Potentiometric Determination of Sulfite Oxidation Rates

By LOUIS C. SCHROETER

Solutions containing hydrogen sulfite, HSO_3^-, and sulfite, SO_3^{2-}, species appear to undergo oxidation at the expense of the divalent anion; this is a consequence of the different strengths of the two acids, HSO_3^- and HSO_4^-. A theoretical equation has been derived which permits calculation of the rate of loss of total sulfurous acid species [$-d(S_t)/dt$] from a pH-time plot of the oxidizing system. Rapid and reproducible rate studies of oxidizing sulfurous acid salt systems have been made possible through the development of an automated apparatus. Applications of the sensitive radical mediated oxidizing sulfite system for detecting presence of a wide variety of compounds in p.p.m. range are discussed. Empirical equations can be used to obtain semiquantitative analyses of compounds which inhibit the rate of sulfite oxidation in low (10^{-6} M) concentrations.

OXIDATION OF AQUEOUS solutions of sulfurous acid salts in the presence of air proceeds by a free radical chain mechanism; compounds capable of breaking the reaction chain serve as effective inhibitors of the oxidative process (1). Organic compounds in rather dilute solution (10^{-6} to 10^{-4} M) representing the following wide variety of classes are known to inhibit sulfite oxidation: acids (2), alcohols, glycols, mono- and polysaccharides (3–6), amines (7–10), amides (11), aldehydes (2), alkaloids (12), ketones (2), indoles (3), phenols and quinones (13, 14). Inorganic anions such as arsenite, antimonite, phosphite, and cyanide (3, 12) may function as chain terminators; the ammonium cation has been reported to be an effective inhibitor (12). Inhibitory action of these diverse compounds on sulfite oxidation which is now generally interpreted in terms of radical chain termination has been known for well over 60 years as shown by Young's observation (12): " . . . this inhibitive action is characteristic to a greater or less degree of all organic matter, and, in addition, of many nitrogen compounds not organic."

Most studies of sulfite oxidative behavior have been carried out in a qualitative manner which reflects the difficulty inherent in designing experiments to study free radical solution reactions in a rigorous manner. However, certain investigators, notably Bäckström (4, 6), have exercised greater care in experimental procedures and have treated data mathematically and offered evidence to support mechanisms. Various empirical equations have been used to describe bisulfite inhibition (15); most take the form

$$-\frac{d(S_t)}{dt} = \frac{A}{B + m}\, k_1 \cdot S_t \qquad \text{(Eq. 1)}$$
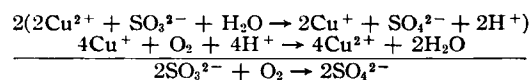
where S_t is the total sulfurous acid species, k_1

is the specific rate constant for the uncatalyzed reaction, and m is the molar concentration of additive. The constants A and B generally have values the order of 10^{-5} and are specific for each compound or class of compounds although the constants, A and B, are equal (10^{-5}) for the case of mannitol inhibition (5).

The relationship shown in Eq. 1 between the observed rate of reaction for sulfite oxidation and the molar concentration of inhibitor present in solution permits one to determine the concentration of inhibitor by comparing the rate constants for the standard k_1 and the inhibited k_2 reaction

$$\frac{k_2}{k_1} = \frac{A}{B + m} \qquad \text{(Eq. 2)}$$

Application of this equation to the determination of organic substances (m) in solution requires a knowledge of the values of the constants A and B. These constants can be evaluated from rate data obtained in systems containing known amounts of organic compound. However, good approximations may be made by assuming $A = B = 10^{-5}$. The oxidizing sulfite system can thus serve as a unique analytical method for the detection and approximate determination of organic compounds in dilute aqueous solution.

Heavy metal ions are known to function as catalysts of the bisulfite oxidative process. The mechanism proposed (1) for the catalytic effect of copper ions is typical

$$\frac{2(2Cu^{2+} + SO_3^{2-} + H_2O \rightarrow 2Cu^+ + SO_4^{2-} + 2H^+)}{\underline{4Cu^+ + O_2 + 4H^+ \rightarrow 4Cu^{2+} + 2H_2O}}$$
$$2SO_3^{2-} + O_2 \rightarrow 2SO_4^{2-}$$

Abel (16) has advanced a strong case for the second sequence as the rate-determining step. The mechanism assumes a steady state production of radicals and a time-dependent reaction with cuprous ion
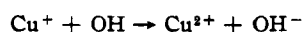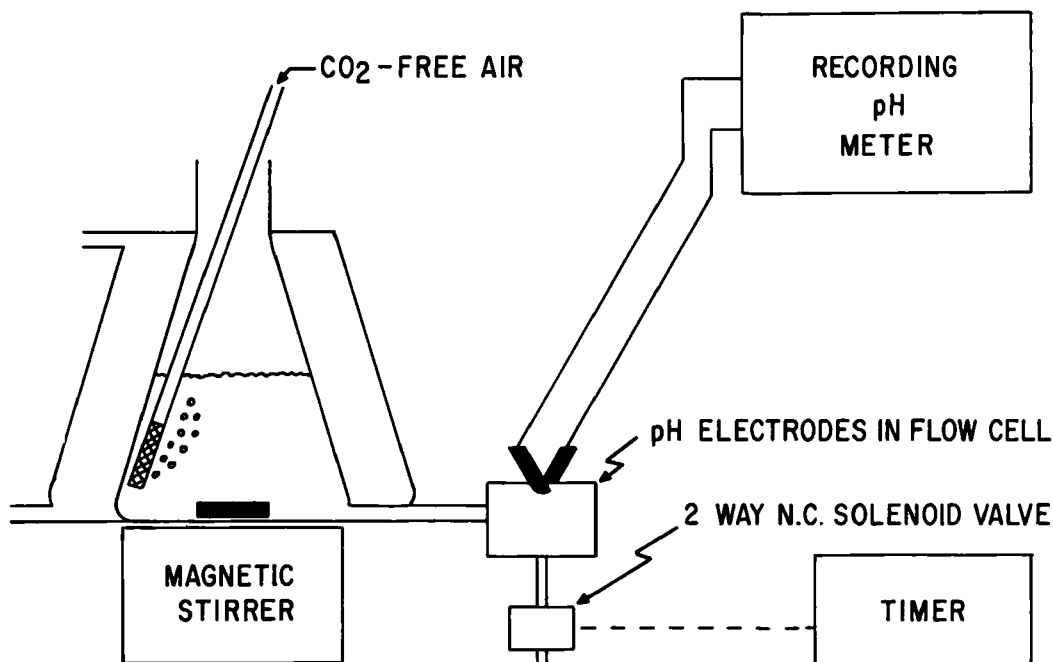
$$Cu^+ + OH \rightarrow Cu^{2+} + OH^-$$

Fig. 1.—Arrangement of oxidation vessel for automatic pH monitoring of samples.

Abel's mechanism is lucid and well documented, but most important, it adequately describes Fuller and Crist's (5) experimental data.

Nonmetal ions may also function as catalysts of the oxidation of sulfurous acid salts. For example, thiosulfate, $S_2O_3{}^{2-}$, may participate in a chain process with bisulfite and sulfite ions (17). Mechanism of the reaction appears to involve trithionate ion, $S_3O_6{}^{2-}$, which arises from the interaction of thiosulfate with a OH, $O_2{}^-$, or HSO_3 radical

$$S_2O_3{}^{2-} + SO_3{}^{2-} + 2OH + 2H^+ \rightarrow S_3O_6{}^{2-} + 2H_2O$$
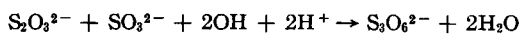
The trithionate ion is readily hydrolyzed to thiosulfate and sulfate

$$S_3O_6{}^{2-} + H_2O \rightarrow S_2O_3{}^{2-} + SO_4{}^{2-} + 2H^+$$

resulting in marked catalysis of the oxidative process.

Systematic investigations of the catalytic and inhibitory effect of various substances on the oxidative rate of sulfurous acid salts have been hampered in the past by the difficulty involved in providing reproducible and reliable systems. It is the purpose of this investigation to describe a reasonably simple yet highly reliable experimental technique and automated apparatus for studying the progress of air oxidation of sulfite systems and to demonstrate the analytical potential of these systems for detecting the presence of very low concentrations of organic compounds in aqueous systems.

TABLE I.—RELATIONSHIP BETWEEN INITIAL AND FINAL[a] BISULFITE CONCENTRATION IN OXIDIZED SULFUROUS ACID SALT SYSTEMS

	$[H^+] \times 10^8$	$[HSO_3{}^-] \times 10^{2b}$	$[SO_3{}^{2-}] \times 10^{2c}$
Initial	11.2	1.13	0.87
Final	3100	1.13	0.00
Initial	7.1	0.91	1.09
Final	2400	0.80	0.00
Initial	3.6	0.60	1.40
Final	2100	0.52	0.00
Initial	1.6	0.33	1.67
Final	1600	0.28	0.00
Initial	0.63	0.14	1.86
Final	1300	0.14	0.00

[a] "Final" means after 1200 seconds' exposure to standard reaction conditions with no further substantial reaction occurring. *c.f.*: Schroeter, L. C., THIS JOURNAL, **52**, 559 (1963). [b] Calculated with the expression

$$[HSO_3{}^-] = S_t - SO_3{}^{2-} = S_t - \left(\frac{S_t}{\frac{[H^+]}{K_{a'2}} + 1} \right)$$

Experimentally determined apparent second ionization constant, $K'_{HSO_3{}^-}$, equals 8.4×10^{-8}. [c] Total sulfurous acid species $[HSO_3{}^-] + [SO_3{}^{2-}]$, determined iodometrically.

EXPERIMENTAL

Apparatus and Material.—Air oxidation of sodium sulfite solutions was carried out in a thermostatted vessel of the design shown in Fig. 1. The sampling port was placed tangentially to the vessel wall to take advantage of the centrifugal pumping action imparted to the liquid by the magnetic stirring bar. Samples were periodically removed from the reaction solution and passed through a flow cell containing micro electrodes (glass-calomel); this was done automatically through the use of a timer-controlled solenoid valve.

Air supplied to the reactor was passed through a filter and molecular sieve to remove suspended solid

and liquid particles. The air stream was then conducted through three gas scrubbing towers in series: *a*, sodium hydroxide solution, *b*, sulfuric acid solution; *c*, triple distilled water; gas scrubbers were maintained in a thermostat at the same temperature as the reactor. Flow rates were measured with a calibrated wet gas flow meter[1] and maintained within 5 cm.³ sec.⁻¹ of the desired flow rate. Tubing was Pyrex glass with short pieces of Tygon used for connections.

Triple distilled water produced from a Pyrex apparatus and stored not longer than 12 hours under nitrogen prior to use was employed for making all solutions and rinsing equipment. Sodium sulfite, reagent grade, was recrystallized twice from distilled water and dried at 90° under vacuum. All other materials were of reagent grade.

The standard reactor was used for all studies. Three-hundred and fifty ml. of sulfite solution was stirred at rates greater than 1000 r.p.m. at 25 ± 0.05° while water-saturated, carbon dioxide-free air was introduced at 50 ± 5 cm.³ sec.⁻¹ through a medium glass frit beneath the surface of the fluid. The pH of samples removed from the reactor by the automatic sampling system was determined manually with a Beckman GS pH meter, or automatically with a recording Beckman Zeromatic pH meter, or with a Sargent model D titrator. Total sulfurous acid salt concentration of samples was determined iodometrically.

RESULTS AND DISCUSSION

Free radical systems such as oxidizing bisulfite solutions are extraordinarily sensitive to the presence of extremely small amounts of compounds which may act either as inhibitors or catalysts. For example, it was found that certain glass-calomel combination electrodes placed directly in the oxidizing system would not alter or affect the rate of sulfite oxidation while other glass-calomel electrodes caused grossly erratic results. Erratic effects appeared to be related to different inherent diffusion rates of electrolyte solution from the calomel reference electrode. For this reason a pH flow cell was used to measure samples withdrawn from the solution. Measurement of pH was generally made with the more sensitive Beckman GS pH meter; however, the arrangement shown in Fig. 1 where a recording pH meter is employed yields reliable data for most studies.

The relationship between initial and final bisulfite concentration in air-oxidized mixed sulfurous acid species (HSO₃⁻ and SO₃²⁻) is shown in Table I. Total sulfurous acid species present in solution, S_t = HSO₃⁻ + SO₃²⁻, was determined by iodometric titration; concentration of bisulfite species was calculated with

$$[HSO_3^-] = S_t - SO_3^{2-} = S_t - \frac{S_t}{\left[\frac{[H^+]}{Ka'_2} + 1\right]}$$ (Eq. 3)

where Ka'_2, the apparent second ionization constant of sulfurous acid experimentally determined in 0.02 M solution at 25°, was 8.4 × 10⁻⁸.
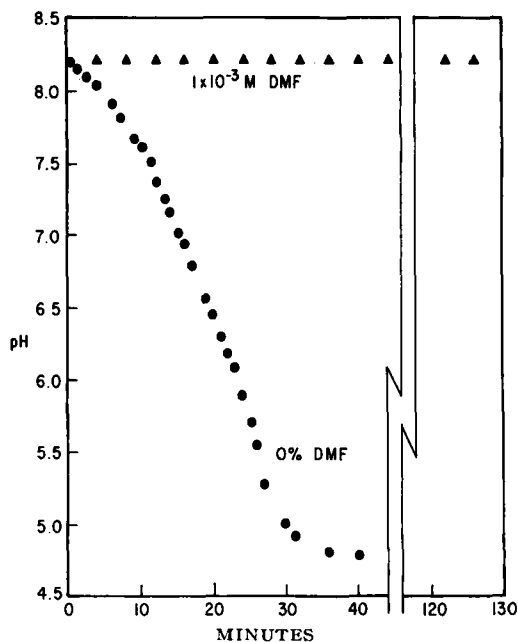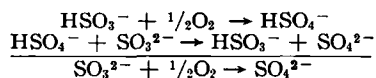

Fig. 2.—The pH of 0.02 M sodium sulfite solutions subjected to standard oxidizing conditions at 25°.

The remarkable constancy of the concentration of bisulfite in these systems, $C_{HSO_3^-}$ initial = $C_{HSO_3^-}$ final, is not a consequence of its inability to be oxidized. This reflects the different strengths of the two acids, $K_{HSO_3^-}$ = 6.24 × 10⁻⁸, $K_{HSO_4^-}$ = 2 × 10⁻².

Solutions containing both bisulfite and sulfite species will always appear to undergo initial rapid oxidation at the expense of the sulfite species

$$HSO_3^- + \frac{1}{2}O_2 \rightarrow HSO_4^-$$
$$\underline{HSO_4^- + SO_3^{2-} \rightarrow HSO_3^- + SO_4^{2-}}$$
$$SO_3^{2-} + \frac{1}{2}O_2 \rightarrow SO_4^{2-}$$

Thus, total sulfurous acid species, S_t, present in the solution at any time, t, will be approximately described by

$$S_t \cong C_{SO_3^{2-}} + C_{HSO_3^-} \text{ initial}$$ (Eq. 4)

or its equivalent form

$$S_t \cong C_{HSO_3^-} \text{ initial } \left[\frac{Ka'_2}{[H^+]} + 1\right]$$ (Eq. 5)

Using Eq. 5, total sulfurous acid salt concentration may be described by potentiometric determination of hydrogen ion activity if the ionization constant is known; initial bisulfite concentration is determined by material balance and Eq. 3.

The assumption of constancy of bisulfite concentration in air-oxidized systems containing both mono- and di-valent sulfurous acid species is strongly supported by experimental evidence presented in Table I. However, it is important to qualify this assumption by indicating that the final bisulfite concentration refers to conditions such that no substantial change in total sulfurous acid salt concentration takes place within the time period of two half-lives of the initial rapid reaction. Otherwise, the assumption would lead to

the inference that a solution containing only bi-sulfite species is not subject to oxidation. This is not the case. The lowered rate of oxidation of sulfurous acid systems containing bisulfite, HSO_3^-, as the predominant species is a consequence of the hydrogen ion effect on the rate equation

$$- \frac{d(S_t)}{dt} = \frac{g[HSO_3^-]}{\sqrt{[H^+]}} \qquad \text{.(Eq. 6)}$$

where g, the catalytic constant, has the value 2.0×10^{-6} (moles/L.)$^{1/2}$ sec.$^{-1}$ in air-oxidized systems (11). Thus, at pH 4 where bisulfite is the dominant species ($[SO_3^{2-}]/[HSO_3^-] = 6.24 \times 10^{-4}$), the rate of loss of sulfurous acid species, $-d (S_t)/dt$, is at least an order of magnitude less than at pH 8 ($[SO_3^{2-}]/[HSO_3^-] = 6.24$).

Change in pH accompanying oxidation of a solution containing initially $1.86 \times 10^{-2} M$ sulfite and $0.14 \times 10^{-2} M$ bisulfite is shown in Fig. 2. The solution containing $1 \times 10^{-3} M$ N,N-dimethyl-formamide (DMF) did not change pH when subjected to the standardized oxidation conditions for a period of over 2 hours, nor did the total available sulfurous acid salt concentration (0.02 M) as determined by iodometric titration of samples change during this period. Inhibitory action of DMF has been reported in a prior communication (11).

The solution containing only sulfurous acid species underwent a change in pH from 8.2 to 4.8 as oxidation progressed. Total available sulfurous acid species, S_t, present in solution as a function of time was determined simultaneously by rapid iodometric titration of samples withdrawn from the reaction as shown in Fig. 3 in which solid circles represent experimentally determined values. Reaction profile shows an initial lag time of about 5 minutes due to stirring effects: the volume of solution contained in the reactor was increased
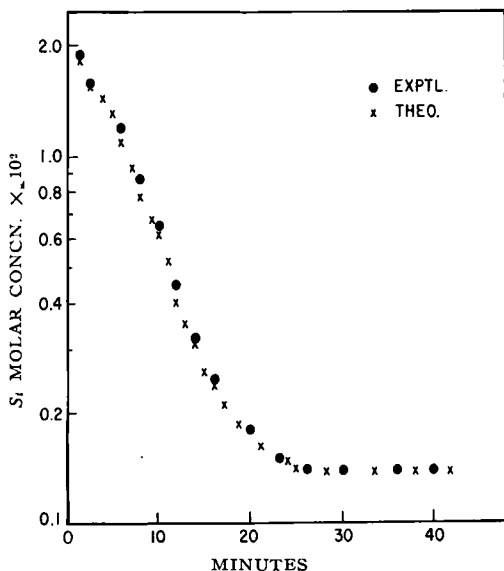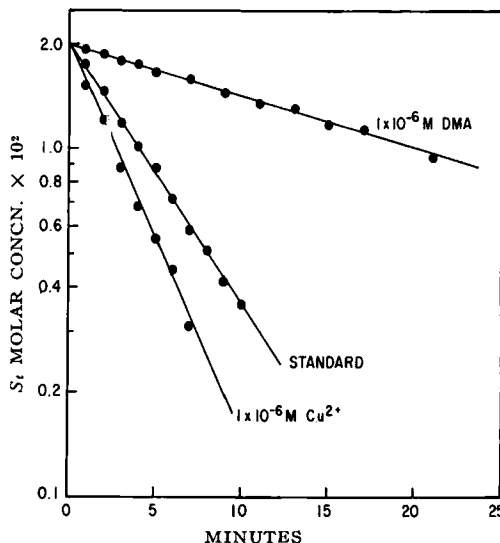

Fig. 4.—Effect of inhibitor ($1 \times 10^{-6} M$ DMA) and catalyst ($1 \times 10^{-6} M$ $CuSO_4$) on rate of sulfite oxidation. Initial solution composition: $[HSO_3^-] = 0.14 \times 10^{-2} M$; $[SO_3^{2-}] = 1.86 \times 10^{-2} M$.

from the standard 350 ml. to 450 ml. to illustrate the importance of controlling not only the stirrer speed but also all other factors (volume, vessel geometry, etc.) which may influence effective rates of stirring and dissolution of oxygen.

After the initial 5-minute lag period, the reaction appeared to follow a first-order dependency on total sulfite for about 75% of the reaction with a specific rate constant (2.67×10^{-3} sec.$^{-1}$) comparable to that found in earlier studies (11). As the solution became more acid, the rate of oxidation slowed rapidly and the total sulfurous acid concentration approached the value $0.14 \times 10^{-2} M$. Since the pH of the solution at this point was 4.8, this value corresponded almost entirely to bisulfite species. This stands in qualitative support of the assumption that bisulfite concentration appears to remain constant. Theoretical points shown as crosses in Fig. 3 were calculated from potentiometric data shown in Fig. 2 using Eq. 5. Good agreement between experimental and theoretical values attests to the validity of Eq. 5 and the soundness of explicit assumptions upon which it is based.

Analytical application of the automated procedure for detecting the presence of very low concentrations of organic compounds or heavy metal ions is shown in Fig. 4. Apparent first-order plots exhibiting no detectable initial lag periods were obtained under standard operating conditions. Ratio of the specific rate constant, k_2, for the reaction containing $1 \times 10^{-6} M$ N,N-dimethylacetamide (DMA) to the specific rate constant, k_1, for the standard reaction was calculated

$$\frac{k_2}{k_1} = \frac{5.75 \times 10^{-4} \text{ sec.}^{-1}}{2.80 \times 10^{-3} \text{ sec.}^{-1}} = 0.205.$$

This ratio and reported values (11) for the constants ($A = 0.25 \times 10^{-5}$; $B = 1.0 \times 10^{-5}$) were used in Eq. 2 to calculate the concentration of DMA. The concentration of DMA found by rate data was

Fig. 3.—Total sulfurous acid salt concn. as a function of time in solution subjected to standard oxidizing conditions. Initial soln. composition: $[HSO_3^-] = 0.14 \times 10^{-2} M$; $[SO_3^{2-}] = 1.86 \times 10^{-2} M$

2.2 × 10⁻⁶ M which agrees within a factor of 2 with the theoretical amount (1 × 10⁻⁶ M) present; this appears to be the practical limit of sensitivity of this kinetic analytical system. While the method is semiquantitative, it will detect the presence of many organic compounds in very dilute (10⁻⁸ to 10⁻⁶ M) solutions and establish concentrations within an order of magnitude of the true value.

CONCLUSIONS

Radical processes such as oxidizing sulfurous acid salt systems are sensitive to many compounds which act to inhibit or accelerate the chain reaction. Characteristically these compounds are effective in significantly modifying the rate of the oxidation even in extremely high dilutions (10⁻⁶ M). Rate determinations of uncatalyzed reactions can be made in a matter of minutes with the automated apparatus; rates observed in air oxidized systems can be increased by a factor of 5 by using pure oxygen (Henry's law).

Establishment of a reproducible sulfite-bisulfite system in a standard automated reactor such as described in this report offers a unique detecting system. A wide variety of compounds including acids, alcohols, glycols, polysaccharides, amines, cldehydes, alkaloids, ketones, indoles, phenols and auinones, and *possibly many other classes of organic qompounds will measurably inhibit the rate of ox-*idation *in very low concentration.* Likewise, heavy metal ions in parts per million can be detected by observing their catalytic effect on the rate of oxidation. Presence of these various compounds in water or air samples (after bubbling through water) can be readily detected. Semiquantitative relationships between concentration of inhibitor and oxidation rate constant may be established with an empirical equation of the form (Eq. 2).

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Role of Serotonin in the Thyroid Action of Reserpine

By B. B. WILLIAMS and S. T. COKER

The possible role of serotonin in the thyroid action of reserpine was studied in intact rats. Thyroid alteration by chronic reserpine medication and by chronic pharmacological treatment designed to alter serotonin levels and activity were compared with controls by three parameters. Direct effects of reserpine and serotonin were also investigated. Some evidence for reduced thyroid activity by chronic systemic reserpine administration was provided. It was found that reserpine directly *in vitro* slightly increased I¹³¹ uptake, and that serotonin in direct *in vitro* tests significantly reduced it.

R EPORTS of alteration of thyroid function by reserpine have been numerous. Contradictory results, however, in different experimental subjects and in use of different parameters are unexplained, and much information on mechanism is yet to be provided.

Mayer, Kelly, and Morton (1) found that a concentration of 0.083 mg./ml. of reserpine reduced the uptake of I¹³¹ by calf thyroid slices.

Their analysis of slices following incubation indicated that the inhibition of thyroid activity was predominantly one of interference with organic binding of iodine. Ershoff (2) reported that doses of desiccated thyroid and reserpine which were nonlethal when given separately to immature rats resulted in 100% mortality within 2 weeks when given concurrently. DeFelice, *et al.* (3), using oxygen consumption as a parameter of thyroid activity, showed that the administration of reserpine for 5 days to a hyperthyroid guinea pig would lower the oxygen consumption to normal. This group also found that the administration of reserpine to euthyroid animals would lower oxygen