hours. The barium oxide first dissolved in the molten barium, and then as the barium evaporated and condensed at the top of the crucible, which was at a much lower temperature, a thick layer of red barium oxide formed at the surface of the solution. This layer consisted of a few large single crystals, and its thickness depended upon the amount of barium evaporated. The crystals were removed from the barium melt by dissolving the barium in a 2:1 toluene-absolute alcohol mixture. The function of the toluene was to retard the precipitation of barium ethylate. There was also a slight reaction between the barium oxide and the absolute alcohol[3]

$$BaO + 2C_2H_5OH \rightleftharpoons Ba(C_2H_5O)_2 + H_2O$$

The water produced in this reaction causes a thin layer of barium hydroxide to be formed on the surface of the BaO crystals. However, this layer of hydroxide is easily removed by polishing the crystals.

Analysis of the Crystals.—In order to eliminate the error due to the occluded globules of barium metal mentioned above, the crystals were ground to a fine powder and washed with dry liquid ammonia, which dissolved the free barium metal without reacting with the barium oxide. The method used for the analysis was a modification of Berdennikowa's[4] method for oxide coated cathodes. The barium was placed into the sample tube of the apparatus shown in Fig. (1). After evacuating and outgassing the system, which has a total volume of 35 cc., the water in the liquid nitrogen trap was distilled into the sample tube. As the barium oxide dissolved, the excess barium reacted with the water to form hydrogen gas

$$Ba + 2H_2O \longrightarrow Ba(OH)_2 + H_2$$

After the barium oxide was completely dissolved, the water was recondensed in the liquid nitrogen trap, and the pressure of the hydrogen gas was measured with a thermocouple vacuum gage. The percentage of excess barium was calculated from the amount of hydrogen gas present. All the work on barium oxide was carried out either in a dry-box or in a water-free atmosphere.

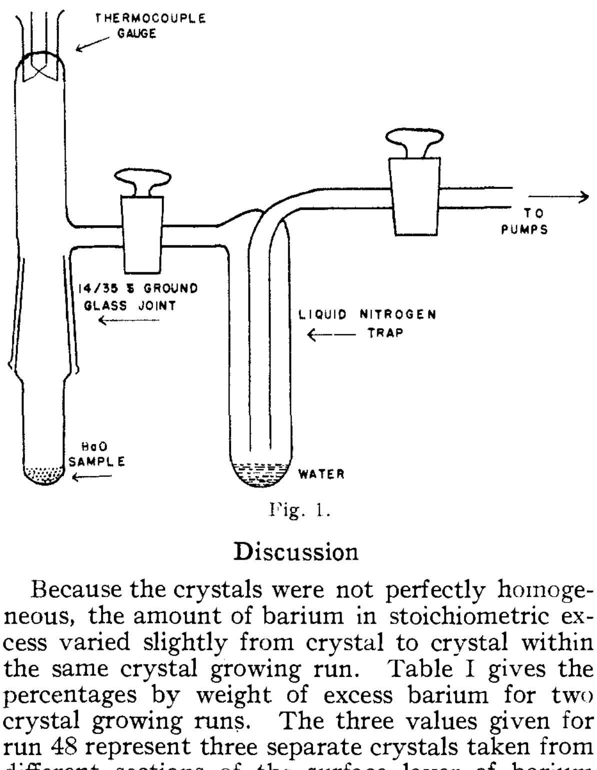

Fig. 1.

Discussion

Because the crystals were not perfectly homogeneous, the amount of barium in stoichiometric excess varied slightly from crystal to crystal within the same crystal growing run. Table I gives the percentages by weight of excess barium for two crystal growing runs. The three values given for run 48 represent three separate crystals taken from different sections of the surface layer of barium oxide.

TABLE I

Run no.	Time, hr.	Temp., °C.	Excess barium, %
35	60	890–910	0.14
48	73	880–905	.091
			.085
			.104

Optical absorption measurements taken on these crystals by Kane[5] indicate that the excess barium is not present as colloidal aggregates, as believed by Schriel, but either as interstitial barium atoms or as oxygen vacancies in the lattice. More recent investigations (to be published soon) at this Laboratory, however, indicate that the interpretation of Kane's experiment may be more complicated.

Acknowledgment.—The author wishes to thank Prof. R. L. Sproull for his advice.

(3) G. F. Smith, *Ind. Eng. Chem., Anal. Ed.*, **1**, 72 (1929).
(4) T. P. Berdennikowa, *Physik. Z. Sowjetunion*, **2**, 77 (1932).
(5) E. O. Kane, *J. Applied Phys.*, **22**, 1214 (1951).

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A Study of the Periodate Method for Determining End-group Values[1]

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The use of periodate for the determination of the degree of branching of certain carbohydrate substances such as starch and glycogen is based on its reaction with those monosaccharide units which contain alcohol groups on three adjacent carbon atoms and the resultant formation of a molecule of formic acid. Since in these polymers only the terminal glucose units have this necessary configuration, the amount of formic acid produced becomes a measure of the average length of the glucose chains, or of the degree of branching of these substances.

Many modifications of the periodate method have been proposed. These differ in the conditions of the oxidation reaction and in the method used for the determination of the formic acid. Formic acid has been determined after separation by steam distillation from the reaction mixture[2] and also iodometrically[3,4] but it is usually titrated directly with alkali after excess periodate is removed by reaction with ethylene glycol. The procedures employing direct titration variously specify that for quantitative recovery of formic acid, titration must be performed with different indicators to end-points of about pH 5.5,[4,5] pH 6.0,[6] pH 8.0,[7] and pH 8.2.[8] However, the titration curve of pure formic acid shows that it is quantitatively titrated at any of these pH values and that the choice of end-point should have little, if any, influence on the quantitative titration. The different modifications appear to give widely different end-group values. In this

(1) This paper is taken from a dissertation presented by M. Morrison in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, Wayne University, 1952. The investigation was supported by a grant from the Griffith Laboratories, Chicago.
(2) Y. Khouvine and G. Arragon, *Bull. soc. chim.*, **8**, 676 (1941).
(3) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).
(4) T. G. Halsall, E. K. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1427 (1947).
(5) A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3488 (1948).
(6) M. Schlamowitz, *J. Biol. Chem.*, **188**, 145 (1951).
(7) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **31**, 1540 (1948).
(8) A. Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

Laboratory the method of Potter and Hassid[5] when applied to maltose, the sugar recommended for standardization, did not give the theoretical yield of 3 moles of formic acid. Furthermore, a fading end-point suggested that something other than free formic acid was included in the titration.

In order to study the reaction further, and to determine to what extent substances other than formic acid are titrated in the periodate method, potentiometric titration curves of the various reaction mixtures were prepared (Figs. 1, 2 and 3) and these were compared with the theoretical titration curves of the formic acid present in these mixtures. The experimental titration curves were corrected for the accumulated hydrogen and hydroxyl ions and for the titration of the iodate and small amounts of silicate in the reaction mixtures. The theoretical titration curve for the formic acid present in each titration mixture was calculated on the basis of a pK' for formic acid of 3.56, as determined by separate titration under the same experimental conditions, and of the amount of substance titrated within one or two pH units[9] of this value. At pH values below about 4.2 these calculated curves coincide with the experimentally determined curves (Figs. 1, 2 and 3). This indicates that over this region formic acid, or acids having the same dissociation constant as formic acid, are being titrated.[10]

At pH values higher than 4.2 the titration curve of the reaction products of periodate and maltose (Fig. 1) does not coincide with the theoretical titration curve for formic acid. In addition, there is a drift of pH values obtained in the titration after each addition of alkali from a relatively high value to a lower pH value (indicated by the double line in the figure). When periodate acts on maltose in the pyranose form it may be shown that, of the three molecules of formic acid produced, one is liberated as an ester. Meyer and Rathgab[7] reported the formation of a similar ester in the periodate oxidation of lactose. Hydrolysis of the ester by alkali and the liberation of formic acid would account for the additional titration and the fading end-point. The amount of formic acid produced as measured by the theoretical titration curve corresponds to about 2.5 moles per mole of maltose. This probably includes formic acid produced by partial hydrolysis of the ester. A titration corresponding to the theoretical 3 moles of formic acid is obtained only at relatively high pH values where the ester is more completely hydrolyzed and where other groups are also included in the titration.

The experimentally determined titration curve of the substances formed in the reaction between periodate and sucrose (Fig. 2) corresponds closely to the calculated curve of formic acid over the pH range below 6.5, indicating that formic acid alone, or acids having the same dissociation constant, are being titrated.[10] The curve differs from that of the maltose reaction mixture in that it is almost vertical

(9) D. D. Van Slyke, *J. Biol. Chem.*, **52**, 525 (1922).

(10) Lactic and oxalic acids have dissociation constants within a few tenths of a pK unit of formic acid and cannot be readily distinguished on the basis of their titration curves. Failure to form an insoluble calcium salt indicated the absence of oxalic acid.

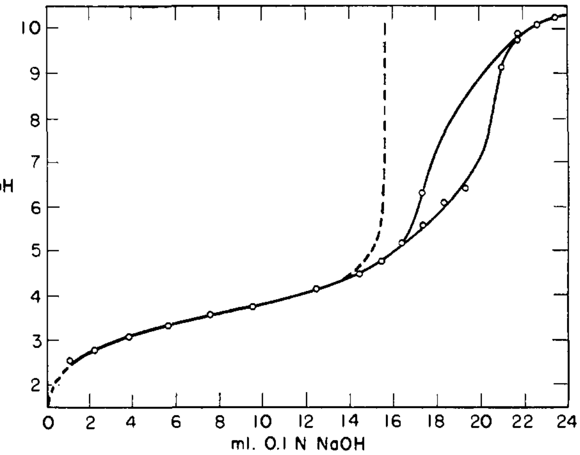

Fig. 1.—Potentiometric titration curve of periodate–maltose reaction mixture: —, experimental curve, the double line indicates a drift in pH; - - - -, theoretical curve of the formic acid present in the reaction mixture.

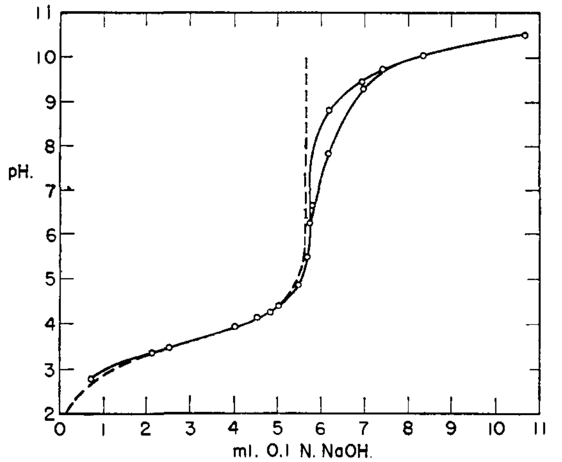

Fig. 2.—Potentiometric titration curve of periodate–sucrose reaction mixture: —, experimental curve, the double line indicates a drift in pH; - - - -, theoretical curve of the formic acid present in the reaction mixture.

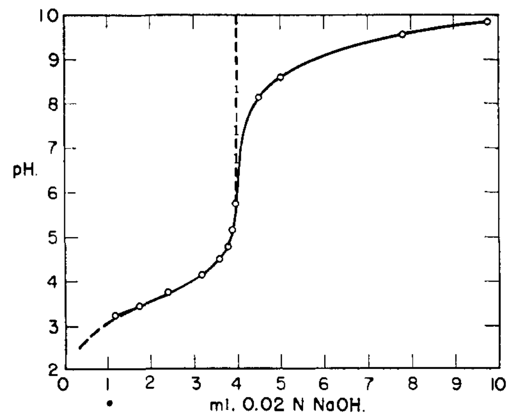

Fig. 3.—Potentiometric titration curve of periodate–glycogen reaction mixture: —, experimental curve; - - - -, theoretical curve of formic acid present in the reaction mixture.

over the pH range of 5.0 to 6.5 and shows a smaller amount of drift of pH in alkaline solution. This drift in pH cannot be attributed to the hydrolysis of an ester, similar to that found in the periodate–maltose reaction mixture. The reaction mixture was made acid with hydrochloric acid and again titrated. The titration curve showed the presence of an increased amount of organic acid corresponding to that liberated during the former titration. The curve was again almost identical with that of an acid of pK' 3.56.

The amount of free formic acid determined in the first titration of the periodate–sucrose reaction mixture to pH 6.0 corresponds to 80–90% of the theoretical value, one mole per mole of sucrose, and depends on the time allowed for the oxidation reaction (Figs. 2, 4). Titration to about pH 8.0 as performed by others[7,8] includes products liberated during the titration and may give a value which indicates a theoretical yield. However, the end-point at this pH is not definite and other groups are included in the titration. When formic acid was added to and incubated with a periodate oxidation mixture, it was quantitatively recovered from the mixture, indicating that this substance is not oxidized by the periodate. The amount of periodate utilized in the reaction remained about constant after 45 hours and corresponded to the theoretical amount of 3 moles per mole of sucrose oxidized. The inclusion of 20 ml. of 0.1 M formic acid in periodate–sucrose or in periodate–glycogen reaction mixtures did not increase the amounts of formic acid liberated during titration in alkaline solution.

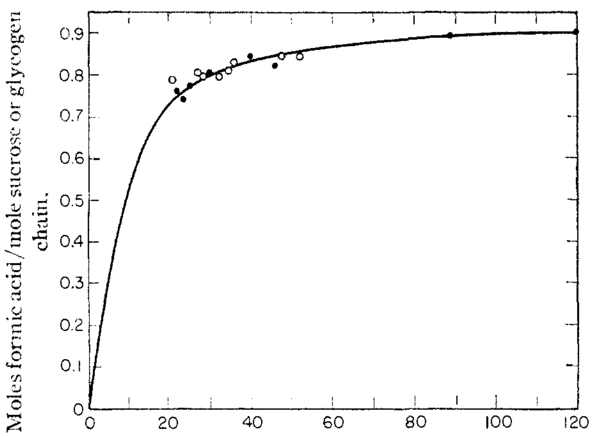

Fig. 4.—Time study of periodate reaction: ●, with sucrose; O, with glycogen.

The titration curve of the reaction mixture of periodate with glycogen (Fig. 3) is similar to that with sucrose except for the presence of much less formic acid and a smaller drift in the alkaline range (not shown in the figure) of only a few hundredths of a pH unit, which is of uncertain significance. Unidentified groups are titrated beginning in the neighborhood of pH 7.0.

The use of the periodate reaction for the determination of end-group values of polysaccharides requires that the conditions of the reaction be standardized on the basis of reaction with a carbohydrate of known composition, and that the reaction occurs in the same manner with this reference substance, as with the polysaccharide. For use as this reference substance, sucrose appears to be preferred to maltose because, like polysaccharides, it is not reducing and because titration of the reaction mixture to pH 6.0 gives a definite, non-fading end-point. The amount of free formic acid produced in the reaction, however, depends on the time allowed for the reaction and amounts to only 80 or 90% of the theoretical value. In applying the reaction to polysaccharides it appears that the most reasonable procedure is to assume the same percentage production of formic acid and to allow a corresponding correction. Support of this procedure is found in the similarity in the rates of reaction (Fig. 4) and the shapes of the titration curves of the reaction products of these two substances (Figs. 2, 3), and also in results obtained by other workers[7,8] who found it necessary to titrate periodate reaction mixtures to high pH values and include substances other than formic acid in order to obtain the theoretical titration value. When a correction was applied, end-group values obtained by the periodate method were in substantial agreement with those obtained by Cori and Larner by an enzymatic method[11] when both methods were run on the same carbohydrate samples.[12]

In view of the foregoing observations, a modified procedure was devised, as outlined under "Experimental." The oxidation was allowed to proceed for 30 hours, and the reaction mixture was titrated with brom cresol purple to pH 6.0. The formic acid measured in this titration was corrected for an average 81% yield as determined with sucrose and the end-group value, or average chain length, was calculated from this corrected figure. End-group values, determined on ten different glycogen samples in this way varied from 12.4 to 14.5 glucose units per end-group.[13]

Experimental

Periodate Oxidation.—0.222 g. of carbohydrate (either sucrose, maltose, arrowroot starch or glycogen) was dissolved in 10 ml. of 3% sodium chloride in a 50-ml. erlenmeyer flask. The flask was placed in a deep-freeze at $-10°$ until a slush formed and then 10 ml. of 0.37 M sodium metaperiodate at room temperature was added and the solution was placed in the refrigerator at 2°. After 30 hours or other desired time interval, the solution was removed from the refrigerator and 2 ml. of ethylene glycol was added. The solution was then kept at room temperature in a dark place for at least half an hour before it was titrated.

Titration of Reaction Mixture.—The reaction mixture was titrated in a CO_2-free atmosphere with CO_2-free sodium hydroxide either with brom cresol purple to pH 6.0, or potentiometrically, using a glass electrode. For potentiometric titration, the reaction mixture was adjusted to 0.4 ionic strength and titrated with sodium hydroxide in a 0.4 M solution of sodium chloride. The titration curves were corrected for the accumulation of hydrogen and hydroxyl ions, for the titration of iodate formed during the oxidation and for small amounts of silicate dissolved from the glassware. The pK' of formic acid was determined from a titration curve of pure formic acid in the presence of the same salt concentration used in the experimental samples.

Periodate consumption was determined by the method of Fleury and Lange as described by Jackson.[14]

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(11) G. T. Cori and J. Larner, *J. Biol. Chem.*, **188**, 17 (1951).

(12) Samples of two polysaccharides were kindly supplied by Dr. G. Cori.

(13) Unpublished experiments.

(14) E. L. Jackson, "Organic Reactions," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1944.