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Kinetics of the sulphite-inhibited browning of fructose: effect of buffer

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The rate of loss of S(IV) in mixtures of fructose–glycine–S(IV) and fructose–S(IV) is increased by the presence of acetate buffer. The conversion of fructose into a reaction intermediate is catalysed by the buffer components. In the presence of glycine, catalysis is due to the acetate ion, and the effect of glycine and acetate on the rate of loss of S(IV) may be additive. The pH of the reaction mixture was found to have a small effect on the rate of S(IV) loss; the order with respect to $[H^+]$ is *c.* 0·4.

INTRODUCTION

The kinetics of the reaction of S(IV) in mixtures of fructose–glycine–S(IV) at pH 5·5 and 55°C were described in a previous paper (Swales & Wedzicha, 1992). The reaction was seen to be of first order with respect to fructose and S(IV) and 1·78 order with respect to glycine. Two distinct mechanisms were identified; one involves only fructose in the rate-determining step, the other requires fructose and both the glycine and S(IV). The noninteger order with respect to glycine was explained by a combination of first- and second-order behaviour.

The Maillard reaction tends to be inhibited at low pH and accelerated by high pH. The rate of browning is increased by the presence of buffer. Lewis *et al.* (1949) found that nitrogen-free carboxylic acids and their salts accelerated the rate of browning of sugars in the absence of amino acids. Burton & McWeeny (1963) observed an increase in browning rate for mixtures of sugar and amino acid in the presence of phosphates. Studies on the effect of added buffer salts on the nonenzymic browning reactions have led to the suggestion that the mode of action of buffers is two-fold (Saunders & Jervis, 1966):

(i) Under mildly alkaline conditions, buffers reduce the fall in pH caused by acidic reaction products, thus helping maintain a higher reaction rate.
(ii) In acid solution the buffer takes part in general acid–base catalysis.

Reynolds (1959) suggested that the Amadori rearrangement of glucosylamines to 1-amino-1-deoxyfructoses, and the subsequent decomposition of the latter is subject to general acid–base catalysis. More recent studies on the effect of phosphate species on the rate of the Maillard reaction showed phosphate to act as a base catalyst during the Amadori rearrangement. Moreover, the effect of the inorganic phosphate as a general base catalyst was shown to follow first-order kinetics, resulting in an increase in the rate of conversion of reactants (Potman & Van Wijk, 1988). We describe here the effects of acetate buffer, and the individual components of the buffer (i.e. sodium acetate and acetic acid), on the kinetics of the fructose–glycine–S(IV) reaction. The effect of pH, which is tied into the effect of buffer components, is also investigated.

MATERIALS AND METHODS

Reaction mixtures were prepared containing fructose (1 M), glycine (0·5 M) and S(IV) (0·0482–0·0554 M) in three series of acetate buffer solutions. One contained a constant acetic acid: acetate ratio, but varying total concentration (0·2–1·0 M). The pH was adjusted to either 4·5 or 5·5 using acetic acid (1·74 M). The second was made up in acetate buffer with varying acetate (0·45–0·05 M) and acetic acid (0·05–0·45 M) concentrations such that the final buffer concentration was 0·5 M. The third series of reaction mixtures was made up in sodium acetate (0·25 M) and the initial pH adjusted to 4·2, 4·7, 5·2 and 5·7, using acetic acid (1·74 M). The series of experiments at pH 5·5 was repeated with glycine absent from the reaction mixtures. Mixtures were sealed in all-glass ampoules and heated at 55 ± 0·1°C. Ampoules were opened and analysed at timed intervals. The S(IV) concentration was determined spectrophotometrically (Humphrey *et al.*, 1970), using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent).

RESULTS AND DISCUSSION

Effect of changing acetate concentration

Concentration–time plots of residual S(IV) were essentially linear over the major part of the reaction, and the rate was measured as the slope.

The rate of loss of S(IV) was plotted as a function of the buffer concentration ([acetic acid] + [acetate]) at pH 4·5 and 5·5 as illustrated in Fig. 1. Initial observations indicate that the fructose–glycine–S(IV) reaction proceeds in the absence of buffer, as the graphs shown in Fig. 1 have significant intercepts, assuming that the rate–concentration data are linear to zero concentration. The presence of the buffer causes an increase in the rate of S(IV) loss.

For a reaction subject to acid–base catalysis, the overall equation for the effect of buffer on the reaction rate is

$$-\mathrm{d}[\mathrm{S(IV)}]/\mathrm{d}t = k_1 + k_2[\mathrm{HA}] + k_3[\mathrm{A}] \tag{1}$$

where k_1 is the rate constant for the buffer-independent reaction. HA and A represent acetic acid and acetate ion, respectively, and k_2 and k_3 the rate constants for the acetic acid- and acetate-catalysed reactions, respectively. Figure 1 indicates that the relationship between rate and buffer concentration is given by

$$-\mathrm{d}[\mathrm{S(IV)}]/\mathrm{d}t = k_1 + k[\mathrm{Buffer}] \tag{2}$$

where k is the rate constant for the buffer-catalysed reaction. For experiments carried out at constant pH, the ratio [HA]/[A] should be relatively constant throughout the measurements and may be calculated from

$$\mathrm{pH} = \mathrm{pK} - \log_{10}[\mathrm{HA}]/[\mathrm{A}] \tag{3}$$

where pK for acetic acid = 4·76. Let [HA]/[A] = r and eqn (1) becomes

$$-\mathrm{d}[\mathrm{S(IV)}]/\mathrm{d}t = k_1 + (k_2 r + k_3)[\mathrm{A}]. \tag{4}$$

Comparison of eqn (4) with eqn (2) gives

$$k(r + 1) = k_2 r + k_3 \tag{5}$$

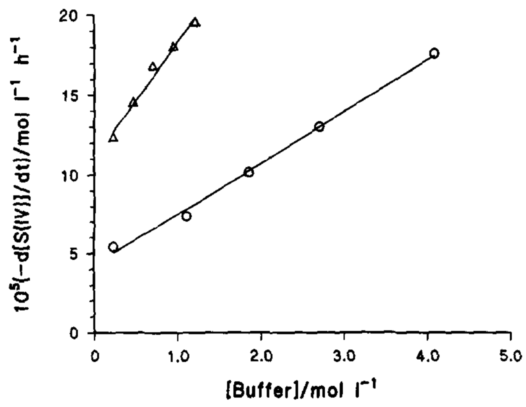

Fig. 1. Rate of S(IV) loss vs. [buffer] for reaction mixtures containing fructose (1 M), glycine (0·5 M), S(IV) (0·0466–0·0554 M), in acetate buffer at (○) pH 4·5 and (△) pH 5·5, heated at 55°C.

The value of k at the two pH values is the gradient of the lines in Fig. 1, and simultaneous solution of eqn (5) at the two pH values gives $k_2 = 0$ and $k_3 = 8{\cdot}82 \times 10^{-5}\ \mathrm{h^{-1}}$. These results indicate that the acetic acid has no effect on rate, whilst the effect of acetate is to catalyse the reaction. Hence, eqn (1) may be simplified to

$$-\mathrm{d}[\mathrm{S(IV)}]/\mathrm{d}t = k_1 + k_3[\mathrm{A}] \tag{6}$$

and a graph of rate of S(IV) loss versus [A] at pH 4·5 and pH 5·5 is shown in Fig. 2.

Contrary to expectation, both graphs appear as shallow curves. The difference in curvature is possibly due to the high concentration (3 M) of acetic acid, particularly when the acetate concentration is high. It is possible that a small, and as yet undetected effect of acetic acid on reaction rate exists and is responsible for the observed curvature. In order to minimise the possible effect of the acetic acid, the slopes of the graphs were estimated as the concentration approached zero. The rate constants for the effect of acetate ion are given in Table 1. The similarity in values of k_3 confirms that the effect of acetate is pH-independent, whilst there is a small effect of the 10-fold change in [H$^+$] on k_1.

Any contribution from acetic acid may be deduced from Fig. 2 and is the difference between the experimental rates and those predicted from eqn (6), using rate constants from Table 1. At the highest concentration of acetic acid (i.e. 1 M acetate) the contribution from acetic acid was found to be equivalent to a first order rate constant $k_2 = 1{\cdot}1 \times 10^{-6}\ \mathrm{h^{-1}}$. The rate constant for the effect of acetate is c. $10^{-4}\ \mathrm{h^{-1}}$ and the value for the effect of acetic acid may be considered negligible. For this reason it was unnoticed in the preliminary analysis of the data. We can conclude, therefore, that the acetate ion is

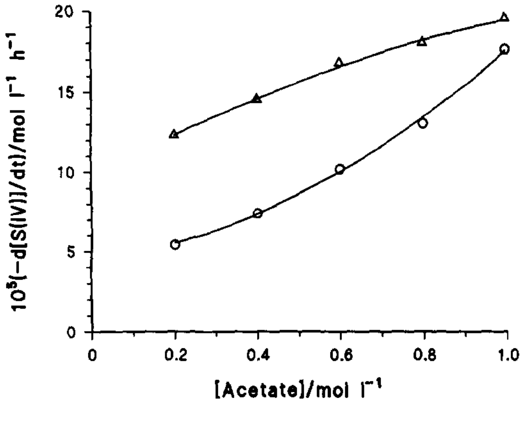

Fig. 2. Rate of S(IV) loss vs. [acetate ion] in buffer for reaction mixtures containing fructose (1 M), glycine (0·5 M), S(IV) (0·0466–0·0554 M) at (○) pH 4·5 and (△) pH 5·5, heated at 55°C.

Table 1. Improved values of rate constants k_1 and k_3 for the buffered fructose–glycine–S (IV) reaction

pH	$10^5 k_1$ (mol litre^{-1} h^{-1})	$10^5 k_3$ (h^{-1})
4·5	3·4	9·9
5·5	2·5	9·3

Table 2. Values of rate constants k_1 and k in the presence and absence of glycine

	$10^5 k_1$ (mol litre^{-1} h^{-1})	$10^5 k$ (h^{-1})
Presence of glycine	11	7·3
Absence of glycine	3·7	4·0

the significant buffer component responsible for catalysing the fructose–glycine–S(IV) reaction.

The rate constants k_1 and k for the reaction in the presence and absence of glycine at pH 5·5 are given in Table 2. These demonstrate that, in the absence of glycine, the reaction rate is further enhanced by increasing the concentration of buffer. It has already been established that S(IV) does not catalyse the conversion of fructose to intermediates in the absence of glycine (Swales & Wedzicha, 1992). Consequently, the effect observed here must be attributed solely to the buffer, or one is seeing a reaction which involves fructose, S(IV) and buffer, where the buffer ions replace the amino acid. Such a situation would arise in the event that the function of the amino acid was simply that of an acid–base catalyst. It appears, therefore, that the 'spontaneous' conversion of fructose into an intermediate is catalysed by the buffer, though no data are available to determine which of the buffer components is responsible for this catalysis. When glycine is present in the reaction mixture the values of k_1 and k are increased.

Dependence of the fructose–glycine–S(IV) reaction on pH

Any contribution of H^+ to the kinetics is included in k_1 (eqn(1)) and thus,

$$k_1'[H^+]^n = -d[S(IV)]/dt - (k_2[HA] + k_3[A]) \quad (7)$$

where k_1' is the pseudo-order rate constant for the effect of $[H^+]$. Taking logarithms of both sides of the equation and plotting $-\log\{-d[S(IV)]/dt - (k_2[HA] + k_3[A])\}$ versus pH results in the graph shown in Fig. 3, whose gradient is the order with respect to [H+], 0·4. Such a kinetic dependence on $[H^+]$ could be the result either of a zero-order reaction running in parallel to one of higher order with respect to $[H^+]$, or the involvement of H^+ and OH^- with different weighting.

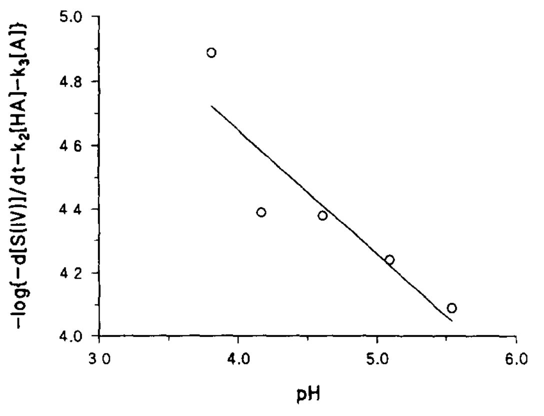

Fig. 3. Graph of $-\log\{-d[S(IV)]/dt - (k_2[HA] + k_3[A])\}$ vs. pH for reaction mixtures containing fructose (1 M), glycine (0·5 M), S(IV) (0·05–0·0524 M), in acetate buffer (0·25 M), pH 4·2–5·7, heated at 55°C.

The value of k_1 for reaction mixtures containing fructose, glycine and S(IV) is seen to decrease as the initial pH decreases, though it is not very significant since a 10-fold increase in $[H^+]$ causes a corresponding change in the value of k_1 by a factor of 1·36. The Lobry de Bruyn–Alberda van Ekenstein transformation (Speck, 1958) involves protonation of the carbonyl oxygen to give the 1,2-enol form, and hence, an increase in rate would be expected as the pH decreases. However, the equilibrium which exists in solution between the hemiacetal ring form and the open chain form of the fructose favours the ring form as the pH decreases (Burton & McWeeny, 1963) and, thus, the reaction is inhibited. The small kinetic effect of H^+ may be attributed to the magnitude of these two effects being similar.

The conversion of fructose into 3-deoxyhexosulose, the precursor of the intermediate which reacts irreversibly with S(IV), is likely to be subject to both general and specific acid–base catalysis. In the reaction mixtures there are a number of both acid and basic species which may catalyse the reaction. In addition to H_2O, OH_3^+ and OH^-, which are present as a result of the aqueous environment, the reaction mixtures contain glycine (mainly in its zwitterionic form, $^+NH_3CH_2COO^-$), acetate ion, hydrogen sulphite and sulphite ion. Although no direct evidence is available as to which of the species present in the reaction mixture is acting as the catalyst at any particular stage, it seems likely that all the catalytic species may be involved at some point.

CONCLUSION

The rate of S(IV) loss from reaction mixtures containing fructose and S(IV) is increased in the presence of acetate buffer. It has been proposed (Swales & Wedzicha, 1992) that fructose is converted into an intermediate by a pathway which involves both glycine and S(IV). The catalytic species at pH 4·5 to 5·5 is acetate ion and both the glycine-independent and glycine-dependent reactions are affected in this way. Apart from the effect of pH on rate as a result of changing buffer composition, the reaction is relatively insensitive to changes in $[H^+]$.

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REFERENCES

Burton, H. S. & McWeeny, D. J. (1963). Non-enzymic browning reactions: consideration of sugar stability. *Nature, Lond.*, **197**, 266–8.

Humphrey, R. E., Ward, M. H. & Hinze, W. (1970). Spectrophotometric determination of sulphite with 4,4'-dithiopyridine and 5,5'-dithiobis-2(nitrobenzoic acid). *Anal. Chem.*, **42**, 698–702.

Lewis, V. M., Esselen, W. B., Jr & Fellers, C. R. (1949). Non-enzymatic browning of foodstuffs. Nitrogen-free carboxylic acids in the browning reaction. *Ind. Engng Chem. (Anal. Edn)*, **41**, 2591–4.

Potman, R. P. & Van Wijk, T. A. (1989). Mechanistic studies of the Maillard reaction with emphasis on phosphate mediated catalysis. *ACS Symp. Ser. (1988)*, 182–95.

Reynolds, T. M. (1959). Chemistry of non-enzymic browning. III. Effect of bisulphite, phosphate and malate on the reaction of glycine and glucose. *Aust. J. Chem.*, **12**, 265–74.

Saunders, J. & Jervis, F. (1966). The role of buffer salts in non-enzymic browning. *J. Sci. Food Agric.*, **17**, 245–9.

Speck, J. C. Jr. (1958). The Lobry de Bruyn–Alberda van Ekenstein transformation. *Adv. Carbohydr. Chem.*, **13**, 63–103.

Swales, S. & Wedzicha, B. L. (1992). Kinetics of the sulphite-inhibited browning of fructose. *Food Addit. Cont.*, **9**, 479–83.