doubtedly be $+I$; then one would expect ethyl cyclohexanoate to saponify considerably slower with a higher activation energy than ethyl cyclohexylacetate. Actually this is not found.

Thus the results of this investigation are in accord with expectations from the standpoint of the ring theory.

Summary

The kinetics of the acid catalyzed esterification of cyclohexanoic acid in dry methanol and of the saponification of ethyl cyclohexanoate in 85% aqueous ethanol have been studied at four temperatures. The effect of joining the terminal carbons of diethylacetic acid with a methylene group to make cyclohexanoic acid is to produce a compound kinetically much like isobutyric acid. This result is in accord with the conception of ring formation in diethylacetic and higher di-substituted acetic acids.

BETHLEHEM, PA. RECEIVED AUGUST 1, 1940

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, CORN PRODUCTS REFINING CO.]

The Relative Reducing Potential of Starch*

BY RALPH W. KERR

Many attempts have been made during the past decade to estimate the molecular magnitude of starch. Results have been variously reported which would indicate a chain length ranging from 20 to 30 glycopyranose units up to a thousand or more and from observations of the colloidal phenomena exhibited by starch pastes, possibly even higher. The following discussion is primarily concerned with the molecular magnitude of corn starch but it should not be interpreted as another attempt at this time to fix precisely the chain length and thus add one more confusing touch to an already confused picture. We intend that our observations be taken as a qualitative confirmation of work that has been done indicating that starch molecules are relatively simple and further to stimulate a more critical inspection of methods and theory that have led to almost incomprehensible magnitudes for molecular size.

Farrow[1] and his co-workers, Hixon[2] and others have attempted to arrive at the molecular magnitude of starch by taking the amount of copper reduced by dispersed starch solutions and comparing this value with amounts found for members of the aldehydic series dextrose, maltose, dextrins and starch. From this comparison, a surprisingly high molecular magnitude is obtained for starch, indicating that it contains possibly as high as one thousand glucopyranose units, or a molecular weight in excess of 150,000.

We suggest that there are considerable possibilities for error to enter the determination of the reducing capacity of a substance such as starch, when comparatively strenuous conditions must be imposed, *e. g.*, boiling for three hours in alkaline medium, to show that capacity. The capacity for reducing is very feeble for these carbohydrates at lower temperature. To induce them to exhibit a measurable one by imposing conditions which must simultaneously cause an extensive change in their constitution and molecular magnitude and to take the results obtained as indicative of original molecular weight, seems illogical.

Furthermore, in determinations such as those made by Farrow, the assumption is made that the power of a reducing group is quantitatively the same, whether it is on the end of maltose or on the end of a longer polysaccharide. Their data show that it is not quantitatively the same for dextrose as compared to the same reducing group on maltose. That the assumption is faulty may be explainable by the fact, as our data below show, that a potential may be estimated for this terminal, reducing group which varies in intensity with chain length and that there is some evidence that there is a relationship between potential and the tendency to react in a particular oxidation–reduction system.

Hence, we believe that there would be less error involved in measuring the comparative potentials of these products and using these values to estimate chain length, especially since we have found a potential for these carbohydrates to be measurable at ordinary temperatures in a few minutes of time.

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* This paper was presented at the 99th meeting of the American Chemical Society, Division of Sugar Chemistry, at Cincinnati, Ohio, April 11, 1940.

[1] Richardson, Higginbotham and Farrow, *J. Textile Inst., Transactions*, **27**, 130 (1936).

[2] C. G. Caldwell and R. M. Hixon, *J. Biol. Chem.*, **123**, 595 (1938).

The determination of the actual reduction potential, E_0, of many organic compounds, including carbohydrates, is a controversial matter. Indeed, it has been frequently stated and at one time accepted[3] that relatively powerful reducing substances, such as aliphatic aldehydes, have no reducing potential. Recently, however, Adkins and Cox[4] as well as other investigators have determined a potential for several of the aliphatic aldehydes by means of the polarograph.

Wurmser[5,6] and others,[7] moreover, many years ago, determined the reducing potential of several of the common sugars, including dextrose, by means of reducible dyes and by the potentials developed by the hypothetical aldehydic form of the sugars in contact with inert electrodes. But Heyrovsky[8] could not demonstrate the presence of a reducing form of dextrose in a polarogram, using $^{1}/_{6}\%$ solutions of the sugar. Earlier work appears to be forgotten and the assumption made that dextrose has no reducing potential.

However, we have observed and will show below that a potential may be estimated for dextrose as well as other sugars under certain conditions even by the polarographic method.

These controversies have not been lost sight of as we present our work.

We present below, first, the potential developed by 1% solutions of the following carbohydrates, buffered with an equal amount of trisodium phosphate at *p*H 11.2 in the Coleman Electrometer, using a gold electrode. The numerical values presented by us are the potentials in excess of the potential given by a 1% phosphate buffer solution at the same *p*H, in the same cell.

Solution	Negative millivolts potential less millivolts for 1% Na_3PO_4 at 11.2 *p*H and 25°
Dextrose	183
Maltose	111
β-Amylose	68
Corn starch	59

The starch used was washed free of soluble material, including residual bisulfites, and 1 g. dispersed in 20 cc. of 0.5 *N* sodium hydroxide at 25° for fifteen minutes, whereupon 80 cc. of water, containing an equivalent of phosphoric acid, was added to adjust the *p*H to 11.2. After fifteen more minutes at 25° the potential was determined with a gold electrode in a Coleman Electrometer.

The sugar solutions were made up in 2% concentration and mixed with equal portions of a 2% sodium phosphate–sodium hydroxide solution at zero time, both solutions at 25°. Potential measurements were read within thirty seconds after mixing and continued for thirty minutes. The maximum value occurs in about six minutes, which then remains more or less constant.

We will not pretend in this work to analyze completely the source of the potential shown by the above carbohydrates. We have assumed that we are dealing with a series of homologous polymers, each with a terminal dextrose unit, and that under the conditions of test, degradation reactions, observable at higher temperatures over longer periods of time, have been eliminated. Whether the variations in the potentials above given result from the aldehydic form of these dextrose terminal units being modified in potential due to the number of glucopyranose units attached to it or whether the potential is developed by the enolic form, ketone form or some other isomer of the end group is relatively less significant to us than the fact that qualitatively, at least, the tendency for the end group to develop a potential is modified by the length of the chain of glucopyranoses attached to the terminal group.

We do advance the hypothesis, however, that in alkaline solution, at least, it is the aldehydic form of these units, which latter dissociate, progressively, the more alkaline the solution, into anions, which are responsible for the potential developed. Thus, it would be the conceivably reversible and conceivably electro-reducible aldehydes in equilibrium with the ionized form in the system

$$(\text{aldehyde}) + ne \rightleftarrows (\text{aldehyde-ion})$$

representing the oxidation–reduction system, which is the origin of the potential measured, rather than the questionable system

$$(\text{aldehyde}) + ne \rightleftarrows (\text{alcohol})$$

If we extend our hypothesis to include the simple aldehydes, then the apparent contradiction between the statement of Gortner, that it is theoretically impossible for lower aldehydes to exhibit a reduction potential and the fact that potentials for aliphatic aldehydes are reported from time to time, also vanishes.

In support of our hypothesis that the members of our carbohydrate series are electro-reducible aldehydes, we offer the following observations on experiments with the Heyrovsky Micro Polarograph.

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[3] Gortner, "Outlines of Biochemistry," 1938, p. 137.

[4] H. Adkins and F. W. Cox, THIS JOURNAL, **60**, 1151 (1938).

[5] R. Wurmser and J. J. Geloso, *J. chim. phys.*, **25**, 641 (1928).

[6] R. Wurmser, *et al.*, *Compt. rend.*, **184**, 407 (1927).

[7] N. Mayer, *ibid.*, **189**, 319 (1929).

[8] J. Heyrovsky and J. Smoler, *Chem. Listy*, No. 26, 479 (1932).

Our first experiments were polarograms made with complex mixtures of the degradation products of corn starch. Various characteristic current steps were observable. Then using exactly the conditions prevailing in our first tests, we examined purified constituents known to be present in the mixture. These latter curves were made using $3^{1}/_{3}\%$ concentration of carbohydrate, made *p*H 9.0 by potassium hydroxide and 0.333 normal potassium chloride at 25°. The Model X Heyrovsky Micro Polarograph was used, applying four volts to the bridge wire and using $^{1}/_{8}$ r. p. m. which produces an increase in voltage across the cell of 0.5 volt per minute. A sensitivity setting of $^{1}/_{20}$ was used (Figs. 1 and 2).

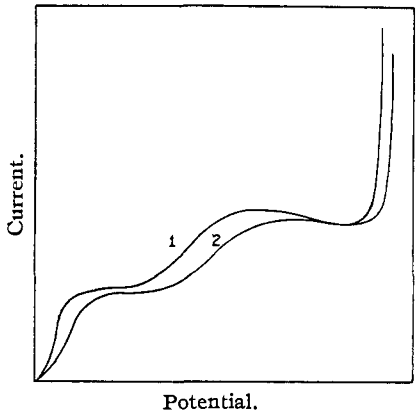

Fig. 1.—Polarograms of: (1) KCl control (1/20); (2) β-amylose (1/20).

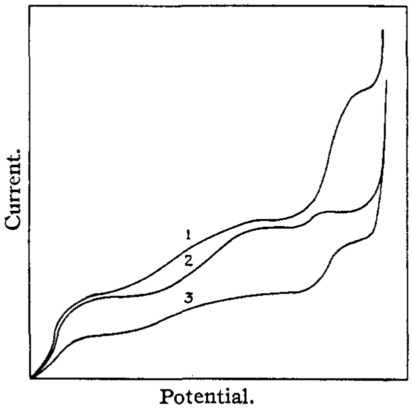

Fig. 2.—Polarograms of: (1) dextrose (1/20); (2) maltose (1/20); (3) dextrose (1/40).

Current waves were observable for both dextrose and maltose from which half wave potentials were estimated at 1.77 and 1.64 volts, respectively. The wave height, or current step, decreases rapidly with increasing chain length of the carbohydrates studied. Even maltose yields a rather unsatisfactory curve for analysis and beta-amylose is too feeble in reducing capacity to register an effect, even at higher alkalinities with the experimental conditions employed.

Since these early tests, we have repeated the polarograms, particularly for dextrose and maltose, many hundred times, altering many experimental variables. We find, for example, that within narrow limits, the current step height is so nearly proportional to the concentration of sugar used that a method has been devised for estimating dextrose in starch conversions which agrees so closely with values obtained by our modified Sichert–Bleyer method[9] for estimating dextrose that the possibility is precluded that the current steps obtained by us could be due to impurities and not to dextrose itself. This method will be detailed, possibly, in a subsequent communication. At dilutions of 2 to 3% dextrose, made *p*H 9.0 by potassium hydroxide–phosphate mixtures and in an atmosphere of hydrogen, we find a current increase of 7.63 × 10^{-7} ampere equivalent to 1% dextrose (Fig. 3).

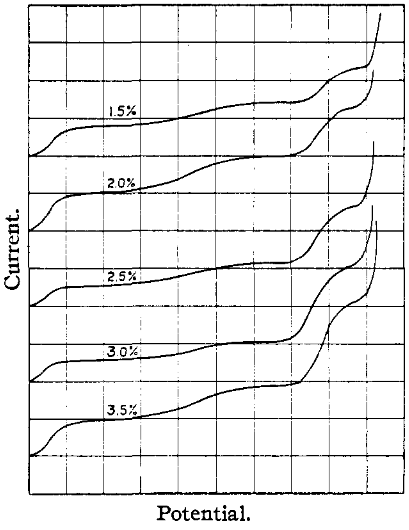

Fig. 3.—Variation in current step height with dextrose concentration. One division equals 1.09 × 10^{-7} amp.

We find also that for any given concentration of dextrose used this current step height increases with rising *p*H which, together with the fact that the half wave potential becomes progressively more negative, for example, 1.66 negative volts at *p*H 7.0, 1.77 at *p*H 9.0 and 1.80 at *p*H 10.8 (using 0.185 *M* dextrose concentration), indicates to

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[9] K. Sichert and B. Bleyer, *Z. anal. Chem.*, **107**, 328 (1936).

us that the reducible form ionizes as an acid in alkaline solution and that the proportion of ionized form is, as might be expected, increased with increase in *p*H, similarly to the behavior of tin, lead and other amphoteric metals.

The proof of our hypothesis is complicated, however, by the fact that the ionization equilibrium is also dependent on other equilibria, *e. g.*, the one between the glucopyranose forms and the free aldehyde form, as well as other equilibria very likely, particularly at higher alkalinities. Thus, we must await further study on these latter questions.

Accepting, however, the limited theory, for which there now appears to be some evidence in support, that the carbohydrates studied can exist as free aldehydes, we may now compare the potentials of the dextrose polymers as measured in the electrometer with the potentials of a simple aldehyde series, members of which we have readily available at suitable purity. We find the potentials of 1% solutions in phosphate buffer at *p*H 11.2 and 25° to be:

Solution	Negative millivolts potential less millivolts for 1% Na_3PO_4 at *p*H 11.2
Formaldehyde	578
Acetaldehyde	402
n-Butyraldehyde	200
n-Valeraldehyde	180

Higher members of this series are soluble with difficulty in water and form decidedly heterogeneous systems. Besides, judging by color and opacity, polymerization of these aldehydes is very rapid at this *p*H. Whereas acetaldehyde appears to be stable for an hour or more, butyraldehyde has evidently condensed within ten minutes and valeraldehyde within one or two minutes. Hence, for valeraldehyde, the initial potential, that is, the value obtained within thirty seconds after mixing with sodium phosphate, is taken as the more accurate estimate of relative potential. Higher members are practically insoluble and give very erratic results.

Comparing the slope of the two curves for carbohydrates and aldehydes, drawn from the electrometer data, it would appear that the position of the potential for starch indicated that the component amyloses were composed of a relatively small number of dextrose units.

It should be pointed out, however, that our carbohydrate series, $(C_6H_{10}O_5)_nC_6H_{12}O_6$ is not strictly comparable with the simple aldehyde series, $H(CH_2)_nCHO$ for among other things for each added increment in the first series we have very nearly doubled the molecular magnitude, whereas in the second we have not. The carbohydrate series would, then, be more comparable to the simple series, $H(CHOH)_nCHO$ of which glycolic aldehyde might be considered as the second member and the monosaccharides are higher members. Most of these products are, unfortunately, difficult to prepare at a purity suitable for potential measurements. Dextrose, however, is the sixth member of this series in addition to being the first member of the starch series. And if we compare the ratio of the potential of dextrose to formaldehyde, we note that we obtain approximately the same ratio as that for the potential of starch to dextrose, confirming our conclusion that the starch molecules are of a relatively low order of molecular magnitude.

The author is indebted to Mr. O. R. Trubell for his valuable assistance with the polarographic work contained herein.

Summary

Relative potentials have been estimated for several simple aldehydes and compared with the potentials found for dextrose, maltose, amylose and corn starch.

Polarographic methods have been applied to these carbohydrates and current steps demonstrated for dextrose and maltose contrary to previous reports that none existed. Half wave potentials are given.

Evidence is submitted that the carbohydrates may exist possibly in the free aldehydic form and the theory proposed that they may in turn ionize as acids.

A comparison of the relative reducing potentials of the carbohydrates studied against potentials of simple aldehydes of known molecular weight supports the view that the elementary starch molecules are composed of relatively few glucopyranose units.

ARGO, ILLINOIS RECEIVED MAY 31, 1940