eral G-negative bacteria but does inhibit the growth of G-positive bacteria, yeasts, and molds.

BEC adds little antibacterial effect to the residual antibiotics present in poliomyelitis vaccine but does contribute antimycotic activity.

Formaldehyde, if not neutralized by bisulfite, is highly antibacterial and exhibits some antimycotic activity.

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Oxidation of Sulfurous Acid Salts in Pharmaceutical Systems

By LOUIS C. SCHROETER

Many aqueous, oxygen-labile pharmaceutical systems may be effectively protected by the antioxidant activity of sulfurous acid salts. The sulfite system is inordinately sensitive to trace amounts of heavy metal catalysts and a wide variety of organic compounds which act as oxidation inhibitors. Mechanism of sulfite oxidation in the presence of known inhibitors at levels normally encountered in pharmaceutical formulations appears to involve heavy metal catalysts. At the concentration (p.p.m.) of heavy metals normally present in formulations, the rate of oxidation and antioxidant activity is directly dependent upon the heavy metal concentration. However, a slow but measurable rate of sulfite oxidation occurs in systems especially purified to remove metal ions. The copper-catalyzed oxidation of sulfurous acid salts in buffered systems containing ethanol as an inhibitor may be described by first-order kinetics. The pH profile of the absolute initial rate of the copper-catalyzed oxidation has been experimentally determined and found to be in reasonable agreement with a theoretically derived curve.

ORGANIC COMPOUNDS in very low concentrations (10^{-6} M) appear to inhibit the rate of oxidation of aqueous solutions of sulfurous acid salts (1). This inhibitory action has been demonstrated by many compounds representing diverse structural features and functional groupings—typical drug molecules may be expected to retard the rate of sulfite oxidation (2). This fact may at first seem paradoxical since it is well known that sulfurous acid salts are employed as pharmaceutical antioxidants (3). Pharmaceutical systems generally contain much more drug than antioxidant: the molar concentration of drug may be as much as two orders of magnitude greater than that of the antioxidant.

Effectiveness of sulfurous acid salts as pharmaceutical antioxidants in most aqueous systems appears to depend on their avidity for free radicals such as OH or simply on the ease with which they are oxidized in comparison with most autoxidizable drugs (3). However, this reveals little about the mechanism of sulfite oxidation in the presence of known inhibitors.

Inhibition of the rate of sulfite oxidation may be described by an equation (1–3)

$$-\frac{d(S_t)}{dt} = \frac{k_1(S_t)A}{B + M} \qquad \text{(Eq. 1)}$$

where S_t is the concentration of total sulfurous acid species, k_1 is the specific rate constant for the uninhibited reaction, M is the molar concentration of additive, and A and B are constants generally of the magnitude 10^{-5}. The specific rate constant, k_1, for the air oxidation (atmospheric pressure, 25°) of pure aqueous sulfite is 2.9×10^{-3} seconds^{-1} under conditions such that the diffusion or dissolution rate of oxygen is not rate limiting; the copper-catalyzed (10^{-6} M Cu^{2+}) reaction yields an apparent rate constant of 5.1×10^{-3} seconds^{-1}. Inhibition of sulfite oxidation by a variety of compounds over

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a wide range (10^{-3} to 10^{-7} M) of inhibitor concentration has been satisfactorily described by Eq. 1 or others of similar form (1–4). Activity of inhibitors in breaking sulfite radical chains appears to be proportional to the inhibitor concentration in dilute ($\leq 10^{-3}$ M) solutions of inhibitor and independent of the primary chain activation. The thermal (dark) and photo-induced reactions of sulfite in the presence of inhibitors both proceed by a radical process and may be described by an equation in which the rate is inversely proportional to the sum of a constant and the molar concentration of inhibitor, *i.e.*, Eq. 1, holds for dilute (10^{-3} to 10^{-7} M) solutions of the inhibitor (5). Chain termination of the sulfite radical in the presence of an inhibitor has been shown (6) to yield traces of oxidized inhibitor. For example, oxidation of sulfite in the presence of benzyl alcohol or isopropyl alcohol yields small quantities of benzaldehyde and acetone, respectively. The inhibitor apparently provides an alternate reaction pathway to the chain propagation process (7) and is itself oxidized to a noncarrier product. This would correspond to a bimolecular reaction with a first-order dependence on inhibitor concentration and first-order dependence on the radical chain carrier. The oxidation of inhibitors through their interaction with radicals generated in systems such as sulfite corresponds to Jorissen's "Induced Oxidation" (8).

Reliable data on the rate of oxidation of sulfite in the presence of high concentrations ($>10^{-2}$ M) of inhibitors are not available. Jorissen (8) reports values for sodium sulfite oxidation in the presence of relatively high concentrations (0.25 M) of inhibitors in pure oxygen. The times required for essentially complete oxidation of sulfite in the presence of 0.25 M mannitol and 0.25 M *n*-propanol were 13 days and 18 days, respectively. The

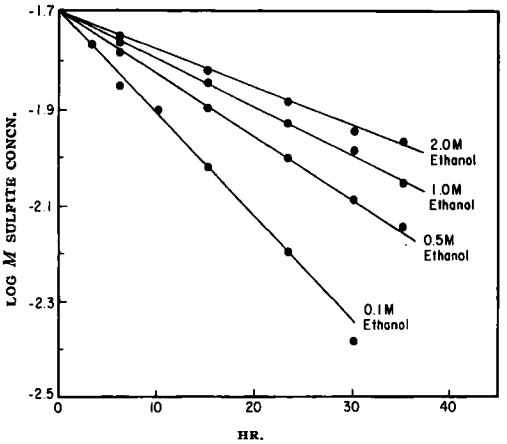

Fig. 1.—Air oxidation of 0.02 M sodium sulfite in the presence of Cu (II) (1×10^{-6} M) and ethanol at 25°C.

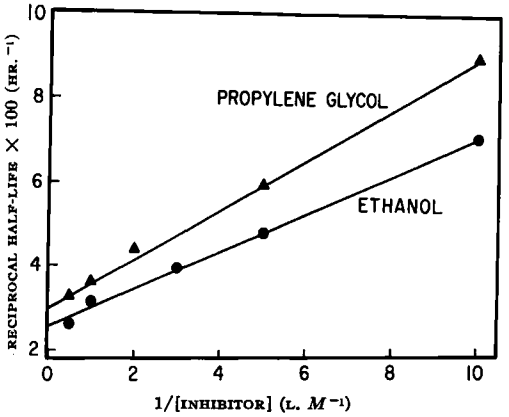

Fig. 2.—Reciprocal half-life for the cupric ion (1×10^{-6} M) catalyzed air oxidation of sodium sulfite (0.02 M) as a function of reciprocal inhibitor concentration at 25°C.

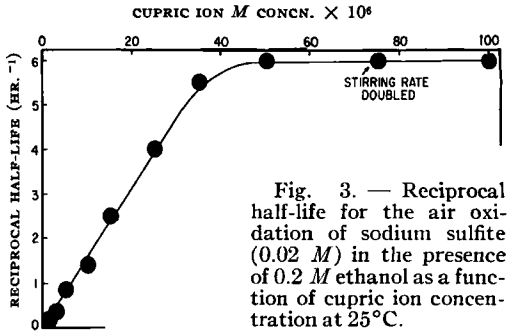

Fig. 3. — Reciprocal half-life for the air oxidation of sodium sulfite (0.02 M) in the presence of 0.2 M ethanol as a function of cupric ion concentration at 25°C.

theoretical value for the time required for 95% ($t_{95\%}$) oxidation of sulfite in the presence of 0.25 M inhibitor may be approximated with

$$t_{95\%} = \frac{2.303}{k_1} \log \frac{100}{5} = \frac{2.99}{k_1} \quad \text{(Eq. 2)}$$

where k_1 is the specific rate constant for the oxidation in pure oxygen in the presence of 0.25 M inhibitor calculated with Eq. 1 using Fuller and Crist's (9) value (1.3×10^{-2} seconds^{-1}) for the noninhibited rate.

$$k_1 = \left(\frac{10^{-5}}{10^{-5} + 0.25}\right) 1.3 \times 10^{-2} \text{ sec.}^{-1} = 5.2 \times 10^{-7} \text{ sec.}^{-1}$$

These approximate calculations yield a value of about 67 days or almost 2 months for the time required for 95% of sulfite to oxidize ($t_{95\%}$) in the presence of 0.25 M inhibitor and pure oxygen. The predicted value, $t_{95\%}$, obtained with Eq. 1 is about five times greater than that found experimentally (13–18 days).

Failure of Eq. 1 to describe the effect of inhibitors

TABLE I.—HALF-LIVES OF THE AIR OXIDATION OF 0.02 M SODIUM SULFITE IN THE PRESENCE OF 0.2 M ETHANOL AT 25°

Additive	Solution pH—Initial	Solution pH—Final[a]	Half-Life
5×10^{-5} M Cu(II)	9.2	8.0	0.167 hr.
1×10^{-3} M NaCN	9.3	8.3	1525 hr.

[a] pH measured after two half-life periods.

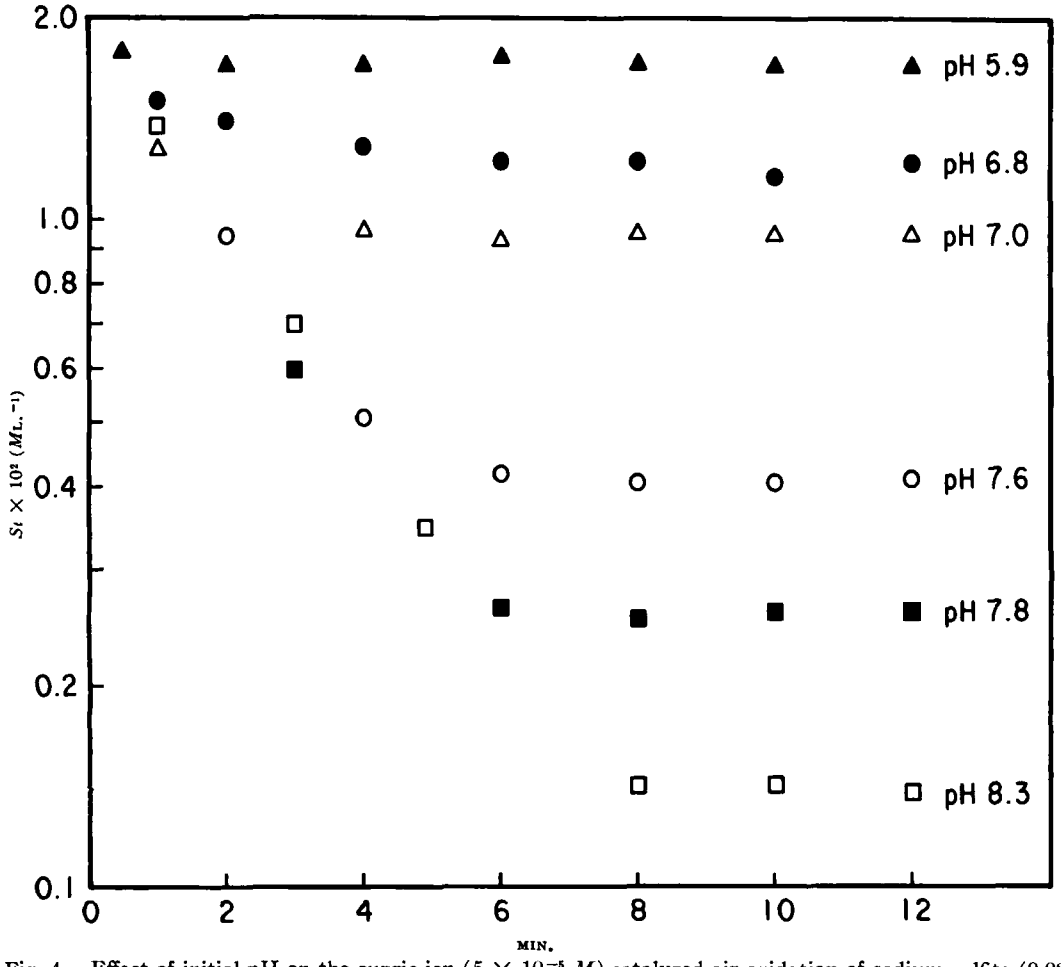

Fig. 4.—Effect of initial pH on the cupric ion (5×10^{-5} M) catalyzed air oxidation of sodium sulfite (0.02 M) in the presence of 0.1 M ethanol at 25°C.

under conditions described above is not wholly unexpected nor without precedent in radical reactions. The form of Eq. 1 is determined by the nature of the chain-breaking process and not by the initiation process (10). The presence of inhibitors generally alters only the rate of chain propagation and not the rate of radical initiation. Thus, the observed decrease in total sulfite which occurs in oxidizing systems containing inhibitors may be accounted for if radial initiation or some fraction thereof leads to oxidized product (sulfate); the radical initiating process becomes the rate-determining step in the overall oxidation scheme when the rate of chain propagation is decreased or, in equivalent terms, when the chain length is reduced. Existence of a measurable reaction rate in radical mediated processes in the presence of overwhelming amounts of inhibitor has been described as the "completely inhibited" rate (10). In the case of nitric oxide inhibition of alkyl free radical gas reactions, the completely inhibited or residual reaction rate has been interpreted in terms of an alternate nonradical mechanism (11); however, there is some evidence of free radicals in these systems (12). Heavy metal catalysis of sulfite oxidation allows another possible mechanism for radical initiation; this pathway for radical initiation may be important in systems containing inhibitors. The catalytic effect of copper ions on sulfite oxidation may be detected in concentrations as low as 10^{-6} M. This is within the permissible range of parts per million of heavy metal contaminants for many compounds of pharmaceutical interest (13); it is a reasonable assumption to consider that most complex pharmaceutical formulations contain catalytic (1 p.p.m.) amounts of heavy metals. It is the

Table II.—Effect of Initial pH on the Cupric Ion (5×10^{-5} M) Catalyzed Air Oxidation of Sodium Sulfite (0.02 M) in the Presence of 0.1 M Ethanol at 25°

pH		Initial[b] $[HSO_3^-] \times 10^2$	Final[c] $S_t \times 10^2$
Initial	Final[a]		
5.9	4.5	1.78	1.70
6.8	4.5	1.32	1.20
7.0	4.7	1.10	0.95
7.6	4.9	0.47	0.40
7.8	5.6	0.32	0.25
8.3	6.0	0.12	0.14

[a] Observation recorded after 10–12 minutes—the plateau region shown in Fig. 4. [b] Calcd: $[HSO_3^-] = S_t - SO_3^{2-} = S_t - S_t/[H^+]/(K_{a2}') + 1$; apparent second ionization constant of sulfurous acid: 8.2×10^{-8}. [c] Total sulfurous acid species, $S_t = [HSO_3^-] + [SO_3^{2-}]$, determined iodometrically.

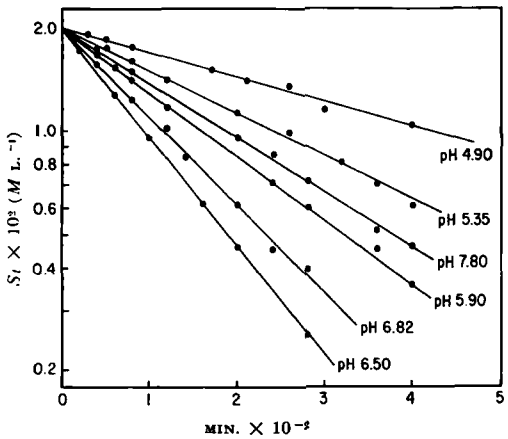

Fig. 5.—Cupric ion (5×10^{-5} M) catalyzed air oxidation of sulfurous acid specie ($S_t = 0.02$ M) in the presence of 0.2 M ethanol at constant pH (McIlvaine buffers 0.3 M; total ionic strength adjusted to 1.0 with KCl).

purpose of this study to describe some of the kinetic and mechanistic aspects of sulfite oxidation in the presence of a known inhibitor (ethanol) and in systems containing both inhibitor and catalyst.

EXPERIMENTAL

Water used in these studies was distilled twice from a Pyrex apparatus and stored not longer than 6 hours under nitrogen atmosphere prior to use. Reagent grade sodium sulfite was recrystallized twice from water. Ethanol was distilled twice from a Pyrex apparatus. All other materials were of reagent grade and used without further purification unless otherwise specified. Rapid ($t^1/_2 \leq 5$ minutes) oxidations were carried out in a vessel described in a prior communication (1). Slower reactions were studied by vigorously agitating the solution in a flask: 25 ml. of solution contained in a 500-ml. Erlenmeyer flask was shaken at a constant rate with a Burrell wrist action shaker[1] in a thermostatted room in which temperature was controlled at 25 ± 1°. Total sulfurous acid content of solutions was determined iodometrically. The pH of each solution was measured at 25 ± 0.05° with a Beckman GS pH meter.

RESULTS AND DISCUSSION

The copper-catalyzed air oxidation of aqueous-ethanol solutions of sodium sulfite exhibits an apparent first-order dependency on total sulfurous acid species as shown in Fig. 1. The apparent first-order rate constant decreases with increasing concentrations of ethanol: 0.1 M ethanol, k = 0.050 hours^{-1}; 0.5 M ethanol, k = 0.030 hours^{-1}; 1.0 M ethanol, k = 0.023 hours^{-1}; 2.0 M ethanol, k = 0.018 hours^{-1}.

Selection of ethanol as the chain propagation inhibitor was made on the basis of its importance in pharmaceutical formulations and the ease with which it can be distilled free from heavy metal contaminants. Cupric ion ($1 \times 10^{-6} M$) catalyst, which was added to each system immediately prior to oxidation, corresponded to less than 0.1 p.p.m. as Cu(II). Total heavy metal content of the oxidation system was not directly determined; however, kinetic oxidative studies with varying concentrations of sodium sulfite in water-ethanol indicated the presence of less than 10 p.p.b. copper ion.

[1] Burrell Corp., Pittsburgh, Pa.

TABLE III.—APPARENT FIRST-ORDER RATE CONSTANTS FOR THE COPPER CATALYZED OXIDATION OF SULFUROUS ACID SALTS AT CONSTANT pH IN THE PRESENCE OF 0.2 M ETHANOL[a]

pH[b]	$\left(\frac{\sqrt{(H^+)}}{K_s' + (H^+)}\right)^c$	$k \times 10^2$ (min.$^{-1}$)
4.90	276	1.65
5.35	465	2.88
5.90	738	4.32
6.50	984	7.08
6.82	962	5.78
7.80	465	3.84

[a] Solutions buffered with 0.3 M McIlvaine buffer; total ionic strength of the system adjusted to 1.0. [b] pH of solutions determined at 25° prior, during, and after oxidation (three half-lives) with Beckman GS pH meter did not vary more than 0.05 pH unit from value shown. [c] Experimentally determined apparent second ionization constant for sulfurous acid, $K_s' = 2.55 \times 10^{-7}$ (25°, $\mu = 1.0$).

Dependence of the copper-catalyzed (1×10^{-6} M) oxidation rate of sodium sulfite on inhibitor concentration is shown in Fig. 2. The linear relationship between the reciprocal half-life for the copper-catalyzed reaction and the reciprocal of the concentration of added inhibitor clearly demonstrates that significant oxidation of the thio compound takes place in the presence of overwhelming amounts of inhibitor when copper is present. The inhibitor appears to have little or no effect on radical initiation; it appears to function primarily by decreasing chain length. The heavy metal catalyzed oxidation of sulfurous acid salts in the presence of high concentrations of inhibitor is especially significant since this simulates pharmaceutical systems in which the compounds are utilized as antioxidants.

The rate of oxidation of sodium sulfite in the presence of 0.2 M ethanol appears to be directly proportional to the concentration of added cupric ion over the concentration range 0 to 30×10^{-6} M Cu(II) as shown in Fig. 3. However, a slow but measurable rate of oxidation of sodium sulfite takes place in the presence of an inhibitor even in the absence of added copper ion. This uncatalyzed rate of sulfite oxidation in the presence of an inhibitor may be approximated with Eq. 1 assuming $A = B = 10^{-5}$ and using the specific rate constant for the oxidation of sodium sulfite in air, $k = 2.8 \times 10^{-3}$ seconds^{-1}. For a sodium sulfite solution containing 0.2 M ethanol as an inhibitor, Eq. 1 predicts an approximate first-order rate constant for the inhibited oxidation: $k_1 = 5 \times 10^{-4}$ hours^{-1}; in terms of half-life, $t^1/_2 \cong 1400$ hours. This theoretical value agrees reasonably well with the experimentally determined half-life (1525 hours) for sulfite oxidation in the presence of 0.2 M ethanol and 1×10^{-3} M sodium cyanide as shown in Table I. Since the uncatalyzed reaction rate is four orders of magnitude less than the maximum copper-catalyzed rate, it is not detected as an intercept value on a rate plot such as Fig. 3. At higher concentrations ($> 40 \times 10^{-5}$ M) of the metal

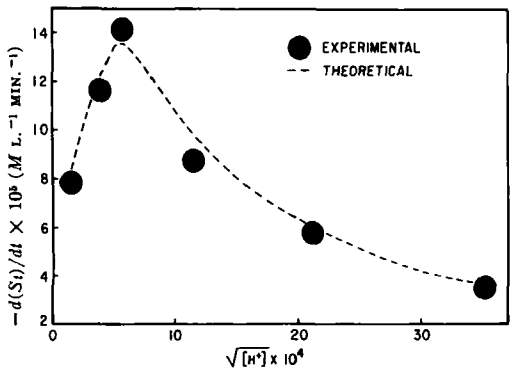

Fig. 6.—Initial rate of loss of total sulfurous acid species ($-d(S_t)/dt$) as a function of square root of the hydrogen ion concentration at 25°C. Initial solution composition: 0.02 M total sulfurous acid species, 0.2 M ethanol, 5×10^{-5} M Cu (II) in 0.3 M buffers. Ionic strength unity ($\mu = 1.0$). Theoretical line calculated with Eq. 4.

ion, the reaction appears to reach a limiting rate corresponding to a half-life for the oxidation of about 10 minutes. Doubling the stirring rate to increase the dissolution of oxygen does not apparently affect the limiting rate under these experimental conditions. This rate-limiting step may be related to the rate of reduction of Cu(II) or its regeneration.

Effect of initial solution pH on the copper-catalyzed oxidation of sulfurous acid salts in aqueous-ethanol solution is shown in Fig. 4. The initial pH of each of these solutions was adjusted to the indicated values with appropriate amounts of standard sodium hydroxide solution; the test solutions contained no other buffer species. Rapid initial oxidation occurred in all solutions at initial pH values from 5.9 to 8.3. The oxidation rates appeared to reach a plateau region in which little or no change occurred during the time of observation. The oxidation of bisulfite proceeds with the production of the more acidic sulfate species and an increase in the hydrogen ion concentration. The apparent effect is to decrease the total sulfurous acid species (HSO_3^- and SO_3^{2-}) wholly at the expense of sulfite as shown in Table II. This phenomenon has been discussed in a previous publication (1). The increase in hydrogen ion concentration in unbuffered oxidizing systems decreases the rate of oxidation in accordance with

$$-\frac{d(S_t)}{dt} = g\frac{(HSO_3^-)}{\sqrt{(H^+)}} \qquad \text{(Eq. 3)}$$

Oxidation of sulfurous acid salts in pharmaceutical systems usually takes place under conditions of constant pH since these compounds are added in small amounts and rarely represent the major buffer species. Oxidation of sulfurous acid salts in strongly buffered systems follows apparent first-order kinetics as shown in Fig. 5. The oxidation was studied in duplicate over about 90% of the reaction course (three half-lives). The relationship between the pH and the slopes of the lines representing the specific first-order rate constants suggests the presence of a maximum in the pH-rate constant profile. Table III indicates that the rate constants increase up to pH 6.5 and decrease at higher pH values. This unique dependency of the oxidation rate on hydrogen ion concentration may be satisfactorily explained by a modified form of Eq. 3. The concentration of hydrogen sulfite may be described in terms of total sulfurous acid specie added to the system, S_t

$$[HSO_3^-] = \frac{S_t(H^+)}{K_s' + (H^+)} \qquad \text{(Eq. 4)}$$

Substituting Eq. 4 into Eq. 3 gives

$$-\frac{d(S_t)}{dt} = g\frac{\frac{S_t(H^+)}{K_s' + (H^+)}}{\sqrt{(H^+)}} = g \cdot S_t \frac{\sqrt{(H^+)}}{K_s' + (H^+)} \qquad \text{(Eq. 5)}$$

The relationship shown above predicts a maximum oxidation rate when $[H^+] = K_s'$ since the terms $(\sqrt{(H^+)}/K_s' + (H^+))$ is maximal at this hydrogen ion concentration.

This further supports Abel's theoretical predictions (14) which were found to describe limited experimental data derived from unbuffered oxidizing sulfite systems. The rather good agreement between experimentally determined absolute initial rate of oxidation in buffered sulfite systems and the theoretical curve from Eq. 5 is shown in Fig. 6. The experimental maximum rate occurs at a hydrogen ion concentration of 3.16×10^{-7} which shows reasonable agreement with the theoretical value 2.55×10^{-7}. The latter value represents the experimentally determined apparent second ionization constant of sulfurous acid at 25° in buffer of total ionic strength equal to unity.

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