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0308-8146(95)00086-0

*Food Chemistry*, Vol. 54, No. 4, pp. 397–402, 1995

0308-8146/95 $9.50 + 0.00

# Kinetics of the oligosaccharide–glycine–sulphite reaction: relationship to the browning of oligosaccharide mixtures

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(Received 10 February 1995; accepted 25 March 1995)

The kinetics of the reaction of sulphite species in mixtures of glucose + glycine, maltose + glycine and oligosaccharide mixtures + glycine are modelled in terms of a mechanism which involves two consecutive rate-determining steps. The rate constant for the first step depends on the dextrose equivalent value of the mixture (range 12–100). The value of the rate constant for the second step is similar for maltose and the oligosaccharides, but slightly greater for glucose. In experiments involving oligosaccharide mixtures, the contribution of oligosaccharides with three or more glucose residues, to the overall rate of reaction, was in the range 60–90%. The rate of browning at 420–490 nm is proportional to the value of the rate constant for the first step except for reaction mixtures containing maltose alone; this disaccharide appears to brown more slowly than expected from the correlation which applies to glucose and the oligosaccharide mixtures.

## INTRODUCTION

Maillard browning is a series of consecutive reactions which give rise to low and high molecular weight products. Sulphite species, S(IV), inhibit the Maillard reaction of monosaccharides by combining irreversibly with the α,ß-unsaturated carbonylic intermediates (McWeeny *et al.*, 1974), which are precursors of flavour volatiles and colour, to form sulphonates. The inhibitor acts at an early stage in the reaction pathway, common to both colour and flavour formation. In the glucose–glycine reaction, there is only one significant reaction product (3,4-dideoxy-4-sulphohexosulose, DSH). This is the result of a nucleophilic reaction of sulphite ion with 3,4-dideoxyhexosulos-3-ene, DDH, which is formed by the loss of a molecule of water from 3-deoxyhexosulose, DH. Kinetically, the involvement of S(IV) in the glucose–glycine reaction may be represented (Wedzicha, 1984; Wedzicha & Vakalis, 1988) by the scheme shown in Fig. 1, where the intermediate I1 has been shown to be DH (Wedzicha & Garner, 1991) and I2 is an unspecified intermediate. The relative rate of the conversion of the latter to the Maillard products in the absence of S(IV) is slower than its reaction with S(IV). Values of $k_1$ and $k_2$ are obtained from measurement of the rate of reaction of S(IV) in these systems (Wedzicha, 1984; Wedzicha & Vakalis, 1988).

It is common practice to follow the progress of the Maillard reaction by measuring the absorbance of reaction mixtures at some wavelength, usually in the range 420–490 nm. From the point of view of reaction kinetics, this approach has a disadvantage in so far as the coloured products are a complex mixture of low and high molecular weight substances, and absorbance-time behaviour reveals that a number of rate-determining reaction steps are involved. Thus, the uncertainty in the measurement and the complexity of the reaction mean that it is not possible to resolve individual rate constants from such data. One approach is, of course, to measure the concentration of individual intermediates but those which can be measured tend to be the more stable and do not necessarily represent the substances which are involved in the rate-determining reactions. An approach which is being developed in the authors' group (Wedzicha *et al.*, 1994) is to use the rate of reaction of S(IV) as a measure of the rate of the reaction which would have taken place in the absence of the anti-browning agent. The measurements allow one to calculate the rate of formation, or the amount of, reactive precursor of colour and flavour under given conditions. Thus, the approach is fundamental and since there are only two rate-determining steps involved, unambiguous interpretation of the kinetics is possible.

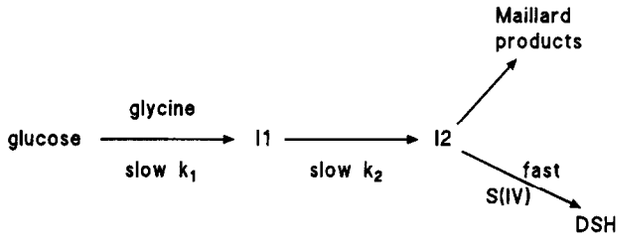



**Fig. 1.** Reaction scheme for the glucose–glycine–S(IV) reaction showing the formation of intermediates I1 and I2 in consecutive rate-determining steps.

One experimental complication is that the reaction step described by $k_1$ is catalysed by S(IV) (Wedzicha & Vakalis, 1988) and $k_1$ is related to S(IV) concentration by

$$k_1 = k_1' + k_1''[\mathrm{S(IV)}]$$

where $k_1'$ and $k_1''$ are the rate-constants for the S(IV)-independent and S(IV)-dependent reactions, respectively. In practice, the rate of the reaction which takes place in the absence of S(IV) can be obtained by extrapolating rate-concentration data to zero concentration. Further, the rate constant $k_1'$ is of first order with respect to glucose and glycine concentration (Wedzicha & Vakalis, 1988; Bellion & Wedzicha, 1993), and the same kinetic behaviour with respect to reducing sugar and glycine has been obtained in studies of the maltose–glycine–S(IV) reaction (Wedzicha & Muller, unpublished). Preliminary experiments (Wedzicha & Kaputo, 1992; Wedzicha *et al.*, 1994) show that values of $k_1'$ correlate with the amount of high molecular weight product formed in the glucose–glycine reaction and with the rate of browning of selected amino acids. The approach has been found also to apply to the fructose–amino acid reaction (Swales & Wedzicha, 1992; Swales & Wedzicha, 1995).

Whilst some knowledge of the intermediates involved in the glucose–glycine–S(IV) reaction is established, the approach described here does not depend strictly on this knowledge.

In this paper we aim to demonstrate the application of this approach to the study of the reaction between oligosaccharides and glycine. Little work has been reported on the role of oligosaccharides in the browning of foods although the conversion of starch into oligosaccharides is known to be important to the colour and flavour of bread and malt. The caramelisation of glucose, maltose and maltotriose (in the absence of amino compound) leads to the formation of hydroxymethylfurfural, HMF, as a result of the progressive fragmentation of di- and trisaccharides (Kroh, 1994). A precursor of HMF is DDH. Whilst the latter is formed in the glucose–amino acid reaction, one cannot draw an analogy between the Maillard reaction of oligosaccharides and the respective caramelisation reaction, on the basis of existing knowledge.

## MATERIALS AND METHODS

All chemicals were of AnalaR grade and were supplied by BDH Chemicals Ltd. Oligosaccharide mixtures were obtained from Roquette (UK) Ltd. They had nominal dextrose equivalents of 12, 21, 33 and 40 and are identified in this paper as DE12, DE21, DE33 and DE40, respectively. Typical oligosaccharide compositions of the DE12, DE21 and DE40 samples were provided by the manufacturer and are illustrated in Fig. 2.

Solutions of S(IV) were prepared by dissolving sodium metabisulphite in water containing ethanol (1 vol%) and were standardised iodimetrically. Glucose, maltose or the oligosaccharide mixtures (18 g) and glycine (3.75 g) were dissolved in acetate buffer (50 ml, 0.5 M $CH_3COONa + CH_3COOH$ to give pH 5.5). S(IV) solution (10 ml, 0.1–1.0 M) was added and the volume made up to approximately 80 ml with water. The pH of the mixture was adjusted to pH 5.5 with HCl (3 M) or NaOH (3 M) and the volume made up to 100 ml. Each reaction mixture was dispensed into several 5 ml sample vials, closed with a screw-cap and submerged in a boiling water-bath.

At time zero and at regular time intervals, a sample vial was removed from the water-bath, cooled in ice and brought to room temperature. The reaction mixture (2 ml) was diluted to 100 ml with aqueous ethanol (1 vol%), an aliquot (1–10 ml) was mixed with DTNB reagent (25 ml, 1 mM 5,5′-dithiobis(2-nitrobenzoic acid) in 0.02 M phosphate buffer, pH 7) and made up to 100 ml with water. The absorbance of the resulting solution was measured at 412 nm against a reagent blank. The analysis was calibrated by mixing iodimetrically standardised S(IV) solutions with the reagent.

The Lane–Eynon titrimetric method was used to determine the reducing power of the oligosaccharide mixtures. Aqueous copper(II) sulphate solution (5 ml, 0.28 M) and sodium potassium tartrate (1.23 M) in NaOH solution (5 ml, 2.5 M) were mixed with the sugar solution (15 ml, 2 g $l^{-1}$) and the mixture boiled for 15 s. A further volume of the sugar solution was added slowly to the boiling mixture from a burette until the

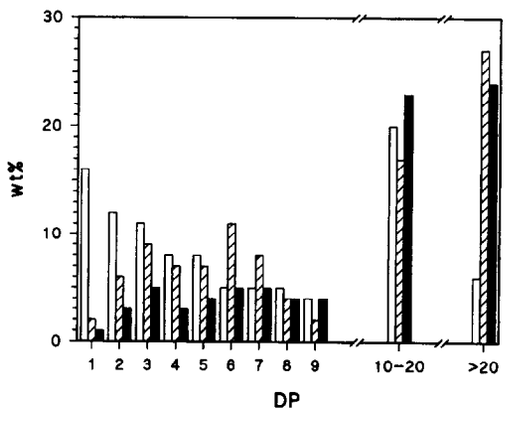



**Fig. 2.** Glucose and oligosaccharide distributions of DE40, DE21 and DE12 oligosaccharide mixtures; data provided by Roquette (UK) Ltd. DP is the number of glucose residues in the oligosaccharide. DE40 □; DE21 ▨; DE12 ■.

blue colour was nearly discharged. Methylene blue indicator was added and the titration continued until the indicator was completely decolourised. The determination was repeated several times adding, before heating, almost all the sugar solution (approximate final titre minus 2 ml), and the titration completed within a total boiling time of 3 min.

In order to measure the rate of browning of oligosaccharide–glycine mixtures, reactions were prepared as described above but omitting the S(IV). At regular time intervals, the absorbance of each sample was measured at 420, 450, 470 and 490 nm against water as a blank.

## RESULTS AND DISCUSSION

The spectrophotometric method for the analysis of S(IV) with DTNB (Humphrey *et al.*, 1970) at pH 7 measures the total S(IV) concentration, i.e. that which is free plus that which is bound reversibly to carbonyl groups, because hydroxysulphonates are decomposed at the pH of the reagent. Any colour (browning) formed in reaction mixtures did not interfere with this analysis. The concentrations of glucose and glycine were chosen to be 1 and 0.5 M, respectively, as those concentrations used previously in model systems to obtain easily measurable rates. The concentrations of maltose and the oligosaccharide mixtures corresponded to the same weight/volume concentration as glucose because there is no systematic way to obtain comparable molar concentrations. Another approach, in which reaction mixtures have the same reducing group concentration, leads to solutions of the lower dextrose equivalent oligosaccharides being prohibitively concentrated. The Maillard reaction causes the release of $H^+$ and two contrasting systems can be set up, with and without pH control. At high temperature, the only feasible method of pH control is to use a buffer. On the other hand, the pH of unbuffered reactions, initially in the range pH 4–6, falls as the reaction proceeds. In this situation, there is not only a change, with time, in $[H^+]$ but also in the concentrations of the acid and conjugate base forms of the amino acid. These may have different tendencies to catalyse the Maillard reaction. It was decided to set up reactions in which the only variable was the concentration of the reactants and a buffered system was, therefore, chosen. It is recognised that the buffer species (acetic acid and acetate ion) are capable of acting as acid–base catalysts in the Maillard reaction (Saunders & Jervis, 1966), but their concentrations were kept constant throughout the investigation. In no experiment did the pH fall by more than 0.1 unit.

Typical concentration–time behaviour for the reaction of S(IV) with glucose + glycine, maltose + glycine and oligosaccharide mixtures + glycine are illustrated in Fig. 3. These raw data show the same characteristic induction phase followed by a gradual increase in rate of loss of S(IV), to a constant value. It is inferred that the reactions generally conform to the stepwise mechanism shown in Fig. 1 and concentration–time

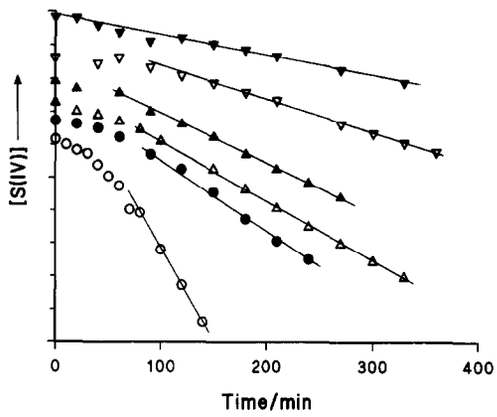



**Fig. 3.** Typical concentration–time data for the reaction of S(IV) with glucose + glycine, maltose + glycine or oligosaccharide mixture + glycine. Reaction conditions: [carbohydrate] = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. The initial concentration of S(IV) was 40–60 mM and successive runs are displaced by arbitrary amounts along the concentration axis for clarity of presentation. Each division represents 10 mmol litre$^{-1}$. Glucose ○; maltose ●; DE40 △; DE33 ▲; DE21 ▽; DE12 ▼.

data fit well the model defined by rate constants $k_1$ and $k_2$. The constant rate of loss of S(IV), which is observed over the major part of the reaction, equals the value of $k_1$ whilst the length of the induction phase is a measure of the relative magnitudes of $k_1$ and $k_2$. Indeed, as the concentration of the higher oligosaccharides in the reaction mixtures is increased, so the induction phase becomes less distinct and the ratio $k_2/k_1$ becomes greater.

Examples of the dependence of $k_1$ on the concentration of S(IV) are shown in Fig. 4 for glucose and three of the oligosaccharide mixtures. These graphs illustrate the quality of the data; results for the other carbohydrate samples have been omitted for clarity of presentation. Values of the rate constants $k_1'$ and $k_1''$ are summarised in Table 1 for all experiments. Whilst there is a general trend for the value of $k_1''$ to increase with

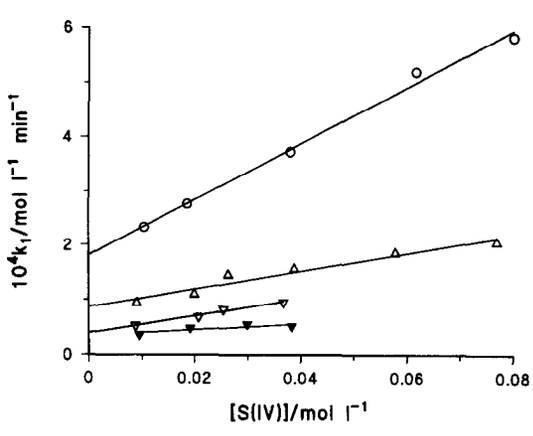



**Fig. 4.** Examples of the relationship between the concentration of S(IV) and the rate constant, $k_1$, for its reaction in glucose + glycine or oligosaccharide mixtures + glycine. Reaction conditions: [carbohydrate] = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. Glucose ○; DE33 △; DE21 ▽; DE12 ▼.

**Table 1. Values of $k_1'$ and $k_1''$ for the S(IV)-independent and S(IV)-dependent reactions, respectively, of saccharide mixtures with glycine and S(IV). Reaction conditions: glucose, maltose or oligosaccharide mixture concentration = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. The detailed oligosaccharide distributions are given in Fig. 2**

| Saccharide mixture | $10^4k_1'$ (mol litre$^{-1}$ min$^{-1}$) | $10^4k_1''$ (min$^{-1}$) |
|---|---|---|
| Glucose | 1.8 | 52 |
| Maltose | 0.91 | 23 |
| DE40 | 0.87 | 16 |
| DE33 | 0.55 | 25 |
| DE21 | 0.39 | 16 |
| DE12 | 0.34 | 5.5 |

**Table 2. Values of rate constant $k_2$ for the reaction of S(IV) in mixtures of glucose, maltose and oligosaccharide mixtures (180 g litre$^{-1}$) with glycine (0.5 M) at pH 5.5 (acetate buffer) and 100°C. The standard deviation $\sigma_{n-1}$ was obtained from n measurements. The significance of $k_2$ is shown in Fig. 1 whilst the specifications of oligosaccharide mixtures DE40, DE21 and DE12 are illustrated in Fig. 2.**

| Sample | $k_2$ (min$^{-1}$) | $\sigma_{n-1}$ | $n$ |
|---|---|---|---|
| Glucose | 0.035 | 0.003 | 4 |
| Maltose | 0.029 | 0.007 | 5 |
| DE40 | 0.030 | 0.006 | 5 |
| DE33 | 0.028 | 0.002 | 3 |
| DE21 | 0.023 | 0.005 | 4 |
| DE12 | 0.030 | 0.010 | 3 |

$k_1'$, the correlation between these is poor and it is not possible to conclude that the two rate constants are mechanistically connected. However, only $k_1'$ is expected to have any significance in relation to browning because $k_1''$ is a special feature of the S(IV)-containing system.

If one assumes that the concentration of reducing sugar and glycine remain constant whilst the loss of S(IV) is being observed, i.e. [sugar] and [glycine] $\gg$ [S(IV)], the rate, $k_1'$, of the first rate-determining step in the reaction is constant throughout the reaction. For the glucose–glycine reaction, it has been demonstrated that the conversion of intermediate I1 (DH) to the species which reacts with S(IV) is of first order with respect to the concentration of this intermediate (Wedzicha & Kaban, 1986). It is not unreasonable to expect that this reaction of intermediates formed from maltose and the oligosaccharide mixtures will also be of first order; i.e. the rate of the subsequent reaction depends on a rate-determining step which involves a molecule of the intermediate. In this situation, and given that a steady-state is set up in which the rate of formation of *I*1 equals its rate of reaction, when a constant rate of loss of S(IV) is observed,

$$[I1]_{ss} = k_1/k_2$$

where $[I1]_{ss}$ is the steady-state concentration of the intermediate (Wedzicha, 1984). A simple graphical approach (Wedzicha & Garner, 1991) to obtain the value of $[I1]_{ss}$ is illustrated in Fig. 5, where this value is

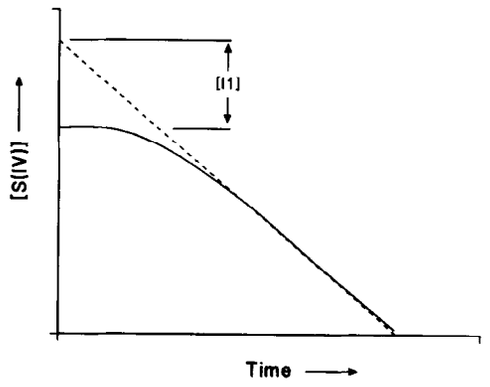



**Fig. 5.** Graphical method to measure the steady-state concentration of intermediate *I*1, $[I1]_{ss}$.

the difference between the value of the intercept ($t = 0$) of a line drawn through the constant rate phase of the reaction, and the initial S(IV) concentration. Thus, the value of $k_2$ is obtained by dividing $k_1$ by $[I1]_{ss}$ from kinetic data obtained at any initial S(IV) concentration. Values of $k_2$ are given in Table 2. That for glucose (0.035 min$^{-1}$) is slightly greater than for the other reactions (range 0.023 –0.030 min$^{-1}$) and, with this exception, no trend in the data can be discerned. Whilst the relative values of $k_1$ and $k_2$ change with the degree of polymerisation of the oligosaccharide, the most significant reason for this change is in the value of $k_1$.

There exists an excellent relationship between the value of $k_1'$ and the concentration of reducing groups in the reaction mixtures as illustrated in Fig. 6; these data include also the values for glucose and it may be concluded that for the first rate-determining step in their reaction with glycine, the reducing moieties in all the carbohydrate samples are equally reactive, irrespective of whether or not there is a substituent (glycosidic bond) in position-4.

It could be questioned as to whether or not the glucose and maltose present in the oligosaccharide mixtures have a dominant effect on the reaction rate;

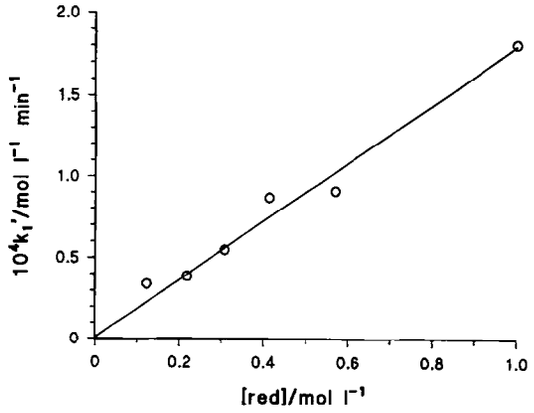



**Fig. 6.** Relationship between the concentration, [red], of reducing groups and the rate constant, $k_1'$, for the reaction of S(IV) with glucose + glycine, maltose + glycine or oligosaccharide + glycine. Reaction conditions: [carbohydrate] = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. The experimental points, in increasing order of [red] are for DE12, DE21, DE33, DE40, maltose, and glucose, respectively.

**Table 3. Contribution of glucose, maltose and oligosaccharides to the value of the rate constant $k_1'$ for the reaction of oligosaccharide mixtures + glycine with S(IV). Reaction conditions: [oligosaccharide mixture] = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. The detailed oligosaccharide distributions are shown in Fig. 2. The units of $k_1'$ are mol litre$^{-1}$ min$^{-1}$**

| Oligosaccharide mixture | DE40 | DE21 | DE12 |
|---|---|---|---|
| [Glucose]/mol litre$^{-1}$ | 0.16 | 0.020 | 0.010 |
| [Maltose]/mol litre$^{-1}$ | 0.063 | 0.032 | 0.016 |
| $10^4\ k_1'$ (glucose) | 0.288 | 0.036 | 0.018 |
| $10^4\ k_1'$ (maltose) | 0.057 | 0.029 | 0.015 |
| $10^4\ k_1'$ (measured) | 0.87 | 0.39 | 0.34 |
| Oligosaccharide reaction (%) | 60 | 83 | 90 |

the results shown in Fig. 6 could simply reflect the changing composition with respect to these two components. The contributions of glucose and maltose to the value of $k_1'$ were obtained from their known concentrations in samples of DE40, DE21 and DE12 and the values of $k_1'$ for the individual reactions of these sugars with glycine S(IV). The following assumptions were made: (a) the rate of the reaction in question is of first order with respect to glucose and maltose, as previously reported, and (b) the overall rate of reaction of mixtures of glucose, maltose and the oligosaccharides is the sum of the individual reaction rates. Table 3 gives a summary of this calculation for the oligosaccharide mixtures, from which we see that the contribution from components with three or more glucose residues is very significant, increasing to 90% of the rate of S(IV) loss in the case of the DE12 sample. This estimate supports the view that the intrinsic reactivity of the reducing groups of all the saccharide components is essentially the same.

Absorbance–time data for the 'browning' of all the reaction mixtures referred to above, but in the absence of S(IV), are illustrated in Fig. 7 for measurements at 420 nm, but similar behaviour was observed at all wavelengths in the range 420–490 nm. The rate of browning was obtained as the slope of each curve when the absorbance value was in the range 1–2 and the curves approximated to straight lines. Figure 8 demonstrates the excellent correlation between this rate of browning and $k_1'$, and is typical of the results at 450, 470 and 490 nm. This implies that $k_1'$ represents the rate-limiting step both in the reaction of S(IV) and in the browning reaction, once browning is under way. If this model is accepted, it follows that the extinction coefficients of the reaction products from the various carbohydrate samples are the same. The only mixture which does not conform to this result is that containing maltose alone; replicate measurements of oligosaccharide and maltose browning were highly reproducible and it appears that, for some reason, the browning of maltose is slower than predicted from the reducing group reactivity. The latter is in line with that of the other saccharide samples. The fact that oligosaccharide mixtures behave according to the correlation shown in Fig. 8, despite containing maltose, does not throw doubt on the result for maltose alone; the contribution from maltose to the overall rate of reaction of the oligosaccharide mixtures with S(IV) (given in Table 3) is only some 4–8%. It is reasonable to expect its contribution to browning to be small.

The most likely mechanism whereby coloured products with the same extinction coefficients are formed from different oligosaccharides is by way of a common intermediate. In the case of the glucose–glycine–S(IV) reaction, the amine-assisted dehydration of glucose gives DH after the first rate-determining step; the corresponding products from the oligosaccharides are 4-substituted-3-deoxyhexosuloses, where the substituent in position 4 is the remainder, *G*, of the oligosaccharide linked by a glycosidic bond. The conversion of the 4-substituted osulose to DDH, by the elimination of the saccharide residue would be analogous to the loss of a water molecule as DH is converted to DDH, as follows:

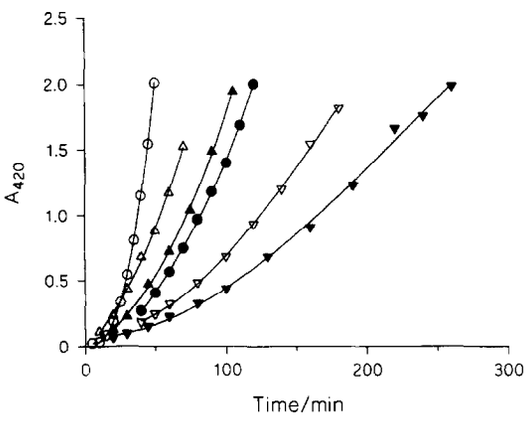



**Fig. 7.** Absorbance–time data obtained at 420 nm for the browning of glucose + glycine, maltose + glycine or oligosaccharide mixture + glycine. Reaction conditions: [carbohydrate] = 180 g litre$^{-1}$, [glycine] = 0.5 M, pH 5.5 (acetate buffer), 100°C. Glucose ○; maltose ●; DE40 △; DE33 ▲; DE21 ▽; DE12 ▼.

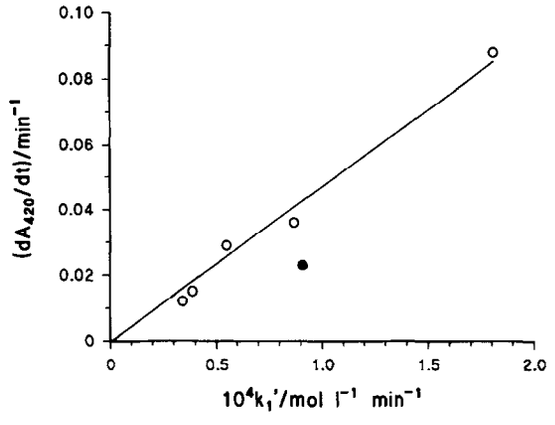
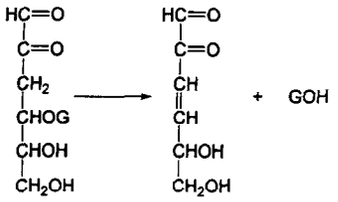



**Fig. 8.** Relationship between the rate of increase of absorbance at 420 nm and the rate constant, $k_1'$, for the reaction of S(IV) in glucose + glycine and oligosaccharide + glycine mixtures. The experimental points (open symbols) in order of increasing $k_1'$ value, are for DE12, DE21, DE33, DE40 and glucose, respectively. The closed symbol is the result for maltose. The reaction conditions for the determination of $k_1'$ are as in Fig. 6 and those for absorbance measurements as in Fig. 7.




DDH is the most reactive known intermediate in Maillard browning and is also the species which is reactive towards S(IV). It is of interest that, at 200°C in the absence of amino acid, the rate of formation of HMF increases in the order maltotriose > maltose > glucose. (Kroh, 1994). The present investigation has indicated that both rate-determining steps in the mechanism leading to the reaction of S(IV) with the oligosaccharides shown in Fig. 1, have rate constants which are not sensitive to the nature of the saccharide component. However, previous work on the DH–glycine–S(IV) reaction (Wedzicha & Kaban, 1986) has indicated that $k_2$ depends also on the concentration of glycine. In the presence of glycine the conversion of *I*1 to *I*2 could involve an interaction between *I*1 and the amino acid. Different mechanisms could well operate in the absence and presence of glycine.

## CONCLUSION

As with any kinetic approach to the study of complex reaction mechanisms, the conclusions of the present investigation are linked to the model which has been used to evaluate the rate constants. At the most elementary level of interpretation, the use of S(IV) to trap reactive intermediates and, thereby, provide quantitative information about their rate of formation, is unambiguous and constitutes a pragmatic approach with few assumptions. On the other hand, the detailed significance of $k_1'$, $k_1''$ and $k_2$, requires faith in the proposed kinetic model. This is, in fact, the most straightforward combination of reaction steps which can be used to explain the observed kinetics and, by virtue of its simplicity, is the most reliable model which could be envisaged.

It is concluded that glucose, maltose and mixtures of oligosaccharides react with glycine, up to the stage at which S(IV) scavenges the reactive intermediates, at a rate independent of saccharide structure but dependent on the concentration of reducing groups in the reaction mixtures. The rate of the first step in this reaction correlates well with the rate of browning of glucose and the oligosaccharide mixtures, but not of maltose alone. Thus, with this exception, a rate-determining step in the browning of oligosaccharides has been measured by this novel approach.

## ACKNOWLEDGEMENTS

The authors are grateful to Dalgety plc for an Advanced Course Studentship to one of us (CK) and particularly to Dr J. Mlotkiewicz for keen interest in the project and helpful discussion. They also appreciate the help of B. A. Borrill, J. Fish and P. K. Kajda with the supervision of the project.

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