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# Effect of glycerol on the kinetics of the glucose–glycine–sulphite reaction

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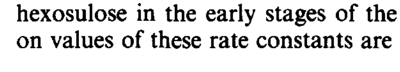
The kinetics of loss of sulphite species, S(IV), from mixtures of glucose, glycine and S(IV) were measured in aqueous solution containing varying amounts of glycerol in the range 0–81·5% (w/w) corresponding to water activities, $a_w$, in the range 1·0–0·45. The data are used to obtain rate constants for the S(IV)-independent and S(IV)-dependent reactions leading to the formation of 3-deoxyhexosulose in the early stages of the glucose–glycine reaction. The effects of $a_w$ on values of these rate constants are discussed.

## INTRODUCTION

Kinetic studies of the loss of sulphite species, S(IV), in the Maillard reaction of glucose and glycine indicate two consecutive rate-determining steps. The first corresponds to a reaction which is catalysed by S(IV) (Wedzicha & Vakalis, 1988), and has been identified as the formation of 3-deoxyhexosulose, DH (Wedzicha & Garner, 1991); the second is the conversion of DH to an intermediate which reacts rapidly with S(IV) to form 3,4-dideoxy-4-sulphohexosulose, DSH. It is possible that the second rate-determining step is the dehydration of DH, because DSH is formed by nucleophilic addition of sulphite ion to 3,4-dideoxyhexosulos-3-ene, DDH. The overall reaction may be described by the following kinetic model:



Rate constants $k'_1$ and $k'_2$ may be obtained by measuring the rate of loss of S(IV), since

$$\text{Rate} = k'_1 + k'_2\,[\text{S(IV)}]$$

When the concentrations of glucose and glycine are large compared with that of S(IV), $k'_1$ and $k'_2$ are pseudo-zero and -first order rate constants, respectively (Wedzicha & Vakalis, 1988). Such conditions are usually adopted when studying the sulphite-inhibited Maillard reaction.

Here we describe the results obtained by measuring the rate of reaction of S(IV) in aqueous glycerol–glucose–glycine–S(IV) mixtures to determine the effect of water activity, $a_w$, on the kinetics of the reaction step leading to the formation of DH.

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## MATERIALS AND METHODS

Reaction mixtures contained glucose (0·2–1·0 M), glycine (0·1–0·5 M), S(IV) (7·8–91 mM) and glycerol (0–81·5 wt%), and were kept in a thermostatically controlled bath at 55°C. The pH was adjusted to pH 5·5 with NaOH. The total S(IV) content of reaction mixtures was determined iodometrically (McWeeny *et al.*, 1969). The $a_w$ of water–glycerol–glucose–glycine–S(IV) mixtures was determined (Labuza, 1984) by allowing samples of the mixtures to come to equilibrium over saturated solutions of the following salts: $MgCl_2$, $a_w = 0{\cdot}30$; NaBr, $a_w = 0{\cdot}50$; $NaNO_3$, $a_w = 0{\cdot}68$; KCl, $a_w = 0{\cdot}81$; and $H_2SO_4$: $H_2O$ mixtures (Murrel & Scott, 1966), at 55°C. The change in weight of the samples was plotted vs $a_w$, and the $a_w$ of the reaction mixture taken as the intercept (i.e. zero weight change).

## RESULTS AND DISCUSSION

The rate of the glucose–glycine–S(IV) reaction was taken as the rate of loss of total S(IV), which was constant over the major part of the reaction (Wedzicha & Vakalis, 1988). Figures 1–3 show that when the reaction medium contains 81·5% (w/w) glycerol, the reaction is of first order with respect to glucose and glycine and obeys the following rate equation:

$$\text{Rate} = (k_1 + k_2\,[\text{S(IV)}])[\text{glucose}][\text{glycine}]$$

where the concentrations are those initially present in

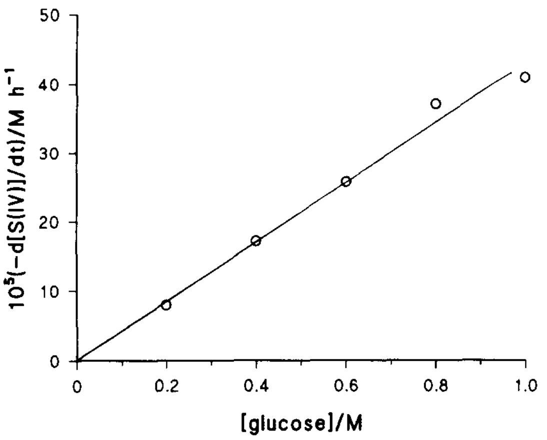


**Fig. 1.** The effect of glucose concentration on the rate of S(IV) loss from mixtures of glucose (0·2–1·0 M), glycine (0·5 M) and S(IV) (0·046 M), containing glycerol (81·5%, w/w, $a_w = 0{\cdot}45$), initial pH 5·5, 55°C.

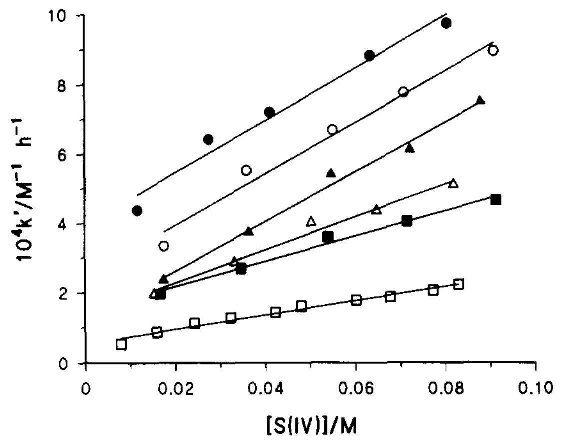


**Fig. 3.** The effect of S(IV) concentration on the pseudo-second order rate constant $k'$ ($k'$ = rate/[glucose][glycine]) at varying concentrations (%, w/w) of glycerol: □, zero, $a_w = 1{\cdot}0$; ■ 40·0%, $a_w = 0{\cdot}85$; △ 46·9%, $a_w = 0{\cdot}79$; ▲ 60·6%, $a_w = 0{\cdot}70$; ○ 70·3%, $a_w = 0{\cdot}64$; ● 81·5%, $a_w = 0{\cdot}45$. Reaction conditions; initial pH 5·5, 55°C.

the reaction mixtures. Since this rate law is also obeyed when the solvent contains only water, it is suggested that the above rate law applies to all mixtures of glycerol + water used in the present investigation, i.e. there is no change in mechanism as glycerol is added. Control experiments containing 81·5% glycerol but no glucose show that, on the timescale of the kinetic experiments described here, no S(IV) is lost due to reactions involving glycerol alone.

Rate constants $k_1$ and $k_2$ were obtained from graphs of the pseudo-second order rate constant $k'$ vs [S(IV)] shown in Fig. 3 where

$$k' = \text{rate/[glucose][glycine]} = k_1 + k_2[\text{S(IV)}]$$

These values are plotted as a function of $a_w$ in Figs 4 and 5 respectively. Standard deviations of the intercepts and slopes of the lines in Fig. 3 were used to generate error bars for the rate constants. The error bars in the $a_w$ direction represent ranges of values determined for each set of reaction mixtures. Measurements involved the use of three samples at the highest and lowest S(IV) concentrations, and at the start and end of the kinetic runs. It was found that the measured $a_w$ (to 2 significant figures) did not change from the start to the end of the period during which the progress of the reaction was followed. Hence, it may be assumed that $a_w$ was invariant during the course of a kinetic run.

The rate constant of the S(IV)-independent formation of DH, $k_1$, is seen to increase as $a_w$ is reduced to 0·45 but the graph does not pass through a maximum in the range of $a_w$ studied. However, since no chemical reaction is likely in an anhydrous system, the graph of $k_1$ vs $a_w$ would show a turning point at $a_w < 0{\cdot}45$.

Eichner and Karel (1972) demonstrated that the extent of browning of glucose–glycine mixtures in glycerol + water depended on the concentration of water when measurements were carried out at constant glycerol content. On the other hand, glycerol acted as a diluent when its concentration was increased while keeping that of water constant. However, the reduction in extent of browning by glycerol was greater than that which could simply be attributed to dilution of the reactants. It seems, therefore, that glycerol is not as good a solvent as water for the overall browning

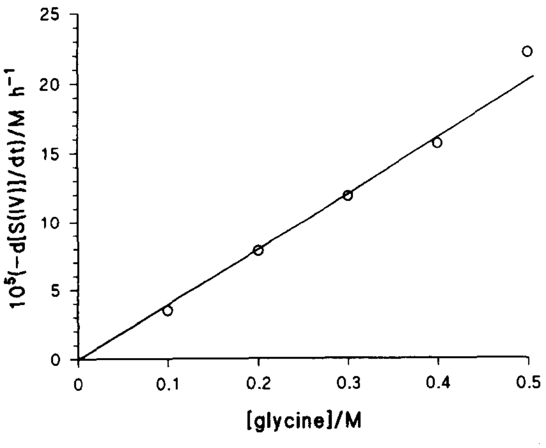


**Fig. 2.** The effect of glycine concentration on the rate of S(IV) loss from mixtures of glucose (0·5 M), glycine (0·1–0·5 M) and S(IV) (0·045 M), containing glycerol (81·5%, w/w, $a_w = 0{\cdot}45$), initial pH 5·5, 55°C.

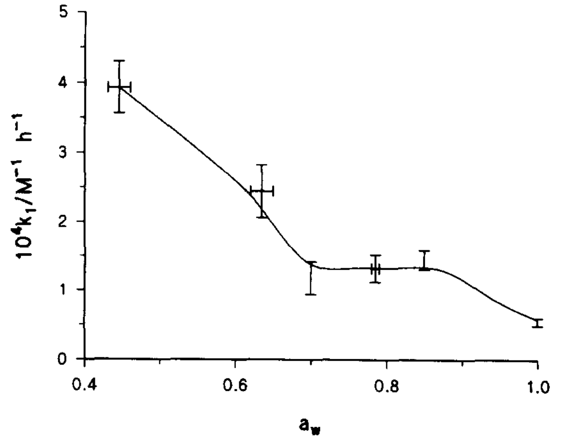


**Fig. 4.** The effect of water activity, $a_w$, on the rate constant, $k_1$, of the S(IV)-independent formation of DH from glucose + glycine. Values of $k_1$ are the intercepts of the lines in Fig. 3.

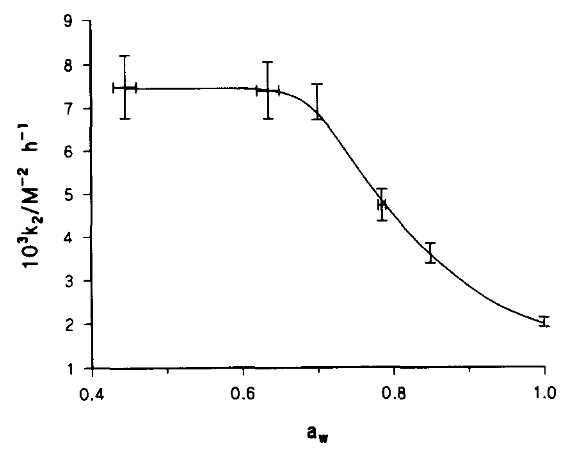


**Fig. 5.** The effect of water activity, $a_w$, on the rate constant, $k_2$, of the S(IV)-dependent formation of DH from glucose + glycine. Values of $k_2$ are the slopes of the lines in Fig. 3.

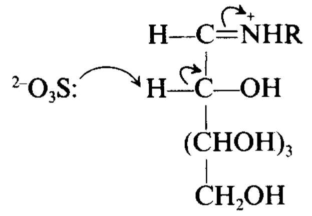
reaction. In the present investigation the reduction of $a_w$ was accompanied by an increase in the concentration of glycerol; this should give rise to an underlying tendency for the rate of reaction, i.e. $k_1$, to be reduced. However, in the range $1{\cdot}0 > a_w > 0{\cdot}7$ this tendency is approximately in balance with the effect, on rate, of the accompanying reduction of the concentration of water. At $a_w < 0{\cdot}7$, the effect of water content probably dominates, giving rise to the marked increase in $k_1$. It is interesting to compare the behaviour of $k_1$ in the range $0{\cdot}7 > a_w > 0{\cdot}45$ with the extent of browning plotted as a function of $a_w$ by Eichner and Karel (1972). The absorbance of glucose–glycine mixtures at 420 nm increased markedly (3·2-fold) in this range; the increase in $k_1$ occurs over the same range of $a_w$ values and is of similar magnitude. At this stage the similarity can only be regarded as fortuitous because Eichner and Karel used substantially more concentrated solutions (*c.* 10 M glucose) and no pH control was included in the procedure. It is, however, reasonable to conclude that the effect of $a_w$ is at least in part due to the effect of water concentration on a reaction step in which water is liberated, e.g. the initial formation of a Schiff base.

The situation in the presence of S(IV) is likely to be more complex. In aqueous solution, S(IV) exists as a mixture of $SO_2$, $HSO_3^-$, $SO_3^{2-}$ and $S_2O_5^{2-}$. Glycerol is a particularly useful choice of humectant for reactions involving S(IV) because addition of it to solutions of $HSO_3^-$ has a relatively small effect on the p$K_a$ of this acid (Wedzicha & Goddard, 1991). However, the law of mass action predicts that a reduction in $a_w$ should cause the following equilibrium for the formation of $S_2O_5^{2-}$ to be displaced to the right:

$$2\ HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O$$

It has been suggested (Wedzicha, 1987) that $S_2O_5^{2-}$ might be a better catalyst of the formation of DH than the other S(IV) species. The possible displacement of the above equilibrium in favour of $S_2O_5^{2-}$ could, therefore, contribute to the observed increase in $k_2$ with glycerol content, illustrated in Fig. 5. However, UV and FTIR spectroscopic data obtained on solutions of $NaHSO_3$ in water–glycerol mixtures (Wedzicha *et al.*, 1992) suggest that less, rather than more, $S_2O_5^{2-}$ is formed on increasing the glycerol content. It seems, therefore, that in this water-humectant system the effect of non-electrolyte is on the catalysis of the glucose–glycine reaction by $SO_2$, $HSO_3^-$ or $SO_3^{2-}$.

The only plausible explanation for the catalytic behaviour of S(IV), available to date, is that sulphite ion assists in the conversion of glucosylamine to ketosamine by removing a hydrogen ion at position 2 of the protonated glycosylamine (Wedzicha & Vakalis, 1988), i.e.



Since the tendency for conversion of $SO_3^{2-}$ to $HSO_3^-$ is relatively insensitive to glycerol content (Wedzicha & Goddard, 1991), the effect of the medium on the catalysis of the reaction by S(IV) could be due to the transition state illustrated above becoming energetically more favourable as the water content is reduced. A reduction in the number of discrete ionic species and the delocalisation of charge over an extended system of bonds occurs as reactants are converted to this transition state; this is expected to reduce the overall requirement for solvation, and the proposed association of $SO_3^{2-}$ with the protonated glycosylamine could, therefore, be enhanced by a reduction in water content.

## CONCLUSION

A method of measuring the effect of glycerol on the rate constant of a kinetically significant early step in the Maillard reaction is described. It is based on the use of S(IV) to suppress subsequent browning reactions and the result is mechanistically less ambiguous than any inferences made from kinetic measurements of colour formation.

Regardless of the description of the mechanisms whereby glycerol affects the values of $k_1$ and $k_2$, it is clear that a small reduction in $a_w$, e.g. to 0·7–0·8, causes a significant enhancement of the S(IV)-dependent reaction but leaves the S(IV)-independent process relatively unaffected. Such differential behaviour must be taken into account in any kinetic model of the reactivity of S(IV) during food dehydration.

## ACKNOWLEDGEMENT

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