to ascertain the precision obtainable at very short reaction times and for immediate titration. The results summarized in Table IV indicate that the reaction of calcium acetate with celluronic acid is very rapid, as all the values agree within experimental error. It seems established, then, that at least for finely ground samples, the reaction liberating acetic acid is so rapid that an aliquot of the filtered reaction mixture may be titrated as soon as desired after the reactants have been combined.

The sample of celluronic acid used in the time series discussed above showed a higher carboxyl value by the carbon dioxide evolution method than by calcium acetate. This phenomenon appears to apply generally to polyuronides, including seven different celluronic acids, oxidized starch and pectic acid (Table V). The same observation applied to alginic acid. Apparently, calcium acetate and uronic acid determinations do not necessarily measure the same chemical entities.

The differences between the carboxyl values obtained by the calcium acetate and those from the carbon dioxide method, as shown in Table V, are noteworthy. We have shown earlier[8] that cellulose nitrate decomposes under the conditions of the latter method to produce carbon dioxide. The presence of combined nitrate groups in certain of the celluronic acids may have produced a part of the divergence in carboxyl values shown in Table V, but we do not believe this accounts for all of the difference. Celluronic acids B and C possessed extremely small amounts of combined nitrogen yet showed real analytical differences. Similar differences were obtained with alginic acid where combined nitrate groups are surely not a factor. We believe that the rigorous hydrolytic conditions of the uronic acid method decomposes all carboxyl groups in uronic acid units, whether free or bound, while the calcium acetate method operating at a *p*H of 5 to 6 measures only the free carboxyl groups. In the absence of combined nitrate groups, this difference represents bound or potential carboxyl which may be in the form of intermolecular anhydride or ester groups or as intramolecular lactone. Many celluronic acids have been observed whose calcium acetate carboxyl values were below that considered necessary for solubility in dilute alkali. Such acids did dissolve, however, and their higher uronic acid carboxyl values were compatible with such behavior. Potential carboxyls of the type just described would be susceptible to alkaline hydrolysis and therefore should become available for solubility effects. Additional evidence for such structures is presented in the succeeding paper of this series.

(8) Taylor, Fowler, McGee and Kenyon, THIS JOURNAL, **69**, 342 (1947).

Summary

1. The calcium acetate method of carboxyl analysis did not produce theoretical values for celluronic acid or other synthetic or natural polyuronides examined.

2. Finely divided samples of celluronic acids may be titrated with precision, using calcium acetate immediately after the reactants are combined.

3. Inter- or intramolecular dehydration could account for the difference between carboxyl content as found by calcium acetate determination and the carbon dioxide evolution method.

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[COMMUNICATION NO. 1099 FROM THE KODAK RESEARCH LABORATORIES]

Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. IV. Potentiometric Titration of Polyuronides[1]

BY C. C. UNRUH, P. A. MCGEE, W. F. FOWLER, JR., AND W. O. KENYON

Celluronic acids, prepared by the oxidation of cellulose with nitrogen dioxide,[1a] have been analyzed for carboxyl groups by several methods but only a few have structural significance. Titration with dilute alkali or pyridine–water solutions (or suspensions) produced erratic results. The reaction was physically heterogeneous for slightly oxidized samples and homogeneous for highly oxidized materials.[2] The modification of titrating a strongly alkaline aqueous pyridine solution of the celluronic acid gave results in excess of theory.[2]

The use of calcium acetate, introduced by M. Ludtke,[3] for determination of carboxyl groups in cellulose gave highly reproducible results.[1a] This method depends on the exchange of ions between celluronic acid and calcium acetate liberating an equivalent amount of acetic acid. It is, in such cases, a heterogeneous reaction. The method appears to measure free carboxyl groups, though it may not show potential uronic acid carboxyl groups.[4]

Application of the carbon dioxide evolution method[1] to celluronic acid invariably produced higher carboxyl values than did the calcium acetate method. The rate of evolution has been

(1) Presented before the Cellulose Chemistry Division at the 110th Meeting of the American Chemical Society, September, 1946, Chicago, Illinois.

(1a) Yackel and Kenyon, THIS JOURNAL, **64**, 121 (1942).

(2) Unruh and Kenyon, *ibid.*, **64**, 127 (1942).

(3) M. Ludtke, *Z. angew. Chem.*, **41**, 650 (1935).

(4) McGee, Fowler and Kenyon, THIS JOURNAL, **69**, 347 (1947).

shown to be of structural significance in certain polyuronides.[5] Other methods of value in characterizing celluronic acids and other "oxidized celluloses" are to be found elsewhere.[6]

Potentiometric titration, as a means of estimating the carboxyl groups in cellulose itself, has been used to determine the apparent dissociation constant of cellulose.[7,8] A Hildebrandt hydrogen electrode with streaming hydrogen was employed with a calomel electrode and a sensitive Tinsley potentiometer. Cation-free cellulose was soaked in a standard calcium acetate solution; the acetic acid liberated by reaction of the "cellulosic acid"[9] was potentiometrically titrated with dilute aqueous barium hydroxide. The dissociation constant for "cellulosic acid" was calculated from these measurements, assuming Donnan equilibrium.

Neale and Stringfellow[10,11] found the amount of dye absorbed by various types of treated and untreated cellulose is a function of sodium chloride concentration in the dye solution. The liberated hydrochloric acid was titrated in the presence of an appropriate indicator. Later, Heymann and Rabinov[12] determined the carboxyl groups of cellulose and cellulose oxidized by sodium hypobromite by treating their samples with potassium chloride solution and titrating the liberated hydrochloric acid by potentiometric means.

This paper describes an investigation of potentiometric titration of celluronic acid which was undertaken to correlate values for carboxyl group content so obtained with those values found by other methods. It seemed possible that titration curves might indicate whether free or potential carboxyl groups other than those disclosed by carbon dioxide evolution and calcium acetate methods are present in celluronic acids. Materials of closely allied structures were studied for comparative purposes. The objective was to obtain further evidence of the celluronic acid structure rather than to develop such titrations as an analytical method. A modification of the titration method of Heymann and Rabinov[12] was employed but using sodium bromide as the electrolyte.

Experimental

Materials.—Celluronic acids and oxidized starch were prepared by the action of solutions of liquid nitrogen tetroxide in carbon tetrachloride on the carbohydrate for appropriate periods of time. The cellulose was dried surgical cotton gauze ground to 100-mesh size. The preparation and characterization of these and other polyuronides or simple organic compounds used for the investigation have been described before.[5] The celluronic acids were analyzed for moisture content by the Fischer method immediately before use and the analytical results are corrected to the dry basis.

Procedure.—The sample, suspended in aqueous sodium bromide solution (or distilled water), was mechanically agitated and titrated with standard sodium hydroxide, using the usual glass and calomel electrodes. The following variations of sample size were used and are later indicated for each series of experiments.

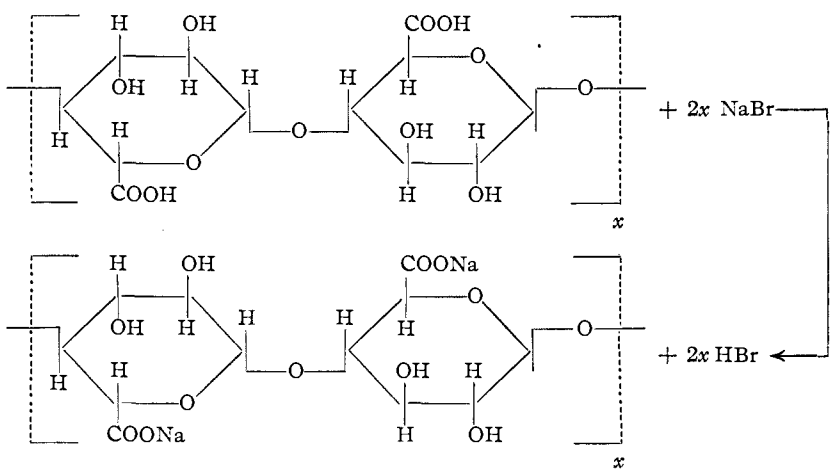
TABLE I

	A	B	C (micro)
Sample, g.	3.0 ± 0.0001	1.0 ± 0.0001	50 mg.
Sodium bromide soln., ml.	75	100	5
Sodium hydroxide, *N*	0.5091	0.1004	0.0502[a]
Potentiometer, Beckman	Model M	Model G	Model G

[a] Graduated 0.2-ml. pipet.

Results and Discussion

When celluronic acid is placed in 1 *N* sodium bromide (Technique B) the *p*H of the solution decreases from about 6.9 to about 2.0. Presumably, this results from the heterogeneous reaction indicated below.



It is believed that this reaction is incomplete but the equilibrium value has not been determined. The hydrobromic acid thus formed is titrated in solution with sodium hydroxide, re-forming sodium bromide, and this homogeneous reaction proceeds until sodium ion is consumed in an amount equivalent to the available carboxyl group present.

The values of Table I show that the presence of sodium bromide facilitates smooth titration of celluronic acid, particularly when present in

(5) Taylor, Fowler, McGee and Kenyon, THIS JOURNAL, **69**, 342 (1947).

(6) Unruh and Kenyon, *Textile Research*, **16**, 1 (1946).

(7) Heymann and Rabinov, *J. Phys. Chem.*, **45**, 1152 (1941).

(8) Heymann and Rabinov, *ibid.*, **45**, 1167 (1941).

(9) The term "cellulosic acid" should not be confused with the name "celluronic acid," as the latter is used specifically to define the class of substances resulting from the action of NO_2-N_2O_4 on cellulose.[6]

(10) Neale and Stringfellow, *Trans. Faraday Soc.*, **33**, 881 (1937).

(11) Hanson, Neale, and Stringfellow, *ibid.*, **31**, 1718 (1935).

(12) Heymann and Rabinov, *ibid.*, **38**, 209 (1942).

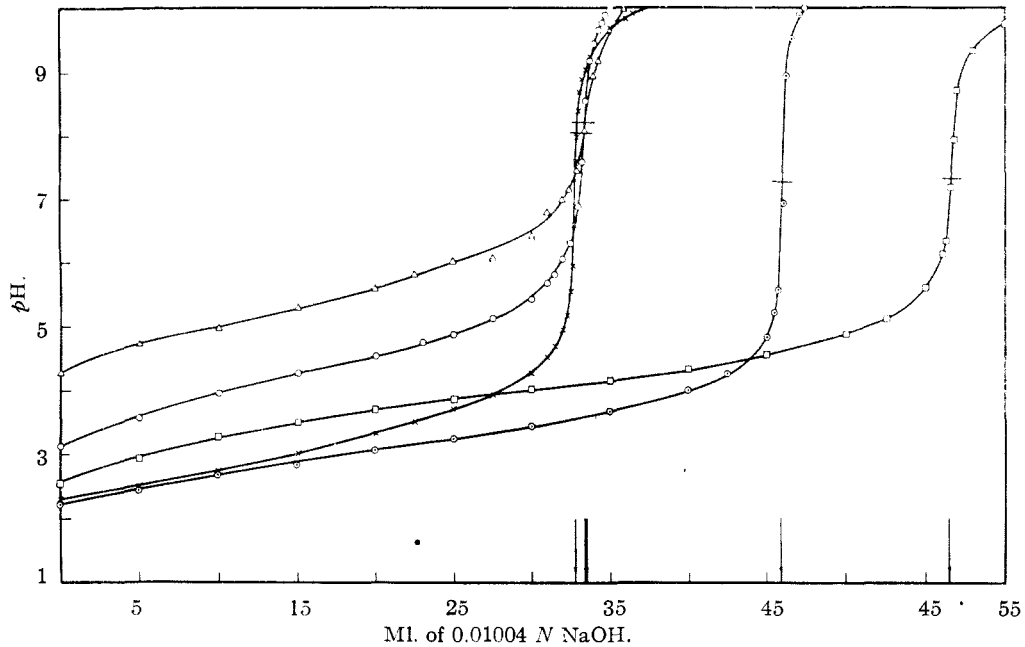


Fig. 1.—Titrations of celluronic acid and simple molecules showing effect of sodium bromide: △, in distilled water; O, in 0.01 *N* sodium bromide; ×, in 1.00 *N* sodium bromide; □, L-ascorbic acid in 1.00 *N* sodium bromide: ⊙ D-galacturonic acid in 1.00*N* sodium bromide.

higher concentrations, but concordant final values were obtained even in the absence of the salt.

TABLE II

EFFECT OF NaBr CONCENTRATION ON POTENTIOMETRIC TITRATION OF CELLURONIC ACID, METHOD B

Method	% COOH (dry basis)	Remarks
Calcium acetate	19.61	
Distilled water only (direct titration)	19.67	Meter needle wavered badly
0.01 *N* NaBr	19.63	Meter needle steady at first; wavered toward end
1.00 *N* NaBr	19.25	Meter needle steady throughout

From the operational standpoint, the normal sodium bromide is advantageous because (a) the *p*H-meter needle does not waver, and (b) the "break" in *p*H at the equivalence point is intensified (see Fig. 1).

The question arose whether the titration of the sodium bromide solution to its original *p*H represents complete neutralization of the carboxyl groups of the celluronic acids or whether the entire titration curve is needed to determine the equivalence point. Selective adsorption of ions was an unknown factor. Calculations on both bases (from Fig. 4 and 5) for a celluronic acid, alginic acid and oxidized starch are summarized in Table III.

In each case there is a small discrepancy, although it is particularly significant that the two values for celluronic acid agree within experimental error and check well with the calcium acetate

TABLE III

CARBOXYL VALUES OF POLYMERS ASSUMING DIFFERENT EQUIVALENCE POINTS

Sample	Technique a at *p*H of NaBr soln.	% COOH	At graphic equivalence, *p*H	% COOH	% COOH by $Ca(OAc)_2$	% Uronic acid
Celluronic acid	6.10	19.54	7.05	19.67	19.42	...
Alginic acid	6.10	22.85	7.25	22.97	22.32	23.49
Oxidized starch	6.09	18.66	7.30	18.76	18.34	22.09

value. Reference to Fig. 4 shows that, for all the various celluronic acids examined, the slope of the curves between *p*H 6.10 and the *p*H of graphical equivalence is almost vertical. Thus, any error introduced in assuming that titration to *p*H of original sodium bromide represents exact neutralization is quite small. The method may be used both as a rapid analytical procedure and for the preparation of sodium celluronate.

To amplify preliminary observations that the carboxyl values obtained by potentiometric titration in the presence of sodium bromide agree with those obtained by the calcium acetate analysis, a variety of celluronic acids were examined. Several other polyuronides, including alginic acid, pectic acid, and oxidized starch, were included, as well as a simple uronic acid and L-ascorbic acid. From the data of Table IV it is apparent that agreement between the two methods is quite general.

The oxidation of cellulose by nitrogen peroxide (and/or its dimer, nitrogen tetroxide) is a heterogeneous reaction when the oxidant is applied as a

TABLE IV

COMPARISON OF POTENTIOMETRIC TITRATION AND CALCIUM ACETATE CARBOXYL VALUES (TECHNIQUE A)

Substance	% Uronic acid by CO_2 evolution	% COOH by calcium acetate	% COOH by NaBr potentiometric titration (dry)
Celluronic acid I	9.99	7.32	7.82
Celluronic acid II	12.46	10.78	10.58
Celluronic acid III	22.70	18.70	18.15
Celluronic acid IV	...	19.42	19.75
Celluronic acid V	24.92	20.29	20.97
Oxidized starch	22.09	18.34	18.80
Alginic acid	23.49	22.32	22.65
Pectic acid	21.47	21.25	20.70
D-Galacturonic acid	29.35	22.79	22.75
L-Ascorbic acid	30.41	27.40	28.02

vapor phase or used in carbon tetrachloride solution. This conceivably could produce topochemical variation in the degree of oxidation from the surface to the interior of the cellulose particle. Gross variations within the cellulose mass could result from lack of uniformity of stirring during the reaction or from local temperature variations.

TABLE V

MICRO-TITRATION OF N_2O_4-OXIDIZED MATERIALS IN PRESENCE OF 1 *N* SODIUM BROMIDE

Sample	% COOH by calcium acetate	Macro-titration Sample wt. (dry)	(B) % COOH	Micro-titration Sample wt. (dry)	(C) % COOH
Celluronic acid	22.70	0.8714	22.42	0.0436	22.94
Oxidized starch	20.52	.9516	20.02	.0476	19.89

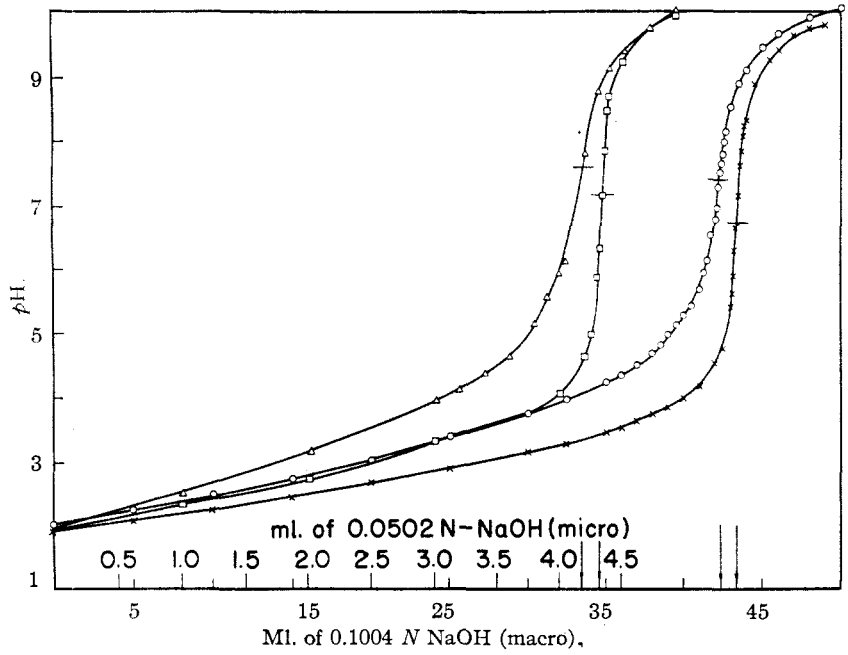


Fig. 2.—Macro- and microtitrations of celluronic acid and oxidized starch in 1 *M* NaBr: ×, celluronic acid (macro); O, oxidized starch (macro); □, celluronic acid (macro): and △, oxidized starch (micro).

The micro-scale titration was developed to permit examination of small portions of oxidized products from various parts of the oxidation system. Comparison of the micro-titration (Technique C) with the macro-scale (B) yields comparative results in Table V and Fig. 2. Good physical homogeneity of the celluronic acid and oxidized starch is indicated.

Celluronic acids having substantial quantities of combined nitrogen evince a spuriously high apparent carboxyl content, as determined by the uronic acid method.[5] The data of Table VI (Fig. 3) indicate that the potentiometric determination of carboxyl groups was not affected (within the limits investigated) by the combined nitrogen content.

TABLE VI

POTENTIOMETRIC TITRATIONS OF CELLURONIC ACIDS CONTAINING COMBINED NITROGEN (TECHNIQUE A)

Celluronic acid	% N	Uronic acid	% COOH (dry basis) $Ca(OAc)_2$	Potentiometric
A	1.20	13.95	6.42	6.56
B	1.20	16.94	10.74	10.94
C	0.96	22.30	16.14	15.82
D	.38	23.80	19.35	19.36

Potentiometric titration of celluronic acid, oxidized starch, and alginic acid, respectively, to a very high *p*H value discloses a strong buffering effect. This phenomenon was not observed in alginic acid titrations. As may be seen from Figs. 4 and 5, the buffering action is considerably more pronounced with titrations in the absence of sodium bromide. With oxidized starch, a *p*H of 10.20 was maintained through the addition of one ml. of 0.5101 *N* sodium hydroxide. At the end of this period the oxidized starch completely dissolved and the titration resumed a normal course. In the case of celluronic acid, a *p*H of 10.80 was maintained through the addition of 1.5 ml. of the alkali at the end of which, solution of the celluronic acid was complete and the titration reverted to a typical path.

These buffering phenomena could be ascribed to a selective adsorption of hydroxyl ions as the *p*H of solution approaches 10. When solution of the polymer is complete, the forces which caused adsorption of the hydroxyl ion have dissipated and the addition of further alkali to the homogeneous system results in regular increments of *p*H. Whether the alkali con-

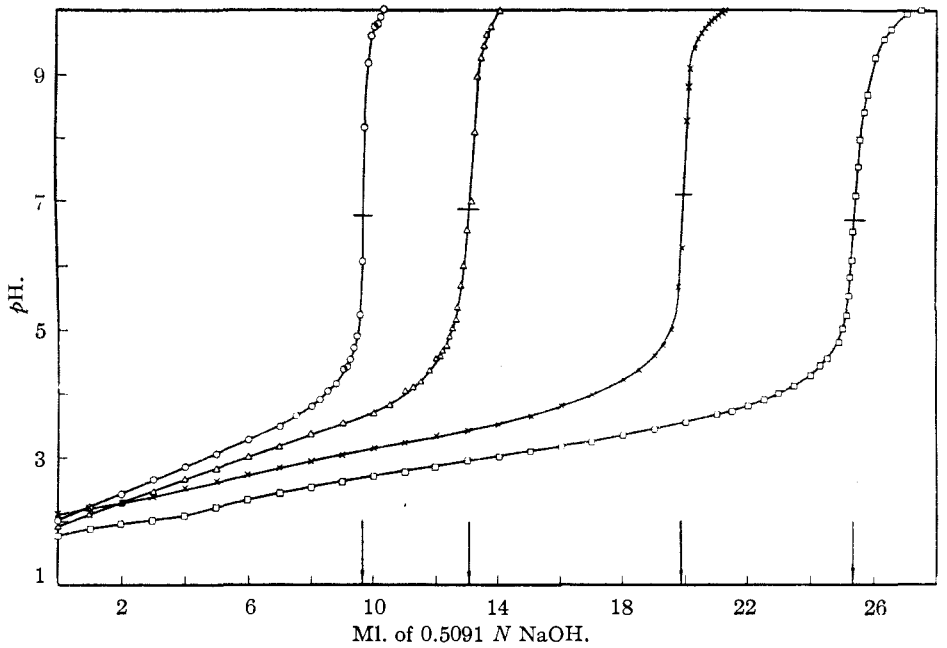


Fig. 3.—Titration curves for various celluronic acids: O, celluronic acid I; △, celluronic acid II; ×, celluronic acid III; and □, celluronic acid V.

sumption at the solution point of celluronic acid (or oxidized starch) represents the *total* carboxyl contents of the polymer, including free and potential carboxyl, is under investigation.

The marked difference in *p*H at which solution of the polyuronides occurs may be ascribed to differences in the method of synthesis. Alginic acid, dissolving at a *p*H of 3.90 to 4.02, is synthesized in seaweed under anything but anhydrous conditions, and it is isolated therefrom by salt formation under alkaline conditions. Intermolecular dehydrations would therefore not be expected. When most of the free carboxyl groups have been neutralized (91% of the carboxyl groups are neutralized at the *p*H of solution), complete solution of the sodium alginate would be expected, and was observed.

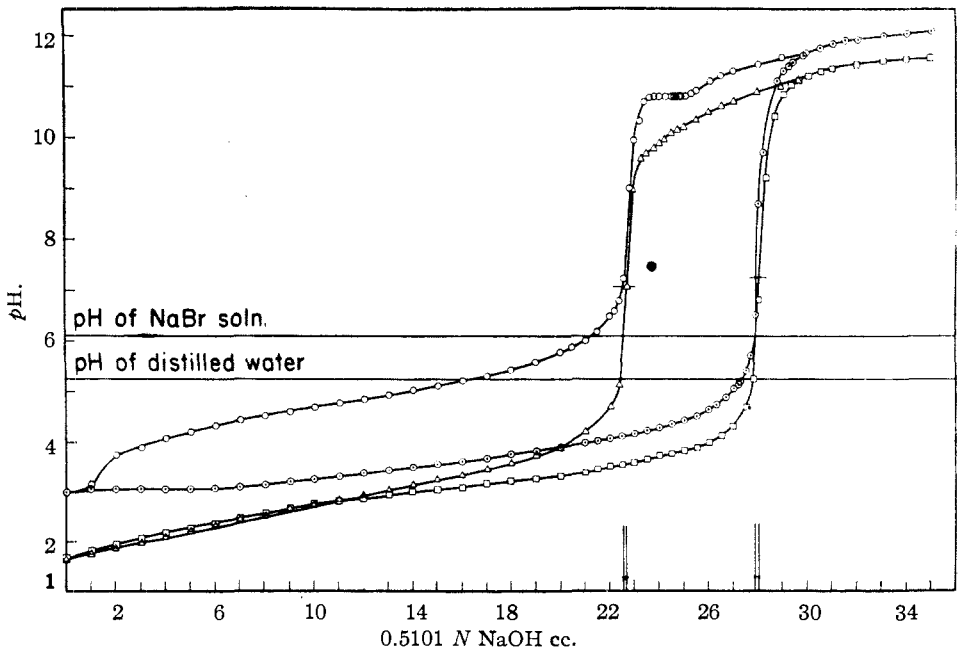


Fig. 4.—Potentiometric titrations of celluronic and alginic acids to high *p*H: O, celluronic acid in distilled water; △, celluronic acid in 1 *N* NaBr; ⊙, alginic acid in distilled water; □, alginic acid in 1 *N* NaBr.

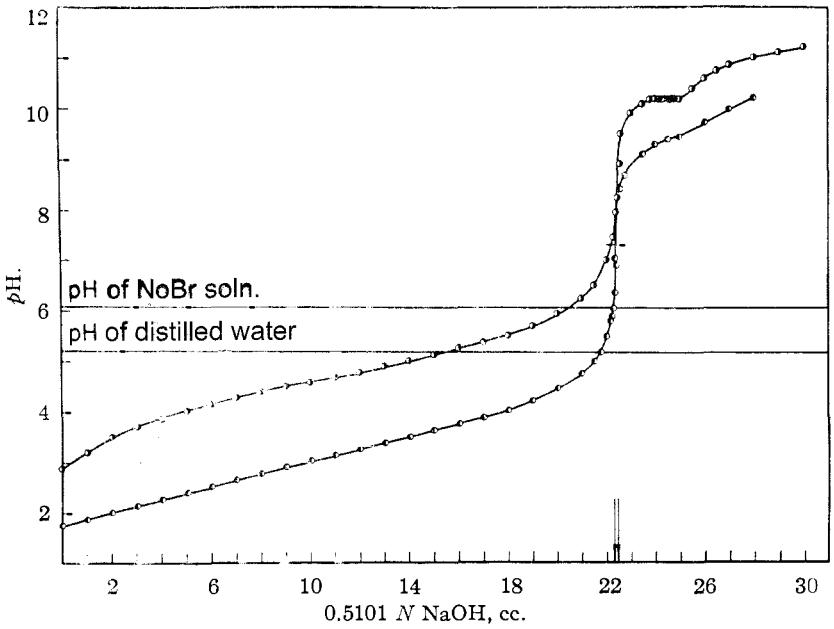


Fig. 5.—Potentiometric titration of oxidized starch: ◑, in distilled water; ◐, in 1 *N* sodium bromide.

Celluronic acids and oxidized starch, on the other hand, are prepared under anhydrous conditions since the nitrogen tetroxide, present in tremendous excess, may be looked upon as the mixed anhydride of nitrous and nitric acids. In these cases it would seem reasonable to assume that at least a limited amount of intermolecular dehydration would occur, giving rise to a partially cross-linked structure through the production of carboxylic anhydride groups. In addition, the possibility exists that dehydration could take place between a carboxyl group of one chain molecule and an hydroxyl group of an adjacent one to produce intermolecular esterification. The presence of either intermolecular link would require a relatively high *p*H for saponification before the synthetic polyuronide would dissolve. This explanation is not entirely satisfactory. Unpublished work in these Laboratories has shown that alginic acid after treatment with nitrogen tetroxide, under the conditions of celluronic acid formation, shows viscosity decrease in alkaline solution but still dissolves in alkali at the same low *p*H value as does alginic acid. Intermolecular condensation is not indicated in this case. Amplification of this investigation is expected to be the subject of a subsequent paper. Whatever may be the structure involved, we believe a linkage in celluronic acid that must be cleaved prior to solution is strongly indicated.

The supposition that an enediol structure,[13] which theoretically could be present in very small amounts in the celluronic acids, produces the break in the titration curve at high *p*H is not corroborated by titrations of ascorbic acid.

Many types of oxidized celluloses show alkali-lability.[6] The potentiometric technique herein described offers a method for determining the carboxyl groups present without exposure to alkaline conditions to produce secondary reactions and spurious values. It should find considerable utility in oxidized cellulose research.

Summary

1. Macro- and microtitrations for carboxyl of celluronic acid, oxidized starch, and other polyuronides in presence of 1 *N* sodium bromide are shown to represent a rapid, convenient method for characterizing these substances.

2. Both celluronic acids and oxidized starch are insoluble in water, in contrast to alginic acid, when neutralized with alkali hydroxide to the equivalence point and a cross-linking reaction(s) possibly involved in their preparation is postulated to explain this observation.

3. Good agreement is obtained between the carboxyl values found by potentiometric titration in presence of sodium bromide and those resulting from the calcium acetate method for a variety of polymeric and monomeric substances containing uronic acid groups.

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(13) Pacsu, *Textile Research*, **15**, 354 (1945).