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# Kinetics of Autoxidation of Sulphur(IV) Oxospecies in Aqueous Ethanol

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## *ABSTRACT*

*The kinetics of autoxidation of sulphur(IV) oxospecies (S(IV)) in ethanol + water (3 + 1 v/v) solvent are reported. Oxidation only proceeds in the presence of metal ion and amine. When Mn(II) and glycine are added, kinetically significant complexes MnGly and* $MnGly_2$ *are formed and the initial rate of oxidation is of first order with respect to complex and S(IV) and of zero order with respect to oxygen. The overall reaction appears to be autocatalytic and is accompanied by the formation of* $^{\cdot}SO_2^-$ *radicals, probably a by-product of reaction. The implications of this reaction to food systems containing S(IV) are considered.*

## INTRODUCTION

It is well known that sulphur(IV) oxospecies (S(IV)), and particularly sulphite ion, react readily with oxygen in a metal ion-initiated free radical reaction (Wedzicha, 1984). This autoxidation also gives rise to oxidising intermediates which may cause the oxidation of organic compounds (Yang, 1984), an example of such an oxidation being the destruction of carotenoids (Peiser & Yang, 1979; Wedzicha & Lamikanra, 1983).

The free radicals formed during the autoxidation of S(IV) are scavenged effectively by alcohols. For example, in a photochemically induced autoxidation, a concentration of 0·1 mM ethanol is sufficient to reduce the depletion of sulphite ion to approximately 30% of its value in alcohol-free

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solution. Possible chain carriers in aqueous solution, which could react at a suitable rate with alcohols, are $SO_4^{\cdot-}$ and $^{\cdot}OH$ (Hayon *et al.*, 1972). It is not surprising, therefore, that aqueous–ethanolic solutions of S(IV) are particularly stable towards autoxidation.

In model studies on the oxidation of carotenoids in the presence of S(IV) it was noted that S(IV) could still undergo autoxidation in solvents containing a high proportion of ethanol (75–80% v/v) if Mn(II) and glycine were also present (Peiser & Yang, 1979; Wedzicha & Lamikanra, 1983). Since the radical chain propagators operating during autoxidation in aqueous solution are scavenged rapidly in the presence of ethanol, it is clear that the mechanism of autoxidation in the medium containing ethanol is likely to be somewhat different from that in water.

The aim of this paper is to report the kinetics of the autoxidation of S(IV) in aqueous ethanol as part of an evaluation of the usefulness of this solvent system for studies of S(IV)-mediated oxidations of organic substrates in homogeneous solution rather than as dispersions of particulate material in water.

## EXPERIMENTAL

### Measurement of rate of autoxidation of S(IV)

The rate of autoxidation of S(IV) in aqueous ethanol (ethanol + water, 3 + 1, v/v) was measured as the rate of loss of dissolved oxygen with no headspace above the reaction mixture. The reaction was therefore studied under homogeneous conditions. Kinetic experiments were carried out in the thermostatted cell compartment of a Clark-type oxygen electrode and emf was displayed on a chart recorder. The maximum reading on the recorder and the base line were set using solvent saturated with oxygen and containing sodium dithionite, respectively. The experiments were prepared by placing buffered reactants, saturated with oxygen, but without S(IV), into the cell and allowing the system to come to thermal equilibrium at 25°C. In all cases the concentration of buffer in the reaction mixture was 0·025M acetate, pH 5·7. The cell cover consisted of a plug which was screwed down to displace air in the headspace and the reaction started by injecting 50 μl of a solution of S(IV) through a narrow opening in the plug. The concentration of oxygen was then recorded as a function of time. In the fastest reaction observed all the oxygen had been consumed after some 15 s and in this case neither the response of the electrode nor that of the recorder was found to be limiting. All experiments were carried out at least three times.

In order to investigate the effect of oxygen concentration on the rate of reaction, the initial amount of oxygen present in the reaction mixture was reduced from saturation by passing nitrogen until the desired electrode response was obtained and the reaction started as before. Since the solubility of oxygen in the reaction medium is not known, all measurements of oxygen concentration are relative to the saturation value which was assigned a reading of 100% in each experiment.

### Esr experiments

In order to obtain evidence for the presence of free radical intermediates the reacting mixture was examined by esr spectroscopy using continuous and stop flow experiments. Reactant solutions were pumped by means of a two-channel peristaltic pump (2–60 ml/min total flow) to a mixer connected to the cell of a Varian E4 spectrometer operating at 9·36 GHz. The delay between mixing and entry into the cell was of the order of 0·15 s at the highest flow rate and the residence time in the cell during continuous flow experiments was similar. All experiments were carried out at 20°C.

## RESULTS

### Initial reaction

Aqueous solutions containing Mn(II) (50 μM), glycine (20 mM) and S(IV) (1 mM), pH 5·7 and saturated with oxygen showed no measurable loss of oxygen over a period of several hours. It is estimated that the concentration of oxygen in the saturated solution was of the order of 1·5 mM (solubility of oxygen in water = 3·16 ml $O_2$/litre at 25°C, 1 atm, Weast & Astle, 1982) and assuming that the stoichiometry of the reaction with S(IV) is given by:

$$SO_3^{2-} + \tfrac{1}{2}O_2 \rightarrow SO_4^{2-}$$

there is a three-fold molar excess of S(IV) over oxygen. The loss of oxygen may, however, be enabled by adding ethanol to the reaction medium as shown in Fig. 1, the initial rate of reaction increasing rapidly with increase in ethanol content above 30–40% v/v. All reaction mixtures were prepared using an acetate buffer (pH 5·7) of constant composition. Whilst the purpose of adding this buffer was to maintain a constant pH when the concentrations of S(IV) and glycine were varied, the actual pH and its significance in media containing a large proportion of ethanol are unknown. The effect of varying the concentration of ethanol on the actual pH is not readily predictable and the observed effect on rate should be

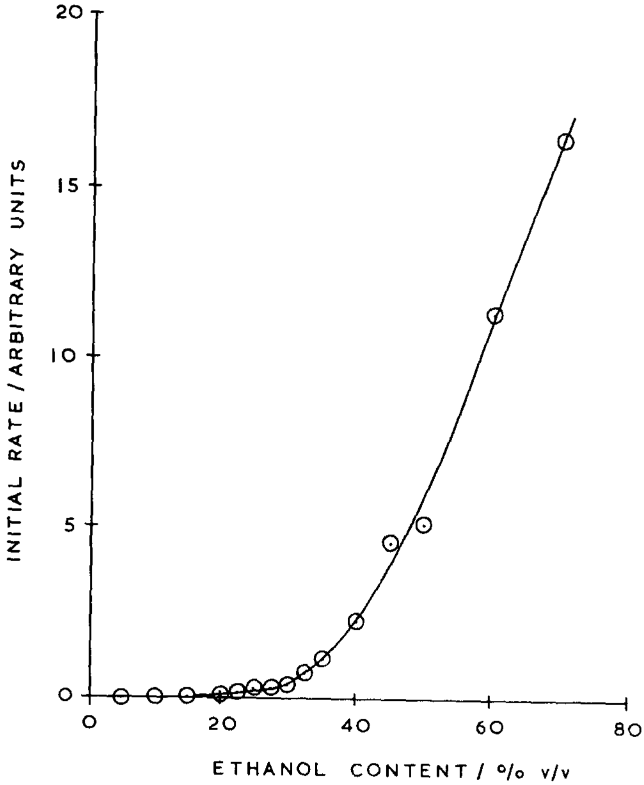


Fig. 1. Effect of concentration of ethanol on initial rate of autoxidation of S(IV). Initial conditions: [Mn(II)] = 50 μM, [glycine] = 20 mM, [S(IV)] = 1 mM, pH 5·7 acetate buffer 0·025 M, 25°C.

considered as a combination of pH and general medium effects. Control experiments, carried out in 75% ethanol, involving reaction mixtures from which Mn(II), glycine and S(IV) were omitted, one at a time, showed that all three components were essential for the reaction to proceed.

When the concentrations of Mn(II) and S(IV) were varied ([Mn(II)] = 5–50 μM, [S(IV)] = 1–4 mM, [glycine] = 1 mM, 75% ethanol), the initial rate of loss of oxygen was found to be of first order with respect to both reactants. However, variation of the concentration of glycine (0·5–5·0 mM) showed the kinetic behaviour to be complex, the initial rate changing with concentration as shown in Fig. 2. These data are consistent with there being a reversible reaction between Mn(II) and glycine to form a kinetically important complex and, over the range of concentrations used, the complex is partially dissociated. It is known that Mn(II) and glycine form 1:1 and 1:2 complexes in aqueous solution and that they are relatively unstable with respective pK values of $pK_1 = 2{\cdot}5–3{\cdot}5$ and $pK_2 = 4{\cdot}3–6{\cdot}6$ at 25°C (Martell, 1964, 1971; Perrin, 1979). In order to test whether the

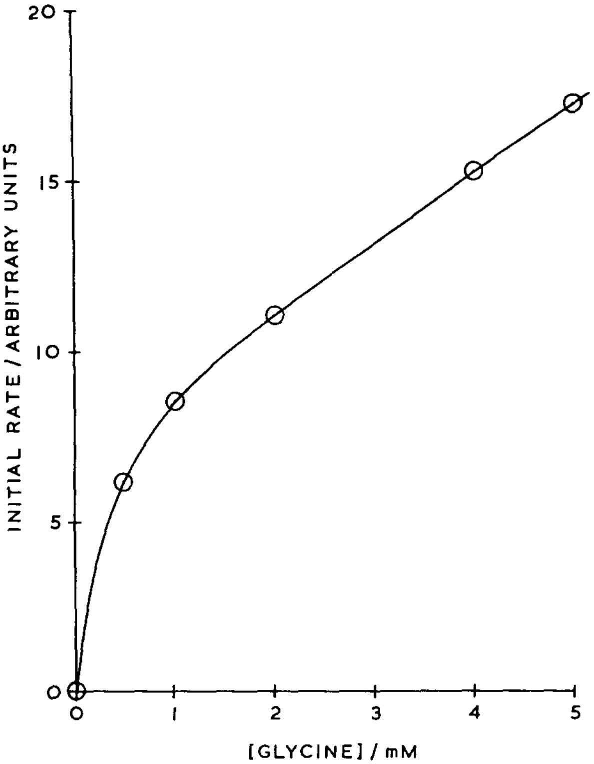


**Fig. 2.** Effect of concentration of glycine on initial rate of autoxidation of S(IV). Initial conditions: [Mn(II)] = 10 μM, [S(IV)] = 1 mM, pH 5·7 acetate buffer 0·025 M, 25°C.

complexes were kinetically important the concentrations of 1:1 and 1:2 adducts were calculated using nominal values of $pK_1 = 3{\cdot}0$ and $pK_2 = 5{\cdot}5$ for kinetic experiments with [Mn(II)] = 5–50 μM and [glycine] = 0·5–5·0 mM. The simplest general rate equation which describes catalysis by complex is as follows:

$$\text{rate} = k_1([\text{Mn(II)gly}] + a[\text{Mn(II)gly}_2])$$

where $k_1$ is a first order rate constant and $a$ is a measure of the relative efficiencies of the 1:1 and 1:2 complexes as catalysts. Table 1 shows experimental and predicted data for $k_1 = 1 \times 10^{-6}$ and $a = 2{\cdot}37$ where the units of $k_1$ are arbitrary. The agreement between observed and predicted data is considered to be very acceptable in view of the ten-fold range of concentration of both complexes involved. The value of $a$ shows that the 1:2 adduct is more than twice as effective as the 1:1 adduct in promoting oxidation. The initial reaction is therefore a result of a reaction which is of first order with respect to complex and S(IV).

The dependence of initial rate of loss of oxygen on the concentration of

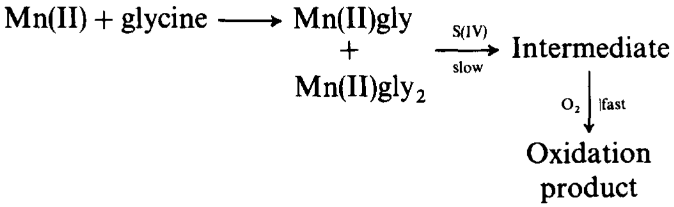
TABLE 1

Calculated and Observed Initial Rates of Autoxidation of S(IV) in 75% Ethanol

| *[Mn(II)]* (μM) | *[Glycine]* (mM) | *[Mn(II)Gly]* (μM) | *[Mn(II)Gly$_2$]* (μM) | *Predicted rate*[a] | *Observed rate*[b] |
|---|---|---|---|---|---|
| 10 | 0·5 | 3·15 | 0·50 | 4·1 | 6·1 |
| 10 | 1·0 | 4·31 | 1·36 | 7·5 | 8·5 |
| 10 | 2·0 | 4·70 | 2·96 | 11·7 | 11·0 |
| 10 | 4·0 | 3·99 | 5·03 | 15·9 | 15·2 |
| 10 | 5·0 | 3·61 | 5·68 | 17·1 | 17·2 |
| 5 | 1·0 | 2·16 | 0·68 | 3·8 | 3·9 |
| 25 | 1·0 | 10·8 | 3·35 | 18·7 | 17·1 |
| 40 | 1·0 | 17·2 | 5·29 | 29·8 | 30·4 |
| 50 | 1·0 | 21·5 | 6·56 | 37·1 | 34·1 |

[a] Arbitrary units. Predicted using: rate = $1 \times 10^{-6}$ ([MnGly] + 2·37[MnGly$_2$])
[b] Arbitrary units.

oxygen was investigated in mixtures with relative oxygen concentrations of 100%, 20% and 4%, all other conditions being kept the same ([Mn(II)] = 10 μM, [glycine] = 1 mM, [S(IV)] = 1·7 mM, 75% ethanol). The respective initial rates were found to be 3·4, 3·2 and 3·5 arbitrary units. Despite the twenty-five-fold change in concentration of oxygen, no significant change in initial rate was evident and the reaction was clearly of zero order with respect to this reactant. The autoxidation of S(IV) in ethanol-free media is also of zero order with respect to oxygen in the range pH 3–7 and of first order with respect to S(IV) and, perhaps, metal ion (Wedzicha, 1984). The essential difference so far identified is the involvement of metal–glycine complex in the ethanolic medium and the mechanism of the initial reaction is as follows:



Some clue as to the function of the metal ion in this reaction may be found from preliminary studies using ions other than Mn(II). It was found that Ni(II), Ce(III), Co(II), Mg(II) and Zn(II) were all able to act in the same way as Mn(II) whilst Fe(II), Fe(III) and Cu(II) ions were ineffective. Since both Fe(II) and Cu(II) form stable complexes with glycine, the ability of the metal ion to form a complex with glycine is not the only prerequisite for catalysis. The most interesting finding is that the role of the metal ion should not be regarded as that of an acceptor of electrons since metal ions such as Mg(II) are not usually involved in easy electron transfers. A number of other nitrogen-containing compounds were tested and found to behave similarly to glycine. These were ammonium chloride, aniline, *N*,*N*-dimethylaniline, pyridine, EDTA and *t*-nitrosobutane (*t*NB).

## Overall reaction

The progress of the overall reaction is shown in Fig. 3 for [S(IV)] = 4·2 mM, [Mn(II)] = 4·9 μM, [glycine] = 0·1 and 15 mM and 75% ethanol. These concentration–time graphs illustrate the apparent autocatalytic nature of the reaction which would be expected if the reaction involved chain-propagating free radicals formed in the initiation stages.

## Esr studies

The free radical nature of the autoxidation of S(IV) in aqueous ethanol is demonstrated by the fact that the reaction is completely inhibited by the

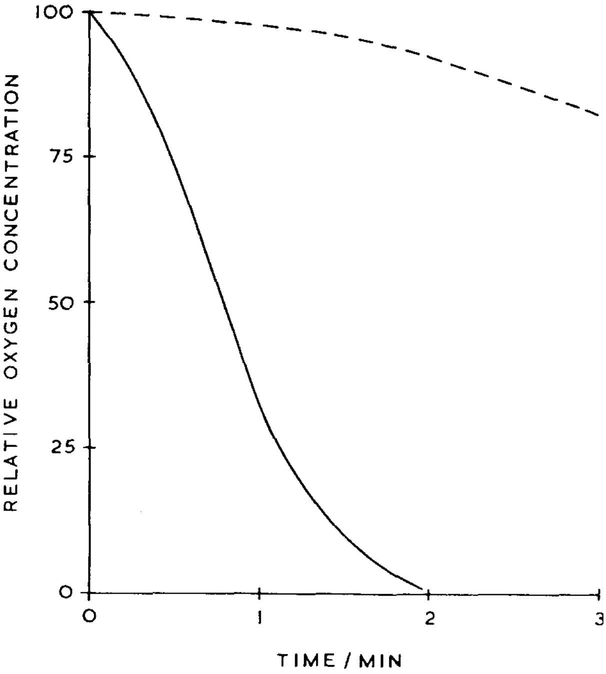


**Fig. 3.** Progress of autoxidation of S(IV) over a large proportion of the reaction. Initial conditions: [Mn(II)] = 4·9 μM, [S(IV)] = 4·2 mM, pH 5·7 acetate buffer 0·025 M, 25°C. [Glycine] = 0·1 mM– – – –, 15 mM ——.

addition of butylatedhydroxytoluene (BHT). However, no esr signals could be detected when a solution of Mn(II) (0·1 mM) and glycine (40 mM) in ethanol + water (3 + 1 v/v) was mixed with an equal volume of a solution of S(IV) (5 mM) in the same solvent. Similarly, no esr evidence could be obtained for free radicals in oxidation in ethanol-free media when a solution of Mn(II) (0·1 mM) in water was mixed with an equal volume of a solution of S(IV) (5 mM) in water. In both case mixtures were run up to the maximum flow-rate of 60 ml/min and examined under stop-flow conditions.

When *t*NB was added to the 75% ethanolic solution of glycine and Mn(II) at a concentration of 0·1% (w/v) subsequent mixing with the solution of S(IV) gave rise to a time-dependent esr signal under stop-flow conditions. Figure 4 shows three successive scans of the esr spectrum, each

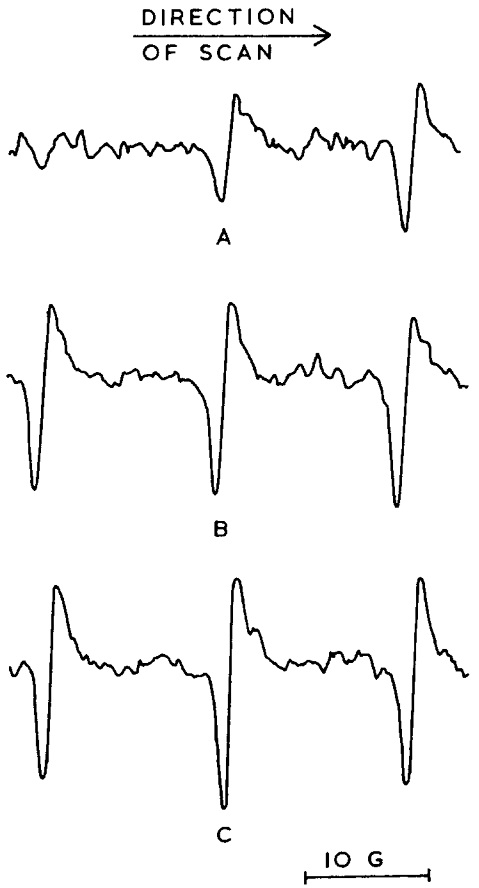


**Fig. 4.** Progressive increase of the nitroxide radical signal during the autoxidation of S(IV) in 75% ethanol in the presence of *t*-nitrosobutane. Peak A was observed after 45 s, B after 3 min and C after 5 min.

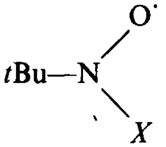
scan taking 2 min, the first being started immediately on stopping the flow of reactants. After some 45 s there is a clear signal which increased in intensity over a period of several minutes. The resulting spectrum is a triplet with a splitting of 14·64 G. A separate experiment, in which the rate of oxygen uptake from solution by such a reaction mixture was measured, showed the loss of oxygen to be on the same time-scale as the appearance of the signal. No signal could be detected when the oxidation of S(IV) was attempted in aqueous solution in the presence of *t*NB.

In control experiments, no signal could be observed when *t*NB and S(IV) or Mn(II), glycine and *t*NB were mixed in aqueous ethanol. A positive result was obtained for mixtures of S(IV), Mn(II) and *t*NB. This is attributed to the ability of the nitroso-compound to behave in a manner similar to that of glycine in so far as it promotes oxidation. A possible radical intermediate is $^{\cdot}SO_3^-$ formed in the first stage of oxidation of S(IV) and it was desirable to attempt to prepare this intermediate and trap it with *t*NB to check if its properties resembled those of the unknown radical product.

A simple method of generating $^{\cdot}SO_3^-$ is by oxidation of S(IV) with Ce(IV) (Ozawa *et al.*, 1971). A solution of ceric ammonium nitrate (10% w/v) in ethanol + water (3 + 1 v/v) was mixed with a solution of S(IV) (0·01 M) in the flow system. A signal attributed to $^{\cdot}SO_3^-$ was clearly observed while the mixture was flowing and persisted for some seconds after the flow was stopped. When the experiment was repeated but with added *t*NB in the S(IV) solution, the signal due to $^{\cdot}SO_3^-$ was still present but no new radical product derived from the nitroso-compound could be detected.

## DISCUSSION

Nitroxide radicals which may be produced by the trapping of sulphur oxy radicals with *t*NB have the following structure:



where $X = SO_2^-$, $SO_3^-$ or $SO_4^-$. The triplet in the esr spectrum indicates that, as expected, the free electron is located on an atom bonded to nitrogen, the signal being split by the nuclear spin of nitrogen. It is unlikely that significant amounts of $SO_4^{\cdot -}$ are present in ethanolic media whilst no evidence for the reaction of $^{\cdot}SO_3^-$ with *t*NB could be found. Waters (1972) and Mulvey & Waters (1974) report a splitting of 16·7 G for the *t*-butylsulphinonitroxide radical. This value corresponds well to 16·64 G observed here and it is likely, therefore, that the $^{\cdot}SO_2^-$ radical has been trapped. This radical is not frequently postulated as an intermediate in the autoxidation of S(IV) in aqueous solution but since the addition of *t*NB does not inhibit autoxidation, it is likely that $^{\cdot}SO_2^-$ is a side product, rather than a radical chain propagator. It is difficult to suggest realistic sources of $^{\cdot}SO_2^-$. The radical was observed by Hayon *et al.* (1972) during photochemically initiated autoxidation of S(IV) in aqueous solution but was regarded as being formed by photolysis of disulphite ion:

$$^-O_3S-SO_2^- \rightarrow {}^{\cdot}SO_3^- + {}^{\cdot}SO_2^-$$

Another instance of the formation of $^{\cdot}SO_2^-$ from S(IV) is when sodium disulphite is heated above 100°C (Janzen, 1972).

In experiments carried out by Yang's group (Yang, 1984) it was found that the destruction of *β*-carotene and chlorophyll during autoxidation of S(IV) in the system Mn(II)–glycine–ethanol–water could be inhibited by the addition of 1,2-dihydroxybenzene-3,5-disulphonic acid (tiron) which is a scavenger of $^{\cdot}O_2^-$ species. This observation has led to the proposal of radical formation steps such as:

$$SO_3^{2-} + O_2 \rightarrow {}^{\cdot}SO_3^- + {}^{\cdot}O_2^-$$

In the presence of unsaturated organic substrates radical transfer involving $^{\cdot}SO_3^-$ leads to organic radicals which may react subsequently with oxygen. Sulphite radicals are known to react also with unsaturated hydrocarbons to form sulphonic acids (Kharasch *et al.*, 1939*a,b*) but there is no evidence of sulphonation during S(IV)-mediated oxidations in ethanol–water mixtures. An alternative pathway to oxidation product involves the direct action of $^{\cdot}O_2^-$ as an oxidising agent. Whilst there is a small amount of evidence for the formation of $^{\cdot}O_2^-$, there is no evidence for the species $^{\cdot}SO_3^-$. An alternative, and equally plausible, radical forming step which gives rise to $^{\cdot}O_2^-$ involves one electron oxidation of disulphite ion as follows:

$$S_2O_5^{2-} + O_2 \rightarrow S_2O_5^{\cdot -} + {}^{\cdot}O_2^-$$

and a simple route to $^{\cdot}SO_2^-$ is then

$$S_2O_5^{\cdot -} \rightarrow SO_3 + {}^{\cdot}SO_2^-$$

An interesting oxidation of S(IV) in non-aqueous systems is observed in dimethylsulphoxide (DMSO) where, for example, it is found that sulphur dioxide reacts with Co(II) sulphite to form Co(II) disulphate (Goodall, 1984). The oxidising agent is probably DMSO. It is suggested that oxidation proceeds by way of disulphite ion. Since the conversion of S—S bonded disulphite to S—O—S bonded disulphate requires a major reorganisation of the atoms, the metal ion could well be stabilising an unconventional intermediate, $MO_2S—O—SO_2$, which is particularly susceptible to oxidation. This type of mechanism is attractive for the system studied in this paper for two reasons. First, the metal ion is not involved in electron transfer and, secondly, the formation of disulphite ion presumably is accompanied by a significant reduction in solvation and would be favoured as the proportion of ethanol in the reaction medium is increased. The formation of disulphite ion, S—S or S—O—S bonded, can also be used to explain the formation of $^{\cdot}SO_2^-$. The main problem concerning a mechanism involving disulphite ion is that the initial rate of autoxidation of S(IV) is of first order with respect to S(IV) and the participation of disulphite ion would lead to second order kinetics. The kinetic data, however, provide no indication of the mechanism after the rate determining step when a second S(IV) species could well be involved. The main limitation in attempting to relate the formation of $^{\cdot}SO_2^-$ to the kinetics of autoxidation is that esr spectra were recorded after the reaction had been proceeding for some time. The kinetics reported here relate to initial rates. However, since one of the applications of these results is to the study of S(IV)-mediated oxidations of unsaturated food components, which takes place over the major part of the autoxidation reaction, the production of $^{\cdot}SO_2^-$ is an important finding.

A number of polyhydroxy compounds (e.g. glycerol, sucrose) may be used to inhibit autoxidation of S(IV) in aqueous solution in the same way as by using small amounts of ethanol. It is interesting to speculate, therefore, whether high concentrations of these polyhydroxy compounds are capable of promoting metal-amine catalysed autoxidation of S(IV). It may be significant that, despite the presence of large amounts of hexoses in vegetables and blanching liquor, the process of air dehydration in the presence of S(IV) leads to the formation of sulphate ion (Wedzicha *et al.*, 1984).

## CONCLUSION

Whilst the reaction medium considered here offers a convenient way of studying the S(IV)-mediated oxidation of food components such as carotenoids which are insoluble in water, the characteristics of the autoxidation of S(IV), which leads to the mediated oxidation, in mixtures of ethanol and water, are very different from those in water. Both aqueous and non-aqueous systems could involve $^{\cdot}O_2^-$ as oxidising agent but the main differences are the formation of $^{\cdot}SO_2^-$ radicals and the need for a metal–amine complex in mixtures of water and ethanol. Zero order dependence on oxygen is common to both systems. It is evident that comparative studies of the oxidation of a given substrate in both solvent systems are now called for.

## ACKNOWLEDGEMENTS

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