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Kinetics of the Sulphite-Inhibited Maillard Reaction: The Effect of Sulphite Ion

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ABSTRACT

The kinetics of the irreversible and reversible binding of sulphur(IV) oxospecies (S(IV)) in the system: glucose–glycine–S(IV) are reported at pH 5·5 and 55°C. The formation of 3-deoxyhexosulose (DH), a reaction intermediate, is of first order with respect to glucose and glycine, but is also catalysed by S(IV) according to:

$$\frac{d[DH]}{dt} = k(1 + 35{\cdot}0[S(IV)]_{free})$$

DH is subsequently converted to 3,4-dideoxy-4-sulphohexosulose (DSH) in a process which is independent of [S(IV)]. In a system containing [glucose] = 1·0M, [glycine] = 0·5M and [S(IV)] = 0·01–0·10M, reversible binding of S(IV) is a result of the formation of hydroxysulphonates of glucose and DSH. No evidence could be found for the formation of the hydroxysulphonate of DH which had been previously demonstrated to play a role in the reaction between DH and S(IV) when studied in isolation. The effect of pH on the rate of loss of total S(IV) is also reported.

Suggestions as to the mechanism of the involvement of S(IV) in the formation of DH are made and reasons for the non-formation of the hydroxysulphonate of DH are considered.

INTRODUCTION

The success of sulphur(IV) oxospecies (S(IV)) in inhibiting the Maillard browning of glucose and glycine lies partly in the ability of sulphite ion to

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react irreversibly with 3-deoxyhexosulose (DH) to give 3,4-dideoxy-4-sulphohexosulose (DSH). It is likely that this reaction proceeds by way of 3,4-dideoxyhexosulos-3-ene (DDH). A kinetic model which describes the loss of recoverable S(IV) in the system glucose–glycine–S(IV), when the concentration of glucose and glycine far exceeds that of S(IV), is shown in Fig. 1 (Wedzicha, 1984*a*) and is based on the experimental data of McWeeny *et al.* (1969). The reaction consists of an induction phase followed by a

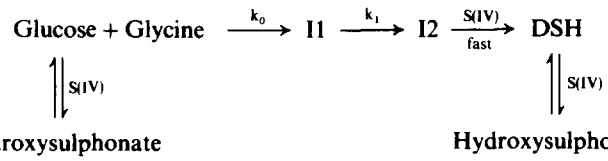


Fig. 1. Kinetic model for the S(IV)-inhibited Maillard reaction of glucose + glycine (Wedzicha, 1984*a*).

constant rate of loss of S(IV) over the major part of the reaction. This constant rate is equal to the rate of the first rate determining step; that is, formation of intermediate I1. Reversible binding of S(IV) is the formation of hydroxysulphonates of glucose and DSH. Although DSH is a dicarbonyl compound, it is likely that it forms only a monohydroxysulphonate (Wedzicha & Smith, 1987).

The kinetic model led to a prediction of the dissociation constant of the hydroxysulphonate of DSH as 0·005M which was later confirmed experimentally as 0·0044M (Wedzicha *et al.*, 1985). Kinetic studies of the reaction between DH and S(IV) showed that its rate is very similar to that of the step in which intermediate I1 is converted to DSH (Wedzicha & Kaban, 1986). It is likely that I1 is DH or some rate determining species derived from it. The study of the reaction between DH and S(IV) suggested some additional features which had not been incorporated into the kinetic model of the S(IV)-inhibited Maillard reaction. The most important of these is that DH appeared to form a relatively stable monohydroxysulphonate with a dissociation constant of 0·004M. This was identified from the observation that the initial reaction of DH with S(IV) was inhibited by S(IV); no such effect could be identified in the available data on the glucose–glycine–S(IV) reaction. Another important observation was that glycine appeared to catalyse the reaction between DH and S(IV). It is possible, therefore, that a more complex model applies to the glucose–glycine–S(IV) reaction, perhaps as suggested in Fig. 2.

The purpose of this investigation is to critically evaluate the role of the hydroxysulphonate of DH in the S(IV)-inhibited Maillard reaction by

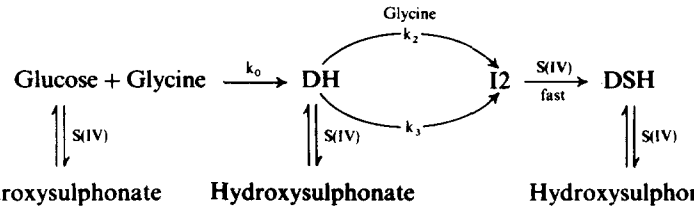


Fig. 2. Kinetic model for the S(IV)-inhibited Maillard reaction of glucose + glycine after additions based on the results of Wedzicha & Kaban (1986).

considering the effect of initial S(IV) concentration on the kinetics of its loss in the system glucose–glycine–S(IV).

EXPERIMENTAL

All reagents were of AnalaR grade and were obtained from BDH Chemicals Ltd. Reaction mixtures contained [glucose] = 0·50–2·0M [glycine] = 0·25–1·0M and [S(IV)] = 0·01–0·1M. For all experiments at constant pH, the pH was adjusted to pH 5·5–5·6 by adding NaOH (1M) before making up the mixtures to their final volumes. In experiments to determine the effect of pH on the kinetics of the reaction, the pH of mixtures was adjusted within the range pH 4·5–6·5 by the addition of HCl (1M) or NaOH (1M), as appropriate. All reaction mixtures were contained in stoppered reagent bottles in a water bath at 55·0 ± 0·1°C.

For measurement of free and reversibly bound S(IV) an aliquot of reaction mixture (2–4 ml) was placed in an Erlenmeyer flask containing distilled water (50 ml). Sulphuric acid (2 ml, 2·5M) and starch solution (1 ml, 1% w/v) were added quickly and the mixture was rapidly titrated with iodine (0·005M) to give the amount of free S(IV) present in the sample. Sodium hydroxide (40 ml, 1M) was added and the mixture left for 10 min to release the reversibly bound S(IV). The solution was re-acidified with sulphuric acid (15 ml, 2·5M), starch solution added (1 ml, 1% w/v) and the mixture rapidly titrated with iodine (0·005M) to give the amount of reversibly bound S(IV).

RESULTS

Preliminary analysis of data

In all kinetic experiments, the concentration–time behaviour of total S(IV) (free + reversibly bound) showed the expected characteristic induction period followed by a constant rate period. Measurement of this constant

rate for experiments in which the concentration of glucose and glycine was varied, keeping all other conditions constant, showed the reaction to be of first order with respect to each of the reactants. According to the original kinetic model (Wedzicha, 1984*a*) and the suggested modifications shown in Fig. 2, the constant rate refers to the rate of the step leading to the formation of DH. This reaction takes place by way of the condensation of glucose and glycine followed by an Amadori rearrangement to monofructoseglycine (MFG). The MFG may then react with a second molecule of glucose and undergo an Amadori rearrangement to give difructoseglycine (DFG). DFG is more reactive towards browning than MFG. It is not unreasonable to expect the formation of MFG to be of first order with respect to glucose and glycine since the original condensation of glucose and glycine involves one molecule of each reactant in the transition step. If DFG is an intermediate in the formation of DH in the system studied here then it is formed in a fast process following the rate determining step and its presence is not kinetically significant. For the purpose of this investigation the reaction intermediate in the formation of DH will be considered to arise from a 1:1 combination of glucose and glycine and could be MFG, a precursor of MFG or some specialised species derived from MFG.

When the initial concentration of S(IV) was varied keeping the concentrations of all other reactants constant ([glucose] = 1·0M, [glycine] = 0·5M), the constant rate was found to vary with concentration as shown in Fig. 3 where the intercept is $1{\cdot}4 \times 10^{-5}$M h^{-1} and the slope, $3{\cdot}51 \times 10^{-4}$ h^{-1}. Using this result and the first order behaviour with respect to glucose and glycine, the rate equation for the formation of DH is:

$$\text{rate/M h}^{-1} = 2{\cdot}8 \times 10^{-5}(1 + 25{\cdot}1[\text{S(IV)}])\ [\text{glucose}]\ [\text{glycine}] \qquad (1)$$

at pH 5·5 and 55°C. This result is surprising as it indicates that the addition of S(IV) actually enhances the rate of its loss. According to the model shown in Fig. 2, an increase in [S(IV)] should cause more DH to be converted to the hydroxysulphonate and hence the rate of irreversible binding of S(IV) should be reduced in the first instance. In previously reported work of McWeeny *et al.* (1969), the concentrations of reactants were [glucose] = 1·0M, [glycine] = 0·5M and [S(IV)] = 0·039M. Substitution of these values into the rate equation gives rate = $2{\cdot}8 \times 10^{-5}$M h^{-1}, which is in reasonable agreement with a value of $3{\cdot}2 \times 10^{-5}$M h^{-1} calculated from the data of McWeeny *et al.* (Wedzicha, 1984*a*) which consisted of only a single kinetic run.

It is difficult to see how the total S(IV) can be the kinetically significant species when a proportion of it is reversibly bound. Also, the reaction is clearly not of zero order with respect to S(IV), unlike what had been suggested previously on the grounds of the linearity of concentration–time

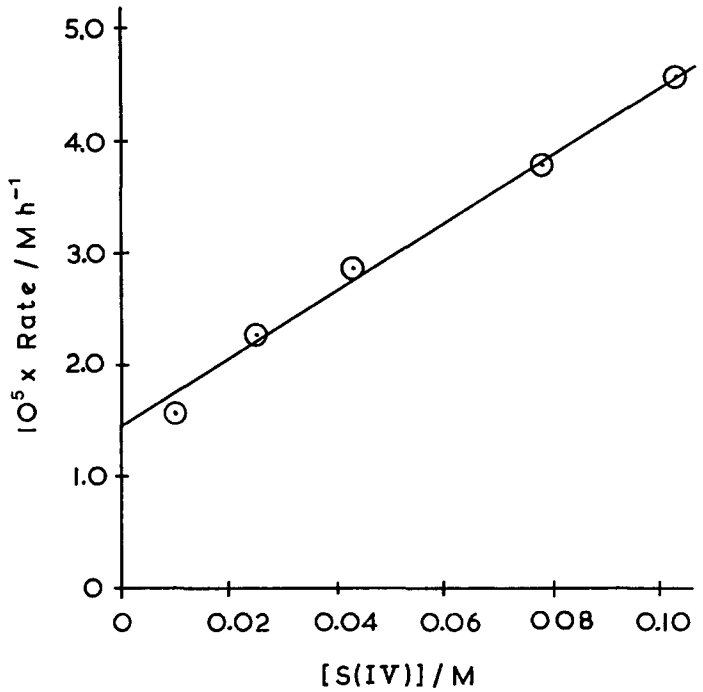


Fig. 3. Effect of total [S(IV)] on the constant rate of loss of total [S(IV)]. Reaction conditions: [glucose] = 1·0M; [glycine] = 0·5M; [S(IV)] = 0·01–0·10M; pH 5·5; 55°C.

plots for total S(IV) over the major part of the reaction. A more detailed analysis of the reaction is therefore required.

The first consideration is whether the kinetically significant species is free or reversibly bound S(IV). Reynolds (1959) considered the effect of S(IV) on the yield of MFG in mixtures of glucose, glycine and S(IV) at high concentration. When 1 mol glycine was heated for 1 h at 100°C with 16 mol glucose and 0·25 mol S(IV) at 10% water content, the yield of MFG was 58%. An increase in the amount of S(IV) to 8 mol but keeping the amounts of other reactants constant gave a corresponding yield of 25%. At such high concentrations it is expected that glucose hydroxysulphonate will be formed in high yield and the reduction in yield of MFG is therefore likely to be due to removal of glucose in this way. The approximate halving of the yield of MFG when approximately half of the glucose is converted to the hydroxysulphonate is, of course, consistent with first order behaviour with respect to glucose. Since DH is derived from MFG any step which limits the formation of MFG will limit the formation of DH. The unreactive nature of the reversibly bound S(IV) implies that the kinetically significant form of S(IV) is, therefore, free S(IV).

The initial amounts of free and reversibly bound S(IV) arise from the

formation of glucose hydroxysulphonate. If the initial amounts of glucose and S(IV) are a and s, respectively, and the amount of hydroxysulphonate present at equilibrium is x then, applying the law of mass action and the condition that the concentration of glucose is much larger than that of S(IV); that is, $a - x \approx a$:

$$x = as/(a + K_1) \tag{2}$$

where K_1 is the dissociation constant of the adduct. Using $K_1 = 1{\cdot}33$M (Wedzicha, 1984*a*) then, for an initial glucose concentration of 1·0M, $x =$ 0·43 s; that is, the concentration of free S(IV) is a constant fraction of the total S(IV) concentration. Hence a plot of constant rate as a function of free [S(IV)] should also be linear. From measurements of free [S(IV)] during kinetic experiments in which the initial [S(IV)] was varied in the range 0·01–0·1M, 55·2 ± 2·4% of the total [S(IV)] was actually found to be free at the first measurement and the rate equation for the constant rate, in terms of free [S(IV)] is, therefore:

$$\text{rate/M h}^{-1} = 2{\cdot}8 \times 10^{-5}(1 + 45{\cdot}5[\text{S(IV)}]_f)\ [\text{glucose}]\ [\text{glycine}] \tag{3}$$

where the subscript f denotes the free form.

So far only the initial concentration of free S(IV) has been used in the rate equation. In any run, the constant rate is established quickly after the induction period is over and the initial part of this constant rate period is indeed taking place under conditions similar to those at zero time. The use of initial concentrations is, therefore, reasonable. However, the constant rate depends on $[\text{S(IV)}]_f$, and this concentration changes during each kinetic run. It is therefore necessary to model the reaction beyond the initial phase.

Kinetic model

The best available model for the glucose–glycine–S(IV) reaction is that shown in Fig. 2 but incorporating the new information found from the present study. Thus, the rate of formation of DH, which is equal to the rate of loss of glucose, is given by:

$$\frac{\text{d[DH]}}{\text{d}t} = \frac{-\text{d[glucose]}}{\text{d}t} = k_1(1 + k_2[\text{S(IV)}]_f)[\text{glucose}]_f[\text{glycine}] \tag{4}$$

The rate of loss of DH is assumed to be equal to the rate of loss of S(IV) and the rate of formation of DSH and is given (Wedzicha & Kaban, 1986) as follows:

$$\frac{-\text{d[DH]}}{\text{d}t} = \frac{-\text{d[S(IV)]}}{\text{d}t} = \frac{\text{d[DSH]}}{\text{d}t} = k_3(1 + k_4[\text{glycine}])[\text{DH}]_f \tag{5}$$

Calculation of the concentrations of free glucose, DH and S(IV) can be carried out using the approach of Wedzicha & Chishya (1983). If, at any instant, the total concentrations (free + reversibly bound) of glucose, DH and DSH are, respectively, a, b and c and at equilibrium the amounts of the respective hydroxysulphonates are x, y and z, for a total S(IV) concentration, s, then application of the law of mass action to each equilibrium gives the following equations:

for the dissociation of glucose–hydroxysulphonate

$$x^2 - x(K_1 + a + s - y - z) + a(s - y - z) = 0 \tag{6}$$

for the dissociation of DH–hydroxysulphonate

$$y^2 - y(K_2 + b + s - x - z) + b(s - x - z) = 0 \tag{7}$$

for the dissociation of DSH–hydroxysulphonate

$$z^2 - z(K_3 + c + s - x - y) + c(s - x - y) = 0 \tag{8}$$

where K_1, K_2 and K_3 are the respective dissociation constants. By initially setting x, y and z to zero and successively evaluating these quantities from eqns (6), (7) and (8), iteration leads to gradually improving values which converge to the equilibrium concentrations.

The time-dependent concentrations of total glucose, DH, DSH, and S(IV) were calculated by numerical integration of eqns (4) and (5) using finite increments. The size of each increment was taken as 1 h; there was no change in the results when smaller increments were used and the derived concentrations were expressed to three significant figures. At each increment the equilibrium concentrations of the hydroxysulphonate species were calculated from eqns (6), (7) and (8), the approximation being refined until successive values differed by no more than 0·1%. This was found to be sufficient when the final results for total and free S(IV) concentrations were expressed to three significant figures.

The initial values of rate and equilibrium constants used were those reported above ($k_1 = 2{\cdot}8 \times 10^{-5}\text{M}^{-1}\ \text{h}^{-1}$, $k_2 = 45{\cdot}5\text{M}^{-1}$, $K_1 = 1{\cdot}33\text{M}$, $K_2 = 4 \times 10^{-3}\text{M}$, $K_3 = 4{\cdot}4 \times 10^{-3}\text{M}$) and calculated from the literature (Wedzicha & Kaban, 1986) as $k_3 = 5{\cdot}38 \times 10^{-3}\ \text{h}^{-1}$ and $k_4 = 4{\cdot}1\text{M}^{-1}$. However, the only conditions under which a satisfactory fit of predicted results to experimental data could be obtained was when K_2 was large; that is, the hydroxysulphonate of DH is highly dissociated. Setting the value of K_2 to 5·0 (the largest value which could be used within the precision of the computation), and adjusting the remaining constants, gave the best fit between experimental and predicted values of total and free S(IV) concentrations when $k_1 = 3{\cdot}2 \times 10^{-5}\ \text{M}^{-1}\ \text{h}^{-1}$, $k_2 = 35{\cdot}0\text{M}^{-1}$, $k_3 = 5{\cdot}38 \times 10^{-3}\ \text{h}^{-1}$, $k_4 = 4{\cdot}1$, $K_1 = 1{\cdot}50\text{M}$ and

TABLE 1

Comparison of Observed and Predicted Total [S(IV)] in Kinetic Experiments. Reaction Conditions: [Glucose] = 1·0M; [Glycine] = 0·5M; [S (IV)] = 0·01–0·10M; pH 5·5; 55°C. The Concentrations are Shown as % of the Initial Total [S(IV)].

Time (*h*)	*Predicted and observed % remaining total S(IV) at initial [S(IV)] (M)*									
	0·010		*0·025*		*0·043*		*0·078*		*0·103*	
	Predicted	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*
100	90·4	84·6	95·1	95·6	96·5	97·8	97·3	98·0	97·6	99·6
200	73·8	69·4	86·6	87·2	90·4	93·7	92·7	93·6	93·4	95·3
300	56·6	54·2	77·8	78·5	84·1	87·0	88·0	88·7	89·2	90·7
400	39·7	39·0	69·4	69·8	78·1	80·2	83·5	83·7	85·1	86·2
500	23·4	23·6	61·3	61·1	72·3	73·5	79·2	78·8	81·2	81·7
600			53·6	52·4	67·0	66·8	75·2	73·9	77·6	77·2
700					61·8	60·1	71·4	69·0	74·2	72·7
800					57·0	53·3	67·8	64·0	71·0	68·2
900					52·3	46·6	64·5	59·1	68·0	63·6
1 000					47·8	39·9	61·3	54·2	65·2	59·1
1 100					43·5	33·2	58·3	49·2	62·5	54·6
1 200					39·3	26·5	55·4	44·3	60·0	50·1
1 300							52·6	39·4	57·6	45·6
1 400									55·3	41·1
1 500									53·1	36·5

$K_3 = 5 \times 10^{-3}$M. The correspondence between observed and predicted values is illustrated in Tables 1 and 2. The most serious deviations are evident when the amount of S(IV) which has been lost is high; that is, in runs at high initial [S(IV)], after a long time. This is not surprising since the kinetic data for the reaction between DH and S(IV) reported by Wedzicha & Kaban (1986) on which the present calculation is partly based applied only to the initial stages of reaction. The later stages are complex and cannot be interpreted in terms of a straightforward mechanism. Overall the quality of the fit shown in Tables 1 and 2 is encouraging, particularly since the values of k_3 (and k_4), K_1 and K_3 correspond well to expected values. The values of k_1 and k_2 are new. The most significant finding is that, for a satisfactory fit, DH must not be involved in hydroxysulphonate formation and the results also confirm the kinetic effect of free S(IV) on the formation of DH. Whilst the order of magnitude of K_1 and the highest value of K_2 tried is the same, the concentration of glucose far exceeds the calculated concentration of DH; thus a significant amount of S(IV) is reversibly bound as glucose hydroxysulphonate whilst the hydroxysulphonate of DH is highly dissociated.

Effect of pH

The effect of pH in the range pH 4·5–6·5 on the constant rate in the glucose–glycine–S(IV) reaction is shown in Table 3. It is clear that the rate is

TABLE 2

Comparison of Observed and Predicted Free [S(IV)] in Kinetic Experiments. Reaction Conditions: [Glucose] = 1·0M; [Glycine] = 0·5M; [S(IV)] = 0·01–0·10M; pH 5·5; 55°C. The Concentrations are Shown as % of the Initial Total [S(IV)].

Time (*h*)	*Predicted and observed % remaining free S(IV) at initial [S(IV)]* (M)									
	0·010		*0·025*		*0·043*		*0·078*		*0·103*	
	Predicted	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*
100	51·4	51·2	55·1	55·9	56·5	55·4	57·7	55·8	58·2	57·6
200	37·5	41·0	46·6	50·9	49·9	52·2	52·5	52·6	53·4	55·3
300	25·1	30·8	38·2	42·5	43·3	48·5	47·1	49·0	48·5	51·5
400	15·2	20·7	30·7	34·4	37·1	44·2	42·1	45·0	43·8	47·0
500	7·7	10·7	24·2	27·5	31·6	39·1	37·4	41·0	39·5	42·4
600			18·8	21·2	26·6	33·0	33·1	36·7	35·4	37·9
700					22·3	26·4	29·2	31·9	31·7	33·3
800					18·5	21·0	25·7	27·5	28·3	29·5
900					15·3	15·9	22·5	23·5	25·2	25·4
1 000					12·6	11·6	19·6	19·5	22·4	21·8
1 100					10·3	7·2	17·0	15·9	19·9	17·8
1 200					8·4	4·3	14·8	12·3	17·6	14·4
1 300							12·8	9·2	15·5	11·0
1 400									13·7	7·6
1 500									12·0	4·5

TABLE 3

Effect of pH on the Constant Rate of Loss of Total S(IV). Reaction Conditions: [Glucose] = 1·0M; [Glycine] = 0·5M; [S(IV)] = 0·044M; 55°C.

pH	$10^5 \times$ *constant rate* ($M h^{-1}$)
4·5	2·54
5·0	2·88
5·5	2·89
6·5	8·20

essentially independent of pH between pH 4·5–5·5 but there is an increase in rate of loss of S(IV) above pH 5·5.

DISCUSSION

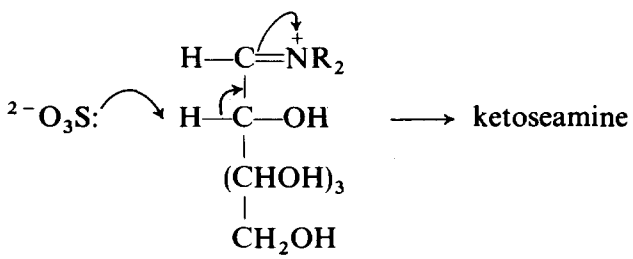
The kinetic effect of S(IV) on the formation of DH could be the result of the involvement of S(IV) in reactions leading to the formation of MFG or in the decomposition of MFG to DH, according to which step is rate determining. It is difficult to envisage a specific reaction between intermediates in the

formation of MFG and S(IV), but it is interesting to note that the formation of MFG is subject to general acid or base catalysis. Reynolds (1959) demonstrated that the addition of orthophosphoric acid + sodium dihydrogen orthophosphate or malic acid + potassium hydrogen malate increased the rate of formation of MFG in concentrated systems. The effect was not due to changes in ionic strength since the addition of sodium chloride had no effect. Rosen *et al.* (1958) also provided good evidence that the Amadori rearrangement of a glucosylamine in methanol at 100°C is acid-base catalysed. There are a small number of cases in which S(IV) have been shown capable of acting as general acid or base catalysts. Perhaps the best example is that of an investigation of the relative abilities of sulphite, hydrogen sulphite and disulphite ions as well as other anions to catalyse the deamination of 1-methyl-5,6-dihydrocytosine (Slae & Shapiro, 1978). The order of effectiveness of the species tried was:

$$SO_4^{2-} < H_2PO_4^- < CH_3CO_2^- < HSO_3^- < HPO_4^{2-} < SO_3^{2-} < S_2O_5^{2-}$$

and it is clear that sulphite and, particularly, disulphite ion are capable of acting as very effective catalysts. An example which may be directly relevant to the reaction being investigated here is the report of Ivanov & Lavrent'ev (1967) that S(IV) act as acid-base catalysts for the interconversion of α and β forms of glucose. An interesting case is the ability of S(IV) to catalyse the hydrolysis of acid anhydrides (Higuchi *et al.*, 1966). Although attempts were made to explain the effect in terms of a specific interaction between the anhydride and S(IV) a straightforward explanation is that of the S(IV) species acting as bases promoting attack on the anhydride by OH^-.

The important steps (Hodge, 1955) in the conversion of glycosylamine to ketoseamine are (1) acid-catalysed protonation of the nitrogen followed by a likely opening of the oxide ring and (2) removal of a proton at C_2. The second step could well be base catalysed by S(IV) as follows:



The observation that DH does not form a stable hydroxysulphonate in the glucose–glycine–S(IV) reaction represents a discrepancy between this system and an experiment in which the kinetics of the reaction between DH and S(IV) were studied directly (Wedzicha & Kaban, 1986). The structures of 3-deoxyosuloses formed during carbohydrate degradations have been

critically reviewed by Wedzicha (1984*b*). Although the structure of DH and other 3-deoxyosuloses is commonly drawn in the keto form at C_2, it is likely that the predominant form of the compound produced in carbohydrate degradations is enol, and the enol appears to undergo further reactions, e.g. dehydration to DDH, without conversion to keto. On the other hand, the sample of DH prepared for kinetic studies on the reaction of DH with S(IV) was obtained by the reaction of benzoylhydrazine with glucose to give, directly, the bis(benzoylhydrazone) of DH. This was subsequently decomposed by a transhydrazonisation reaction with benzaldehyde (Madson & Feather, 1981). It is likely that the keto-product is formed preferentially during this preparation.

The likely absence of a hydroxysulphonate of DH had been suggested previously (Wedzicha, 1984*a*) on account of the good apparent zero order behaviour with respect to S(IV) of the constant rate phase of individual runs of the glucose–glycine–S(IV) reaction. Similar zero order behavior has also been observed for the irreversible binding of S(IV) in the system ascorbic acid–glycine–S(IV) (Wedzicha & McWeeny, 1974). In this case ascorbic acid decomposes spontaneously in a rate determining process to give 3-deoxypentosulose (DP) which then reacts rapidly with S(IV) to form 3,4-dideoxy-4-sulphopentosulose. Graphs of total [S(IV)] as a function of time are linear from zero time to at least 75% reaction, indicating that there is no accumulation of DP as a hydroxysulphonate, to be released in the later stages of reaction. There is, however, some circumstantial evidence that both DH and DP extracted from S(IV)-containing mixtures (Maillard and ascorbic acid browning, respectively) do exist as hydroxysulphonates since they are retained by basic ion exchange resins (Knowles, 1971; Wedzicha & Imeson, 1977). Also, Ingles (1961) succeeded in identifying a relatively unstable hydroxysulphonate of DH when DH was mixed with an equimolar amount of S(IV) in concentrated solution (200 g water/100 g solids). Although this sample of DH was prepared by means of a browning reaction, the degree of dissociation of any adduct formed is lowered by the high concentration used and this result is not strictly applicable to the relatively dilute system used here.

As the pH of the glucose–glycine–S(IV) reaction is changed from pH 4·5–5·5 the concentration of sulphite ion increases approximately tenfold whilst the concentration of hydrogen sulphite ion ($pK_a = 7{\cdot}18$) remains approximately constant. The absence of a large kinetic effect of pH suggests that the catalysis by S(IV) is not caused by the small quantity of sulphite ion present. The rate of browning is independent of pH in the range pH 4–6 (Wedzicha, 1984*b*). Since the rate at which hydroxysulphonate adducts are formed and decomposed is of approximately first order with respect to OH^-, the lack of an effect of pH on the rate of loss of S(IV) confirms the

important assumption that the rate of hydroxysulphonate formation and dissociation is rapid on the time scale of the kinetic experiments and it is correct to consider the system as in equilibrium with respect to the formation of hydroxysulphonates at all times. At pH > 6 the rate of browning increases and the observed increase in rate of loss of S(IV) under these conditions could be related to a more rapid production of reactive intermediates. It is, of course, possible that the nature of the reactive intermediates in browning could change with increasing pH, thereby modifying the reactivity of the system towards S(IV).

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