)	0.113(0.977)
B	1.585(0.989)	0.156(0.984)
C	2.093(0.995)	0.213(0.993)
D	2.058(0.996)	0.206(0.992)
E	2.055(0.995)	0.208(0.992)
F	2.129(0.998)	0.216(0.997)
PDDA	2.158(0.999)	0.220(0.997)

* correlation coefficient

humic acid and humic extract are almost identical. The latter values are nearly the same as that obtained of PDDA which has a very high charge density(about 6 meq/g). This is unlike previously published results which show a stoichiometric relationship(Terrayama, 1952; Bernhardt, 1993). The optimum dosage for the removal of humic acid and humic extract does not always correspond to 1:1 charge neutralization between anionic and cationic groups(i.e., there is not apparent charge neutralization of humic acid or humic extract with the polymers), when the polymers of lower charge density are used. Polymers of high charge density(above 2.5 meq/g) should be used in order to achieve a complete charge neutralization. When the polymers of high charge density are used, the average values of 2.099 ± 0.059 meq/g are obtained, which gives the relative standard deviation of 2.14% for humic acid, and 0.213 ± 0.007 meq/g and 2.70% are obtained for humic extract.

The pH dependent titration curves of humic acid and humic extract by back titration using PDDA are shown in Fig. 8 and 9. As discussed previously(Kam, 1998), the charge densities of cationic polymers A-F, are pH dependent, which

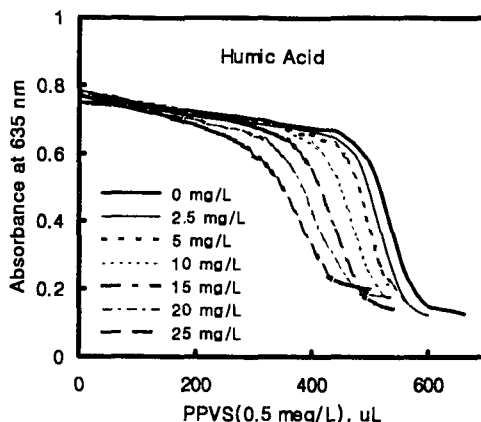


Fig. 6. Spectrophotometric titration curves of polymer C against PPVS in the presence of different humic acid concentrations at pH 7($C_{\text{polymer C}} = 50$ mg/L)

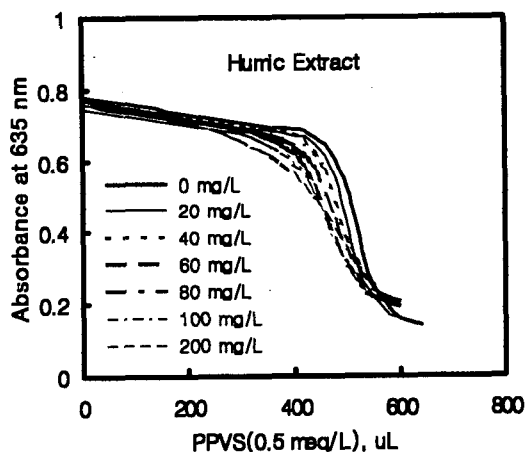


Fig. 7. Spectrophotometric titration curves of polymer C against PPVS in the presence of different humic extract concentrations at pH 7($C_{\text{polymer C}} = 50$ mg/L).

decrease with increasing pH, considered due to some hydrolysis of the cationic units of the DMAEA of the copolymers, but that of PDDA are pH independent, and so PDDA was used in order to examine the effect of pH on the charge densities of humic acid and humic extract. After a defined amount of PDDA(25 mg/L) was added to the solution containing humic acid(20 mg/L) or humic extract(200 mg/L), the excess PDDA was

Table 3. The charge density values of humic acid and humic extract with pH.

pH	Charge density(meq/g)	
	Humic acid	Humic extract
3	0.974	0.060
4	1.430	0.150
5	1.886	0.200
6	2.070	0.210
7	2.158	0.220
8	2.287	0.243
9	2.418	0.263
10	2.523	0.286

titrated against PPVS in the presence of *o*-Tb(0.001%). It can be known that the equivalence volume of the titrant decreases as pH increases. This is ascribed to higher concentration of negatively charged humic acid and humic extract. The charge density values obtained are summarized in Table 3.

5. Conclusions

In order to determine the charge densities of

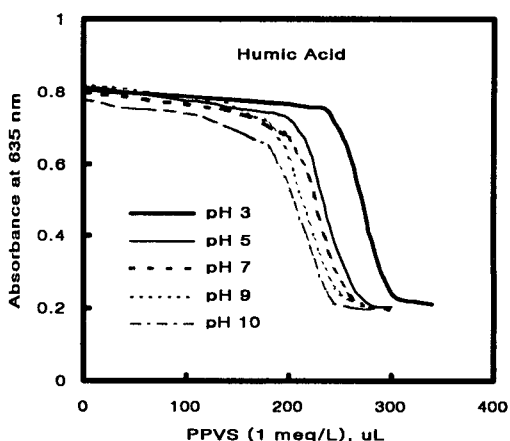


Fig. 8. The pH dependent titration curves of humic acid(20 mg/L) using PDDA(25 mg/L). Because of complications, the curves at pH 4 and 8 are omitted.

humic acid that is a major component and humic extract from natural waters, spectrophotometry has been used as the method of end point detection and back titration has been applied using several cationic polymers of different charge density and a cationic indicator *o*-Tb, and the results compared.

The charge densities of humic acid and humic extract obtained depend on the charge densities of cationic polymers used for the titration. When the polymers of lower charge density are used, lower apparent charge density values of humic acid and humic extract are obtained, but for polymers of high charge density(above 2.5 meq/g), the measured charge density values of humic acid and humic extract are almost identical but also nearly the same as that obtained for PDDA which has a very high charge density(about 6 meq/g). It is considered that the optimum dosage does not correspond to 1:1 charge neutralization between anionic and cationic group(i.e., there is not an apparent charge neutralization of humic extract with the polymers) when the polymers of lower charge density are used. Polymers of high charge density(above 2.5 meq/g) should be used in order

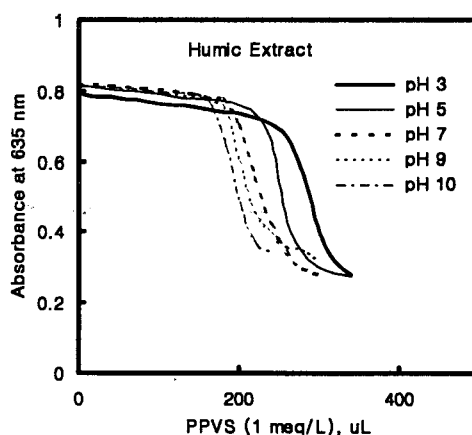


Fig. 9. The pH dependent titration curves of humic extract(200 mg/L) using PDDA(25 mg/L). Because of complications, the curves at pH 4 and 8 are omitted.

to achieve a complete charge neutralization. For polymers of high charge density, the average charge density values and relative standard deviation obtained are 2.099 ± 0.059 meq/g and 2.14% for humic acid, and 0.213 ± 0.007 meq/g and 2.70% for humic extract.

As humic acid and humic extract are weakly dissociable acids, their ionization is heavily pH dependent and so higher charge density values are obtained with increasing pH.

Acknowledgements

We would like to express our thanks to Prof. N.J.D. Graham, Dept. of Civil Engineering, Imperial College, UK, for the donation of humic extract and his helpful comments, and Allied Colloid Co. for the support of cationic polymers.

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