THE TITRATION OF HYDROXY ORGANIC ACIDS IN THE PRESENCE OF FERRIC AND CUPRIC SALTS

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In previous work (1, 2) it was pointed out from theoretical reasoning that when a hydroxy organic acid combines with a metal such as iron to form an unionized compound the acid properties of the alcoholic hydroxyl group must be increased. There is abundant evidence in the literature which indicates that the hydrogen of such hydroxyl groups may be replaced by a metal. References to most of this work may be found in the handbooks of Gmelin (3) and Abegg *et al.* (4). There is apparently no direct evidence, however, showing how strongly acidic these groups become. The only titration curves with which the writer is familiar are those of Wark and Wark (5) on the titration of the normal copper salts of several such acids with sodium hydroxide. These authors followed the potential of their solutions during the titration by means of a hydrogen electrode. It is apparent from the potential values that they obtained, that they were not measuring pH but an oxidation-reduction potential instead. The present paper is the result of an attempt to follow, by means of a glass electrode, the titration of representative hydroxy organic acids alone and in the presence of ferric chloride and of cupric chloride.

The glass membranes were made in the manner described by MacInnes and Dole (6). The glass used was obtained from the Corning Glass Company, their No. 015. The MacInnes type of permanently sealed electrode containing 0.1 M HCl and a Ag, AgCl wire was used. The membranes were calibrated against buffers of known pH and this calibration was checked at frequent intervals. The electromotive force was measured by means of a

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vacuum tube potentiometer arrangement of the type described by Partridge (7). The readings obtained were accurate to 1 millivolt.

The results are presented in Figs. 1 to 6. Curves are included for ferric chloride, for cupric chloride, for each of the following acids, lactic, glycollic, oxalic, malic, tartaric, and citric, and for each of

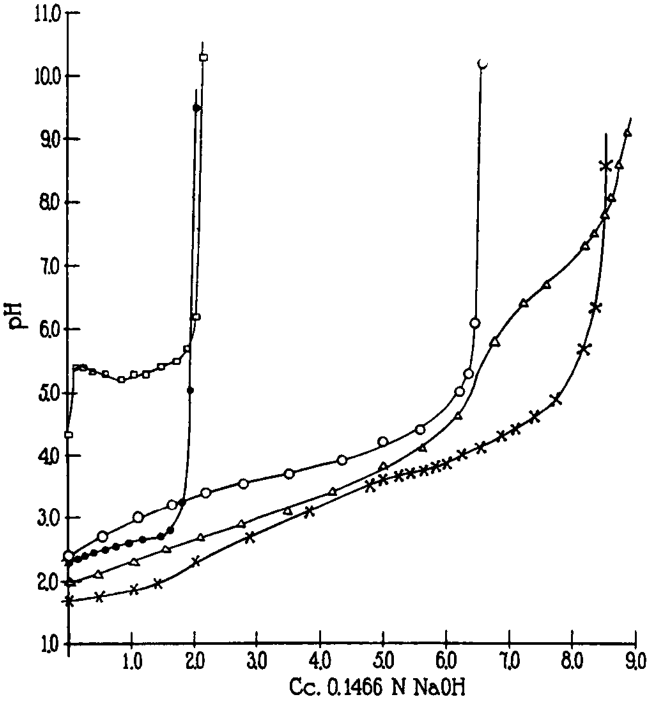


FIG. 1. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. lactic acid, △ 5.0 cc. lactic acid + 1.0 cc. $CuCl_2$, × 5.0 cc. lactic acid + 1.0 cc. $FeCl_3$.

these acids in the presence of ferric chloride and in the presence of cupric chloride.

It may be seen from each of the figures that the mixtures of acid and metal chloride are considerably more acidic than either of these substances alone. The beginning of each curve where iron is present clearly indicates that we are titrating some strong acid. We interpret this as an indication of the fact that the metal and the acid combine to form a complex with the liberation of free hydrochloric acid.

Let us examine the results with lactic acid (Fig. 1) in some detail. It may be seen that the quantity of cupric chloride used requires 2.0 cc. of base for its neutralization. At this point all the copper has been precipitated as cupric hydroxide. The quantity of lactic acid used requires 6.5 cc. of base for its neutralization. Now, if the cupric chloride and lactic acid are mixed and the mixture is titrated it requires an amount of base equal to the sum required for the two separate solutions. At this point the copper has again been precipitated as cupric hydroxide. Thus, under the conditions of these experiments lactic acid is unable to prevent

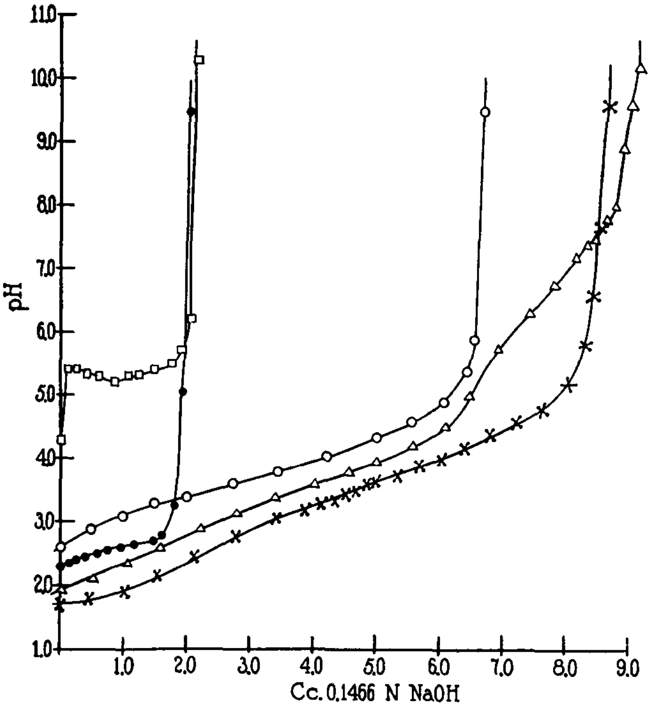


FIG. 2. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. glycollic acid, △ 5.0 cc. glycollic acid + 1.0 cc. $CuCl_2$, × 5.0 cc. glycollic acid + 1.0 cc. $FeCl_3$.

the precipitation of copper as the hydroxide in a neutral or alkaline solution. If we add to the lactic acid an amount of ferric chloride equivalent to the cupric chloride previously added we get a quite different curve. Again the end-point comes when an amount of base equivalent to the two separate solutions has been added, but at this point there is no precipitation of ferric hydroxide. It should be noticed that there is a distinct break in this curve at a point corresponding to pH 3.5. The significance of this break will be discussed later.

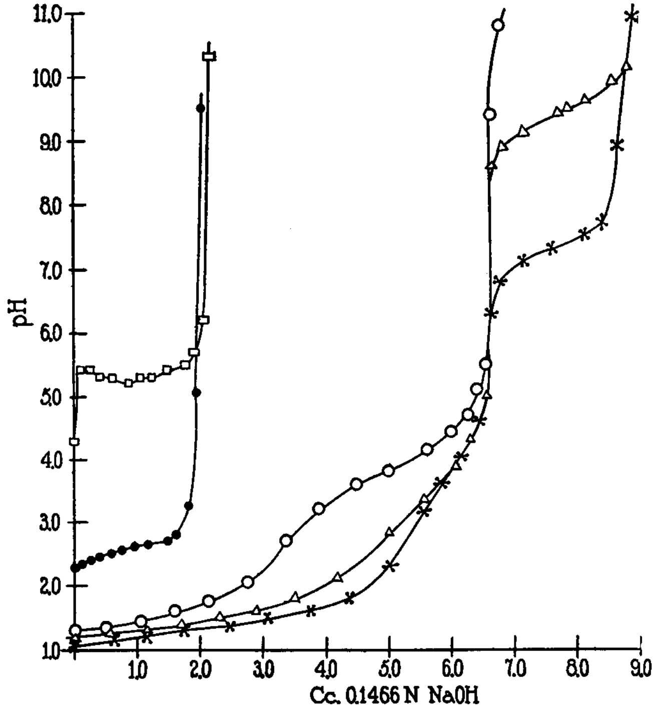


Fig. 3. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. oxalic acid, △ 5.0 cc. oxalic acid + 1.0 cc. $CuCl_2$, × 5.0 cc. oxalic acid + 1.0 cc. $FeCl_3$.

The results with glycollic acid are presented in Fig. 2. In general they are the same as those with lactic acid. The break in the curve for acid plus iron is less marked but still definitely perceptible.

It may be well to examine, at this point, the curves obtained with some acid that forms a complex with metals, but which does not possess an alcoholic hydroxyl group. Such an acid is oxalic. Fig. 3 presents the curves obtained with it. It may be seen that the first end-point occurs at the end-point of the oxalic acid itself and this is followed by the precipitation of the ferric or cupric hydroxide. Under the conditions of these experiments this acid does not prevent the precipitation of either ferric or cupric hydroxide in an alkaline solution. An important point to be observed is that that part of the curve which corresponds to the second constant of the oxalic acid lies in a much more acid range when either iron or copper is present than in their absence.

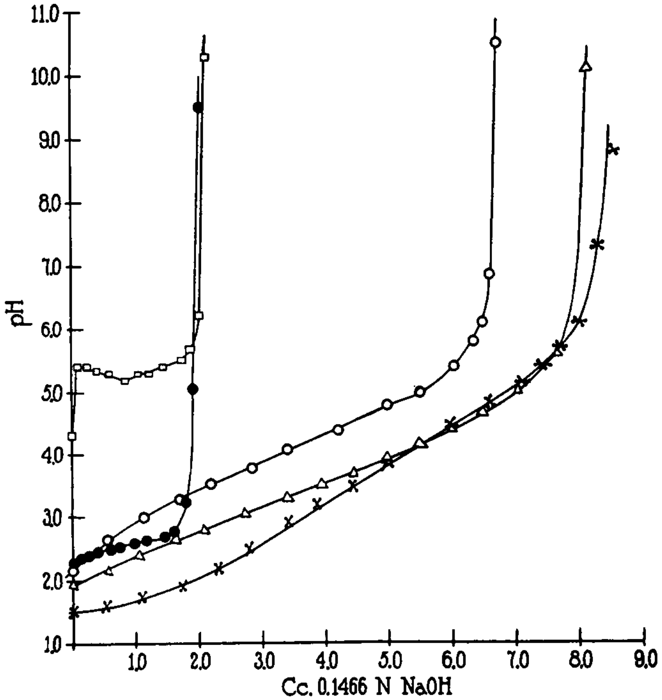


Fig. 4. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. malic acid, △ 5.0 cc. malic acid + 1.0 cc. $CuCl_2$, × 5.0 cc. malic acid + 1.0 cc. $FeCl_3$.

Figs. 4 to 6 present the results obtained with malic, tartaric, and citric acids, respectively. It may be observed that with these acids the behavior of iron and of copper is similar. In each case the amount of base required to titrate a mixture of acid plus metal chloride is very considerably more than that required for the acid alone and in no case is there any precipitation of metal hydroxide. It should also be noted that in no case does the titration of such a mixture require an amount of base as large as the sum required for the titration of the two solutions separately.

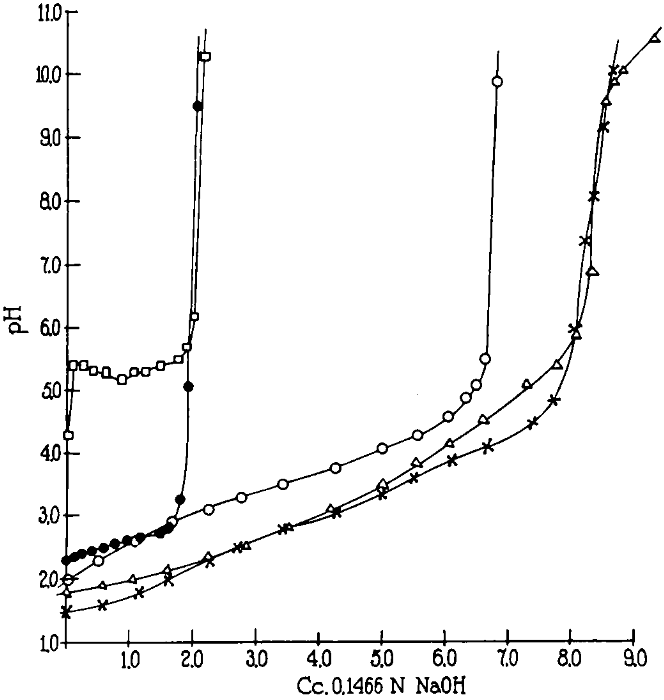


FIG. 5. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. tartaric acid, △ 5.0 cc. tartaric acid + 1.0 cc. $CuCl_2$, × 5.0 cc. tartaric acid + 1.0 cc. $FeCl_3$.

DISCUSSION

Let us consider the results with lactic acid first. We saw that the mixture of lactic acid and ferric chloride required for its neutralization an amount of base equal to that required for the two solutions separately. When the solutions are titrated separately all of the iron is precipitated as ferric hydroxide, but when the mixture is titrated no precipitate is formed. The question is, how did we use up so much base without precipitating any ferric hydroxide. There are at least two possible answers. The first of these is that ferric hydroxide was really formed, but it remained in colloidal solution instead of precipitating. One fact seemingly in favor of this view is that these solutions are not readily diffusible through a collodion bag. However, if one examines the various compounds reported by Gmelin (3) another explanation suggests itself for this lack of diffusibility. This is that the compounds which exist in solution have high molecular

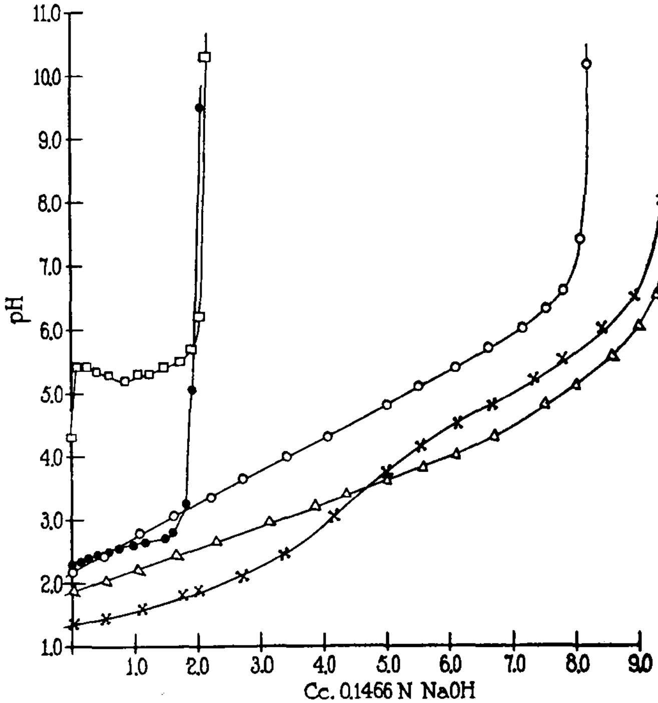


Fig. 6. □ indicates the curve for 1.0 cc. $CuCl_2$, ● 1.0 cc. $FeCl_3$, ○ 5.0 cc. citric acid, △ 5.0 cc. citric acid + 1.0 cc. $CuCl_2$, × 5.0 cc. citric acid + 1.0 cc. $FeCl_3$.

weights. Many of those reported contain 3 or more iron atoms and a corresponding number of organic residues. We have tested the diffusibility of the alkaline solutions of iron with each of the acids studied. In each case it is very slow, but in each case some iron passes through the collodion bag, in contrast to the complete lack of diffusibility with an ordinary sol of ferric hydroxide.

There are other difficulties in the way of accepting this colloidal explanation. These solutions are not sensitive to electrolytes. Any one of them may be saturated with ammonium sulfate without producing any precipitate. Relatively large amounts of calcium chloride do produce a precipitate, but this precipitate is not ferric hydroxide. It would seem to be the calcium salt, analogous, in the case of lactic acid, to the sodium, potassium, and ammonium salts isolated by Hofmann (8) to which he assigned the formula $M[Fe(CH_3CHOCO_2)_2]$, where M represents either sodium, potassium, or ammonium.

If these solutions contain the iron as colloidal ferric hydroxide then we have the problem of determining why an alcoholic hydroxyl group is so very important for the maintenance of the colloidal state. Thus lactic acid and glycollic acid would be good peptizing agents whereas propionic acid and acetic acid are of no avail. Similarly, neither oxalic acid nor succinic acid can produce the necessary action, but malic acid works very nicely and tartaric acid is exceedingly effective.

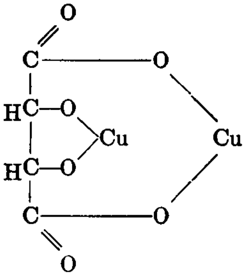
The other explanation is that when the lactic acid and iron combined to form a complex the alcoholic hydroxyl group took part in this combination. Such an action must make the oxygen of this group more positive and as a result the hydrogen must become more acidic. Then to the extent that iron is present lactic acid will behave as a dibasic acid. This will account nicely for the amount of base used without making it necessary to assume the formation of ferric hydroxide. Furthermore, it will account nicely for the two steps in the titration curve for lactic acid plus ferric chloride. According to this interpretation the pK_a for this alcoholic hydroxyl group is about 3.85. We may point out that this interpretation is in accord with our previous contention (2) that in dehydrogenation reactions the hydrogen separates from the molecule as hydrogen ion and the process of activation is simply a process of increasing the acid dissociation constant of the group involved.

An interesting observation concerning the acidity of such hydroxyl groups has recently been reported by Hölzl (9). He found that if the ester of salicylic acid was dissolved in absolute alcohol the hydroxyl group then exhibited appreciably acid properties.

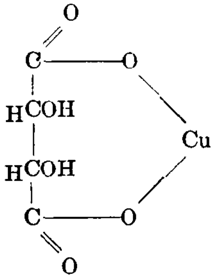
The explanation given for the results obtained with lactic acid will apply equally well to those obtained with glycollic acid. Let us see now how it will apply to oxalic acid. If this free acid forms an unionized compound with iron, and the curves (Fig. 3) show that it does, we would expect that the OH group of the undissociated carboxyl would take part in the combination. The result should be that this second carboxyl group would become a stronger acid than it is in the absence of a metal. The curves show that this is markedly true.

The same general explanation will apply to the results with malic, tartaric, and citric acids. Here we have the added complication, however, that the amount of base required for the neutralization of a mixture of any one of these acids with ferric or cupric chloride is not quite as large as the sum required for the neutralization of the two separate solutions. The explanation for this must lie in the formation of complex basic salts. This is in agreement with the results obtained by Packer and Wark (10) and by Wark and Wark (5) in their work on copper tartrates, as well as with the results of many other investigators (3, 4). Dumanskii and Chalisew (11), however, regard these copper tartrates as colloidal solutions of copper hydroxide. Similarly, Dumanskii and Yakovlev (12) regard solutions of ferric iron and various hydroxy acids as colloidal solutions of ferric hydroxide.

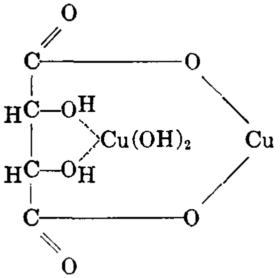
We may inquire here just what the difference is between the colloidal and the non-colloidal view-point regarding these solutions. Jellinek and Gordon (13) have obtained a crystalline copper salt of copper tartrate. They assigned to it this formula:



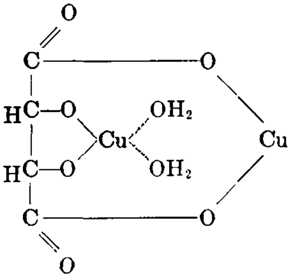
Dumanskii and Chalisew (11) have prepared what they believe is an identical salt and they assign to it this formula: Cu$T \cdot Cu(OH)_2 \cdot H_2O$. The T indicates tartrate. Let us see how this differs from Jellinek and Gordon's formula. We may write the CuT as



To this is attached a $Cu(OH)_2$ by means of a point (·). We must interpret this point as some kind of an attraction and this attraction must be furnished by the OH groups of the tartrate, for one cannot substitute succinic acid for the tartaric acid. By incorporating this into the formula we have



We may rewrite this as



This now differs from Jellinek and Gordon's formula by 2 molecules of water. If Jellinek and Gordon's compound be taken to represent the non-colloidal point of view and Dumanskii and Chalisew's compound to represent the colloidal point of view then, remembering that basic compounds may also be formed in which there is one copper attached to each hydroxyl group, and that one copper may be attached to a hydroxyl group in each of 2 tartrate molecules, there is really no difference as far as present data are concerned between the two views.

The application of these results to the titration of any one or any mixture of the hydroxy acids considered is clear. If one were to determine by titration the amount of these acids present in a solution containing proportionately as much iron, or in some cases as much copper, as the solutions used here, and were to use the turning point of phenolphthalein as end-point, the result would be in error by over 30 per cent.

SUMMARY

Titration curves, obtained by means of the glass electrode, are reported for lactic, glycollic, oxalic, malic, tartaric, and citric acids, and for each of these acids in the presence of ferric chloride and in the presence of cupric chloride.

It is shown that the presence of these metal salts greatly alters the titration curves. The nature of this change is discussed and the application of the results is pointed out.

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BIBLIOGRAPHY

1. Smythe, C. V., and Schmidt, C. L. A., *J. Biol. Chem.*, **88**, 241 (1930).
2. Smythe, C. V., *J. Biol. Chem.*, **90**, 251 (1931).
3. Gmelin, Handbuch der anorganischen Chemie, Berlin, 8th edition (1930).
4. Abegg, R., Auerbach, F., and Koppel, I., Handbuch der anorganischen Chemie, Leipsic (1930).
5. Wark, E. E., and Wark, I. W., *J. Chem. Soc.*, 2474 (1930).
6. MacInnes, D. A., and Dole, M., *Ind. and Eng. Chem., Anal. Ed.*, **1**, 57 (1929).
7. Partridge, H. M., *J. Am. Chem. Soc.*, **51**, 1 (1929).

8. Hofmann, K. A., *Ber. chem. Ges.*, **53**, 2224 (1920).
9. Hölzl, F., *Monatsh. Chem.*, **57**, 375 (1931).
10. Packer, J., and Wark, I. W., *J. Chem. Soc.*, **119**, 1348 (1921).
11. Dumanskii, A., and Chalisew, A. A., *Kolloid-Z.*, **47**, 121 (1929).
12. Dumanskii, A., and Yakovlev, A. G., *Bull. Soc. chim.*, series 4, **47**, 1211 (1930).
13. Jellinek, K., and Gordon, H., *Z. physik. Chem.*, **112**, 207 (1924).

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