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A Kinetic Model for the Sulphite Inhibited Maillard Reaction

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ABSTRACT

The time-dependent concentrations of free and total sulphur(IV) oxoanion present during the sulphite inhibited Maillard reaction of glucose and glycine have been found to fit a simple kinetic model. The irreversible combination of the additive follows the scheme:

$$\textit{reactants} \xrightarrow{k_1} \textit{intermediate 1} \xrightarrow{k_2} \textit{intermediate 2}$$

$$\textit{intermediate 2} \xrightarrow[S(IV)]{\textit{fast}} \textit{products}$$

In this the sulphur(IV) oxoanion does not participate in the reactions involving k_1 *and* k_2. *The former proceeds at constant rate when the concentrations of glucose and glycine are both high compared with the concentration of sulphur(IV) oxoanion, whilst the latter is of first order with respect to intermediate 1. When the reaction is carried out at pH 5·5 and 55 °C and the initial concentrations of glucose and glycine are 1* M *and 0·5* M, *respectively,* $k_1 = 3{\cdot}2 \times 10^{-5}$ M h^{-1} *and* $k_2 = 5{\cdot}5 \times 10^{-3}\ h^{-1}$. *Reversible binding of the additive takes place as a result of hydroxysulphonate formation with glucose and with the final product of reaction, 3,4-dideoxy-4-sulphohexosulose. The dissociation constant of the hydroxysulphonate of the latter is deduced to be* 5×10^{-3} M *under the reaction conditions stated above. The model does not include hydroxysulphonate formation involving intermediates in the reaction leading to irreversible combination of the additive, and fits concentration–time data with an initial sulphur(IV) oxoanion concentration of 0·039* M *to 90% loss of the additive, the latter representing the limit of the available experimental data.*

INTRODUCTION

The amounts of sulphur(IV) oxospecies present in the free form (referred to collectively as S(IV)) and that converted to hydroxysulphonate adducts of carbonylic components (reversibly bound S(IV)), as a function of time, in the system glucose–glycine–S(IV), are shown in Fig. 1 (McWeeny *et al.*, 1969). The total S(IV), that is the sum of that which is free and that which is reversibly bound, decreases with time as a result of irreversible combination of the additive with components of the mixture. The initial condition, when some 43% of the total S(IV) is reversibly bound, arises from hydroxysulphonate formation with glucose. As the reaction proceeds the amount of reversibly bound additive remains reasonably constant, while the amount of free additive falls. Since, over the timescale used, the system is expected to stay near equilibrium with regard to the carbonyl–S(IV) reaction, the stability of the hydroxysulphonate adducts

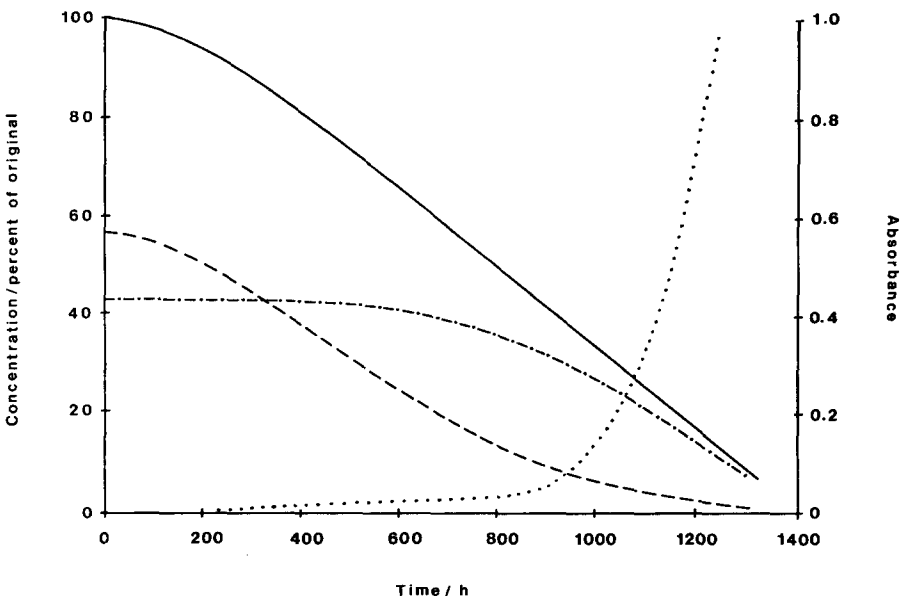


Fig. 1. Concentration–time behaviour of total, reversibly bound and irreversibly bound sulphur(IV) oxoanion during the inhibited reaction of glucose and glycine. Absorbance data superimposed. Reaction conditions: [glucose] = 1 M; [glycine] = 0·5 M; [S(IV)] = 0·039 M; pH 5·5; 55 °C. Graphs plotted from the data published by McWeeny *et al.* (1969) to reproduce figure shown by these authors. Key: ——— total S(IV); – – – – free S(IV); –·–·– reversibly bound S(IV); ···· absorbance at 490 nm.

appears to increase with time. This observation can only be explained by the formation of carbonyl compounds which bind the additive more strongly than does glucose. Conversion of S(IV) to an irreversibly bound form eventually leads to the decomposition of the hydroxysulphonate adducts.

In the case of the glucose–glycine–S(IV) reaction the most abundant stable S(IV)-derived product is 3,4-dideoxy-4-sulphohexosulose (DSH), obtained by sulphonation of 3,4-dideoxyhexosulos-3-ene (DDH), a dehydration product of 3-deoxyhexosulose (DH). The inhibition of Maillard browning by S(IV) is attributed to the lower reactivity towards browning of DSH than DH and DDH and the likelihood of dicarbonylic intermediates forming particularly stable hydroxysulphonates, existing possibly as diadducts. This explains also the increased tendency for reversible binding of the additive with time, as shown in Fig. 1 (McWeeny *et al.*, 1974).

Despite the good understanding of the mechanism of the inhibition of the Maillard reaction by S(IV), there is still a lack of information concerning the specific carbonyl compounds which contribute to the reversible binding of the additive and of the relationship of these intermediates to colour formation once inhibition is over. This paper considers a kinetic model to account for the observations shown in Fig. 1, thereby providing a more detailed description of the glucose–glycine–S(IV) reaction.

KINETIC MODEL

The concentrations of glucose and glycine (1 M and 0·5 M, respectively) in the experiment of McWeeny *et al.* (1969) are very largely compared with the concentration of total S(IV) (0·039 M). If it is accepted that, for successful inhibition of browning, the amount of S(IV) added to the system and the amount of reactive intermediate formed from glucose must be of similar magnitude, then the amount of glucose which has undergone reaction by the time browning commences will be small. The apparent dissociation constant of glucose hydroxysulphonate (1·33 M) in the glucose–glycine–S(IV) system during the first 100 h of reaction is very similar to that measured using glucose and S(IV) under similar conditions, but without glycine (McWeeny *et al.*, 1969). Therefore, the glucose is present in a free form rather than, say, in the form of

aldosylamine. Since not more than one mole of glycine is involved in the reaction of one mole of glucose, then it is reasonable to make the assumption that the concentrations of glucose and glycine will effectively remain constant during the period of the observations. Therefore, irrespective of its kinetic order, the primary reaction between glucose and glycine will proceed at constant rate during this period.

Considering the experimental data for the irreversible combination of S(IV) shown in Fig. 1, it is striking that, if the initial period during which the reaction is being established is neglected, the rate of irreversible loss of additive is constant over the major part of the reaction, despite a very large change in the concentration of the reactant, free S(IV). This zero order kinetic behaviour suggests that the formation of intermediate in the Maillard reaction is rate determining as far as irreversible loss of S(IV) is concerned and the reaction between the additive and the intermediate in question is, therefore, fast on the timescale of the experiment. The intermediate involved in this final reaction with S(IV) cannot be the product of the primary reaction between glucose and glycine, since the combination of the two zero order processes would lead to the constant rate of loss of total S(IV) being observed at zero time. The induction phase, during which the rate of irreversible combination of the additive is increasing and which takes place during the first 400 h of reaction, could be attributed to a mechanism involving two consecutive reactions: the first being a constant rate process involving glucose to give, say, intermediate 1, whilst the second is a reaction of non-zero kinetic order with respect to intermediate 1 to form a new intermediate, intermediate 2, which is the substrate for rapid reaction with S(IV). Thus, the proposed scheme is:

$$\text{reactants} \xrightarrow{k_1} \text{intermediate 1} \xrightarrow{k_2} \text{intermediate 2}$$

$$\text{intermediate 2} \xrightarrow[\text{S(IV)}]{\text{fast}} \text{products}$$

where the S(IV) does not participate in the reactions involving k_1 and k_2. It will be assumed that the conversion of intermediate 1 to intermediate 2 is of first order with respect to intermediate 1. It is difficult to see how such a reaction may involve more than one molecule of intermediate in the transition step but it may, of course, involve other reactants which are not kinetically significant. The overall scheme has the characteristics of zero rate of loss of total S(IV) at zero time, i.e., when there is no intermediate 1 or 2 present, a steady increase in rate of irreversible

combination of the additive as the concentration of intermediate 1 increases and a final steady state situation with the concentration of intermediate 1 constant and, hence, rate of formation of intermediate 2 and rate of formation of final product constant. Qualitatively, this is seen to be consistent with the concentration–time data shown in Fig. 1.

To derive an integrated rate expression for this model, the net rate of change of concentration of intermediate 1 at any time, t, is given by:

$$\frac{d[I]}{dt} = k_1 - k_2[I]$$

where [I] is the concentration of intermediate 1. Integrating and imposing the conditions that $[I] = 0$ at $t = 0$, the following result is obtained:

$$[I] = \frac{k_1}{k_2}\{1 - \exp(-k_2 t)\}$$

Since the rate of formation of final product is equal to the rate of formation of intermediate 2, it is possible to write:

$$\frac{d[P]}{dt} = k_2[I] = k_1\{1 - \exp(-k_2 t)\}$$

where [P] represents the concentration of the final product at time t. Integrating and imposing the condition that $[P] = 0$ at $t = 0$, the time-dependent concentration of final product is obtained as follows:

$$[P] = k_1 t - \frac{k_1}{k_2}\{1 - \exp(-k_2 t)\} \quad (1)$$

Knowles (1971) suggested that the reaction of compounds containing an α-dicarbonyl group, with S(IV), should lead to the formation of dihydroxysulphonates which are stabilised through hydrogen bonding. The type of structure proposed is illustrated for the dihydroxysulphonate of pyruvaldehyde in Fig. 2 where the relevant atoms of the corresponding sulphonate and hydroxyl group and the carbon–carbon bond are drawn coplanar for clarity. Rotation about the carbon–carbon bond is, in fact, required, to achieve an acceptable hydrogen bonding distance. Whilst this proposal is attractive, Salomaa (1956) reports that, at pH 7·3, the dissociation of the dihydroxysulphonate of glyoxal takes place much more readily than dissociation of the monoadduct.

Four reasons why dihydroxysulphonate formation may be less favourable than monohydroxysulphonate formation may be advanced. First,

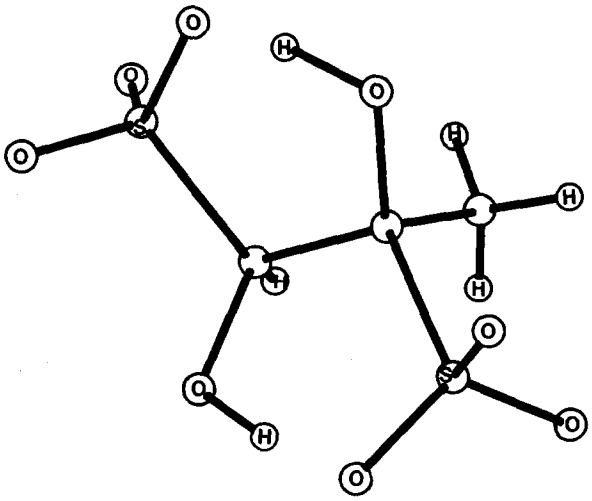
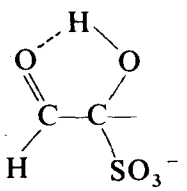


Fig. 2. Model of the dihydroxysulphonate of pyruvaldehyde. Unmarked atoms represent carbon.

the addition of S(IV) to a monohydroxysulphonate needs to be stereospecific and is, therefore, less probable. Secondly, the monohydroxysulphonate may already be stabilised through hydrogen bonding between the free carbonyl group and the adjacent hydroxyl group, thus:



This type of stabilisation is thought to exist in the hydrate of glyoxal. Thirdly, the hydrogen bonds in the dihydroxysulphonate will be formed between a negatively charged sulphonate group and a hydroxyl group. Since most hydrogen bonds are basically electrostatic, the situation in the diadduct will lead to a larger negative charge being induced on the hydroxyl oxygen than if the hydrogen bond had been to, say, an uncharged carbonyl group. The development of negative charge on the oxygen of the hydroxyl group of the hydroxysulphonates is the first step in the alkaline hydrolysis of these compounds. It may be significant that, although the diadduct of glyoxal is less stable than the monoadduct,

it is formed and decomposed more rapidly than the latter (Salomaa, 1956). The hydroxysulphonate-type adducts of monocarbonyl compounds become similarly labile as pH is increased. Fourthly, the polarity of the diadduct will be reduced through hydrogen bonding and, therefore, formation of the diadduct could be more favourable in less polar solvents. It may be relevant that the diadducts of glyoxal and of hydroxypyruvaldehyde may be prepared in the crystalline state (Ronzio & Waugh, 1944; Ingles, 1961). An additional reason for the preferential formation of monohydroxysulphonates from dicarbonyl compounds exists when one of the carbonyl groups is involved in hemiacetal formation. This is demonstrated by monohydroxysulphonate formation with D-*threo*-2,5-hexodiulose and 2,5-diketogluconic acid and is probably the reason why L-*erythro*-pentosulose only forms a monoadduct with S(IV) (Burroughs & Sparks, 1973). In the case of intermediates in the Maillard reaction, DH probably exists in the form of pyranose, and the *cis*-unsaturated intermediate derived from it may, likewise, be cyclic. There is no reason why DSH should not consist of an open chain structure in equilibrium with cyclic forms. On the basis of the equilibrium data for the formation of hydroxysulphonate adducts with glyoxal and the tendency for the formation of cyclic structures by the osuloses, and notwithstanding some possible desirable features of dihydroxysulphonate formation, it is concluded that there is no justification for assuming that dihydroxysulphonate adducts are formed in preference to the monohydroxysulphonates and it is, indeed, more likely that the latter will predominate. There are no published equilibrium data for the reversible addition of S(IV) to dicarbonylic intermediates or products in Maillard browning.

The good zero order kinetic behaviour with respect to S(IV) of irreversible combination of the additive, over a large part of the reaction, suggests that S(IV) does not interact significantly with the carbonyl groups of the precursors of DSH. Therefore, the only known compounds which need to be considered in their capacity to bind S(IV) reversibly are glucose and DSH. The choice of the latter as the important carbonylic component which binds S(IV) reversibly can be supported by considering published data on the comparison of the rates of colour formation in the glucose–glycine reaction, with and without added S(IV) (Burton *et al.*, 1962). It is evident from Fig. 1 that the formation of colour begins when there is a release of free carbonyl compound, as the S(IV) concentration falls. Despite there being a release of carbonyl compound, it is found that the rate of colour development in the S(IV)-containing system never

exceeds that in the system containing no additive. If the amount of intermediate or product which has accumulated in the form of hydroxysulphonate adduct reflects the integrated amount of an important intermediate in the browning reaction, which would have been formed in the absence of S(IV), then, in the later stages of inhibition of browning, this amount of intermediate or product will be released into the system in approximately half the time taken for its formation. In addition, more of this compound will be continuously produced by the reacting system. If the released compound were, indeed, an important intermediate in the S(IV)-free browning reaction, then it is expected that this additional release of the intermediate would give rise to a rate of browning in excess of that observed for glucose and glycine alone. The comparable or slower rate implies that the released compound is less reactive towards browning and this condition is satisfied by DSH. It is proposed, therefore, that the increase in stability of hydroxysulphonate adducts with time during the glucose–glycine–S(IV) reaction is due to the formation of DSH.

To calculate the extent of hydroxysulphonate formation g, p and s are assigned to the concentrations of glucose, DSH and total S(IV), respectively, at any given time. If the equilibrium constants for the dissociation of hydroxysulphonate adducts of glucose and DSH are K_1 and K_2, respectively, and the equilibrium concentrations of these adducts are x and y, respectively, application of the law of mass action but allowing for no change in glucose concentration gives the following expressions:

$$K_1 = \frac{g(s-x-y)}{x}$$

$$K_2 = \frac{(p-y)(s-x-y)}{y}$$

Rearranging and expressing in terms of y, the following result is obtained:

$$K_1 y^2 - \{K_1(p+s+K_2) + K_2 g\} y + K_1 ps = 0 \qquad (2)$$

from which the amount, y, may be calculated for given initial concentrations and equilibrium constants. The result may be used to calculate x using:

$$x = \frac{g(s-y)}{K_1 + g} \qquad (3)$$

from which the concentration of reversibly bound additive may be

determined as $x + y$. Therefore, use of eqns (1), (2) and (3) allows the amounts of total and reversibly bound S(IV) to be calculated and the amount of free additive is then found by difference.

CALCULATIONS AND RESULTS

The assignment of values to rate and equilibrium constants is a prerequisite to calculation. Rate constants may be deduced simply from experimental data as follows. When the reaction is well advanced, the concentration of intermediate 1 will have reached its steady-state concentration and the rate of its formation will, therefore, equal the rate of its loss, which will also equal the rate of formation of DSH. Since the rate of formation of intermediate 1 is the constant rate step, the value of k_1 will equal the rate of irreversible loss of total S(IV) once the reaction has become established. The slope of the linear portion of the concentration–time curve for the irreversible combination of total S(IV) is $3{\cdot}2 \times 10^{-5}$ M h^{-1} and this value is assigned to k_1. The value of k_2 may be found by fitting experimental concentration–time data, from the first 400 h of reaction, to eqn (1), after substituting the value of k_1, and the best fit gives the result, $k_2 = 5{\cdot}5 \times 10^{-3}\ h^{-1}$. The dissociation constant of glucose hydroxysulphonate is calculated from the initial concentrations of free and reversibly bound S(IV) and found to be 1·33 M. The only remaining unknown is the dissociation constant of the hydroxysulphonate of DSH. Since there are no reported values of the latter and it is not possible to deduce the value from the kinetic data, the calculation of the progress of the glucose–glycine–S(IV) reaction, using eqns (1), (2) and (3), was carried out as a function of K_2, and excellent correspondence between predicted and experimental data, illustrated in Fig. 3, was obtained using a value of 5×10^{-3} M.

Having successfully modelled the kinetic data, it is informative to show how the concentrations of the individual components of the reacting system vary with time. The concentrations of both hydroxysulphonate adducts are available from eqns (2) and (3) and, using the result for the extent of irreversible combination of the additive, the concentration of free DSH may be found. These results are plotted in Fig. 4. During the first 400 h of reaction, the apparent constant amount of reversibly bound S(IV) is the result of the formation of one hydroxysulphonate adduct at the expense of the other, this apparent balancing of contributions being

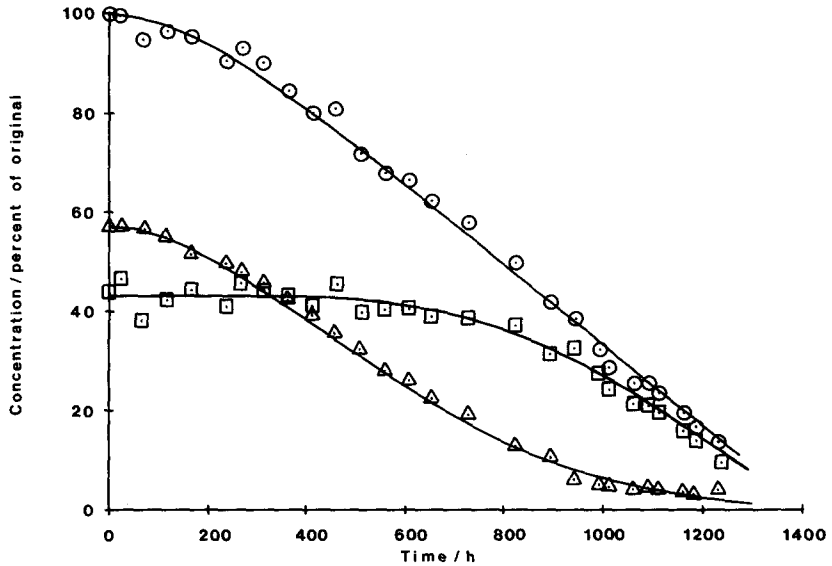


Fig. 3. Comparison of data predicted by kinetic model with experimental data of McWeeny *et al.* (1969). Lines denote calculated values. Reaction conditions as in Fig. 1. Key to experimental data: ⊙ total S(IV); △ free S(IV); ⊡ reversibly bound S(IV).

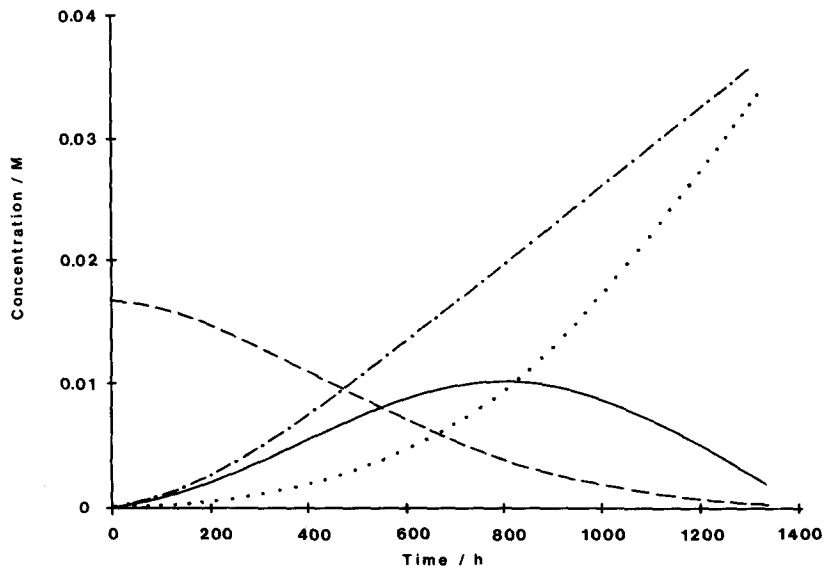


Fig. 4. Graph showing the concentrations of hydroxysulphonate adducts and stable products during the sulphur(IV) oxoanion inhibited Maillard reaction of glucose and glycine. Reaction conditions as in Fig. 1. Key: ——— 3,4-dideoxy-4-sulphohexosulose hydroxysulphonate; – – – – glucose hydroxysulphonate; –·–·– total 3,4-dideoxy-4-sulphohexosulose; ···· free 3,4-dideoxy-4-sulphohexosulose.

fortuitous. The removal of DSH in the form of hydroxysulphonate is also most efficient at this stage but is far from being complete. For example, after 400 h of reaction some 25% of the sulphonate remains in the free form. The efficiency with which DSH is converted to hydroxysulphonate decreases with increasing reaction time, and it is a combination of this reduced efficiency and the relatively fast decomposition of glucose hydroxysulphonate which causes the start of the reduction in concentration of reversibly bound S(IV) observed after approximately 500 h (Fig. 1). The concentration of the hydroxysulphonate of DSH does not begin to fall until after 800 h have elapsed and, at this stage, the rate of formation of free DSH exceeds the rate of irreversible combination of S(IV) ($3{\cdot}2 \times 10^{-5}$ M h^{-1}), the calculated rate of production of the free compound increasing to a value of $5{\cdot}5 \times 10^{-5}$ M h^{-1} after 1300 h. The data in Fig. 1 show the rate of colour development to increase rapidly at reaction times between 800 and 900 h, this period being preceded by the phase in which the rate of formation of DSH increases markedly.

It is possible to calculate the steady-state concentration of intermediate 1 by equating its rate of formation to its rate of loss. The concentration of intermediate is then given by the ratio of rate constants, k_1/k_2, and is equal to $5{\cdot}8 \times 10^{-3}$ M using the derived values of k_1 and k_2.

DISCUSSION

The kinetic model proposed for the glucose–glycine–S(IV) reaction is found to agree very well with the kinetic data of McWeeny *et al.* (1969). It is consistent with the cause of reversible combination of S(IV) being hydroxysulphonate formation with glucose and with one other component whose concentration increases at the same rate as the irreversible combination of S(IV). The latter component is presumed to be DSH, although the success of the model does not depend on its identity. The existence of hydroxysulphonate adducts of 3,4-dideoxy-4-sulphosuloses has been reported in the literature. Anet & Ingles (1964) report the formation of the hydroxysulphonate of DSH as the product of the preparation of DSH by the action of sulphur dioxide on 3,4-dideoxyhexosulos-3-ene in aqueous solution. The hydroxysulphonate of 3,4-dideoxy-4-sulphopentosulose is known to exist from the fact that, when mixtures of the latter and S(IV) are subjected to ion exchange chromatography on a strongly basic resin, the products mixture appears to contain two components. One is the free sulphonate whilst the other is

its hydroxysulphonate adduct (Wedzicha & Imeson, 1977). The evidence presented here suggests that the hydroxysulphonate adduct is, in fact, a monoadduct and its dissociation constant of 5×10^{-3} M reflects only moderate stability. It is, however, possible that the apparent dissociation constant is affected by the presence of a high concentration of glycine which will compete for the carbonyl groups, or the hydroxysulphonate adduct may be of the type reported by Ingles (1967) for glyoxal and diacetyl, involving both glycine and S(IV).

Reversible interactions between intermediates in the reaction leading to irreversible combination of S(IV), and S(IV), are not included in the mechanism and, within the resolution of the experimental data, there is no reason for modifying the proposal to include such interactions. However, during the analysis of glucose–glycine–S(IV) reaction mixtures, Knowles (1971) reports the appearance of 3-deoxyhexosulose as an apparently acidic product when separations of reaction products are carried out by ion exchange chromatography. This behaviour was presumed to be a result of hydroxysulphonate formation. Similarly, in work on the S(IV) inhibited browning of ascorbic acid under anaerobic conditions, which proceeds through 3-deoxypentosulose to the sulphonated product, 3,4-dideoxy-4-sulphopentosulose, reaction mixtures are found to contain some of the hydroxysulphonate of 3-deoxypentosulose (Wedzicha & Imeson, 1977). It is therefore evident that some reversible combination between S(IV) and precursors of DSH cannot be ruled out but any such adduct formation does not appear to be kinetically significant. The suggestion that 3-deoxyhexosulose does not react to a significant extent directly with S(IV) was originally made by McWeeny & Burton (1963) on the basis of semiquantitative observations of the rate of browning of 3-deoxyhexosulose in the presence of glycine, with and without added S(IV). Whereas, in the absence of additive, the browning reaction commenced immediately, the effect of the additive was to delay the start considerably, and not merely to reduce the rate of browning.

The kinetic model places DSH as the most important compound, not only for the inhibition of browning in the glucose–glycine reaction, but also in the initial formation of colour once the period of inhibition is over, there being a significant increase in concentration of free sulphonate prior to the increase in rate of colour development.

As shown in Fig. 4, the efficiency of removal of DSH, in the form of hydroxysulphonate, is relatively poor and leads to an increase in concentration of the free compound which is not as abrupt as the increase

in optical density shown in Fig. 1. It cannot be automatically assumed, therefore, that the release of free DSH and the formation of colour are directly linked.

The polymeric nature of melanoidins implies that they arise from combination of monomers and oligomers. If the formation of dimer, D, and trimer, T, from DSH can be expressed by the following rate equations, respectively,

$$\frac{d[D]}{dt} = k_3[DSH]^2$$

and

$$\frac{d[T]}{dt} = k_4[D][DSH]$$

where k_3 and k_4 are the respective second order rate constants, [D] and [T] at any time may be calculated by numerical integration of concentration–time data for free DSH in Fig. 4. If by way of a postulate, it is stated that, in the early stages of colour formation the most important component which absorbs at 490 nm is the trimer, the dimer absorbing only in the ultraviolet region and the reaction not being sufficiently

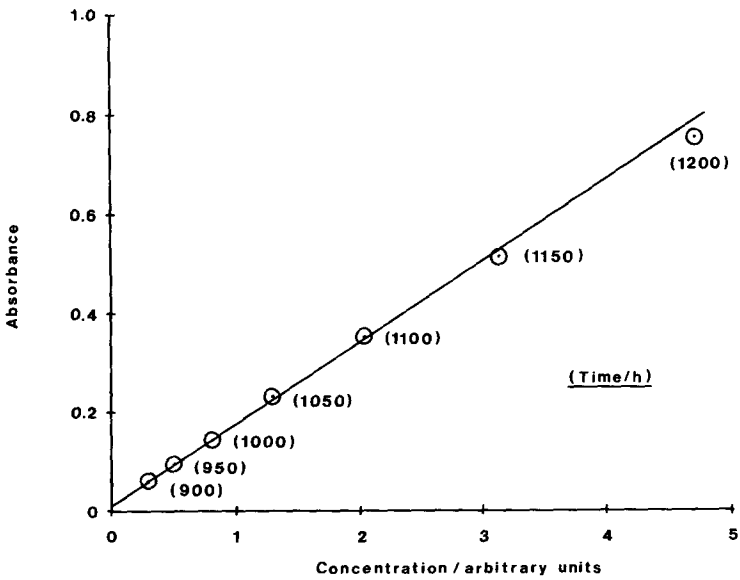


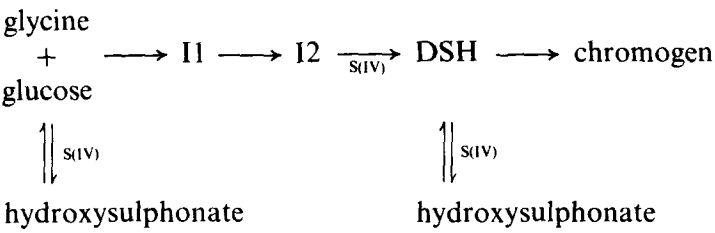
Fig. 5. Relationship between measured absorbance and calculated concentration of trimer of 3,4-dideoxy-4-sulphohexosulose at the given times.

advanced for significant presence of tetramer, the feasibility of a connection between the concentration of free DSH and absorbance may be demonstrated by plotting experimental absorbance (from Fig. 1) as a function of [T]. Such a graph is shown in Fig. 5 with an indication of the reaction time at which the points were observed. The concentration is given in arbitrary units since the absolute values are dependent on the choice of k_3 and k_4; the linear relationship is independent of these constants providing that the overall conversion of DSH to dimer and trimer is small.

These results show that the concentration–time data for free DSH are capable of being directly related to the absorbance–time data, giving support to the role of DSH as the principal source of coloured product once the period of inhibition of browning is over. Of course, once the S(IV) has been exhausted, much more reactive intermediates in browning will take part in the colour-forming reactions, probably bypassing the sulphonate.

CONCLUSION

The kinetic model proposed for the inhibition by S(IV) of glucose–glycine browning is the minimum requirement to explain the observed kinetic data and provides a single scheme to account for irreversible and reversible combination of the additive as follows:



where I1 and I2 are intermediates. An attractive feature of this model is that it leads to the following predictions which will allow the most important conclusions to be tested:

1. The dissociation constant of the hydroxysulphonate of DSH is 5×10^{-3} M under the specified conditions.
2. The melanoidin produced in the early stages of colour development should contain one sulphur atom for every six carbon atoms derived from glucose.

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