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ELECTROMETRIC TITRATIONS OF HUMIC ACIDS

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An account is given of investigations into the possibility of distinguishing different acid groups in synthetic and soil humic acids by means of electrometric titration. The potentiometric and conductometric titrations in aqueous and non-aqueous solutions and the high frequency titration in non-aqueous media are discussed. Conductometric and especially high frequency titration of humic acids in dimethylformamide with sodium isopropylate showed a distinct maximum in the titration curve which is assumed to be the equivalence point for the less weak acid groups, presumably carboxyl groups. The high frequency titration described is therefore proposed as an accurate and rather convenient method to determine this type of acid groups.

The minimum in the H.F. titration curves appeared to correspond more or less with the amount of very weak acid groups (probably phenolic hydroxyl groups) judging from the total exchange capacity determined by other methods. The reproducibility of the maximum, however, appeared to be better than that of the minimum. The ratio between the amount of different acid groups thus determined appeared to diverge for different humic acids.

The difference in exchange capacity when determined by potentiometric and conductometric titration with different bases in aqueous media was demonstrated not to be caused by steric effects.

INTRODUCTION

The main purpose of the present investigation was to determine exactly whether and in what ratio different acid groups are present in humic acids. In literature several methods to study this question are proposed which can be classified in two groups:

1. Organic-chemical methods (*e.g.* Halle 1935, Broadbent & Bradford 1952 and Forsyth 1947): This includes "exhaustive" methylation of "phenolic hydroxyl groups" with dimethyl sulphate, and determination of the increase of methoxyl content, or acetylation and determination of the increase in acetyl content, and also "exhaustive" methylation with diazomethane, which reacts with "all" active hydrogen, including those of carboxyl groups. Determination of active hydrogen was also carried out with CH_3MgBr. The difference between the values obtained with one of the first two methods and the value obtained by the diazomethane treatment is supposed to give the carboxyl groups content. An estimation of the latter is made directly by determining the increase of methoxyl upon esterification with $CH_3OH-HCl$. The complications of these methods include tediousness, incompleteness of the methylation or acetylation, or (with diazomethane) side reactions with other groups.

2. Inorganic methods (*e.g.* Ubaldini 1937, see also Kononova 1958): The sum total of the acid groups is determined with alkali or with barium hydroxide, usually by means of potentiometric or conductometric titration in aqueous solutions or suspensions. Some investigators suppose the amount of carboxyl groups to be equivalent to the amount of CO_2 liberated by humic acid solutions from calcium carbonate. Others take the amount of acetic acid set free by humic acids in a calcium acetate solution. A determination of COOH-groups by means of titration with a weak base such as ammonia was also proposed. Some investigators simply suppose that the acid groups which have not reacted with a

base at pH 7 are phenolic hydroxyl groups and that those were neutralized are carboxyl groups. Others take as this limit pH 8.2. However, when applying this to a titration curve of humic acids, the content of phenolic OH-groups would be a function of the concentration of the humic acid solution; more acid groups being dissociated at a certain pH the more the solution is diluted.

Combinations of a method of Group 1 with one of Group 2 were also applied.

It is evident that several of these methods, but especially those of Group 2 for determination of one type of acid groups, are entirely empirical. It is therefore not surprising that the determined values often do not agree. Applying electrometric titration to distinguish between the different kinds of acid groups would have a distinct advantage over other methods, while not only the inflexion points or maxima and minima are important, but also the course of the curves in between these points can give interesting additional information. Several authors give titration curves of humic acids in aqueous media, *e.g.* Gillam (1940), Puustjärvi (1955) and Sandhoff (1954). However, the usual inflexion, about half-way along the curves obtained with potentiometric titration with sodium hydroxide, is too indistinct for drawing conclusions.

In a former publication (van Dijk 1959) it has been reported that attempts to distinguish between the different kinds of acid groups by means of potentiometric and conductometric titrations with tetrabutylammonium hydroxide in non-aqueous solutions had failed. Prolonged experiments, however, gave better results. An account of these investigations is given below.

Auerbach & Smolczyk (1924) calculated that, when dibasic acids are titrated, no inflexion point can be detected at the first equivalence point unless the ratio of the two dissociation constants is larger than 16. Sims & Peters (1957), however, calculated that the ratio K_1/K_2 should be larger than $59.7a_1/a_2$ to be able to perform a differential titration of a mixture of two acids; a_1 and a_2 are the concentrations of the stronger and the weaker acid, respectively. For a dibasic acid ($a_1 = a_2$) the ratio of K_1/K_2 thus should be larger than 59.7. In practice the detection of an inflexion point is difficult at a ratio smaller than 100.

As is well known, a polybasic acid exhibits a series of dissociation constants, which decrease as more protons are dissociated off. Thus, when one wants to distinguish between these respective dissociation constants, the necessity of a rather wide ratio of these constants sets a rather narrow limit to the amount of acid groups per molecule.

With humic acids, we have to deal with polybasic acids with, as is supposed, at least two different types of acid groups, viz. carboxyl and phenolic hydroxyl groups. The carboxyl function is more acidic than the phenolic hydroxyl function. So, accordingly, it would only be possible to distinguish between these types of acid groups by titration when the pK of the last of the dissociating carboxyl groups differs sufficiently from the pK of the first of the dissociating phenolic hydroxyl groups. As both groups are weakly acidic, it is not surprising that a differential titration in aqueous solution fails. So we are committed to other solvents where a weak acid behaves as a stronger acid in water; in other words, solvents with a higher affinity for protons, *i.e.*, a basic solvent. However, this solvent must not be too basic, for otherwise the protons of both types of acid groups would react completely with the solvent molecules according to the equation:

$$\begin{array}{ccccccccc} \text{HA} & + & \text{S} & \longrightarrow & \text{SH}^+ & + & \text{A}^- \\ \text{acid} & & \text{basic} & & \text{solvated} & & \text{conjugate} \\ & & \text{solvent} & & \text{proton} & & \text{base of acid} \end{array}$$

In this case both acid functions would seem to be equally strong. This is called the "levelling effect" of the solvent. For example, a strong "levelling solvent" such as ethylene diamine, as used by Brockmann & Meyer (1953), is often unsuitable for differential titrations of weak acids.

Other requirements for the solvent are:

It must dissolve the humic acids; there should be no disturbing side-reactions; the products of titration should be soluble or at least precipitates should not be highly absorptive, as these cause low results and fading end-points.

For a more detailed treatise on acid-base titrations in non-aqueous media the reader is referred to Jones (1959), where further references are given.

EXPERIMENTAL

Materials and reagents

Humic acids were isolated from several soil types and also prepared synthetically. For the methods of isolation and purification of the soil humic acids, reference is made to a former publication (van Dijk 1959). The synthetic humic acids were prepared according to Flaig (private communication) by oxidizing hydroquinone, pyrocatechol and pyrogallol in alkaline and in ammoniacal medium with gaseous oxygen.

Dimethylformamide (D.M.F.; Merck) was used without further purification. Pyridine was dried over sodium hydroxide (pellets) and distilled. Isopropyl alcohol (I.P.A.) was dried over metallic sodium and distilled.

Tetrabutylammonium hydroxide (T.B.A.H.) 0.3 N was prepared according to Harlow, Noble & Wyld (1956): 30.75 g. of tetrabutylammonium iodide (Schuchardt) was dissolved in I.P.A., converted into the hydroxide by percolating over a column of Amberlite I.R.A.400 in OH-form (previously rinsed with pure I.P.A. to remove water) and diluted with I.P.A. to 250 ml. The solution was standardized potentiometrically against benzoic acid in D.M.F.

Sodium isopropylate 0.3 N was prepared according to Bruss & Harlow (1958): 3.45 g. of clean-cut sodium was dissolved in 250 ml. of I.P.A. in a N_2-atmosphere and diluted with pyridine to 500 ml. The solution cannot be stored for a long time. The solution was standardized against benzoic acid in D.M.F. using crystal violet as an indicator. Standardization by adding 10 ml. of titrant solution to 25 ml. of water and titrating with 0.1 N HCl in water with phenolphtalein as an indicator gives the same result.

We chose a higher normality of the titrant solutions than most other investigators in order to minimize the increase of volume during titration, for which we did not correct.

Several other solvent-titrant combinations were tried without improvement on the above procedure.

Apparatus

The potentiometric titrations were made with a Radiometer, pH-meter Nr 22, equipped with the G 202 BT glass electrode and a saturated calomel bridge with an aqueous agar-salt bridge. In non-aqueous media there are no special complications when T.B.A.H. is used as a titrant. Platinum electrodes as were used by Harlow, Noble & Wyld (1956) and Brockmann & Meyer (1953) appeared to offer no improvement here.

The conductometric titrations were carried out with the Philips measuring bridge GM 4249, operated at 1,000 c/sec. For the electrode system the platinized platinum electrodes belonging to the dip-type measuring cell PR 9512/900 were used.

The titration vessel used for the potentiometric and conductometric titrations was constructed with a cooling mantle to maintain constant temperature. Stirring is accomplished with a magnetic stirrer.

The high frequency measurements were made with a made-up oscillometer. A block diagram is given in Figure 1 together with a detailed diagram of the high frequency circuit. The feeding is highly stabilized. The oscillometer can be employed at three frequencies, viz. approximately 3.5, 8, and 30 megacycles

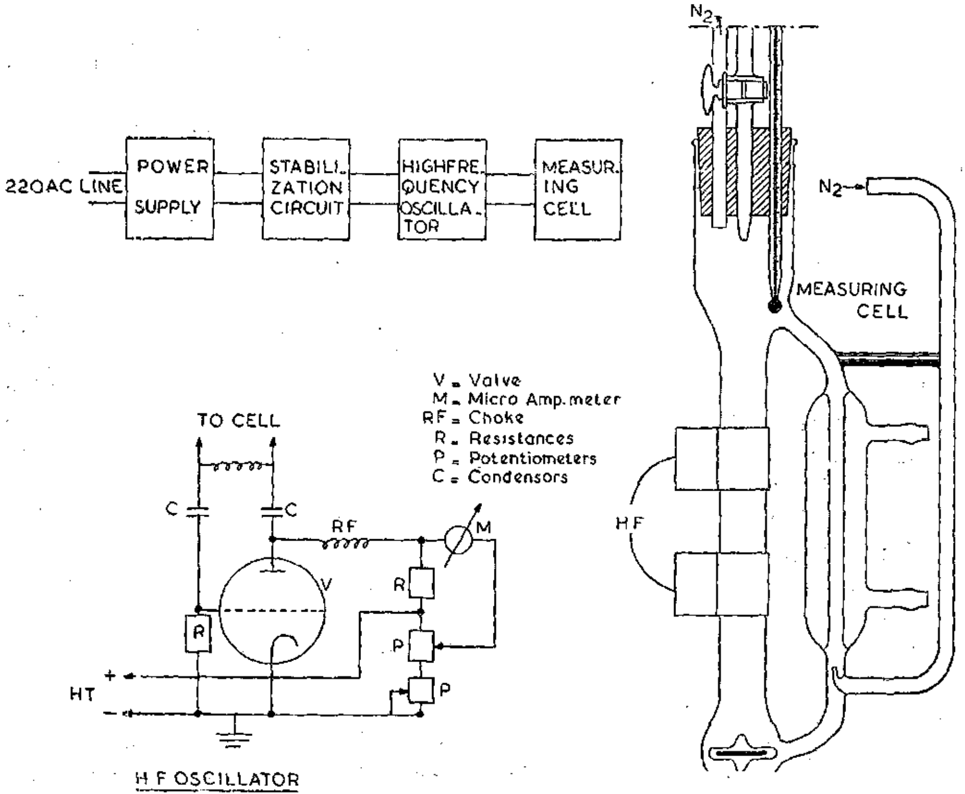

Figure 1. High Frequency titrator and measuring cell.

Figure 1 also gives a drawing of the measuring cell used. The nitrogen gas flow, entering in the side-arm, to avoid oxygen and carbon dioxide, serves at the same time as a stirring device. This is supplemented by a magnetic stirrer. By circulating water of constant temperature through the small cooler on the side arm, the temperature in the cell could be held at 25°C ± 0.1. A 5 ml. microburet, divided in 0.01 ml. was used for all titrations.

Titration procedure

As dry humic acids do not readily dissolve in water, titration of suspensions of the dry compounds in aqueous solution often gives erroneous results (*e.g.* Puustjärvi 1955). We found, for example, that a rapid determination of the exchange capacity of a suspension of dry, finely ground humic acids gave a value which was about 60% too low. Therefore, some investigators recommend a back titration of a sodium humate solution with hydrogen chloride. In this case, however, the course of the curve is somewhat altered because of a small but perceptible salt effect. In our experiments all titrations in aqueous solution were carried out with solutions which were prepared by dissolving the dry humic acids in a small amount of diluted sodium hydroxide and removing the sodium ions by percolating this solution over a column of Amberlite IR 120 in H-form. From the stable solution of humic acid thus obtained (containing 10 g. per l.) 10 ml. was pipetted into the titration vessel and diluted with 50 ml. of water. The titration could then be carried out rapidly and accurately.

For the titrations in non-aqueous solution: 100 mg of dry humic acids was weighed accurately in a tube or directly in the titration vessel and 10 ml. of D.M.F. was added. The solubility of the humic acids in D.M.F. is dependent on their ash content. Humic acids with a high ash content, *e.g.*, those from clay soils, may be difficultly soluble or even partly insoluble in D.M.F. When the humic acids are sufficiently swollen and dissolved, the solution is diluted or, when predissolved in a tube, rinsed over in the titration vessel with 50 ml. of D.M.F.

All other organic solvents tried displayed an insufficient solvent capacity. Thus only mixtures of solvents could be tried. This was achieved by dissolving the dry humic acids in 10 ml. of D.M.F., whereafter this solution was diluted with 50 ml. of other solvents, *e.g.*, pyridine, ethylene diamine, acetonitrile, etc. In this way precipitation of the humic acids often occurred. All the titrations were carried out in a nitrogen atmosphere and at constant temperature of 25°C.

In several titrations, especially those in non-aqueous media, a precipitate is formed during the titration. In such cases equilibrium was sometimes reached more slowly.

RESULTS AND DISCUSSION

Figure 2 shows the curves of some humic acids titrated potentiometrically in aqueous solution with sodium hydroxide. With other monovalent bases the course of the curve is not significantly different, except, of course, with a weak base as ammonia, which cannot give a pH higher than about 9. With barium hydroxide the weak inflexion in the first part of the curves obtained with NaOH disappeared and the exchange capacity seemed to be somewhat higher. It is, however, in all these titrations difficult to determine the end-point accurately.

The difference in exchange capacity, when determined with different bases is ascribed by Broadbent & Bradford (1952) partly to steric effects. Heimann (1929) reports that the exchange capacity of humic acids, determined with quinine and quinidine, was respectively 30 and 70% lower, as with alkali hydroxide. This could be caused by steric hindrance. If this were the cause indeed, it would give valuable information about the structure of humic acids. Heimann, however, worked with a suspension of dry humic acids, and he,

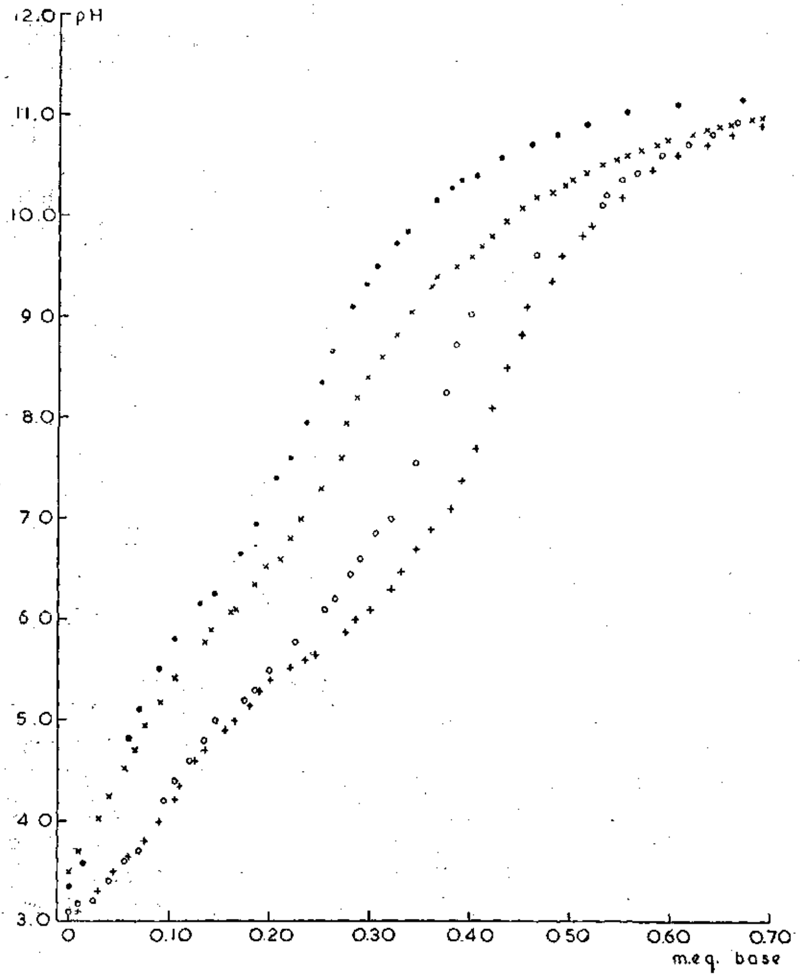

FIGURE 2. Potentiometric titration of 100 mg. of humic acids in 60 ml. of water with 0·3 N NaOH.

. . . . humic acids from an old black sphagnum moor-peat.
x x x x humic acids prepared from hydroquinone in ammoniacal medium.
o o o o humic acids from a woody bog-peat.
+ + + + humic acids from a diluvial sand soil covered with heath.

The ash contents of these humic acids are 0.64, 0.00, 1.00 and 1.24%, respectively.

presumably, did not realize that these tertiary amines are too weakly basic to react completely with the humic acids. We checked the possibility of steric hindrance by titrating an aqueous solution of humic acids with the quaternary ammonium bases methylquininium hydroxide, methylbrucinium hydroxide, and

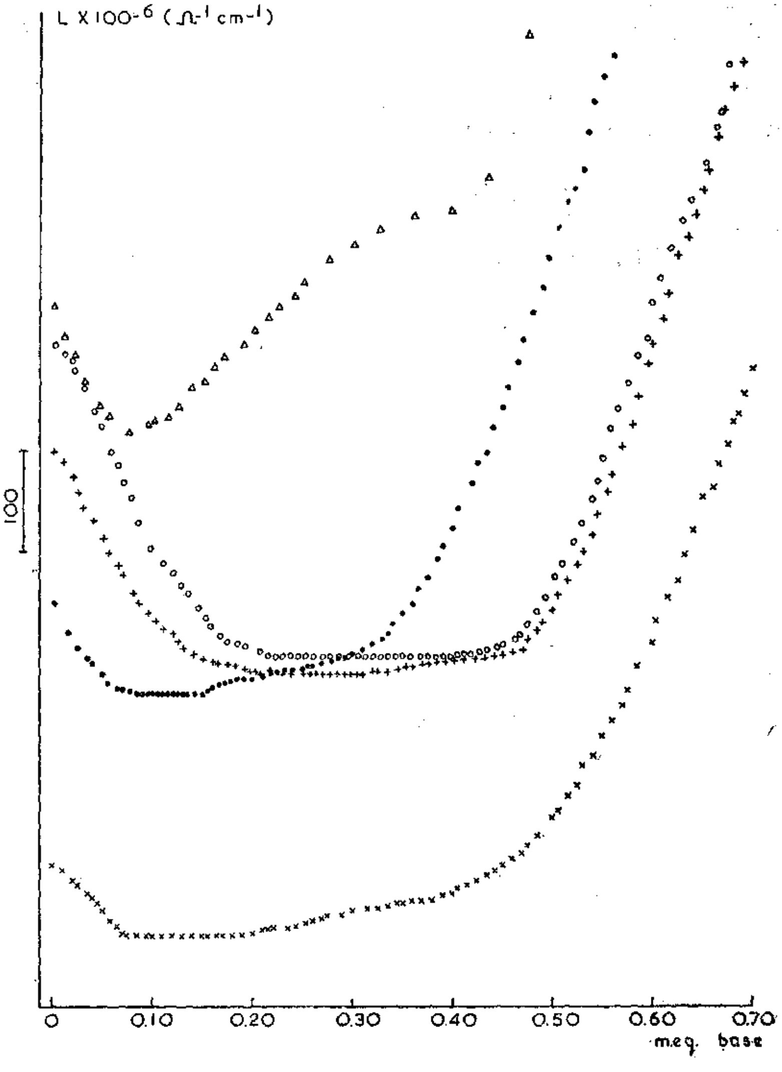

FIGURE 3. Conductometric titration of 100 mg. of humic acids in 60 ml. of water.
△△△△ humic acids from a woody bog-peat, titrated with 0.3 N KOH.
o o o o the same humic acids, titrated with 0.2 N $Ba(OH)_2$.
++++ humic acids from a diluvial sand soil covered with heath, titrated with 0.2 N $Ba(OH)_2$.
. . . . humic acids from an old black sphagnum moor-peat, titrated with 0.2 N $Ba(OH)_2$.
x x x x humic acids prepared from pyrocatechol in ammoniacal medium, titrated with 0.25 N $Ba(OH)_2$.

The ash contents of these humic acids are 1.00, 1.24, 0.64 and 0.00%, respectively.

tetrabutylammonium hydroxide. The first two bases were easily prepared by adding methyl iodide to an ethereal solution of quinine and brucine, respectively, and converting the quaternary iodides obtained into the corresponding bases by percolating an aqueous solution of the iodides over Amberlite IRA 400 in the OH-form. The titration curves, however, appeared to be almost identical with those obtained with NaOH. So the difference in exchange capacity when determined by potentiometric and conductometric titration with different bases in aqueous medium appears to be not caused by steric effects.

The titration curves of synthetically prepared humic acids, of which one has been drawn in Figure 2, do not differ essentially from those of the soil humic acids.

Figure 3 shows some curves obtained with conductometric titration of an aqueous solution of humic acids with barium hydroxide. The end-point of the titration can be determined much better than by potentiometric titration. Here

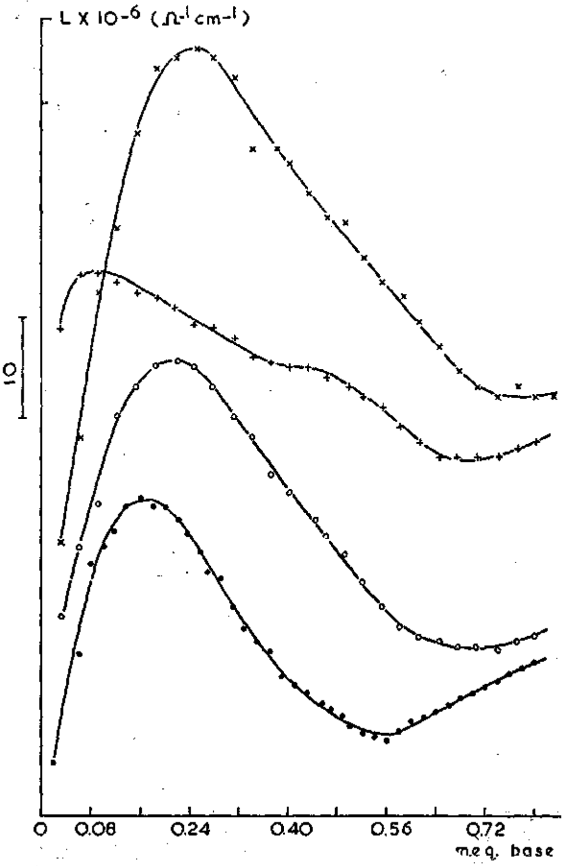

Figure 4. Conductometric titration of 10 mg. of humic acids in 60 ml. of D.M.F. with 0.3 N $NaOC_3H_7$iso.

• • • • humic acids from a reclaimed peat soil.
o o o o humic acids from a woody bog-peat.
++++ humic acids prepared from pyrocatechol in ammoniacal medium.
x x x x humic acids prepared from pyrocatechol in alkaline medium.

The ash contents of these humic acids are 1.88, 1.00, 0.00 and 0.51% respectively.

no esential differences were found between synthetic and soil humic acids. As the alkali humates are much better ionized in aqueous solution than the barium humates, the conductivity of a K-humate solution is much higher than of a Ba-humate solution, as is shown in Figure 3. Therefore, the end-point of the titration with alkali is more difficult to determine. Here also the exchange capacities calculated from the curves obtained with Ba $(OH)_2$ are higher than those obtained with NaOH.

Potentiometric titration of humic acids in non-aqueous media appeared to give no more information about different acid groups, notwithstanding the many attempts made with different solvent-titrant combinations. The best that can be said is that the end-point often could be better determined than with the potentiometric titration of aqueous solutions. One typical curve is shown in Figure 6.

Figure 4 shows some curves of humic acids dissolved in D.M.F. and titrated conductometrically with sodium isopropylate in I.P.A.-pyridine. These curves indicate that a differential titration, enabling different acid groups to be distinguished, is possible. One has, however, to be cautious with the interpretation in view of the similar titration curves which Bruss & Harlow (1958) and van Meurs & Dahmen (1958) obtained from monobasic acids. Bruss & Harlow found in some cases that when a solvent was employed with an intermediate or low dielectric constant, a conductance maximum at about the mid-point of the titration curve is obtained. This they ascribe to an acid-anion complex which is ion-paired to the titrant cation. The presence of small amounts of strong hydrogen-bonding solvents as isopropyl alcohol annihilated this mid-point maxima while destroying the acid-anion complex. In our titrations of humic acids with addition of every drop of titrant an excess of I.P.A. is added, compared with the added amount of sodium isopropylate. Therefore, the formation of such an acid-anion complex seems improbable. Besides, in our curves the maxima occur much earlier than at mid-point of the titration, and the dielectric constant of D.M.F. (36.7) is much higher than those of the solvents employed by Bruss & Harlow. Van Meurs & Dahmen, however, explain the N-shape of their curves from precipitate formation, which is very likely to play an important role indeed. In our conductometric titrations of humic acids, however, precipitate formation usually started long before the maximum in the curve was reached. We are, therefore, of the opinion that the maximum in our curves corresponds with the equivalence point of a certain type of acid grouping in humic acids, presumably carboxyl groups. Other indications for this are given below. The minimum in the curves was determined with less accuracy and was also less reproducible. Conductometric titrations of humic acids in D.M.F. with T.B.A.H. in I.P.A. did not show an end-point.

Much better results than with conductometric titrations were obtained with the high frequency titration of humic acids in D.M.F. using sodium isopropylate as a titrant. One of the advantages of this method is that the electrodes are not in direct contact with the solution. Also, the apparatus appeared to respond very sensitively. According to Blaedel & Petitjean (1956) the oscillator response depends in a rather complicated way on the dielectric constant and the specific conductance, so it is not so simple to translate the response, here measured in

μA, in terms of chemical properties of the investigated system. If, however, a maximum and a minimum occur in the titration curves, then they can be expected to correspond with a conductance maximum and minimum. So in this sense it is not surprising that the curves obtained with high frequency titration correspond to a rather high degree with those obtained with conductometric titration of the same humic acids in the same medium.

In Figure 5 some high frequency titration curves are shown. It is important

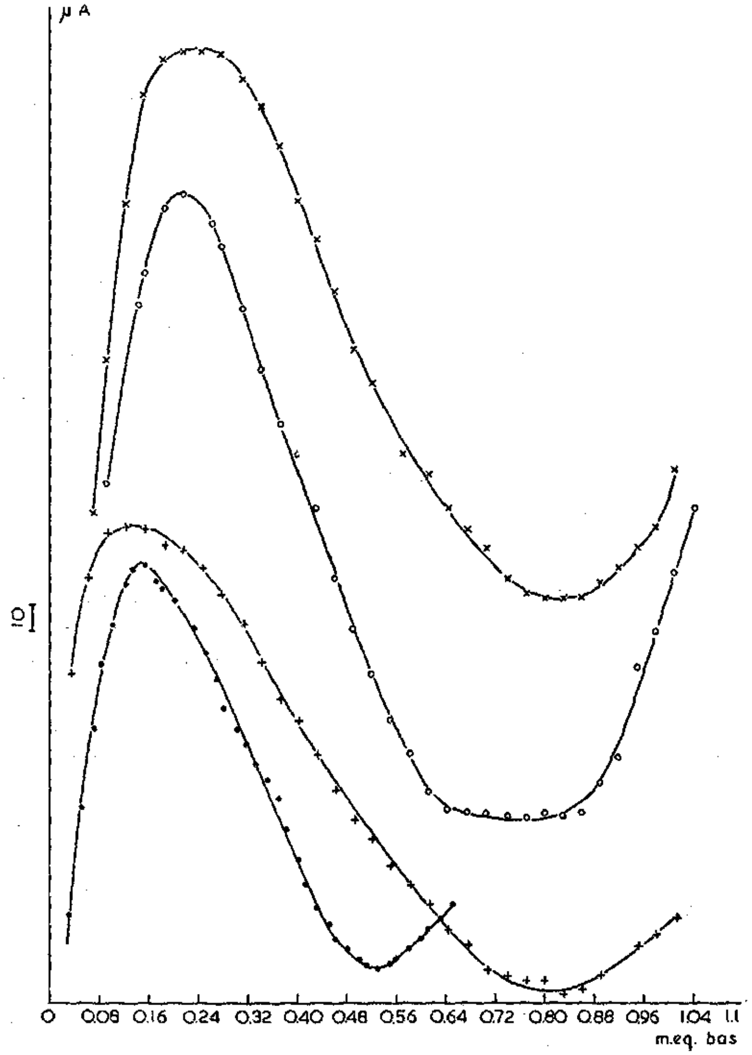

FIGURE 5. High frequency titration of 100 mg of humic acids (calculated on ash-free base) in 60 ml. of D.M.F., titrated with 0·3 N NaOC$_3$H$_7$iso at 8 megacycles.

• • • • humic acids from an old black sphagnum moor-peat.
++++ humic acids prepared from pyrocatechol in ammoniacal medium.
o o o o humic acids from a woody bog-peat.
x x x x humic acids prepared from pyrocatechol in alkaline medium.

The ash contents of these humic acids are 0·64, 0·00, 1·00 and 0·51 %, respectively.

to note that the positions of the maximum and the minimum in these curves are much better determined than with conductometric titration. As can be seen further, the ratio between the amounts of base consumed at the maximum and at the minimum is not the same for different humic acids. As to what extent this ratio is an indication of the degree of "ripening" of humic acids remains to be further investigated.

In Figure 6 five kinds of titration curves of one humic acid are shown

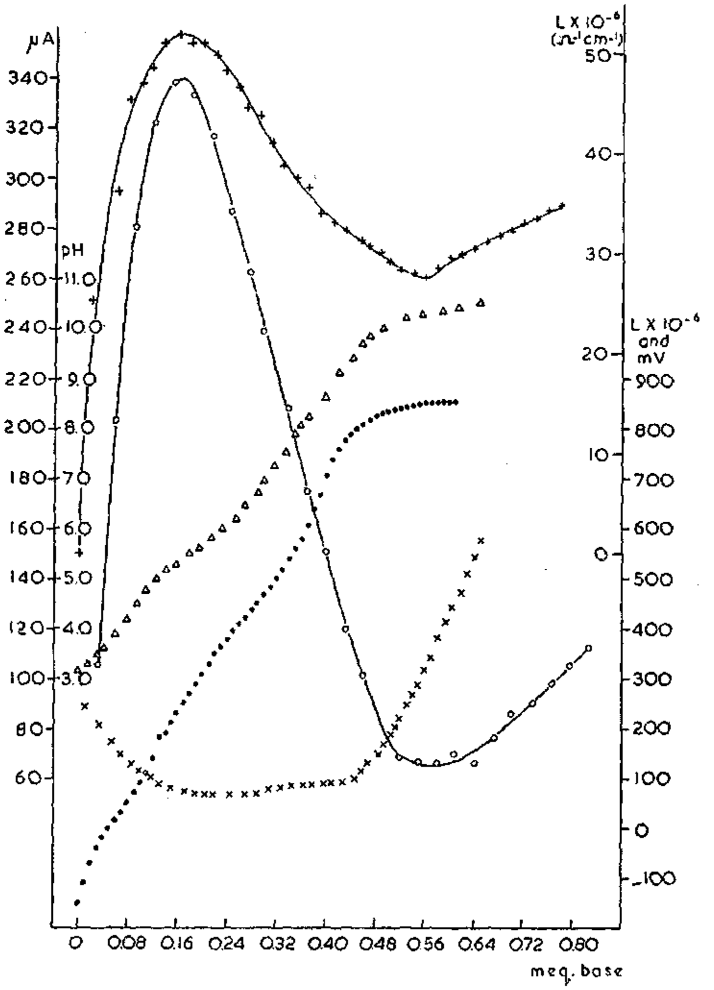

FIGURE 6. Five types of titration curves of 100 mg. of humic acids from a reclaimed peat soil in 60 ml. of solvent.

△ △ △ △ potentiometric titration in water with 0·3 N NaOH (pH-scale).
x x x x conductometric titration in water with 0·2 N Ba(OH)$_2$ (conductivity-scale on right side of right-hand ordinate).
· · · · potentiometric titration in D.M.F. with 0·3 N T.B.A.H. (mV-scale).
+ + + + conductometric titration in D.M.F. with 0·3 N NaOC$_3$H$_7$iso (conductivity-scale on left side of right-hand ordinate).
o o o o high frequency titration in D.M.F. with 0·3 N NaOC$_3$H$_7$iso (micro Amp.—scale).

together. What we want to emphasize especially is the coincidence of the maximum in the curves obtained with high frequency titration and conductometric titration in D.M.F. with the weak inflexion in the curve obtained with potentiometric titration in aqueous solution. This seems to be another indication that these maxima are real equivalence points. The occurrence of only one maximum and one minimum in the titration curves of humic acids is considered to be the result of a levelling of the mutual differences between the dissociation constants of the carboxyl groups in D.M.F., while also the mutual differences between the dissociation constants of very weak acid groups obviously have disappeared.

As for the minimum in the curves, it is more difficult to draw exact conclusions. This minimum is less reproducible than the maximum, which is perhaps partly due to the fact that at this point the humates are almost com-

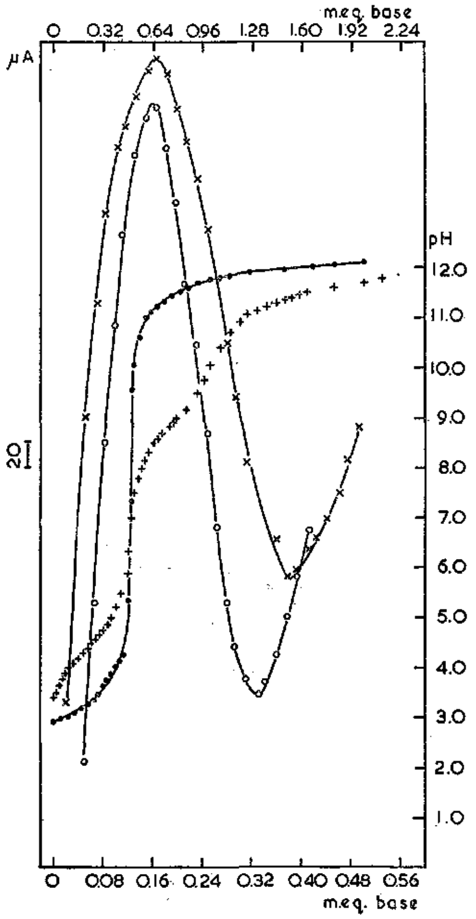

FIGURE 7. Titration curves of salicylic and gallic acid. Potentiometric titration of 0·5 mMole in 60 ml. of water with 0·3 N NaOH (pH against m. eq. base, top scale) : .... salicylic acid, ++++ gallic acid.
High frequency titration of 0·13 mMole in 60 ml. of D.M.F. with 0·15 N NaOC$_3$H$_7$iso (micro-Amp. against m. eq. base, lower scale) : oooo salicylic acid, xxxx gallic acid.

pletely coagulated. Obtaining a reproducible minimum also makes higher demands upon the quality of the titrant solution than in the case of reproducing the maximum. The minimum, taken as an end-point of the titration, corresponds with a somewhat higher "exchange capacity" than the end-point determined with conductometric titration in aqueous solution with barium hydroxide. Possibly this is caused by a more complete reaction of the sodium alcoholate with weak acid groups. Here it has to be remembered that in D.M.F. more groups can be proton-donating than in aqueous solutions.

On the other hand, it is not permissible to assume that the minimum gives the amount of phenolic hydroxyl groups which are totally present in humic acids. There may be phenolic OH-groups remaining which do not react with the alcoholate at all, as is shown in Figure 7. Salicylic and gallic acid were taken as model substances of humic acids. Salicylic acid behaves as a monobasic acid upon potentiometric titration with sodium hydroxide in aqueous medium. When titrating on the high frequency method and also conductometrically (van Meurs & Dahmen 1958) both equivalence points could be determined. Gallic acid, when titrated potentiometrically in aqueous solution, shows a distinct first equivalence point for the carboxyl group and one more indistinct at well over twice the value. High frequency titration of gallic acid in D.M.F. with sodium isopropylate shows a sharp maximum for the carboxylic group and a minimum at three equivalents. So there remains one hydroxyl group for which no equivalence point could be determined.

Furthermore, in the high frequency titration of gallic acid also a precipitate is formed before the first equivalence point is reached.

So, we think that in high frequency titration we have found an accurate and rather convenient method to determine the amount of less weak acid groups, presumably carboxyl groups, in purified humic acids. Whether the same can be said for the amount of very weak acid groups which are assumed to be phenolic hydroxyl groups is not yet definitely established. Perhaps the determination of the total amount of groups which react as acids in aqueous solution is best accomplished with conductometric titration with barium hydroxide. Then the content of very weak acid groups can be obtained by subtraction.

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