# The dissociation constants of the carboxyl and hydroxyl groups in some insoluble and sol-forming polysaccharides

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In order to establish the conditions under which the buffering of insoluble substance may most usefully be studied, suspensions of fibre cellulose, hemicellulose and xylan have been examined to determine the relationship between pH and the degree of dissociation of their carboxyl groups. The materials were suspended in normal potassium chloride solution, in order to confine local gradients of hydrion concentration to the immediate vicinity of each charged buffer group. Under these conditions the dissociation curves are all of the simple Henderson type with a common constant $pK = 2.95$ (N/100 hydrochloric acid in normal potassium chloride being taken as pH 2).

Sols of arabic and pectic acid follow the same dissociation curve, showing that the dissociation of the carboxyl groups is not necessarily influenced by the state of aggregation. Within the accuracy of these measurements, uronic and gluconic carboxyls have the same $pK$. The dissociation of pectic acid departs from the Henderson curve in the same general way as the dissociation curve of maleic acid differs from that of fumaric acid. It is suggested that some of the carboxyls in pectic acid are linked by hydrogen bridges. To a less extent such linkage may also occur in alginic acid below pH 3 when a gel is formed.

The $pK$ for the first stage of acid dissociation of the hydroxyl groups in wheat starch is close to 13.3. The same value was obtained whether potassium hydroxide, calcium hydroxide or barium hydroxide was used. The alkalis were made up in normal solutions of the corresponding chlorides. The same constant holds for alginate within the limits of experimental error. The dissociation in cellulose up to pH 13.5 is much less than in starch.

Using a new method based on a determination of the ratio of chloride ions to hydroxyl ions, the dissociations of starch and cellulose were measured in 5N alkali (approximately pH 14.7). The second $pK$ of starch is estimated at roughly 15.0. The cellulose, largely mercerized at this pH, exhibited a dissociation not much less than that of starch, and agreeing closely with the prediction of Neale.

An insoluble buffer substance suspended in an electrolyte solution is a two-phase system. The hydrion dissociation can be determined in two-phase systems over a greater pH range than is possible in one-phase systems.

## INTRODUCTION

The investigation described in this paper is part of a programme of work on the buffer action of the plant residues which make up the soil organic matter. At the outset it was necessary to establish the conditions under which the buffer action of solid matter can most profitably be studied. From theoretical considerations set out below it appears that the solid should be suspended in a solution of electrolyte, strong enough to confine local gradients of hydrion concentration to the immediate vicinity of each charged buffer group. In order to obtain the simplest conditions it was desirable to study some suspensions in which the buffering over a range of, at least, 5 pH units is due to buffer groups of one kind only. Several polysaccharides were found that fulfil this condition. These contain carboxyl groups which exert buffer action below pH 6, and hydroxyl groups of the primary alcohol type which exert buffer action above pH 10. The two buffer regions are so widely separated that each can be studied independently of the other.

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The theoretical reasoning shows that the buffer curve, obtained by titrating such a solid suspended in a salt solution, should have the same shape as the Henderson curve for a weak monobasic acid or base in true solution. The results set out in the following pages completely support the theory. They also give the first precise value for the dissociation constant of carboxyl groups in polysaccharides, and show that, provided sufficient electrolyte is present, the same constant holds whether the substance remains undissolved or disperses to form a sol.

A study has also been made of the buffering due to the hydroxyl groups of polysaccharides. The results again show the desirability of carrying out the titration in the presence of sufficient electrolyte, for then a Henderson curve can be fitted to the experimental points. In this way a fairly precise constant is obtained for the first stage of the dissociation of the hydroxyl groups.

Finally, a procedure has been developed in which solvation does not interfere with the measurement of hydrion dissociation at very high pH values. In this way an estimate has been made of the constant of the second stage of dissociation of the hydroxyl groups in starch.

The most tangible result of these investigations has been the evaluation of three dissociation constants for polysaccharides. Of more general importance, however, is the demonstration that, such constants can be evaluated for substances in the solid state. The way is now open for similar studies of more complex substances. As a method of analysis, the study of buffer action has the great advantage that it makes use of a reversible reaction which does not involve destructive breakdown of the substance investigated.

### INFLUENCE OF ELECTROLYTES

When a solid, which exerts buffer action, is suspended in pure or nearly pure water, there is a gradient of hydrion concentration in the diffuse electric double layer surrounding each particle. If the particles are negatively charged, the hydrion concentration increases towards their surface; if they are positively charged, it decreases towards their surface. Under these conditions it is difficult, if not impossible, to establish any quantitative relationship between the degree of dissociation of the buffer groups (the chemical groups responsible for the buffer action) and the hydrion concentration measured by a glass electrode or equivalent device, in which a liquid junction is made with a salt solution.

In the presence of sufficient electrolyte, these local gradients of hydrion concentration are confined to the immediate vicinity of the charged groups; so that the region round one charged group in which the hydrion concentration differs from that in the bulk of the solution does not embrace any of the neighbouring buffer groups. Moreover, if the salt used in the standard half-cell is the same, and at the same concentration, as that in the suspension, there is no junction potential to introduce uncertainty in electrometric pH measurements. When these conditions are fulfilled in the suspension of a solid containing only one kind of buffer group, the pH will be given by the equation

$$\text{pH} = pK + \log r, \tag{1}$$

in which $r$, the dissociation ratio, is the ratio of the number of buffer groups that are dissociated to the number that are undissociated, and $pK$ is logarithmic form of the dissociation constant. Writing $\alpha$ for the degree of dissociation so that

$$r = \alpha/(1-\alpha),$$

and substituting, then $\qquad \mathrm{pH} = pK + \log \alpha/(1-\alpha),$ $\qquad\qquad$ (2)

which has the same form as the Henderson equation for the dissociation of a weak monobasic acid or base in true solution.

Whenever the titration of a solid suspended in a salt solution furnishes a curve of pH against $\alpha$ conforming to the Henderson equation (2) it may be concluded:

(1) that sufficient electrolyte is present to confine local gradients of hydrion concentration to the immediate vicinity of each charged group;

(2) that all the buffer groups that can exert buffer action within the range (4–5 pH units) covered by the curve are equivalent to one another.

## POLYSACCHARIDES CONTAINING CARBOXYL GROUPS

Measurements were made of the carboxylic buffering in fibre cellulose, xylan, hemicellulose, arabic acid, alginic acid and pectic acid. The first three substances are insoluble throughout the range (pH 1–6) in which the carboxylic buffering occurs. The sodium salts of the last three disperse as clear sols at pH 6. Arabic acid is not precipitated by the addition of hydrochloric acid, but alginic acid and pectic acid are precipitated in this way.

*Cellulose* is represented in the well-known structural formula as consisting of long chains formed by $\beta$-gluco-pyranose rings joined by glucoside linkages through the $1:4$ positions. This formula makes no provision for the carboxyl groups which are present in small amounts in all natural cellulose. Starting with an ideal cellulose, carboxyl groups could be introduced either by oxidation of some of the —$CH_2OH$ groups to form uronic carboxyls, or by oxidation of some of the reducing groups which should be present, one at one end of each chain, to gluconic carboxyls.

*Xylan* has a structure similar to that of cellulose, but is built of xylo-pyrazone units. Thus the linked pyranose units have no —$CH_2OH$ side-chains that could be oxidized to uronic carboxyl groups. The carboxyls occurring in xylan are presumably attached at the ends of the chains in the same way as the gluconic carboxyls of cellulose. The chains are relatively short.

*Hemicellulose* is a complex and variable substance in which pyranose rings of the gluco-xylo and the galacto-arabino types occur. Uronic carboxyls are attached to some of the rings. The chains are shorter than those of cellulose, so there is more opportunity for the presence of carboxyls of the gluconic type.

*Alginic acid* consists of mannuronic acid (pyranose) units linked through the $1:4$ positions (Hirst, Jones & Jones 1939). This structure may be derived from the cellulose formula by substituting uronic carboxyls for all the —$CH_2OH$ groups and interchanging the —H and the —OH attached to each $C_2$ carbon. According to this ideal formula there is a uronic carboxyl attached to each ring.

*Arabic acid* is a complex polysaccharide which gives on hydrolysis *l*-arabinose, *l*-rhamnose, glucuronic acid and the disaccharide 3-*l*-galactoside-*l*-arabinose (Smith 1939, 1940). Thus arabic acid contains uronic carboxyls. Smith found 1:3 and 1:6 as well as 1:4 linkages between the units.

*Pectic acid* is a polysaccharide which is hydrolysed to galacturonic acid, galactose and arabinose. It owes its acidic properties to the presence of galacturonic acid units. The details of its structure are not known. In pectin, some of the uronic carboxyls are combined as methyl esters. When these are hydrolysed by dilute alkali the salt of pectic acid is formed. Acetic acid is also liberated from some pectins during hydrolysis.

### PREPARATION OF MATERIALS

With the exception of the alginic acid the materials were prepared by Dr A. G. Norman at the Rothamsted Experimental Station some years ago. The methods he used are only outlined here.

Cellulose was separated from jute, from ramie and from silver-fir wood, by treating the raw materials with boiling 1 % sodium hydroxide, gaseous chlorine and alkaline sodium sulphite.

Hemicellulose was prepared from beechwood by extracting with 2 % sodium hydroxide for 4 hours at 80° C, followed by 4 % sodium hydroxide at 100° C for 4 hours. The combined extracts were made acid by adding glacial acetic acid. The precipitate was centrifuged out and dried from alcohol. This fraction is usually called 'Hemicellulose A' to distinguish it from that not precipitated by acetic acid.

Xylan was obtained by treating oat hulls as for the extraction of hemicellulose. The xylan was precipitated from the clear liquid, separated in the centrifuge from hemicellulose A, by adding to it half its volume of acetone. It was purified by re-dissolving and reprecipitating and finally dried from alcohol. In general, material separated in this way is classed as 'Hemicellulose B', but with oat hulls the material consists almost entirely of xylan.

Arabic acid was prepared from a solution of raw gum arabic in warm water by acidification with hydrochloric acid and precipitation by alcohol. The acid thus separated was dried from alcohol.

Pectic acid was prepared by hydrolysing a commercial citrus pectin in cold N/10 sodium hydroxide, acidifying with acetic acid and precipitating calcium pectate with calcium chloride. The precipitate of free pectic acid, obtained by the action of hydrochloric acid on the calcium pectate, was washed and dried from alcohol.

Alginic acid was obtained as the sodium salt under the trade name 'Manucol', and this was used without further purification.

Cotton (Texas) had received the following treatment at the Cotton Industry Research Association whence the samples were obtained. Sample 336 was boiled for 4 hours in 1 % sodium hydroxide solution at 35–40 lb./in.$^2$ pressure in excess of atmospheric. Sample 341 was boiled for 4 hours in 1 % sodium carbonate solution at

5–10 lb./in.² pressure in excess of atmospheric. Air was excluded during the boiling in both cases. Methylene blue absorptions were 1·06 in sample 336 and 1·58 in sample 341.

<div style="text-align:center">MEASUREMENTS OF CARBOXYLIC BUFFERING</div>

The immediate object of the measurements was to obtain experimentally the relationship between the pH in the salt solution and the degree of dissociation, $\alpha$, of the carboxyl groups. Potassium chloride was preferred to other salts because its use eliminates junction potentials in the glass electrode cell. Normal concentration was chosen for convenience, and it is evident from the results that this strength is sufficient to confine the local gradients of hydrion concentration. The glass electrode pH meter was set to read pH 2 for a solution of N/100 hydrochloric acid in normal potassium chloride solution.

The experimental procedure differed according to whether the material was insoluble throughout the range of pH to be investigated or whether, as the sodium salt, it dispersed to form a sol.

<div style="text-align:center">*Insoluble materials—cellulose, hemicellulose and xylan*</div>

One g. samples ($\frac{1}{2}$ g. for hemicellulose) were weighed into a set of resistance glass test-tubes $6 \times 1\frac{1}{4}$ in. fitted with rubber bungs, and shaken with 10 ml. of N/10 sodium hydroxide on a rotary shaker overnight. This pre-treatment was to complete the hydrolysis of any ester linkages that might otherwise have disturbed the measurements. To each tube a different amount of hydrochloric acid was then added so as to give a series of pH values ranging from pH 1 to 6. Enough water and strong potassium chloride solution was added to bring the total liquid to 50 ml. and make it normal in potassium chloride. The tubes were shaken for a further 24 hours. The pH values were then read from the glass-electrode pH meter, the electrode being dipped into each tube in turn while the solid was in suspension all around it. The tubes were then centrifuged. One-half (25 ml.) of the clear liquid (the extract) was pipetted out and titrated separately with alkali to pH 6. The remaining liquid with the solid in suspension (the remainder) was titrated in each tube to pH 6. These end-points could be obtained quite sharply with bromo-cresol purple. All the solutions were made up with boiled distilled water so as to avoid disturbance from carbon dioxide.

The excess of the titre of the remainder over the titre of the extract evidently gives the amount of alkali which reacts with the solid between the pH measured by the glass electrode and pH 6. When the initial reaction was below pH 2, the volumes of liquid in the extract and remainder were checked by weighing, so that allowance could be made for any small inequality.

The results of the measurements are plotted in figure 1. The ordinates are the pH values given by the glass electrode before titration. The distance of each point from the right-hand edge (i.e. the value of $1 - \alpha$) was obtained by dividing the measured difference between extract and remainder by the limiting difference for very low pH

values. This limiting difference was, of course, a measure of the total hydrion dissociation of the solid at pH 6.

The curve in figure 1 was drawn to conform to the equation (2) taking $pK = 2.95$. The points for jute cellulose, ramie cellulose and xylan all fall close to the curve, and show no systematic departure from it. Thus the hydrion dissociation at pH 6 in these substances is solely due to carboxyl groups of $pK$ 2.95. The carboxyl contents so determined are given in table 1.
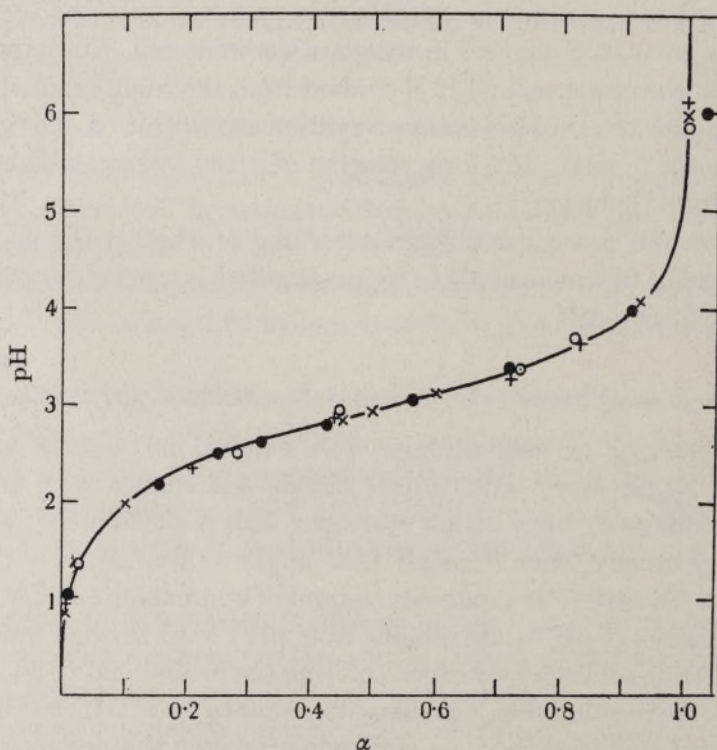


FIGURE 1. Carboxyl groups of insoluble polysaccharides in N KCl.
× jute cellulose; ○ xylan; + ramie cellulose; ● hemicellulose.

TABLE 1. TOTAL CARBOXYL CONTENT EXPRESSED IN MILLIEQUIVALENTS
PER 100 G. OF DRY MATERIAL

| | | | |
|---|---|---|---|
| jute cellulose | 16·0 | hemicellulose A | 28·6* |
| ramie cellulose | 9·2 | xylan | 21·5 |
| wood cellulose | 1·30 | alginic acid | 438† |
| cotton 336 | 0·5 | arabic acid | 65 |
| cotton 341 | 1·25 | pectic acid | 528 |

\* Hydrion dissociation at pH 6, 29·5. † Per 100 g. free acid.

Examination of the results for hemicellulose showed that the buffering between pH 4 and 6 was a little greater than would be the case if carboxyl groups of $pK$ 2.95 alone were present. The total dissociation at pH 6 was 29·5 m.equiv. per 100 g. The values of $\alpha$ plotted in figure 1 were obtained by taking the carboxyl content as

28·6 m.equiv. The additional 0·9 m.equiv. which develops between pH 4 and 6 may be due to a small amount of lignin present as impurity. With this adjustment the points for hemicellulose below pH 4 fit the curve well.

The carboxylic buffering was very small in the wood cellulose and in the two cottons. The estimated carboxyl contents are included in table 1.

### Sol-forming materials—arabic, alginic and pectic acids

In the case of the polyuronides, samples were weighed out into tubes as already described—0·5 g. of arabic acid, 0·2 g. of sodium alginate (Manucol) and 0·1 g. of pectic acid. The alginic acid being already neutralized with sodium hydroxide, 15 ml. of water only was at first added to each tube, which was shaken until a clear sol had been formed. To the arabic acid just enough N/20 sodium hydroxide was added to form a clear sol at pH 6. To obtain a clear sol of the pectic acid it was necessary to go to pH 8 with alkali. The sol was then brought to pH 6 by adding a few drops of N/20 hydrochloric acid without causing any precipitate to form.

The samples were then brought to a series of pH values by adding a different amount of hydrochloric acid to each tube. In the case of arabic acid no precipitate formed. Enough strong potassium chloride solution was added to bring the potassium chloride concentration to normal, and the pH was determined after a brief period of shaking. With the aid of blank experiments the amount of hydrochloric acid needed to bring the same volume of normal potassium chloride to the same pH was found. The amount of acid that had reacted with the arabate to form free arabic acid was obtained by difference, and could be obtained in this way with sufficient accuracy down to pH 2·3.

With alginic acid, the addition of acid tends to precipitate a gel. One ml. of N/10 acid was, therefore, introduced at a time, and the tube was shaken so as to give the gel that was precipitated an opportunity to redissolve before the next 1 ml. was added. With the larger total additions a stage would be reached when the gel would not redissolve. The tube was then shaken for 24 hours between each addition of acid until the whole had been introduced. Finally, enough water was added to bring the total volume added to 25 ml. together with 25 ml. of 2N potassium chloride. The tube was again shaken for 24 hours, and the pH was then taken.

Down to pH 3·5 the material remained as a sol, and the quantity of acid that had reacted was obtained as in arabic acid. Below pH 3·3 all the material could be thrown down in the centrifuge, and so the amount of reaction could be obtained from the difference in titre of extract and remainder as for the insoluble materials. The gel easily redissolved on the addition of alkali to give a sharp end-point at pH 6.

With pectic acid the precipitation caused by the addition of acid is progressive, and it is not easy to determine the pH at which it is complete. Moreover, the precipitate did not redissolve on back titration with alkali until pH 8 was reached, although acid could then be added to pH 6 without causing precipitation. For both reasons it is considered that down to pH 2·4 the amount of acid that had reacted

was more accurately given by the direct method. A few values below pH 2·7 were obtained by back titration.

The total carboxyl content of the arabic acid is computed from the amount of alkali required to bring it to pH 6. The total carboxyl content of the pectic acid was found by subtracting from the alkali needed to make a sol at pH 8 the acid required to bring it back to pH 6. The total carboxyl content of the alginic acid was found by adding sufficient hydrochloric acid to the neutral sol to bring it to pH 1, and back titrating extract and remainder as for the insoluble materials.
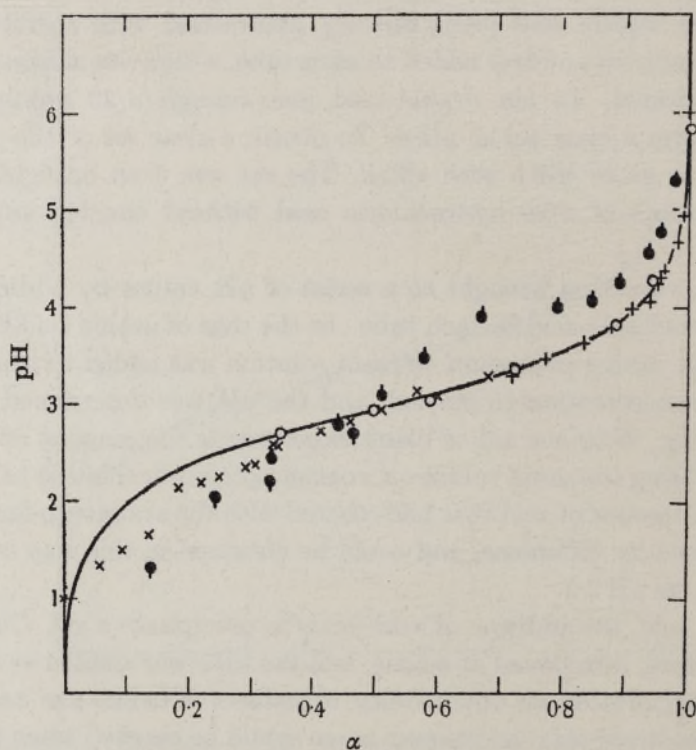


FIGURE 2. Carboxyl groups of sol-forming polysaccharides in N KCl.

| by direct titration | by difference |
|---|---|
| + alginic acid | × alginic acid |
| ● pectic acid | ● pectic acid |
| ○ arabic acid | |

Figure 2 shows the results for the sol-forming materials. As in figure 1, the curve conforms to equation (2) (p. 433) with $pK = 2·95$. The points for arabic acid, which remained as a sol throughout, all agree well with the curve. Alginic acid is a sol above pH 3·4, and the points relating to the sol condition also fall close to the curve. Below pH 2·8 there is an unmistakable discrepancy. It is just possible that, in spite of the time allowed, equilibrium had not been established throughout the lumps of gel, but it is more probable that the equilibrium condition is not here represented by the Henderson curve.

The departure of the points for pectic acid is more striking. Below pH 3 it is in the same sense as for alginic acid. Making all possible allowance for the experimental difficulties it is considered that this departure is real. Above pH 3 the departure is in the opposite sense, and is quite outside the limits of experimental error.

## DISCUSSION

The results set out in figures 1 and 2 show that the carboxyl groups of the two celluloses, the hemicellulose, the xylan, the arabic acid and the alginic acid, have the common dissociation constant $pK = 2.95$. In xylan the carboxyls are presumably of the gluconic type, and no chain can have more than one of them. The carboxyls of alginic acid and arabic acid are of the uronic type. In the sample of alginic acid used in this investigation more than three-quarters of the rings had uronic carboxyls attached, whereas in the arabic acid the proportion was only about one in nine. The close approximation of the points for both substances to a common Henderson curve can only mean that, even where uronic carboxyls are attached to neighbouring rings, as must largely occur in alginic acid, they are far enough apart on the molecule to dissociate independently. Although these acids are polybasic in the sense that more than one carboxyl is attached to each molecule, their dissociation is characterized by a single constant. It was disappointing to find that the $pK$ for gluconic carboxyl is so close to that for uronic carboxyl. Had a significant difference been found it would have been possible to estimate the proportion of gluconic to uronic carboxyls in the celluloses and the hemicellulose. A separation of the gluconic and uronic carboxyls in cotton might have provided information about the chain length. No doubt there is a small difference in the dissociation of these groups, but it is evident that very exact measurements will be needed to determine it.

Our results show beyond doubt that a colloidal system can yield a dissociation curve of the simple Henderson type defined by a single constant. The governing factor is not the degree of dispersion of the system, but the equivalence and independence of the active groups. It is clear that the dissociation constant of an active group attached to a large molecule is not governed by the size of the molecule. Nor is it altered when the molecule becomes part of a crystalline or semi-crystalline aggregate so long as the immediate environment of the group is not altered thereby. Crystallization would, for instance, quite alter the environment if it prevented free access of ions to the active group. Our results thus show that the crystalline state of the cellulose, the hemicellulose and the xylan allowed free access of ions to gluconic and uronic carboxyl groups.

In seeking the cause of the departure of the points for pectic acid from the Henderson curve we are handicapped by lack of knowledge regarding the chemical structure of this substance. The best that we can offer is a hypothesis which appears to fit the facts. We suggest that some of the carboxyl groups come together in pairs, the hydrogen of one —COOH group forming a 'bridge' to an oxygen of another —COOH group or of a —COO⁻ group.

We further suggest that essentially the same complication arises in maleic acid. Dissociation curves for fumaric and maleic acids were obtained by titration in N potassium chloride. These are shown in figure 3. Measurements were not made below pH 2·3. The broken portions of the curves were obtained by symmetry.
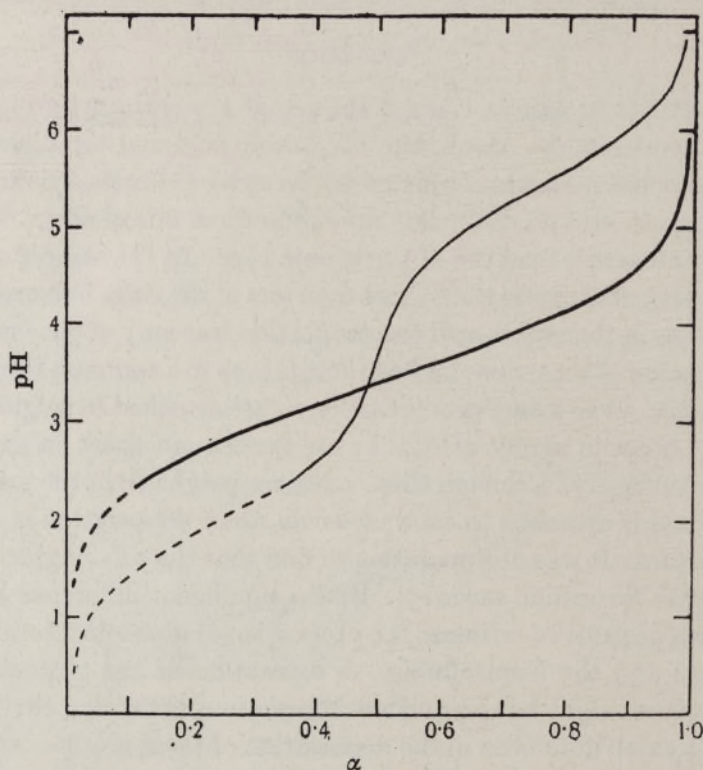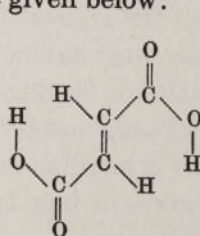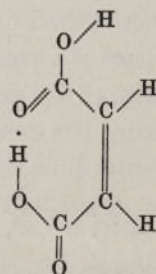


FIGURE 3. Carboxyl groups in fumaric and maleic acids in N KCl.
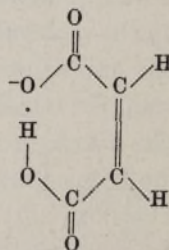———— fumaric acid; ———— maleic acid.

Structures for the fumaric acid and maleic acid molecules and for the acid maleate anions are given below:



fumaric acid          maleic acid          acid maleate
molecule              molecule             anion

Before the theory of the hydrogen bridge had been developed, it was difficult to see why the first dissociation of maleic acid is stronger than the first dissociation of fumaric acid. It was easier to see why the second dissociation of maleic acid is

weaker than the second dissociation of fumaric acid. The negative charge on the first carboxyl contributes more to the attraction by which the second hydrion is held to the molecule, the closer the two carboxyls are to each other.

Both facts are readily accounted for if a hydrogen bridge forms both in the maleic acid molecule, and in the acid maleate anion. The weakening of the second dissociation is then attributed to the added stability which the hydrogen bridge confers on the acid maleate anion. The strengthening of the first dissociation is to be interpreted as showing that the decrease in free energy due to hydrogen bridge formation is considerably greater in the acid maleate anion than in the maleic acid molecule.

The way in which the points for pectic acid in figure 2 depart from the Henderson curve is strikingly similar to the way in which the curve for maleic acid in figure 3 departs from the curve for fumaric acid. The effect on the dissociation curve would be the same whether the carboxyls that become linked are attached to the same molecule or to different molecules, but the magnitude of the effect in pectic acid, and its persistence up to high values of $\alpha$, inclines us to the view that, in this substance, the links are mainly between carboxyls attached to the same molecule.

In the case of alginic acid, there is no reason to think that linkage occurs between carboxyls attached to the same molecule, since, in the sol condition above pH 3·5, the points for this substance fall on the Henderson curve. Below pH 3, when a gel forms, it is possible that some linkage occurs between carboxyls attached to different chains. Indeed, such linkage may be a factor in causing the gel to form.

These ideas may prove fruitful in explaining the power of pectin to form jellies at about pH 3. In this substance some rings have carboxyl groups attached to them, but more have $—COO.CH_3$ groups. Pectic acid is obtained when all the latter groups are hydrolysed. The formation of a jelly by pectin suggests that considerable linkage is taking place between carboxyls of different chains, presumably because there is less opportunity for linkage between carboxyls in the same chain when many of the rings have $—COO.CH_3$ groups attached. This aspect of jelly formation deserves further study.

When making the measurements on pectic acid, difficulty was experienced in obtaining reproducible results. This was at first put down to slow attainment of equilibrium, but the fundamental cause is probably the dependence of the amount of the hydrogen bridge formation on the exact way in which the substance is handled. This complication does not arise in maleic acid which has a reproducible dissociation curve defined by two constants.

If our views about the formation of hydrogen bridges in pectic acid are correct, it follows that the Henderson curve is only obtained where such bridges are not formed. We can regard this condition as part of the general requirement that all the carboxyls must be equivalent and able to dissociate independently.

### *Measurement of hydroxylic buffering between* pH 11 *and* 13·5

Although it has long been recognized that the hydroxyl groups in polysaccharides have a very weak acidic nature, the pH range in which they dissociate is so high that

ordinary methods cannot be used to determine the dissociation constants. Previous investigators have also been handicapped by their concentration on the investigation of cellulose. It is now clear that native cellulose is peculiar as regards the dissociation of its hydroxyl groups. The present work shows that the groups in starch and alginate exhibit a normal behaviour.

### Measurements on alginate

0·5 g. portions of the sodium alginate were placed in each of a series of 100 ml. resistance glass boiling tubes containing 15 ml. carbon dioxide-free distilled water. The tubes were shaken until all the solid had dispersed to form a clear sol, which was found to be neutral. A different measured quantity of carbonate-free N/20 potassium hydroxide solution was then added to each tube, and the volumes were made up to 50 ml. by addition of carbon dioxide-free distilled water and enough potassium chloride solution to make a normal solution. Actually the water and potassium chloride solution were added first so as to minimize the uptake of carbon dioxide, and the tubes were immediately closed by rubber bungs after each addition. A second set of tubes similarly filled but containing no sodium alginate served as blanks.

Although the pH values were too high to be obtained directly from the readings of the glass electrode, it was possible by direct comparison to find the blank that gave the same reading as a tube containing the alginate. The pH was computed from the hydroxyl-ion concentration in the blank, taking N/100 as pH 12. To find the hydrions that had dissociated from the alginate, the difference was taken between the alkali added to the sol and that added to the blank of the same pH.

According to the ideal structure of alginic acid in which a uronic carboxyl is attached to each pyranose ring, the first stage of hydroxyl dissociation should yield 568 m.equiv. of hydrions per 100 g. of free acid or 505 m.equiv. per 100 g. of sodium alginate. The carboxyl content given in table 1 is only 438 m.equiv. per 100 g. of free alginic acid. This shows that not all the rings in the sample used had carboxyl groups attached. If the other rings have —$CH_2OM$ groups attached, the dissociation of one hydroxyl group per ring should yield 532 m.equiv. per 100 g. of the sodium alginate. The measured hydrion dissociation expressed as milliequivalents per 100 g. of the sodium alginate were, therefore, divided by 532 to obtain $\alpha$.

The results for the alginate are plotted in figure 4 and are discussed below in conjunction with those for starch. The measurements do not extend beyond pH 12·4, as the glass electrode appeared to be unreliable above this pH.

### Measurements on starch and cellulose

The procedure was slightly different in measurements on wheat starch and cellulose. The starch was a commercial sample, and was used without further treatment. 0·5 g. portions were added to each of a series of tubes containing 50 ml. of solution normal in potassium chloride and containing graduated quantities of potassium hydroxide. The tubes were closed with rubber bungs and shaken overnight, after

which the starch was allowed to settle. 25 ml. of clear solution was then extracted from each tube and titrated with N/20 hydrochloric acid, using bromo-thymol blue as indicator. The 25 ml. remaining in each tube with the starch in suspension were then similarly titrated.

The pH values were calculated from the hydroxyl-ion concentration of the 25 ml. of clear extract. The amount of hydrion dissociation was found by subtracting the titre of the 25 ml. of clear solution from that of the 25 ml. with the starch in suspension. Expressing the result as milliequivalents per 100 g. of dry substance and dividing by 617 we obtain $\alpha$, the degree of dissociation.
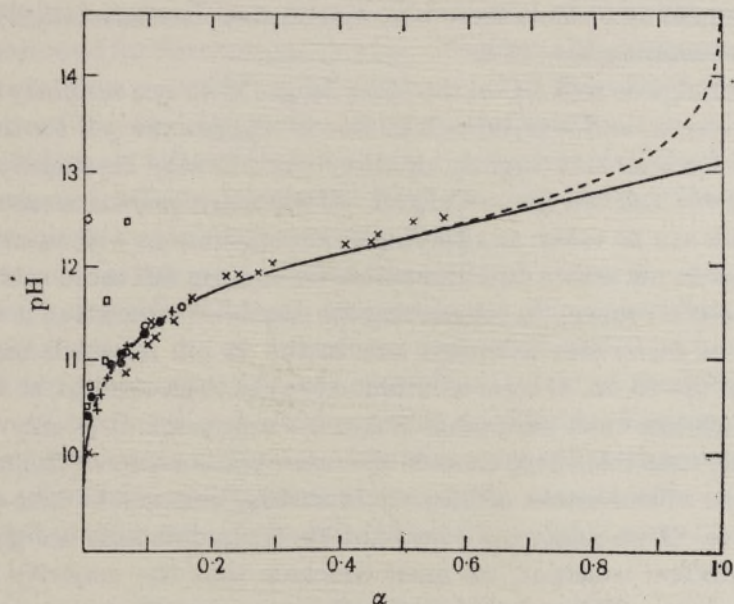


FIGURE 4. Hydroxyl groups of polysaccharides. + starch in N KCl; + starch in N BaCl$_2$; O starch in N CaCl$_2$; ● alginate in N KCl; □ cotton 337 in N KCl; ◇ methylated cotton 337 in N KCl.

This method could not be used above pH 12·5 with potassium as cation, because the starch tended to go into solution. It did not, however, dissolve when barium hydroxide in normal barium chloride solution was used, and measurements in this solution could be made up to the limit, pH 13·5, set by the solubility of barium hydroxide. A few measurements were also made with calcium hydroxide in normal calcium chloride solution.

Since starch consists essentially of $\alpha$ gluco-pyranose units linked through the 1 : 4 positions, the first stage of dissociation of the hydroxyl groups should yield 617 m.equiv. of hydrion per 100 g. of dry starch. The measured hydrion dissociations were, therefore, divided by 617 to get $\alpha$. The results are plotted in figure 4.

Similar measurements were made on the samples of cellulose used in the investigation of carboxylic buffering. The dissociation was not the same in the different

celluloses, but in every case it was much less than in starch. A detailed examination of these results is outside the scope of this paper. To indicate the order of magnitude, points for one of the cottons are included in figure 4.

The full-line curve in figure 4 was drawn assuming $pK = 13\cdot3$ for the first stage of dissociation and $pK = 15\cdot0$ for the second. The dotted curve would result if there were only one stage of dissociation for which $pK = 13\cdot3$.

Considering first the points for starch between pH $11\cdot5$ and $12\cdot5$, it will be seen that those for potassium and calcium are generally in close agreement with the theoretical curve, and show no systematic departure from it. Several of the points for barium are equally close, but others fall below. The reason for the discrepancy is not known. Taking these results as a whole there is no doubt that the dissociation is well represented by the constant $pK = 13\cdot3$.

The points for alginic acid fall in the same range. They are certainly less accurate than those for starch, and it is difficult to decide whether the $pK$ for this substance is the same as for starch or slightly smaller. The $pK$ may be slightly affected by substitution of —COO Na for —CH₂OH. Until more precise measurements are made $pK = 13\cdot3$ can be taken as applying to the alginate as well as starch.

With increase in pH above $12\cdot5$ the points for barium fall more and more to the left of the theoretical curve. In calculating the degree of association it was assumed that there are as many free hydroxyl ions in the 25 ml. in which the starch was suspended as in the 25 ml. of clear solution. Actually there are less, so the degree of dissociation is greater than the points in figure 4 represent. It is only above about pH 13, however, that this effect exceeds the other inaccuracies of the method.

The behaviour of the cotton cellulose is in striking contrast to that of the starch and the alginate. If we reject the view that the hydroxyls here are governed by a different dissociation constant, we must conclude that the majority of them are built into the native cellulose crystal in such a way that they are not able to dissociate normally. The observed dissociation is then due to a fraction only of the hydroxyls which, owing to crystalline imperfections and exposed surfaces, are accessible, and can dissociate normally. The effect of mild methylation with dimethyl sulphate is greatly to reduce the dissociation, while the treatment with alkali (mercerization), which is known to open up the structure, increases it. These observations do not provide a basis for precise conclusions, but they are in keeping with the interpretation here advanced.

### Measurements of dissociation at about pH 14·7

In order to get a rough estimate of the second dissociation constant for starch it was necessary to make measurements above pH 14. For these to be of any value it was useless to calculate the dissociation on the assumption that the water in the starch contains free alkali at the same concentrations as the surrounding solution. This was the assumption originally made by Vieweg (1907), in computing the amount of alkali that combines with cellulose. Neale (1929, 1931), applying the principle of the Donnan membrane equilibrium, showed that the water in the cellulose must

contain a lower concentration of free hydroxide than the surrounding solution. He computed that in 20 % sodium hydroxide solution (approximately 5N), the amount of combined alkali is close to one equivalent per glucose anhydride unit and not half this amount as computed by Vieweg.

In the present investigation use was made of a new principle. Assuming the general correctness of Neale's idea that the free hydroxyls are less concentrated within the starch or cellulose because of the electrical potential difference set up in the Donnan equilibrium, it follows that the concentration of any other small univalent anion will be similarly affected. In order to determine how much alkali remains uncombined when $A$ equivalents are added to a measured quantity of starch or cellulose, a small amount, $D$ equivalents, of chloride was also added. 24 hours was allowed for the attainment of equilibrium, and a volume of clear extract was found to contain $a$ equivalent of alkali and $d$ of chloride. Assuming that the concentrations of free hydroxyl and chloride are affected in the same ratio by the Donnan equilibrium it follows that the total free hydroxyl is $Da/d$. The hydroxyls which have reacted with hydrions that have dissociated from the polysaccharide are, therefore, given by the expression $A - Da/d$.

It was fortunate for our purpose that wheat starch shows no sign of dissolving in 5N potassium hydroxide solution. Measurements were, therefore, made with a solution approximately 5N in potassium hydroxide and 0·05N in a potassium chloride. The weight composition was such that 11·84 g. of the solution contained 48·4 m.equiv. of hydroxyl and 0·498 m.equiv. of chloride. The measurements and results are set out in table 2. The amounts of hydrions that dissociated are given in the last two columns in milliequivalents per gram of substance as taken, and in equivalents per glucose anhydride unit (162 g. dry weight) respectively.

TABLE 2

| substance weight, *w*, and moisture content | solution added | | | solution extracted | | dissociation | |
|---|---|---|---|---|---|---|---|
| | weight *W* g. | alkali *A* m.equiv. | chloride *D* m.equiv. | alkali *a* m.equiv. | chloride *d* m.equiv. | per *w* $A - Da/d$ m.equiv. | per 162 g. dry weight equiv. |
| 3 g. starch | 58·94 | 240·9 | 2·479 | 46·58 | 0·530 | 23·8 | 1·39 |
| moisture | 58·78 | 240·3 | 2·473 | 48·48 | 0·526 | 21·7 | 1·31 |
| 11·5 % | 58·86 | 240·6 | 2·476 | 46·55 | 0·525 | 21·1 | 1·27 |
| 4 g. cotton | 58·87 | 240·6 | 2·476 | 45·62 | 0·515 | 21·2 | 0·93 |
| 337 mois- | 58·98 | 241·1 | 2·481 | 45·79 | 0·5225 | 23·6 | 1·04 |
| ture 8·7 % | 58·93 | 240·9 | 2·479 | 45·85 | 0·5175 | 21·3 | 0·94 |
| 4 g. cotton | 59·11 | 241·6 | 2·486 | 45·82 | 0·5225 | 23·5 | 1·04 |
| 342 mois- | 59·10 | 241·5 | 2·485 | 45·72 | 0·5187 | 22·4 | 0·99 |
| ture 9·3 % | 59·02 | 241·2 | 2·482 | 45·82 | 0·5225 | 23·5 | 1·04 |

The results for the two samples of cotton are remarkably close to the calculation of Neale that one equivalent reacts per glucose anhydride unit in 5N alkali. In the case of starch the amount of reaction is greater, but the dissociation of cellulose is

approximating to that of starch in this strong alkali (pH 14·7 approximately). The cellulose had largely changed from the native to the mercerized state. It is possible that a cellulose completely mercerized would have the same dissociation constants as starch.

In the case of starch the first stage of dissociation is complete and the second stage has proceeded to about one-third by pH 14·7. Thus at pH 15·0 the second stage would be roughly half complete, and the second $pK$ is, therefore, approximately 15·0. This is not sufficiently removed from 13·3 for any inflexion to occur as the dissociation curve is traced from the first stage to the second. The theoretical course of the curve to the end of the first stage, based on these dissociation constants, is given by the full-line curve in figure 4.

### Interpretation of the $pK$ values for hydroxylic dissociation

The chemical structure of the gluco-pyranose ring suggests that the strongest hydroxyl is that on $C_2$, because this carbon atom is bonded to $C_1$ with two oxygen atoms attached and $C_3$ with one oxygen atom attached. The next is that on $C_3$ which is bonded to $C_2$ and $C_4$, both with one oxygen atom attached. The weakest is on $C_6$, bonded only to $C_5$ with one oxygen atom attached. It is assumed that no hydrogen bridges are formed.
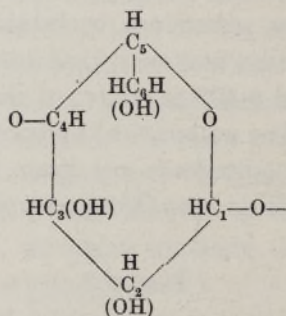


$\alpha$-gluco-pyranose ring (diagrammatic)

The completion of the first stage of dissociation merely indicates that the total dissociation is equivalent to the loss of one hydrion per ring. It does not necessarily mean that every ring has lost one hydrion. No doubt a few have lost two, and the same number none (neglecting the possibility that any ring has lost three at this stage). Amongst those that have lost one, the largest fraction will have dissociated at the $C_2$ hydroxyl, the next at the $C_3$ hydroxyl, and the smallest at the $C_6$ hydroxyl. Thus the $pK$ determined for the first stage of dissociation is a property of the whole ring structure, and not of one identifiable hydroxyl group. The same is true of the $pK$ for the second stage.

The structure of sucrose is such that one would expect the hydrion dissociation of one molecule of sucrose to be very similar to that of two $\alpha$-gluco-pyranose rings in starch. The value, $pK = 13·0$, reported for sucrose (Britton 1929) relates to the dissociation of one hydrion per molecule. The two rings in the sucrose molecule

presumably dissociate their hydrions almost independently, and to about the same degree, so that the $pK$ for the first stage of dissociation of either ring must be close to 13·3.

### CONCLUSION

This investigation has shown that insoluble buffering materials can be found which, when suspended in a neutral salt solution, exhibit the same form of relationship between degree of dissociation and hydrion concentration as is found with weak acids and bases in true solution. The dissociation of the carboxyl groups in cellulose, hemicellulose and xylan is of the simplest type corresponding to the dissociation of a weak monobasic acid in true solution. The dissociation of the hydroxyl groups of starch corresponds to that of a polybasic acid. The reaction of these substances with alkali is due to the dissociation of hydrions from identifiable buffer groups that behave in the same way as they do when attached to small molecules in true solution. Hydroxyl ions of the added alkali combine chemically with hydrions as they dissociate; the corresponding cations are held to the material by electrostatic attraction. The 'sorption' of the alkali may thus be said to be both chemical and physical.

The fact that the suspension of an insoluble buffer substance in an electrolyte solution is a two-phase system permits us to determine the hydrion dissociation over a greater range of hydrion concentration than is possible in the one-phase system given by soluble buffer substances. Similar determinations would be possible with a sol if we could obtain the electrolyte solution in equilibrium with it through a semipermeable membrane. It is through the study of two-phase systems that we may hope to extend our knowledge of the behaviour of buffer groups that dissociate at very low and very high hydrion concentrations.

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