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Potentiometric Analysis and Fundamental Technique

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The formose reaction, the calcium hydroxide-catalyzed condensation of formaldehyde to monosaccharides, was potentiometrically analyzed. The oxidation-reduction potential varied in a characteristic way and gave information of considerable importance. Some appreciable transition points in the oxidation-reduction potential curve showed satisfactorily the occurrence of a phase change such as an induction period and saccharide-forming and saccharide-decomposing stages. With regard to the fundamental operating conditions, vigorous stirring and an increase in the dissolved oxygen elongated the induction period. Furthermore, the latter diminished the yield of saccharide under the lower limit of the critical range of the calcium hydroxide concentration.

The formose reaction, in which monosaccharides result from formaldehyde in the presence of an alkaline catalyst in an aqueous medium, was first reported by Butlerow.[1] Thereafter, much attention has been directed to studies of catalysts for saccharide-forming reactions. Of these, some alkali earth- and alkali-hydroxides and organic bases, such as pyridine and picoline, have been recognized as active catalysts.[2,3] Especially, $Ca(OH)_2$ is widely used for the investigation of the above reaction. From their experiments using a dilatometer, Belezin and Surykina[4] observed the existence of a time lag prior to the saccharide condensation; this period was shortened by adding glucose or fructose.

Pfeil and Schroth[5] prepared saccharides using TlOH as a catalyst, and recognized the formation of substances which accelerate the formose reaction during the induction time by pursuing the consumption of formaldehyde or products, followed by the Cannizzaro reaction. Subsequently, they supposed that the substances would form a calcium complex and contributed to the saccharide formation. Furthermore, they demonstrated that the extent of formose reaction is greatly affected by the kind of alkaline cations used.

Recently, in their series of studies of the formose reaction, Weiss *et al.*[6-9] followed the reaction in a continuously stirred tank reactor. They derived a kinetic expression for the formose condensation, and pointed out the instabilities observed in both the Cannizzaro reaction and the formose reaction were obviously attributable to changes in the $Ca(OH)_2$ concentration. Fujino *et al.*[10] have obtained evidence for the formation of soluble complexes consisting of alkali earth-saccharides or alkali earth-formaldehyde and recognized the presence of a relationship between their catalytic activity and their complex-forming ability.

In certain situations, the oxidation-reduction potential (ORP) has often been employed to estimate a state or a mechanism of reaction systems.[11,12] As has been mentioned above, with regard to the formose synthesis, much information concerning the activity of catalysts and the reaction mechanism has been accumulated. However, there have been few attempts to elucidate the nature of this reaction by employing ORP estimation. In an earlier paper[13] dealing with the potentiometric analysis of the formose reaction, it was shown that the changes in ORP show a good correlation with the characteristic reaction phases. The object of this paper is to present more details of the previous work and to describe some additional experiments concerning the fundamental techniques in the reaction.

Experimental

Procedure. Under N_2 an aqueous formaldehyde was treated with $Ca(OH)_2$ in an apparatus attached to a potentiometer.

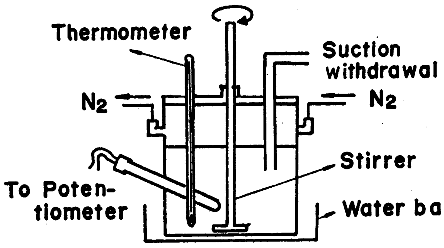

Fig. 1. Apparatus.

These are shown in Fig. 1. The bath temperature was kept in the range of 60±1 °C, and the rate of stirring remained at 320 rpm through the experiment. A representative method of the reaction was as follows. The reactor, which contained 90 ml of 37% formalin (commercial) and 790 ml of water, was pre-equilibrated at 60±1 °C in a thermostat; the reaction was then initiated, followed by the addition of 8.5 g of $Ca(OH)_2$. At convenient intervals, *ca.* a 10-ml aliquot was withdrawn into a 50 ml flask and immediately acidified with 1M HCl; these aliquots were analyzed for formaldehyde, the total saccharides, and organic acids as follows.

The formaldehyde was determined by the method of Bricker *et al.*,[14] only the optical density being measured at 579 nm. The calculation of the formaldehyde concentration was done by means of a previously prepared calibration curve.

The total saccharides were determined using the phenol-sulfuric acid method,[15] the concentration being calculated as glucose from a calibration curve. The organic acids was determined by back titration with 1M KOH after the aliquot had been acidified. When A ml of 1M HCl was added to the solution and subsequently B ml of 0.1M KOH was consumed to neutralize the solution, the organic acid concentration, x M, was given by the following equation:

$$x = 2 \times 8.5 \times 1000/74 \times 880 + 0.01B - 0.1A$$

The dissolved oxygen was estimated by following a modification of the Winkler method,[16] while the stirring speed was measured using a stroboscope.

The UV spectra were measured using a Shimadzu Spectrophotometer, MPS-50L. A sample solution withdrawn from the reaction mixture was cooled immediately in a dry ice–acetone bath to about 0 °C, at which the reaction would virtually not proceed at all. Next, the solution was centrifuged under a high speed to remove the turbidity of the medium. Even when the sample was simply cooled and not acidified, it was stable enough for the determination of the UV spectra so long as it was stored in a refrigerator.

Results and Discussion

As is shown in Fig. 2, a representative ORP curve for the formose reaction apparently has four characteristic steps. The first step: Almost no change in ORP is observed, and no production of saccharides can be detected with a little consumption of formaldehyde. This step may be an induction period, after which only a small amount of organic acids (mainly formic acid) is detected. The period of this step varies depending upon the reaction conditions. For example, it is shortened when a known promoter, such as glucose,[5] fructose,[17] xylose, or glycolaldehyde,[18] is added. The second step: After the induction period, the ORP curve begins to turn downward and reaches to a minimum point (the time up to this point is termed T_{min}). At this point, the yield of saccharides is only approximately 3% (as glucose based on formaldehyde), and the formaldehyde condensation to saccharides is initiated slowly, whereas as much as 20—30% of the formaldehyde is consumed. The rate of organic acid production is similar to that in the previous step. The third step: The ORP curve turns upwards from the minimum point and reaches a small maximum (the time up to this point is termed T_{max}), whereupon a little drop is observed, while the color of the solution simultaneously turns to pale yellow (the so-called yellowing point). As the ORP of the solution increases, the reaction proceeds rapidly with a rise in the temperature up to the maximum, 70—75 °C. At the termination of this step, the formaldehyde consumption is raised to 95% and the yield of the saccharides amounts to 50—70%. The fourth step: A steady state appears again in ORP, while the saccharides formed begin to decompose and the total amount of saccharides decreases successively as time passes. The temperature of the mixture slowly becomes lower, on the other hand, the color of the mixture changes from light yellow to light brown or brown.

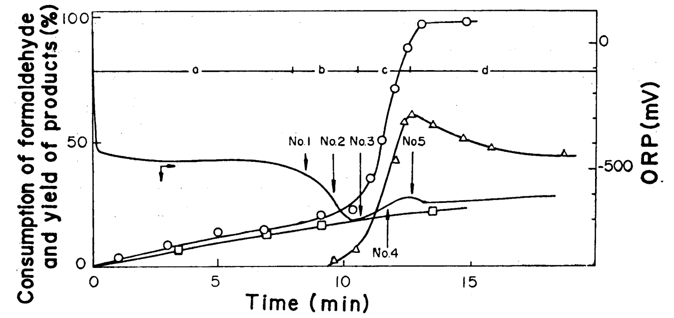

Fig. 2. Potentiometric and product analyses of the formose reaction.
[HCHO]=1.4 M; [Ca(OH)$_2$]=0.13 M; ○, Formaldehyde; △, Total sugars; □, Organic acids; T_{min}= a+b; T_{max}=a+b+c; ΔORP=maxORP−minORP.

In order to ascertain the optimum conditions for the saccharide formation, some basic experimental conditions, such as the influences of the stirring speed and the dissolved oxygen, were investigated. The stirring speed has a negligible influence on the saccharide yield within the rage of 200—1200 rpm. However, vigorous stirring causes the ΔORP (the ORP difference between T_{max} and T_{min}) to decrease (Fig. 3) and results in an increase in the period up to both T_{min} and T_{max} (Fig. 4). The results indicate that the stirring speed need not necessarily be high; hence, 320 rpm would seem an adequate speed for usual experiments.

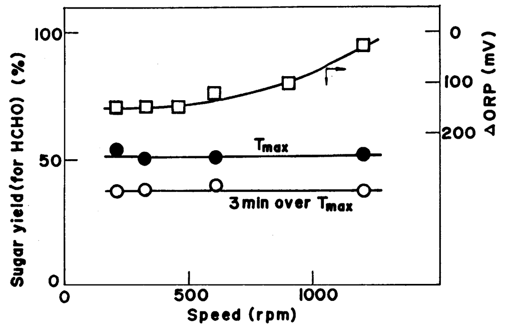

Fig. 3. Effect of agitation on sugar yield and ΔORP.
[HCHO]=2.0 M; [Ca(OH)$_2$]=0.2 M.

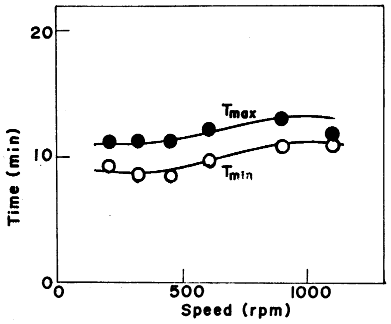

Fig. 4. Effect of agitation on T_{min} and T_{max}.
Reaction condition; Same as Fig. 3.

Concerning the influence of dissolved oxygen, a set of experiments were carried out in which the periods up to T_{min} and T_{max} were determined with an increase in the concentration of dissolved oxygen (Fig. 5). The oxygen concentrations were adjusted by introducing limited amounts of oxygen and nitrogen into the reactor. As may be seen in Fig. 5, slight elongation in the time up to both T_{min} and T_{max} is observed with an increase in the oxygen concentration. On the other hand, at a comparatively lower level of the Ca(OH)$_2$ concentration (0.17 M), the dissolved oxygen at a high concentration diminishes the sugar yield, while at a little higher concentration (0.20 M), the sugar yield remains unchanged within this range of oxygen concentration (Fig. 6). The results show that oxygen acts as a weak inhibitor of the formose reaction. Therefore, the progress of the reaction under a nitrogen atmosphere seems to be favorable for obtaining saccharides in a high yield.

The ORP measurements of the formose reaction were carried out under various conditions. Whenever the

Table 1. Changes of UV absorption in the formose reaction

Sample number[a]	Supernatant			Neutralized			Acidified		
	pH	λ_{max}(nm)	Abs.	pH	λ_{max}(nm)	Abs.	pH	λ_{max}(nm)	Abs.
No. 1	11.2	272	—	4.2	272	0.33	1.0	272	0.31
No. 2	11.2	272	—	6.0	272	0.24	1.2	272	0.20
No. 3	11.2	288	0.35	5.2	288	0.43	1.0	288	0.38
No. 4	11.1	290	1.90	6.1	290	1.99	1.0	290	1.73
No. 5	11.0	292	5.96	6.2	287	4.48	1.0	282	3.03

a) Shown in Fig. 2.

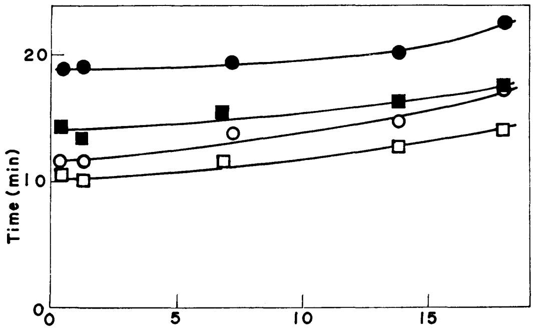

Fig. 5. Effect of oxygen on T_{min} and T_{max}.
T_{min} (-○-) and T_{max} (-●-); [HCHO]=2.0 M and $[Ca(OH)_2]=0.17$ M. T_{min} (-□-) and T_{max} (-■-); [HCHO]=2.0 M and $[Ca(OH)_2]=0.2$ M. HCHO; Prepared from paraformaldehyde.

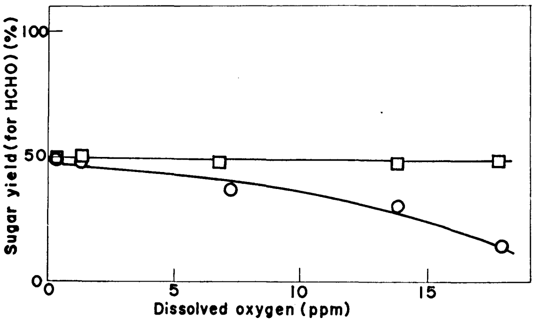

Fig. 6. Effect of oxygen on sugar yield.
Reaction conditions; Same as Fig. 5. $[Ca(OH)_2]$: ○, 0.17 M; □, 0.20 M.

conditions under which the formose reaction took place smoothly to give saccharides were employed, the minimum (2nd step) and maximum (3rd step) points necessarily appeared. On the contrary, under such conditions where the formose reaction would not take place, when, for example, the reaction was initiated under the lower limit of the critical of the $Ca(OH)_2$ concentration, the ORP did not show significant change but, rather, rose slowly. In these cases, the pH of the mixture was gradually lowered because of the organic acids resulting from the Cannizzaro reaction.

With regard to the saccharide formation, trioses may be produced at the early stage of the second step, followed by the additional formation of tetroses at the minimum ORP point, and then higher saccharides are accumulated up to the maximum ORP point, as may be seen from the paperchromatographic analysis of aliquots.[13]

The UV spectra data with samples withdrawn intermittently during the course of the reaction are shown in Table 1. Some absorption maximum at 290 nm is first observed at T_{min}, and the amount of optical absorption continues to increase until T_{max}. Immediately after the yellowing point (T_{max}), λ_{max} is shifted beyond the wavelength of 300 nm. The substance with the λ_{max} value of 290 nm may be considered to be an intermediate produced during the induction period and may play some role in the subsequent step of sugar formation. However, the substance has not yet been successfully isolated, and its nature remains to be elucidated. In view of the above facts, emphasis may be placed on the utility of ORP measurements in the formose reaction. Although there may not be completely satisfactory arguments for the observed ORP changes, it should be noted that some important features of the formose reaction, such as the initiation and the end of the reaction, can be characterized by means of the ORP curve; this is worthy of future investigation. For example, the visual method (yellowing point), which has been employed for obtaining the highest yield of saccharides, might better be placed by ORP measurements because of some disadvantages with the operation and accuracy of the former method.

In conclusion, the ORP-determination method might make some contributions to future studies of the formose reaction in searching for the optimum reaction conditions and for active catalysts; moreover, it might well be used in technological development.

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