Archives of Biochemistry and Biophysics
Vol. 367, No. 2, July 15, pp. 289–296, 1999
Article ID abbi.1999.1264, available online at http://www.idealibrary.com on IDEAL®

Reactive Oxygen Species in the Aerobic Decomposition of Sodium Hydroxymethanesulfinate

Sergei V. Makarov,* Claudius Mundoma,† Serge A. Svarovsky,§ X. Shi,†
P. M. Gannett,‡ and Reuben H. Simoyi§,[1]

*Department of Physical Chemistry, Academy of Chemistry and Technology, Engels Street 7, Ivanovo, 153460 Russia;
†National Institute for Occupational Safety and Health, 1093 Willowdale Road, Morgantown, West Virginia 26506;
‡Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, West Virginia 26506; and
§Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506-6045

Received January 5, 1999, and in revised form April 20, 1999

Sodium hydroxymethanesulfinate, ($HOCH_2SO_2Na$, HMS) is relatively stable in aqueous alkaline environments, but rapidly decomposes in acidic medium to give a variety of products that include sulfur dioxide. A detailed kinetic and mechanistic study of the decomposition of HMS in slightly acidic medium has shown a process that produces dithionite, $S_2O_4^{2-}$, which is preceded by an induction period which persists for as long as molecular oxygen is present in the reaction solution. The complete consumption of molecular oxygen is a prerequisite for the formation of $S_2O_4^{2-}$. Among some of the intermediates detected in the decomposition of HMS is the sulfite radical, SO_3^{-}. Comparisons are made between the decomposition mechanisms of thiourea dioxide (aminoiminomethanesulfinic acid) and HMS.

Key Words: **hydroxymethanesulfinate; thioureas oxides; aminoiminomethanesulfinic acid; sulfoxylate ion; sulfur dioxide radical ion; sulfite radical ion; reactive oxygen species.**

Many thioureas are known toxins. Thiourea, itself, causes liver and thyroid tumors in rats (2). It has been, however, difficult to predict the toxicity of specific thioureas. There have been several theories postulated on the varying degrees of toxicity of thioureas. One possibility is the nature of the metabolic nitrogen-containing end products of the thiourea. In some cases, thioureas are desulfurized to nontoxic ureas (3). In general, those thioureas that are metabolized to ureas are more toxic than those that retain the thione group. For example, *N*, *N*-diphenylthiourea, which is nontoxic, is excreted primarily as the aromatic ring hydroxylated products but with the thione group intact (4).

A possible way of examining the toxicity of thioureas is to study the sulfur leaving groups and their reactivities. The toxicity could then be determined by secondary reactions of the sulfur leaving groups. Sulfoxylate, for example, is known to react rapidly with molecular oxygen to produce the radical anion $SO_2^{-\bullet}$ and superoxide (5):

$$SO_2^{2-} + O_2 \rightarrow SO_2^{-\bullet} + O_2^{-\bullet}. \quad [1]$$

$SO_2^{-\bullet}$ also rapidly reacts with oxygen (6):

$$SO_2^{-\bullet} + O_2 \rightarrow SO_2 + O_2^{-\bullet}. \quad [2]$$

Sulfoxylate is a product of the heterolytic cleavage of the C-S bond in aminoiminomethanesulfinic acid (AIMSA)[2] in alkaline medium (7):

$$(NH_2)_2CSO_2 + OH^- \rightarrow (NH_2)_2CO + HSO_2^- \quad [3]$$

The highly reactive superoxide can generate other reactive oxygen species such as the hydroxyl radical and peroxides due to its reactions with sulfoxylate and sulfur dioxide anion radical (8) and disproportionation (9). Reactive oxygen species are extremely toxic and

[1] To whom correspondence should be addressed. Fax: +1-304-2934904. E-mail: rhsimoy@wvu.edu.

[2] Abbreviations used: AIMSA, aminoiminmethansufinic acid; HMS, hydroxymethanesulfinate; SOD, superoxide dismutase; DMPO, 5,5,-dimethyl-l-pyroline *N*-oxide.

0003-9861/99 $30.00

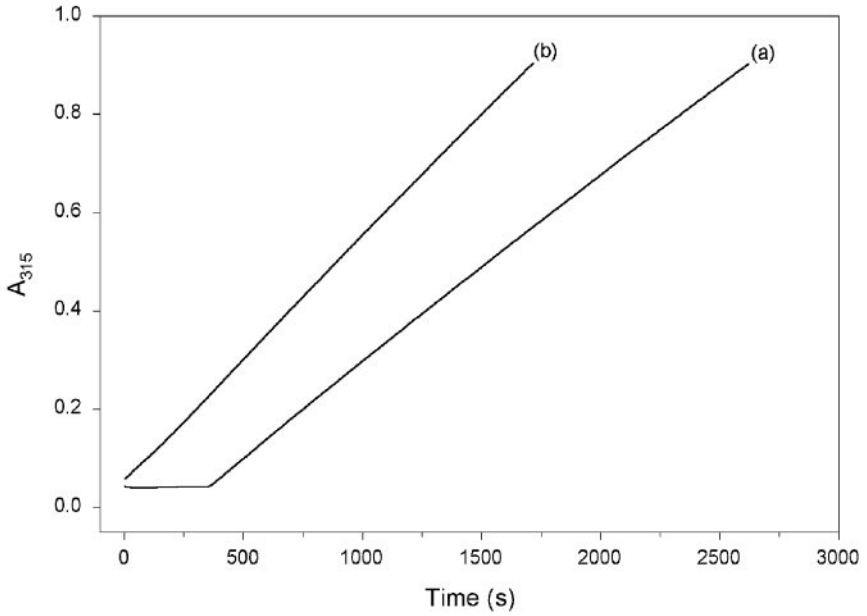

FIG. 1. Dithionite formation during (a) aerobic and (b) anaerobic decomposition of 0.104 M HMS at pH 6.5.

would contribute to the observed toxicity of the parent thiourea.

Thioureas are known for their strong reducing properties, especially the *S*-oxides, sulfenic, sulfinic, and sulfonic acids, which are supposed to be the precursors in the metabolic pathways that convert thioureas to ureas or to the thione compound (10). The accepted mechanism for the oxidation of thioureas involves successive oxidation of the sulfur center, finally leading to the cleavage of the C-S bond in the sulfonic acid to give sulfate and the urea residue (11). Recent studies on the oxidation of aminoiminomethanesulfinic and aminoiminomethanesulfonic acids have shown that sulfinic acids are much more easily oxidized than the corresponding sulfonic acids (12). Furthermore, the oxidation of sulfinic acids does not necessarily pass through the sulfonic acid before formation of sulfate as expected from the accepted oxidation mechanism. Instead, sulfinic acids tend to decompose in aqueous alkaline medium to give sulfoxylate ion, HSO_2^-, which is later oxidized to sulfate (12). The elimination of HSO_2^- or HSO_3^- appears to be the most important step in the formation of guanidines from the reactions of sulfinic and sulfonic acids with amines (13).

The strong reducing properties of hydroxymethanesulfinate (HMS) also are known to be associated with formation of sulfoxylate and sulfur dioxide anion-radical (7). Thus, the oxidation of HMS is a well-known procedure for initiation of polymerization reactions (14) and is likely to be a free radical process. HMS is also used in anticancer formulations as an antioxidant stabilizator (15).

We report, here, a study on the aerobic decomposition of HMS. Preliminary results have shown that HMS decomposes in batch environments to form dithionite, suggesting that its decomposition might share the same mechanism as that for the aminoiminomethanesulfinic acid (8). The intermediates formed during the decomposition were also closely monitored by ESR spectroscopy.

EXPERIMENTAL

Materials

Doubly distilled deionized water (Millipore) was used for the preparation of all the stock solutions and their subsequent dilution. All solutions were prepared fresh unless otherwise stated. Sodium HMS, sodium dithionite, and sodium sulfite were purchased from Aldrich Chemical Co (Milwaukee, WI). HMS was recrystallized twice from 50:50 ethanol:water prior to each use. Sodium dithionite and sodium sulfite were used without further purification. Superoxide dismutase (SOD), catalase, and sodium formate were from Sigma (St. Louis, MO). The following trap was of the highest purity possible: 5,5-dimethyl-1-pyroline *N*-oxide (DMPO) (Sigma). DMPO was purified using activated charcoal until free radical impurities disappeared as verified by ESR spectroscopy.

Instrumentation

Absorptivity coefficients were measured on Perkin–Elmer Lambda 2S UV-Vis spectrophotometer interfaced with a minicomputer. A Hi-Tech Scientific SF-DX2 stopped-flow spectrophotometer interfaced with a minicomputer was used to follow dithionite formation at 315 nm (ϵ = 8043 M^{-1} cm^{-1}) (16). KinetAsyst 2.1 software was used for data acquisition and analysis. Temperature control was maintained with NesLab RTE-101 thermostat. ESR measurements were run on a Varian E109 EPR spectrometer with a flat cell assem-

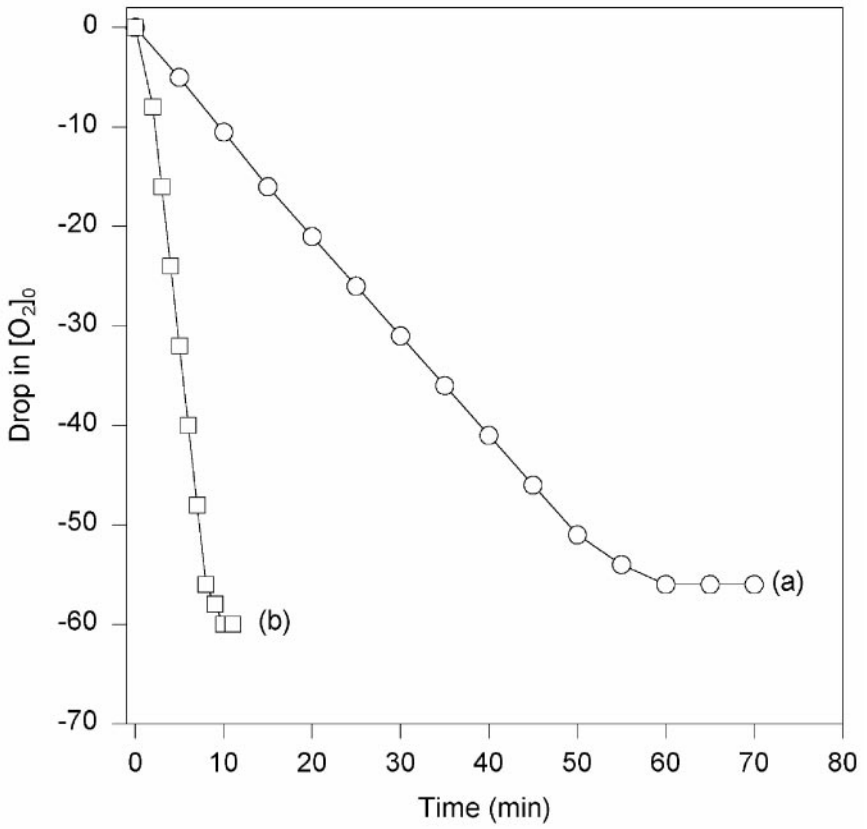

FIG. 2. Plot of oxygen consumption with time. The scale of the *y*-axis represents the number of 1-cm squares on the oxygraph and the points were taken at 2-min intervals. HMS: (a) 0.065 M and (b) 0.130 M (pH 6.8) buffer.

bly. The instrument is interfaced to a computer and an EPR DAP 2.0 program was used for data acquisition and analysis.

Oxygen uptake measurements were done at room temperature on a Gilson Oxy 5/6 oxygraph. A YSI 5331 oxygen probe was inserted into a stirred and thermostatically controlled reaction vessel coupled to an amplifier and recorder.

Methods

UV-Vis measurements. All experiments were performed at 25°C. Reactions were run at a constant ionic strength of 0.5 M (NaCl). A mixture of acetic acid, phosphoric acid, and boric acid plus NaOH (Britton–Robinson system) (17) was used for the preparation of buffer solutions. Deaeration was performed by passing argon through the buffer for 30 min prior to addition of HMS.

ESR measurements. ESR spin trapping was used to detect short-lived free radical intermediates. This technique involves the addition reaction of a very reactive short-lived free radical with a diamagnetic compound (spin trap) to produce a relatively long-lived free radical adduct which can be studied by ESR. The following settings were used for a typical run: receiver gain, 2.5×10^4; time constant, 250 ms; modulation amplitude, 1.0 G; scan time, 4 min; and magnetic field, 3360 $\pm$ 50 G. All measurements were taken at room temperature and successive scans taken at 4-min intervals unless otherwise stated.

A typical reaction mixture for ESR consisted of 2 mg HMS in 2 ml of pH 6.8 buffer, 10 mM spin trap (DMPO). When used, the concentrations of catalase and SOD were 2000 and 5000 U/ml, respectively. Each run is composed of 20 successive scans taken at 4-min time intervals. The HMS was dissolved in pH 6.8 buffer followed by addition of the spin trap, and the mixture was vortexed for rapid mixing and then introduced to the ESR spectrometer. A time lag of 60 $\pm$ 10 s was allowed before the introduction of the reaction solutions to the ESR spectrometer.

Oxygen consumption. The oxygen probe was a complete polarographic system consisting of a platinum cathode, silver anode, and KCl solution held captive around the electrodes by a Teflon membrane fastened with an O-ring. The response time is 90% in 10 s and it takes 30 s to reach steady state mainly because of the membrane. Since the probe is a complete system by itself, it is relatively unaffected by, and does not offset, its external environment. Adequate stirring eliminated reading error due to oxygen depletion in the vicinity of the membrane.

RESULTS

Unless otherwise noted, the experimental results reported here were obtained from reactions run in a buffer of pH 6.8 and at 25°C. HMS crystals were quickly dissolved in the air-saturated buffer solutions and sealed in a cuvette to prevent further absorption of molecular oxygen into the solution. Air-saturated solutions contained 2.4×10^{-4} M molecular oxygen (18). The reaction mixture was monitored at 315 nm. Only

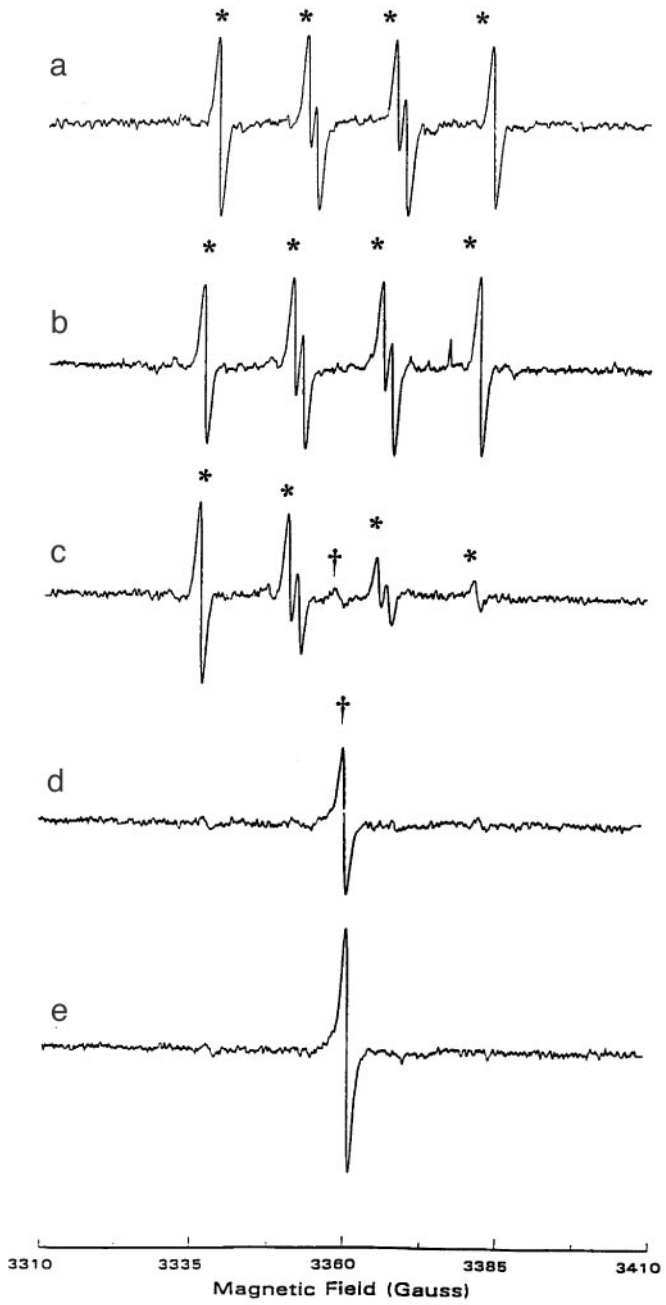

FIG. 3. HMS (0.123M) in pH 6.8 buffer, 10 mM DMPO. Mixture introduced into ESR spectrometer after 2 min. Scans were taken at 4-min intervals and 20 scans were taken. Spectra (a)–(e) represent the signals obtained as the reaction progresses: (a) 2 min, (b) 6 min, (c) 10 min (asymmetry is due to rapid decay during the sweep time), (d) 14 min, (e) 26 min. Spectrometer settings: receiver gain, 2.5×10^4; modulation amplitude, 1.0 G; center field, 3600 G; scan width, 100 G; scan time, 4 min. The measurements were taken at room temperature. The asterisked (*) peaks are due to the DMPO–SO_3^- radical adduct, whereas the crossed (†) peak is untrapped SO_2^- anion-radical.

dithionite absorbs at this wavelength. Figure 1 shows that in standard air-saturated solutions, the decomposition of HMS shows an induction period before a zero-order rate of formation of dithionite is observed (curve a). Buffer solutions degassed with argon or nitrogen displayed immediate formation of dithionite without an induction period (curve b). The induction period itself is strictly controlled by the amount of oxygen in solution. Partially degassed buffer solutions gave shorter induction times, and solutions bubbled with oxygen gave very long induction periods.

Figure 2 shows the rate of consumption of molecular oxygen from the reaction solution. Higher HMS concentrations gave much more rapid oxygen consumption rates and reduced the length of the induction period (curve b). Combining this information with the data in Fig. 1 shows that complete consumption of molecular oxygen is needed before dithionite can accumulate.

The reaction could also be followed by the ESR spectroscopy. The ESR data using DMPO as a radical trap in air-saturated solutions of HMS is shown in Fig. 3. During the induction period, the successive spectra show a clean spectrum of the DMPO–$SO_3^{-\bullet}$ radical adduct (19). This radical quickly reaches its steady-state concentration, which is maintained through the induction period (spectra a and b). It dies down rapidly, during the sweep time of 4 min, at the end of the induction period (spectrum c), and simultaneously a strong signal of untrapped $SO_2^{-\bullet}$ radical emerges (spectra d and e). The time-dependencies for these radical ions are shown in Figure 4. Just before the rapid collapse of the sulfite radical, it shows a small increase (curve a) whose maximum coincides with the end of the induction period. The $SO_2^{-\bullet}$ radical, in air-saturated solutions, is formed at the end of the induction period, just like dithionite (Figure 1). ESR experiments were performed in a wide range of DMPO concentrations (5mM to 0.1M); no concentration effect has been observed. Degassed solutions gave immediate formation of the $SO_2^{-\bullet}$ radical without an induction period. No $SO_3^{-\bullet}$ radical has been detected under anaerobic conditions.

The effect of sulfite on the rate of decomposition of HMS in the presence of oxygen is shown in Figure 5. Addition of sulfite increased rate of dithionite formation and reduced the induction period before formation of dithionite, and at sufficiently high enough sulfite, the induction period could be completely eliminated. Under anaerobic conditions added sulfite also increased rate of dithionite formation.

Figure 6 shows the effects of formate, superoxide dismutase, and catalase on the rate of formation of the $SO_2^{-\bullet}$ radical. Effect of formate was minor, if any. In all other cases, the additives lengthened the induction period. Superoxide dismutase gave the strongest effect and nearly doubled the induction period.

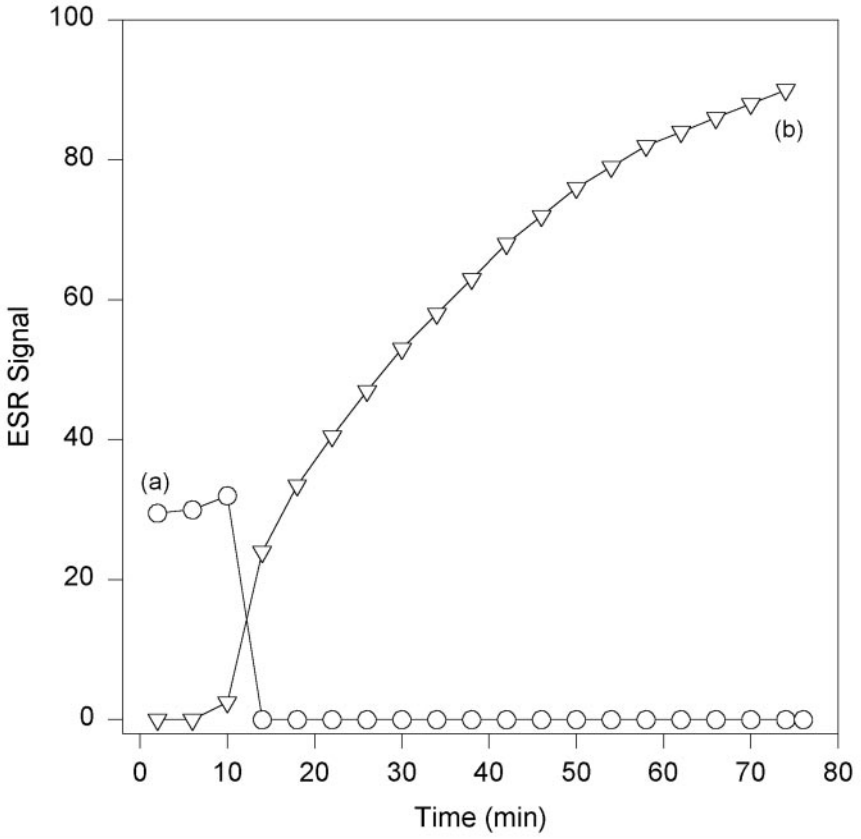

FIG. 4. Plot of ESR signal intensity with time for the conditions in Fig. 3. (a) DMPO–SO_3^- radical adduct; (b) untrapped SO_2^- anion-radical. The plot represents all the data from the traces not shown in Fig. 3.

DISCUSSION

There are two possible mechanistic pathways of decomposition of HMS (7). The first is a heterolytic–as shown in R4-R7:

$$HOCH_2SO_2^- \leftrightarrow HSO_2^- + CH_2O \quad [4]$$

$$3\,HSO_2^- \rightarrow 2HSO_3^- + HS^- \quad [5]$$

$$HSO_2^- + HSO_3^- \leftrightarrow S_2O_4^{2-} + H_2O \quad [6]$$

$$HSO_3^- + CH_2O \leftrightarrow HOCH_2SO_3^- \quad [7]$$

and a homolytic–as shown in R8-R9:

$$HOCH_2SO_2^- \leftrightarrow {}^{\cdot}CH_2OH + SO_2^{-\cdot} \quad [8]$$

$$2SO_2^{-\cdot} \leftrightarrow S_2O_4^{2-} \quad [9]$$

The mechanisms of dithionite formation are substantially different in these two cases (cf. R6 and R9). In case of homolytic mechanism, dithionite is formed by coupling of two $SO_2^{-\cdot}$ radicals (R9). In case of heterolytic mechanism, dithionite is formed via disproportionation reaction of two anions, sulfoxylate and sulfite (R6). Since added sulfite dramatically increases the rate of dithionite formation in HMS solutions (Fig. 5), we assume that the heterolytic pathway is more plausible than homolytic.

Interestingly, earlier we were unable to observe any influence of sulfite during the decomposition of AIMSA in strongly alkaline solutions (8) (0.5M NaOH), but in weakly alkaline media the additives of sulfite increase the rate of dithionite formation (20). At pH near 7 HMS (this work) and AIMSA (20) produce dithionite both in aerobic and anaerobic conditions. It is reasonable to assume that in neutral solutions dithionite is formed after appearance of sulfoxylate and follow-up reactions R5 and R6. The mechanism is markedly changed in strongly alkaline solutions. Here both sulfoxylate and sulfite are completely deprotonated; therefore reaction between two dianions (SO_2^{2-} and SO_3^{2-}) should be much slower than between two monoanions (R6). In these solutions, AIMSA produces dithionite only under aerobic conditions (R1 and R9).

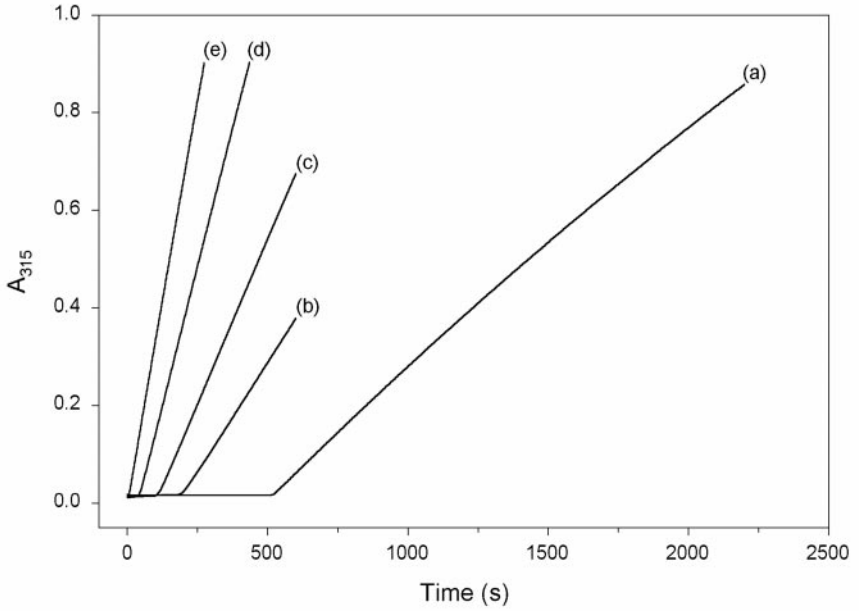

FIG. 5. Effects of Na_2SO_3 additives on the aerobic decomposition of 0.104 M HMS at pH 6.5: (a) none, (b) 0.015 M, (c) 0.031 M, (d) 0.063 M, (e) 0.084 M Na_2SO_3.

Due to high stability of HMS in alkaline solutions, we did not observe formation of dithionite even in concentrated solutions of HMS (3.2 M) at a pH $>$ 11.

Oxygen consumption rates shown in Figure 2 conform to our proposed mechanism. The rate of formation of the SO_2^{2-} will determine the rate of oxygen consumption. For a fixed amount of oxygen in solution (at pH 7 $[O_2]_0 = 2.4 \times 10^{-4}$ M) (18), it is expected that higher HMS concentrations will deplete oxygen at a much faster rate and hence reduce the induction period. The formation of dithionite also coincides with the complete consumption of oxygen.

In addition to reactions R6, R7, R1, and R2 in neutral solutions of HMS, the interaction of sulfite and O_2 also mops up molecular oxygen to form the sulfite radical and superoxide anion:

$$SO_3^{2-} + O_2 \rightarrow SO_3^{-\bullet} + O_2^{-\bullet} \qquad [10]$$

The trapping of the sulfite radical (Figure 3) was very important in proving our mechanism. Without addition of sulfite into the reaction mixture, ESR data show that there is almost an instant formation of the sulfite radical. The formation of sulfite may be associated with reactions R1, R2 and R5. The sulfite radical concentration remains constant during the induction period and shows a small increase before a rapid shutdown at the end of the induction period (Figure 4). There are several deactivating mechanisms that can consume sulfite and sulfite radical. Sulfite may be consumed by reactions R6 and R7. Decomposition products of HMS have not shown the formation of sulfate, which suggests that the predominant pathway by which the sulfite radical is consumed is via the formation of $S_2O_5^{2-}$ (21):

$$SO_3^{-\bullet} + SO_2^{-\bullet} \rightarrow S_2O_5^{2-} \qquad [11]$$

The exhaustion of oxygen at the end of the induction period causes the observed instant consumption of the sulfite radical.

The data in Figure 6 again show the importance of superoxide in this reaction mechanism. Superoxide dismutase (SOD) effectively doubles the induction period by converting superoxide to oxygen (9):

$$2O_2^{-\bullet} + H_2O + SOD \rightarrow O_2 + HO_2^- + OH^- \qquad [12]$$

The powerful effect of SOD suggests that the formation of superoxide is the predominant pathway of this decomposition mechanism. Our inability to trap superoxide radical in ESR experiments may be explained by instability of DMPO-superoxide radical-adduct under our reaction conditions of pH6.8. This limitation of DMPO has been addressed in numerous publications (22). Thus, the DMPO-superoxide adduct is known to be rapidly converted to DMPO-hydroxyl radical adduct which can be easily reduced, even by superoxide, into diamagnetic species (23). In addition, reaction of DMPO with superoxide is rather slow, ranging from 10 M^{-1} s^{-1} at pH 7.8 (24) to 1.2 M^{-1} s^{-1} at pH 7.4 (25). It

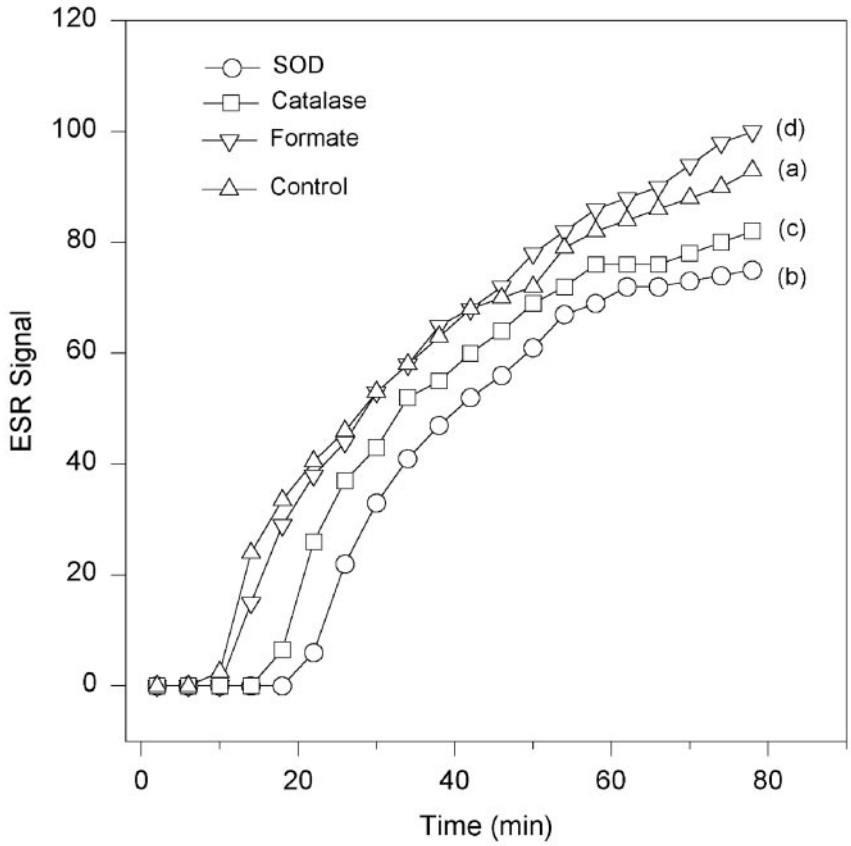

FIG. 6. Effect of various reagents on the decomposition of HMS. Initial conditions of (a) 0.058 M HMS in pH 6.8 buffer, 10 mM DMPO and (b) same as (a) but with 5000 U/ml SOD; (c) same as (a) plus 2000 U/ml catalase; (d) same as (a) plus 20 mM sodium formate.

is not surprising, therefore, that in the presence of a variety of powerful reducing sulfur-containing species we were unable to observe the signal from DMPO-superoxide radical adduct.

The other reactive oxygen species, peroxide and hydroxyl radical do not seem to be as significant as superoxide as evidenced by the effects of catalase and formate, respectively. Catalase traps peroxide and converts it to oxygen (9), but in this case, the effect is smaller than that of SOD (Figure 6). Formate, a hydroxyl radical trap, is very ineffective in this decomposition reaction.

TABLE I

$HOCH_2SO_2^- \leftrightarrow HSO_2^- + CH_2O$	M1
$3\ HSO_2^- \rightarrow 2HSO_3^- + HS^-$	M2
$HSO_2^- + HSO_3^- \leftrightarrow S_2O_4^{2-} + H_2O$	M3
$HSO_3^- + CH_2O \leftrightarrow HOCH_2SO_3^-$	M4
$HSO_2^- \leftrightarrow H^+ + SO_2^{2-}$	M5
$SO_2^{2-} + O_2 \rightarrow SO_2^{-\bullet} + O_2^{-\bullet}$	M6
$SO_2^{-\bullet} + O_2 \rightarrow SO_2 + O_2^{-\bullet}$	M7
$2\ O_2^{-\bullet} + H_2O \rightarrow O_2 + HO_2^- + OH^-$	M8
$SO_2^{2-} + O_2^{-\bullet} \rightarrow SO_2^{-\bullet} + O_2^{2-}$	M9
$SO_2^{-\bullet} + O_2^{-\bullet} \rightarrow SO_2 + O_2^{2-}$	M10
$O_2^{2-} + H_2O \leftrightarrow HO_2^- + OH^-$	M11
$HO_2^- + H_2O \leftrightarrow H_2O_2 + OH^-$	M12
$H_2O_2 + HO_2^- \leftrightarrow H_2O + O_2 + OH^-$	M13
$SO_2^{2-} + O_2^{2-} + 2H_2O \rightarrow SO_2^{-\bullet} + 3OH^- + OH^{\bullet}$	M14
$SO_2^{-\bullet} + O_2^{2-} + 2H_2O \rightarrow SO_2 + 3OH^- + OH^{\bullet}$	M15
$2\ SO_2^{-\bullet} \leftrightarrow S_2O_4^{2-}$	M16

CONCLUSION

The decomposition of HMS is an extremely pH dependent reaction system. In high pH conditions HMS is very stable as the anion. As pH drops, it becomes unstable and will decompose to give predominantly SO_3^{2-}, $S_2O_4^{2-}$ and $S_2O_5^{2-}$ plus formaldehyde. In neutral solutions dithionite formation will proceed in either aerobic or anaerobic conditions. Oxygen is very important and controls the time taken before formation of dithionite. Both sulfinic acid derivatives–HMS and AIMSA reveal a common mechanism of decomposition in aqueous solutions (Table I).

ACKNOWLEDGMENTS

We thank the Ivanovo Academy of Chemistry and Technology for giving a leave of absence to S.V.M. S.V.M. is grateful to the Russian

Foundation for Basic Research for financial support (Grant No. 98-03-32802). C.M. is grateful to Steve Leonard for helping with ESR experiments. This work was sponsored by the National Science Foundation (Grant No. CHE-9632592).

REFERENCES

1. Makarov, S. V., Mundoma, C., Penn, J. H., Petersen, J. L., Svarovsky, S. A., and Simoyi, R. H. (1999) *Inorg. Chim. Acta* **286,** 149.
2. Boyd, M. R., and Neal, R. A. (1976) *Drug. Metab. Dispos.* **4,** 314.
3. Smith, R. L., and Williams, R. T. (1961) *J. Med. Pharm. Chem.* **4,** 147.
4. Smith, R. L., and Williams, R. T. (1961) *J. Med. Pharm. Chem.* **4,** 97.
5. Zhang, J. Z., and Millero, F. J. (1992) *in* Environmental Geochemistry of Sulfide Oxidation (Alpers, C. N., and Blowes, D. W., Eds.), pp. 393–409, ACS, Washington, DC.
6. Huie, R. E., Clifton, C. L., and Altstein, N. (1989) *Radiat. Phys. Chem.* **33,** 361.
7. Budanov, V. V., and Makarov, S. V. (1994) Chemistry of Sulfur-Containing Reducing Agents, Khimia (Chemistry), Moscow [*In Russian*]
8. Makarov, S. V., Svarovsky, S. A., and Simoyi, R. H. *Chem. Res. Toxicol.,* submitted for publication.
9. Bertini, I., Mangani, S., and Viezzoli, M. S. (1998) *in* Advances in Inorganic Chemistry (Sykes, A. G., Ed.), Vol. **45,** pp. 127–249.
10. Ziegler, D. M. (1982) *in* Metabolic Basis of Detoxification (Jacoby, W. B., Bent, J. R., and Caldwell, J., Eds.), pp. 171–184, Academic Press, New York.
11. Epstein, I. R., and Simoyi, R. H. (1987) *J. Phys. Chem.* **91,** 5124.
12. Makarov, S. V., Mundoma, C., Penn, J. H., Svarovsky, S. A., and Simoyi, R. H. (1998) *J. Phys. Chem. A* **102,** 6786.
13. Miller, A. E., Bischoff, J. J., and Pae, K. (1988) *Chem. Res. Toxicol.* **1,** 169.
14. Lin C. C., Patat F., and Staude E. (1969) *Angew. Makromol. Chem.* **8,** 28.
15. Nassar, M. N., Reff, M. J., and Aghankar, S. N. (1996) *US Patent 5,508,268.*
16. Mc Kenna, C. E., Gutheil, W. G., and Wei, S. (1991) *Biochim. Biophys. Acta* **109,** 1075.
17. Dean, J. A. (1992) Lange's Handbook of Chemistry, 14th ed., p. 8.110, McGraw-Hill, Inc., New York.
18. Linke, W. F. (Ed.) (1965) Solubilities Inorganic and Metal-Organic Compounds, Vol. 2, ACS Washington, DC.
19. Shi, X. (1994) *J. Inorg. Biochem.* **56,** 155.
20. Polenov, Yu. V., Makarov, S. V., and Budanov, V. V. (1986) *Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.* **29,** 30.
21. Janzen, E. G. (1972) *J. Phys. Chem.* **76,** 157.
22. Frejaville, C., Karoui, H., Tuccio, B., Le Moigne, F., Culcasi, M., Pietri, S., Lauricella, R., and Tordo, P. (1995) *J. Med. Chem.* **38,** 258.
23. Finkelstein, E., Roson, G. M., and Rauckman, E. J. (1982) *J. Mol. Pharmacol.* **21,** 262.
24. Finkelstein, E., Roson, G. M., and Rauckman, E. J. (1980) *J. Am. Chem. Soc.* **102,** 4994.
25. Yamazaki, I., Piette, I. H., and Groover, T. A. (1980) *J. Biol. Chem.* **255,** 652.