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Kinetics of dextrose degradation under autoclaving conditions

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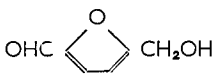
The kinetics of the decomposition of dextrose have been investigated over a temperature range of 106° to 127°. The reaction is shown to exhibit an induction period with respect to 5-hydroxymethylfurfural production which is due to the formation of an intermediate compound. A reaction mechanism is proposed which appears consistent with the experimental measurements. Rate constants are calculated for the various reaction steps and the activation energies associated with these steps are reported.

Dextrose decomposes under autoclaving conditions, one of the main decomposition products being 5-hydroxymethylfurfural (5-HMF).



The kinetics of this decomposition have been extensively studied usually by measuring the rate of dextrose depletion or the rate of 5-HMF production. Most investigators have equated these rates. The rate of the decomposition reaction is relatively small and previous workers have accelerated the decomposition by working in solutions of fairly high acid concentration. These measurements have, in general, shown that the reaction is first order in dextrose concentration and that it is catalysed by hydrogen ion. Kinetic treatment of the decomposition has been made on a reaction mechanism represented by

$$\text{Dextrose} \longrightarrow \text{5-HMF} \longrightarrow \text{Products}$$

The products of the second step in the reaction have been assumed to be formic and laevulinic acids.

It has been shown (Wolfram, Schuetz & Cavalieri, 1948) on the basis of spectroscopic evidence that there is an intermediate compound produced during the formation of 5-HMF from dextrose. These observations would appear to indicate that, under conditions of autoclaving, fairly neutral solutions of dextrose decompose according to a mechanism

$$\text{Dextrose} \longrightarrow \text{Intermediate} \longrightarrow \text{5-HMF} \longrightarrow \text{Products}$$

The purpose of the present investigation was to study the effect of changes in dextrose concentration, temperature and pH on the kinetics of dextrose decomposition actually encountered during autoclaving and to determine if the mechanism is adequately represented by either of the above schemes. The interest in such a reaction lies in the concentration of the various decomposition products arising as a function of time rather than the concentration of dextrose decomposed.

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METHODS

The dextrose degradation reaction was followed by sealing 10 cm^3 samples of 0·278 M dextrose solution, made up with deionized water, into glass ampoules. These were heated in an air oven, the temperature of which was controlled to $\pm 0{\cdot}1°$. At suitable intervals of time one ampoule was removed, cooled quickly, and diluted to produce absorbance readings of between 0·2 and 1·5 at wavelengths 284 and 228 nm. At these wavelengths unheated dextrose solutions showed negligible absorption. Absorption measurements made were on Unicam SP800 and Hilger Uvispek spectrophotometers using 10 mm silica cells.

The decomposition of 5-HMF was followed in a similar fashion by sealing samples of 6×10^{-4} M 5-HMF solution which had previously been adjusted to pH 4 by the addition of HCl, into ampoules and analysing as above. The absorption at $\lambda = 284$ nm is assumed to be due exclusively to 5-HMF. The molar absorptivity of 5-HMF was determined by preparing several solutions of 5-HMF over a concentration range of 1×10^{-5} to 1×10^{-4} M, and measuring their absorbance. These solutions were found to obey Beer's Law and a molar absorptivity of $1{\cdot}66 \times 10^4$ was calculated. This value was used to calculate molar concentrations of 5-HMF from absorbance measurements. It is in good agreement with literature values of 1·68; 1·67 and $1{\cdot}69 \times 10^4$ (Turner, 1954). The absorption at $\lambda = 228$ nm is postulated as being due to both 5-HMF and an intermediate decomposition product. The method of calculating the absorbance due to the intermediate product is described below.

The reproducibility of the 5-HMF assay was determined by heating ten ampoules of dextrose solution for a fixed time at a temperature of 117° and measuring the absorbances of all the samples at the two wavelengths. This procedure produced the following results: $A_{284} = 0{\cdot}911$ with a coefficient of variation of 8%, $A_{228} = 0{\cdot}273$ with a coefficient of variation of 7·5%. pH measurements were made using a Pye 78 pH meter with a combined glass electrode. This was standardized using commercial buffer sachets of pH 4 and pH 7 obtained from E.I.L.

The dextrose was obtained from Brown and Polson Ltd. and the 5-HMF from Sigma Chemicals.

RESULTS AND DISCUSSION

The variation of 5-HMF concentration with time in solutions originally 0·278 M in dextrose is shown (Fig. 1) as a function of time at several temperatures. All of the curves show an increase in the rate of change of 5-HMF concentration with time at the beginning of the reaction. The data at higher temperatures also show a much smaller decrease in this rate at longer reaction times. Such behaviour indicates that, under our experimental conditions, the rate of formation of 5-HMF cannot be described by a straightforward reaction first order in dextrose concentration as has previously been treated kinetically (Heimlich & Martin, 1960) and that the concentration of the intermediate product must be considered.

Fig. 2 shows representative absorption spectra of dextrose, degraded dextrose and 5-HMF. It was established by experiment using 5-HMF that the ratio A_{228}/A_{284} was 0·170 while degraded dextrose samples yielded values of this ratio from 5·0 to 0·4 depending on the extent of the degradation reaction. This indicated that the 228 nm peak is not due entirely to 5-HMF. It has already been suggested that this peak is partially due to an intermediate, structures for which have been postulated (Wolfram

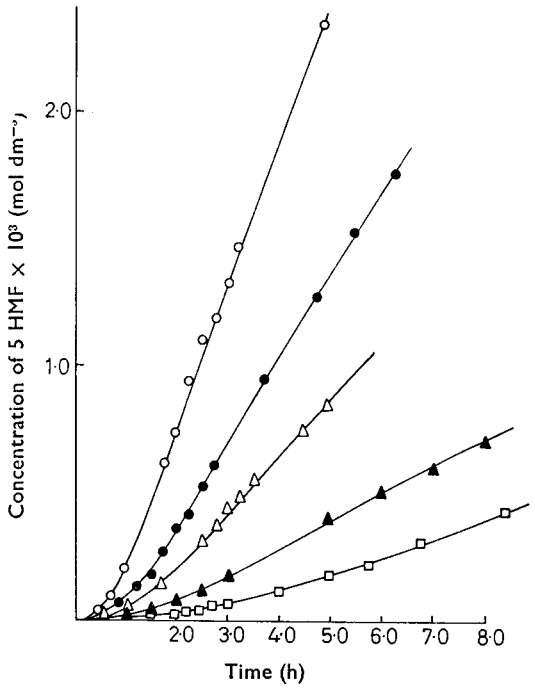

FIG. 1. Plots showing the variation of 5-HMF concentration as a function of time at various temperatures. ○–127°; ●–121·5°; Δ–117°; ▲–111·5°; □–106°.

& others, 1948). Using the A_{228}/A_{284} ratio for 5-HMF it is possible to calculate the absorbance at $\lambda = 228$ nm due to the intermediate compound, P_1, by the following equation

$$A_{P_1} = A_{228} - 0{\cdot}17\, A_{284}$$

where A_{P_1} is the absorbance due to P_1 at $\lambda = 228$ nm. The absorbance of P_1 was calculated as a function of time at the temperatures used. The results are represented

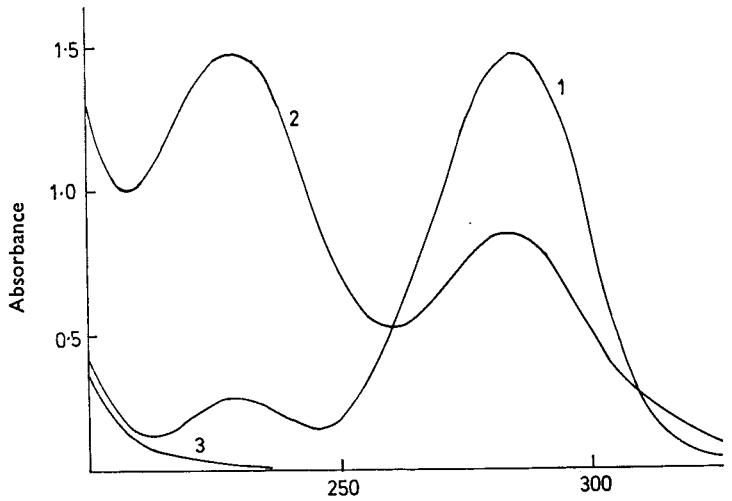

FIG. 2. Plots showing representative ultraviolet spectra of (1) 5-HMF, (2) degraded dextrose, (3) unheated dextrose.

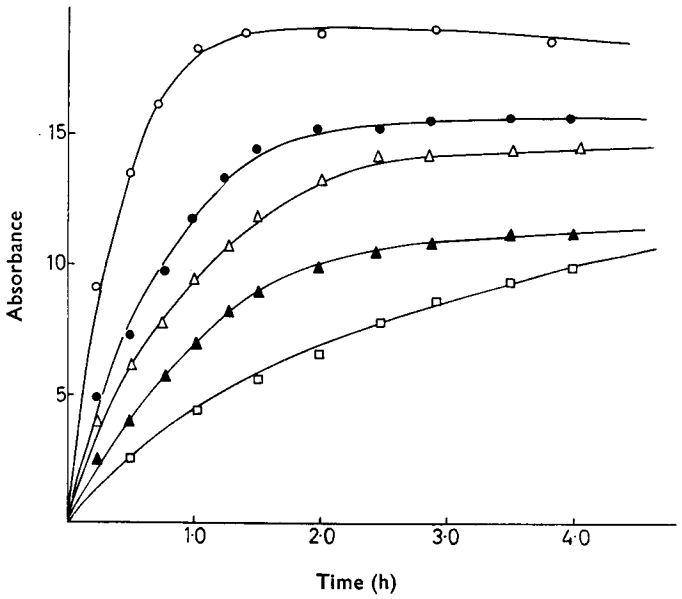

FIG. 3. Plots showing the absorbance of intermediate compound P_1 as a function of time at various temperatures. ○–127°; ●–121·5°; △–117°; ▲–111·5°; □–106°.

in Fig. 3. It is apparent from Fig. 3 that at each temperature there is a limiting value for the concentration of P_1. At 127° this steady state is reached in a little over 1 h while at 106° over 6 h are required. It is also significant in considering the data shown in Figs 1 and 3 that, while the absorbance of 5-HMF after say 3 h varies widely with temperature—from 1·2 at 106° to 20 at 127°, the absorbance of P_1 over the same range in temperature changes from only 0·9 to 1·9. These results indicated that the rate of change of 5-HMF concentration with time at a given temperature is dependent upon the concentration of the intermediate as well as on any subsequent degradation of 5-HMF. This led to the kinetics of formation of P_1 and the decomposition of 5-HMF being examined separately.

Formation of intermediate compound P_1

The dependence of the rate of formation of P_1 on dextrose concentration could not be determined by simultaneous measurement of the rate of change of P_1 with time from Fig. 3 and estimation of the dextrose concentration remaining because of the very small decrease in dextrose concentration. The magnitude of this decrease is estimated from the concentration of 5-HMF formed which, under our experimental conditions, never exceeded 2×10^{-3} M at the highest temperature.

To establish that the rate of formation of P_1 with time depended on the dextrose concentration, ten samples of dextrose solutions were prepared ranging in concentration from 0·0555 to 0·555 M. These were heated in sealed ampoules in the usual way at constant temperature for 1 h after which time the absorbances at 228 and 284 nm were measured. The arbitrary 1 h period was chosen to be long enough to allow measurable concentrations of P_1 to be produced while being short enough to make the correction for 5-HMF formation small compared to the effect of the ten-fold change in dextrose

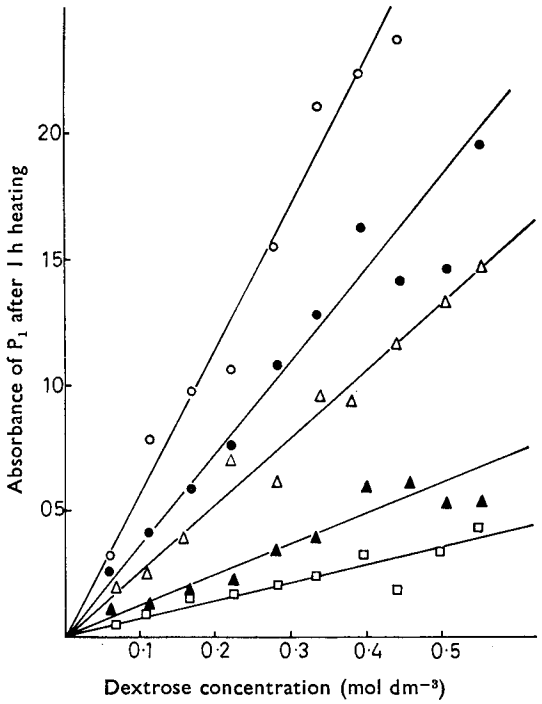

FIG. 4, Plots showing the absorbance of P_1 after 1 h heating at various temperatures as a function of initial dextrose concentration, Key as Fig. 3.

concentration on the initial reaction rate. This correction was a maximum of about 20%. The absorbance of P_1 after 1 h is taken as an estimate of the initial rate of P_1 formation. Fig. 4 shows plots of dA_{P_1}/dt obtained in this way as a function of dextrose concentration. These results show a reasonable first order dependence. While rate constants could be determined from these results, they have not been quoted here because of the averaging of the rate over a 1 h period which produces larger errors at the higher than at the lower temperatures. These rate constants are obtained by an alternative method below.

pH changes during dextrose degradation

Dextrose solutions during autoclaving have already been shown to undergo a decrease in pH (Wing, 1960). It has hitherto been assumed that this decrease is due to organic acids formed during the decomposition of 5-HMF. We found that a small decrease in pH occurred during the early stages of the reaction when only a small concentration of 5-HMF and subsequent degradation products would be present. The pH drop was measured in the following way.

The pH of 0·278 M dextrose solution was determined before it was sealed in ampoules and heated for 1 h at the different temperatures. The pH of the solution before heating was 5·25 and after heating ranged from 3·80 to 106° to 3·61 at 127° (Table 1). These results would appear to indicate that hydrogen ion is produced during the first stages of the reaction as well as the intermediate P_1 and that the reaction scheme might be represented

$$\text{Dextrose} \longrightarrow P_1 + H^+$$

Decomposition of 5-HMF

The decomposition of 5-HMF at the temperatures used for the dextrose degradation was followed by the method described above. The results were found to conform to a rate equation first order in 5-HMF at all of the five temperatures. First order rate constants were calculated by the method of least squares together with their standard deviations. The results are shown in Table 1 as values of k_3. The Arrhenius activation energy for this reaction was calculated by the method of least squares to be 108 k J mol^{-1} with a standard deviation of 7 k J mol^{-1}.

Table 1. *Summary of principal kinetic results at various temperatures.*

Temperature	$k_1 \times 10^4$ h^{-1}	$k_c \times 10^{-3}$ dm^3 mol^{-1} h^{-1}	Standard deviation for $k_c \times 10^{-3}$	$k_3 \times 10^4$ h^{-1}	Standard deviation for $k_3 \times 10^3$	$\epsilon_1 \times 10^{-3}$ for P_1	pH of dextrose solutions after 1 h heating
106·0	2·77	4·07	0·17	3·22	0·30	7·2	3·80
111·5	3·59	5·16	0·10	5·60	0·62	9·3	3·75
117·0	7·91	6·45	0·39	9·85	0·32	9·1	3·65
121·5	12·60	9·34	0·21	13·9	0·66	7·4	3·65
127·0	22·3	—	—	18·5	1·9	6·6	3·61

Kinetics of the dextrose decomposition

From the foregoing data it appears that an intermediate step in the production of 5-HMF from dextrose under autoclaving conditions produces an intermediate compound P_1 and hydrogen ion. That P_1 is involved in a subsequent reaction is indicated by the limiting value of P_1 concentration obtained at any one temperature. It is known that the dextrose decomposition is sensitive to pH and in the simplified reaction scheme suggested below it is assumed that the hydrogen ion produced has a catalytic effect on the formation of 5-HMF.

$$D \xrightarrow{k_1} P_1 + H^+ \qquad P_1 \underset{k_2}{\overset{[H^+]\,k_c}{\longrightarrow}} \text{5-HMF} \qquad \text{5-HMF} \xrightarrow{k_3} P_2$$

where k_1, k_2, k_3 are the rate constants for the reaction steps indicated and k_c is the catalytic constant for the second reaction. D represents dextrose and P_2 the unspecified decomposition products of 5-HMF. The above reaction scheme is represented by the rate equations:

$$-\frac{d[D]}{dt} = k_1[D] \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$\frac{d[P_1]}{dt} = k_1[D] - \{k_2 + k_c[H^+]\}[P_1] \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$\frac{d[5\text{-HMF}]}{dt} = \{k_2 + k_c[H^+]\}[P_1] - k_3[5\text{-HMF}] \quad .. \quad .. \quad (3)$$

$$\frac{d[P_2]}{dt} = k_3[5\text{-HMF}] \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

To show that the above reaction scheme is consistent with the results obtained and to obtain estimates of the individual reaction rate constants, the formation of 5-HMF may be divided into two stages.

(*a*) the stationary state with respect to P_1 concentration.

(*b*) the non stationary state when the concentration of P_1 is increasing with time.

(*a*) At the stationary state

$$\frac{d[P_1]}{dt} = 0 \text{ and from equation (2)}$$

$$\{k_2 + k_c [H^+]\} [P_1] = k_1 [D]$$

Substituting k_1 [D] for $\{k_2 + k_c [H^+]\} [P_1]$ in equation (3), the equation describing the nett rate of appearance of 5-HMF is

$$\frac{d [5\text{-HMF}]}{dt} = k_1 [D] - k_3 [5\text{-HMF}] \quad .. \quad .. \quad .. \quad (5)$$

This indicates a first order dependence of 5-HMF formation on dextrose concentration which has previously been reported by other workers (Heimlich & Martin, 1960; Saprano & Khavin, 1967). Under the present experimental conditions the concentration of dextrose is essentially constant during all the decompositions, the greatest decrease in concentration obtained being 0·002 M. Equation (5) may be written as

$$\frac{d [5\text{-HMF}]}{dt} = 0{\cdot}278\, k_1 - k_3 [5\text{-HMF}] \quad .. \quad .. \quad .. \quad (6)$$

This equation was used to determine k_1 at the various temperatures of measurement by determining the slopes of the curves in Fig. 1 at their maximum value and calculating the appropriate k_3 [5-HMF] term. The reaction was not continued to large enough values of 5-HMF concentration to be able to determine a proportionality between the rate of formation of 5-HMF and its concentration although this has already been well demonstrated (Heimlich & Martin, 1960). The values of k_1 obtained by the above procedure are shown in Table 1. These values at the different temperatures were used in the Arrhenius equation and the activation energy of the process at the stationary state was determined by the least squares method. From this a value for the activation energy of 132 k J mol^{-1} (standard deviation 11 k J mol^{-1}) was obtained—a value in good agreement with that obtained by Heimlich & Martin (1960) for the postulated first order decomposition reaction of dextrose.

As can be seen from equation (2), at the beginning of the reaction the rate of production of P_1 is equal to the rate of degradation of dextrose. Thus by measuring the initial slopes of the plots in Fig. 3 values of d A_{P_1}/dt were obtained. By substituting these values and the k_1 values obtained above in the equation

$$\frac{1}{\epsilon_1} \frac{dA_{P_1}}{dt} = k_1 [D] \text{ at } t = 0$$

estimates were made of the molar absorptivity ϵ_1 of the compound P_1 at each temperature. These are shown in Table 1. The fact that these values are similar is confirmation that degradation during the steady state does follow the reaction scheme

given above and that the initial rate of formation of P_1 does have an activation energy of 132 k J mol^{-1}.

(*b*) In the non stationary state of the reaction equation (3) may be written

$$\frac{d\,[5\text{-HMF}]}{dt} = k_2\,[P_1] + k_c\,[H^+]\,[P_1] - k_3\,[5\text{-HMF}] \quad .. \quad .. \quad (7)$$

where $k_2\,[P_1]$ is the uncatalysed rate of formation of 5-HMF and $k_c[H^+][P_1]$ the catalysed rate. If it is assumed that

$$k_c\,[H^+][P_1] \gg k_2\,[P_1]$$

$$\text{then } \frac{d\,[5\text{-HMF}]}{dt} + k_3\,[5\text{-HMF}] = k_c\,[H^+][P_1] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

Under our experimental conditions when no acid is added to the system on the evidence that hydrogen ion is produced during the initial stages of the reaction we may write that

$$[H^+] = [P_1] \text{ at any time and that}$$

$$\frac{d\,[5\text{-HMF}]}{dt} + k_3\,[5\ HMF] = k_c\,[P_1]^2 \quad .. \quad .. \quad .. \quad (9)$$

The data of Figs 1 and 3 were used to verify the form of this equation mainly with respect to the dependence of the rate of 5-HMF concentration increase on $[P_1]^2$ as follows. Gradients of the lines in Fig. 1 were determined as a function of time at the various temperatures and the term k_3 [5-HMF] added. The values of P_1 concentration at corresponding times were determined from Fig. 3 using an average value for ϵ_1 of $7{\cdot}9 \times 10^3$. The left hand side of equation (9) was plotted against $[P_1]^2$ as shown in

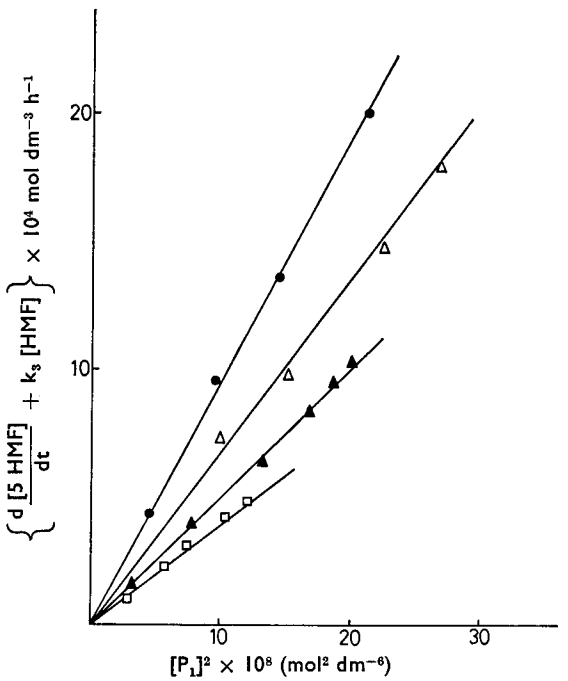

FIG. 5, Plots showing the dependence of the rate of formation of 5-HMF on the square of the concentration of intermediate P_1 during the non-stationary state of 5 HMF production. Key as Fig. 3.

Fig. 5. The gradients of these calculated by the method of least squares are shown as values of k_c in Table 1. For the highest temperature low values of k_c were obtained and it is thought that the very rapid approach of P_1 concentration to its stationary state rendered the $[P_1]$ term inaccurate. The temperature dependence of the k_c rate constant yielded an activation energy of 65 k J mol^{-1} (standard deviation 8 k J mol^{-1}) considerably lower than that of k_1.

CONCLUSIONS

From the results described above the concentrations of the various decomposition products produced during dextrose autoclaving may be calculated for any conditions of time and temperature. For conditions of 30 min at 117° as an example the concentrations of P_1 and 5-HMF are 1×10^{-4} and 2×10^{-5} M respectively. At longer times of heating the concentration of 5-HMF will rapidly exceed that of P_1. Under all our experimental conditions the concentration of 5-HMF breakdown products is very small compared to 5-HMF concentration. While attempts have been made to isolate and identify the intermediate compound P_1 by several chromatographic techniques no such separation methods have been effective and evidence for the existence of such an intermediate rests on the time dependent appearance of the absorption maximum at $\lambda = 228$ nm.

It is apparent from the present results that the decrease in pH, known to occur during autoclaving accompanies the first step in the dextrose degradation scheme.

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