CHEMICAL STUDIES ON SOIL HUMIC ACIDS
VI. Absorbance-pH Curves of Humic Acids

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The relationship between the ultraviolet and visible absorbance and the pH value of solution of each 3 samples of A, B, and Rp type humic acids and the Pg fraction of a P type humic acid was investigated. The absorbance of humic acids decreased stepwise with decreasing the pH value. The pK_a values of dissociative groups, which were deduced from the inflection points of the absorbance-pH curves, were distributed discontinuously over a very wide range from 3.5 to 13 in the humic acids with low degree of humification. The absorbance-pH curves of humic acids with high degree of humification were smoother, especially, in the pH region lower than 7, suggesting that the distribution of pK_a values are more continuous. This may be due to the electrostatic interaction among dissociative groups which is intensified with increasing content of dissociative groups. The pattern of dissociation of phenolic hydroxyl groups in the Pg pigments was similar to that of polyelectrolytes. Therefore, Pg pigments were presumed to exist as polymers in humic acids.
Additional Index Words: ultraviolet and visible absorption, dissociation constant, humic acids, polyelectrolyte.

To clarify the relationship existing between the chemical structure of humic acids and the degree of humification or soil characteristics, we have investigated the elementary composition (4), the functional groups (6), and the products of acid hydrolysis (7, 8) and the degradation with KOH (9) of various humic acids. From the results, it was presumed that humic acids are formed by the contribution of various natural polymers such as lignin, polysaccharides, and protein, and thereafter undergo oxidation, polymerization and the degradation of easily degradable fraction, to form dark-colored amorphous polymers. Ultraviolet and visible absorption of humic acid, especially visible absorption, is presumed to be highly contributed by such dark-colored polymer in humic acids. Therefore, this as well as the subsequent studies are aimed to provide information on the chemical structure of the dark-colored polymer and are focused on the relationship between the ultraviolet and visible absorption and the chemical structure. In this report, the contribution of the dissociative groups to the ultraviolet and visible absorbance of humic acids is discussed.

MATERIALS AND METHODS

1. Humic acid samples. Humic acid samples and their carboxyl and phenol

Table 1. Humic acid samples.

Type	No.	Sample	Original soil	COOH (me/g)	Phenolic OH (me/g)
A	2	Temmondai	Kuroboku (humic volcanic ash) soil	4.94	2.50
A	4	Inogashira	ditto	5.68	0.83
A	14	Nagara OH	Rendzina-like calcareous soil	5.64	2.27
B	18	Kuragari	Brown forest soil	3.69	1.94
B	19	Kinshozan F	Rendzina-like calcareous soil	4.84	1.64
B	21	Higashiyama (A)	Brown forest soil	4.39	2.73
Rp (2)	30	Higashiyama (FH)	F-H layer of brown forest soil	2.95	2.82
Rp (1)	32	Kinshozan OH	Rendzina-like calcareous soil	3.44	1.00
Rp (1)	34	Ishimakisan OH	ditto	2.81	2.63
P_{+++}	1	Tsubame Pg	Humic buried soil	4.78	3.69

Carboxyl group content was determined by calcium acetate method and phenolic OH group content was obtained by subtracting carboxyl group content from total acidity. *cf.* Ref. *6*.

contents are shown in Table 1. Other analytical properties of these samples are available in our previous papers (*4, 6–9*).

2. *Absorbance-pH curves.* Twenty-five milliliter portion of humic acid solution in 0.5 N NaOH (carbon 30 mg/100 ml for A type and P_{+++} type humic acids or carbon 60 mg/100 ml for B type and Rp type humic acids) and 25 ml of mixed buffer were taken into each of two 250-ml volumetric flasks and filled up with water. The mixed buffer was prepared by dissolving 40.2 g $Na_2HPO_4 \cdot 12H_2O$, 14.3 g $Na_2B_4O_7$, and 22.0 g sodium citrate·$2H_2O$ in water, adjusting to pH 13 with conc. NaOH, and making to 1 liter with water. The whole solution in one of the two flasks was titrated potentiometrically with 2 N HCl using Hiranuma RAT 11 autotitrater. The solution in the other flask was also titrated with the same 2 N HCl titrant, and the absorbance was measured at 300, 400, 500, and 600 nm at intervals of 0.2–0.3 ml titrant with Hitachi 124 spectrophotometer. The absorbance of Tsubame Pg solution was measured at 280, 410, 460, 570, and 620 nm. After each measurement, the solution was returned to the flask. The absorbance was corrected for the dilution by the titrant. By matching the absorbance-titre curve and the pH-titre curve, the absorbance-pH curve was obtained.

RESULTS AND DISCUSSION

The absorbance of humic acids at every wavelength (300, 400, 500, and 600 nm) decreased stepwise with decreasing pH value till the flocculation of humic acids occurred (Figs. 1, 2, and 3). The absorbance-pH curves for Rp type humic acids (Fig. 3) had many inflection points, and showed an irregular behavior in certain pH ranges, for example, the curve 34-300 showed this behavior between pH 8.9 and pH 10.2 and
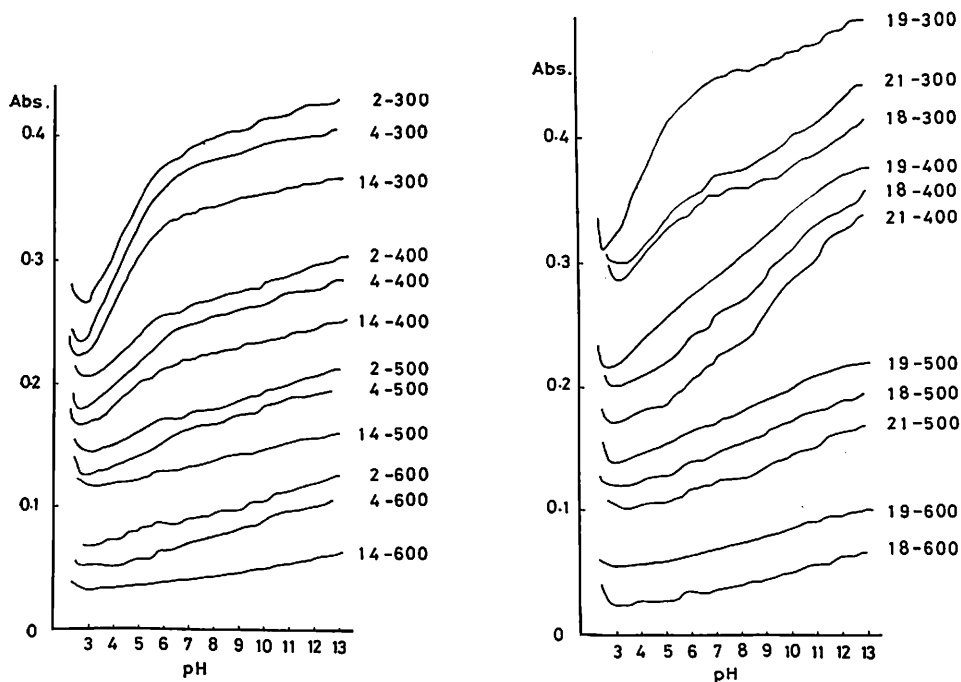
Fig. 1.   Absorbance-pH curves of A type humic acids.
The numbers 2, 4, and 16 represent the sample numbers of Temmondai, Inogashira, and Nagara OH, respectively, and 300, 400, 500, and 600, the wavelengths (nm). Concentrations of the solutions are fixed at 30 mg carbon/liter.   The curves are shifted parallel to the axis of the ordinates by arbitrary distance.

Fig. 2.   Absorbance-pH curves of B type humic acids.
The numbers 18, 19, and 21 represent the sample numbers of Kuragari, Kinshozan F, and Higashiyama (A), respectively, and 300, 400, 500, and 600, the wavelengths (nm).   Concentrations of the solutions are fixed at 60 mg carbon/liter.   The curves are shifted parallel to the axis of the ordinates by arbitrary distance.

the curves 30-400 and 30-500 around pH 8.   These irregular ranges were experimentally reproducible.   Such irregular change in absorbance might be due to some unknown factors other than the dissociation of dissociative groups.   On the other hand, the curves of the highly humified humic acids such as A and B type humic acids were smooth, with less inflection points (Figs. 1 and 2).   In addition, the absorbance-pH curves had generally more inflection points in the alkaline pH range than in the acidic pH range.

The pH-dependency of the ultraviolet and visible absorption of soil humic acids is thought to be due to the change in dissociation forms of carboxyl and phenolic hydroxyl groups in humic acids, the dispersion and association phenomena of humic acid particles, the complex formation with metals, *etc*.   However, all the factors depend
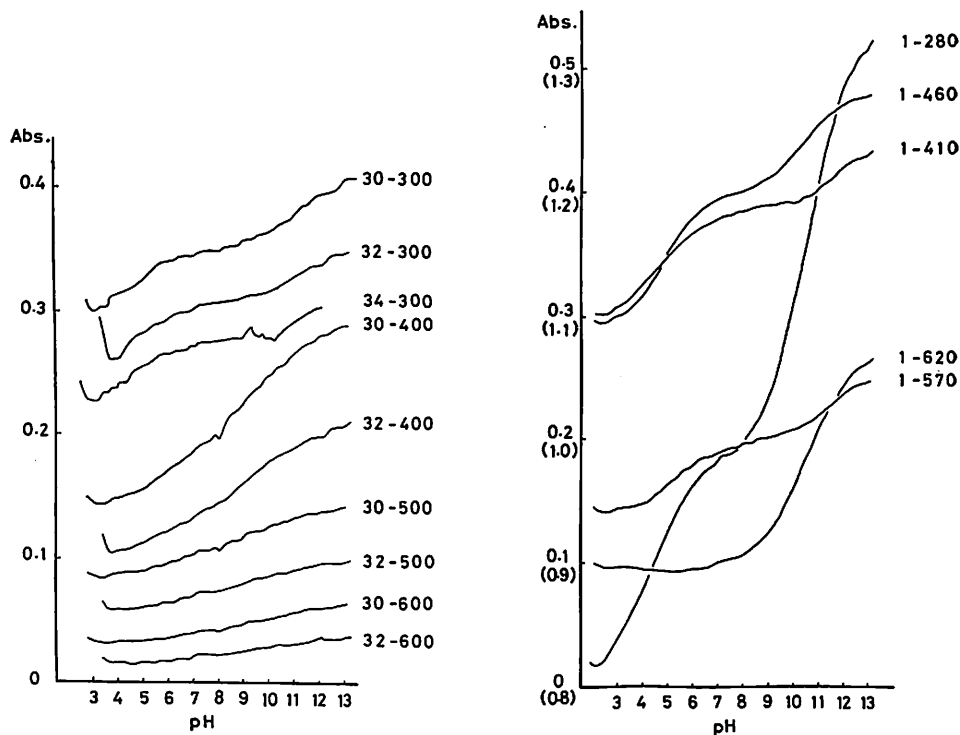
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Fig. 3. Absorbance-pH curves of Rp type humic acids.
The numbers 30, 32, and 34 represent the sample numbers of Higashiyama (F-H), Kinshozan OH, and Ishimakisan OH, respectively, and 300, 400, 500, and 600, the wavelengths (nm). Concentrations of the solutions are fixed at 60 mg carbon/liter. The curves are shifted parallel to the axis of the ordinates by arbitrary distance.

Fig. 4. Absorbance-pH curves of Tsubame Pg.
The numbers 280, 410, 460, 570, and 620 represent the wavelengths (nm). Concentration of the solution is 30 mg carbon/liter. The absorbance for 1-280 is in parentheses.

on the dissociation of the dissociative groups in humic acids, and therefore the inflection points in the absorbance-pH curves of soil humic acids are presumed to correspond to the change in the dissociation forms of carboxyl and phenolic hydroxyl groups.

Carboxyl group content in soil humic acids increased with increasing degree of humification (6). Therefore, electrostatic interaction among the carboxyl groups in humic acids is also presumed to increase with increasing degree of humification. Because electrostatic interaction enlarges the dissociating range of the dissociative groups, the dissociating ranges of many dissociative groups may overlap for highly humified humic acids, resulting in a decrease in the number of inflection points in the absorbance-pH curves especially in the acidic pH range.

In addition, absorbance-pH curves may also become smooth if humic acids have very simple composition in dissociative groups like Tsubame Pg (Fig. 4). However, also for Tsubame Pg, electrostatic interaction influenced the dissociation of the two kinds of phenolic hydroxyl groups in the Pg pigment as would be mentioned later.

The fact that absorbance-pH curves have many inflection points may suggest that dissociative groups in humic acids dissociate independently of each other on account of the weak electrostatic interaction among the groups. Therefore, electrostatic interaction among the dissociative groups in Rp type humic acids may be weaker, because they had more inflection points in the absorbance-pH curves than the other types of humic acids. This assumption corresponds to the small content of carboxyl groups in Rp type humic acids. The large number of inflection points in the alkaline range more than in the acidic range of the curves may be due to the smaller content of phenolic hydroxyl groups than carboxyl groups in humic acids.

The absorbance-pH curves of Tsubame Pg, which is a fraction of Pg pigment semi-purified from Tsubame humic acid, had two inflection points in acidic and alkaline regions (Fig. 4). These two inflection points may correspond to the first and the second dissociation of 4,9-dihydroxyperylene-3,10-quinone which is the main chromophore of Pg pigment. The strong acidity of the first dissociative phenolic hydroxyl group may be due to the delocalization of the negative charge of the phenolate anion on the carbonyl group and the aromatic ring by resonance. The weak acidity of the second dissociative group may be attributed to the stability of the semiquinone form caused by the tautomeric effect of the hydroxyl group at the opposite side of the phenolate anion. The carboxyl content of Tsubame Pg by calcium acetate method was 4.78 me/g and phenolic hydroxyl content was 3.69 me/g according to the difference between the total acidity and the carboxyl content. The calcium acetate method is pK-dependent, and is not specific to only carboxyl groups. Therefore, the first dissociative phenol of the dihydroxyperylenequinone may be included in the "carboxyl content" of Tsubame Pg.

KATCHALSKY and SPITNIK (3) found that a single titration curve of a polymeric acid may be described empirically by two constants, pK and n, according to the equation,

$$pH = pK - n \log (1-i)/i \qquad (1)$$

where i denotes the degree of ionization of the acid. From the mass action equation,

$$pK_a = pH - \log i/(1-i) \qquad (2)$$

from (1) and (2),

$$pK_a = pK + (n-1) \log i/(1-i) \qquad (3)$$

The degree of ionization can be calculated from the absorbance as follows,
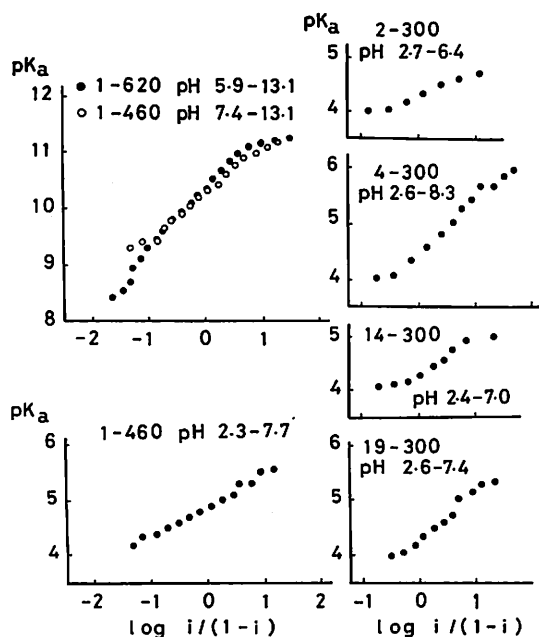
$$i = (E - E_{AH})/(E_A - E_{AH}) \qquad (4)$$

Fig. 5. The relationship between pK_a and log $i/(1-i)$.
The numbers 1, 2, 4, 14, and 19 represent the sample numbers of Tsubame Pg, Temmondai, Inogashira, Nagara OH, and Kinshozan F, respectively, and 620, 460, and 300, the wavelengths (nm). The values of pK_a and log $i/(1-i)$ are calculated from the values of absorbance and pH in the pH range given in the figures.

where E_{AH} denotes the absorbance of undissociated form, E_A the absorbance of dissociated form, and E the absorbance at the degree of ionization i. Therefore, the pK_a and the log $i/(1-i)$ values were calculated from the absorbance-pH curves of Tsubame-Pg at 460 nm for the first dissociation region, and at 460 and 620 nm for the second dissociation region. The values were also calculated from the curves of Temmondai (No. 2), Inogashira (No. 4), Nagara OH (No. 14), and Kinshozan F (No. 19) humic acids at 300 nm in the acidic region, and these values were plotted (Fig. 5). Positive significant linear correlations were found between the pK_a and the log $i/(1-i)$ values in all cases. There have been two explanations of this relationship on humic acids. One of the two (*1*) ascribed the linear relation of Eq. (3) with n larger than 1 to the polyelectrolytic nature of humic acids, and another (*2, 5*) to the Gaussian distribution of pK_a values and refuted the electrostatic interaction of the dissociative groups in humic acids. Although this study was not designed to clarify this problem, the contents of dissociative groups in these humc acids are presumed to be too high to refute the electrostatic interaction among the dissociative groups, because the contents of

dissociative groups in these humic acids (Table 1) were almost as high as that of pectic acid (5.71 me/g) or polyaspartic acid (7.82 me/g) which are used as the representative samples for polyelectrolyte. Therefore, for Tsubame Pg the linear relationship of Eq. (3) with n larger than 1 seems to indicate that Pg pigments exist as polymer in humic acids. The high significance of the correlation for Tsubame Pg may be due to Pg pigments which highly contribute to the ultraviolet and visible absorption of Tsubame Pg. The linear positive correlation between the pK_a and the $\log i/(1-i)$ for Temmondai, Inogashira, Nagara OH, and Kinshozan F humic acids were not so high as that of Tsubame Pg. This may be because the composition of the dissociative groups in these humic acids is more complex than that of Tsubame Pg. Many inflection points existing in the absorbance-pH curves of these humic acids suggest that there are various carboxyl and phenolic groups differing in pK_a values in these humic acids. Furthermore, the discontinuous distribution of these pK_a values implies that the pK_a values of the dissociative groups in these humic acids do not always follow the Gaussian distribution.

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