

# Precautionary Note for Use of Bisulfite in Pharmaceutical Formulations

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**Abstract** □ The effect of sodium bisulfite on aspirin hydrolysis was studied at 40° in the pH range of 6.5–7.5. Significant catalytic activity by the sulfite ion was observed. Second-order rate constants were calculated for this catalysis and compared to other buffer species. The sulfite ion was a much more efficient catalyst than acetate, phosphate, or carbonate.

**Keyphrases** □ Sodium bisulfite—effect on aspirin hydrolysis, various pH values □ Aspirin—hydrolysis, effect of sodium bisulfite, various pH values □ Hydrolysis—aspirin, effect of sodium bisulfite, various pH values □ Antioxidants—sodium bisulfite, effect on aspirin hydrolysis, various pH values

Sodium bisulfite, a widely used antioxidant, is known to react reversibly or irreversibly with various functional groups in drug molecules such as aldehyde, ketone, and alkene (1, 2). The inactivation of epinephrine and other drug molecules by bisulfite (3, 4) and the addition of bisulfite to a carbon-carbon double bond in uracil-type molecules (5) also were reported.

However, the catalytic effect of bisulfite on ester hydrolysis has not been recognized fully. Since many pharmaceutical preparations that employ bisulfite as an antioxidant may also contain esters as active ingredients or preservatives, the catalytic effect of bisulfite, if any, on ester hydrolysis should be examined.

Aspirin was chosen as the model ester because its rate and mechanism of hydrolysis have been investigated thoroughly.

## EXPERIMENTAL

**Materials**—All reagents were either analytical reagent grade or USP and were used without further purification. All water was double distilled in glass. All sodium bisulfite solutions were prepared fresh in double-distilled water saturated with nitrogen. Nitrogen was passed through a series of gas washing bottles containing vanadous oxychloride to remove trace amounts of oxygen.

**Methods**—All pH measurements were made at 40.0° using a research pH meter<sup>1</sup> and a combination electrode with a silver-silver chloride reference electrode. Standard pH buffers were prepared according to Bates (6). All spectral measurements<sup>2</sup> were made at 296 nm using distilled water as the blank. Temperature control was maintained to  $\pm 0.1^\circ$  using circulating water baths<sup>3</sup>.

**Determination of pKa for Sodium Bisulfite**—The pKa for the dissociation was determined potentiometrically. A stock solution of sodium bisulfite was prepared in nitrogen-saturated distilled water. Sufficient sodium chloride was added to produce an ionic strength of 1.0 in the region where the bisulfite was half-neutralized. A potentiometric titration was then performed at 40° under nitrogen atmosphere using 0.1 M NaOH containing 1 M NaCl. The pKa was calculated using the titration data near the half-neutralization point.

**Determination of Rate Constants for Aspirin Hydrolysis**—Aliquots of stock solutions of sodium bisulfite, sodium chloride, and ethylenediaminetetraacetic acid (I) were mixed such that the final ionic strength at 40° and at the pH given would be 1.0 M. The bisulfite concentrations

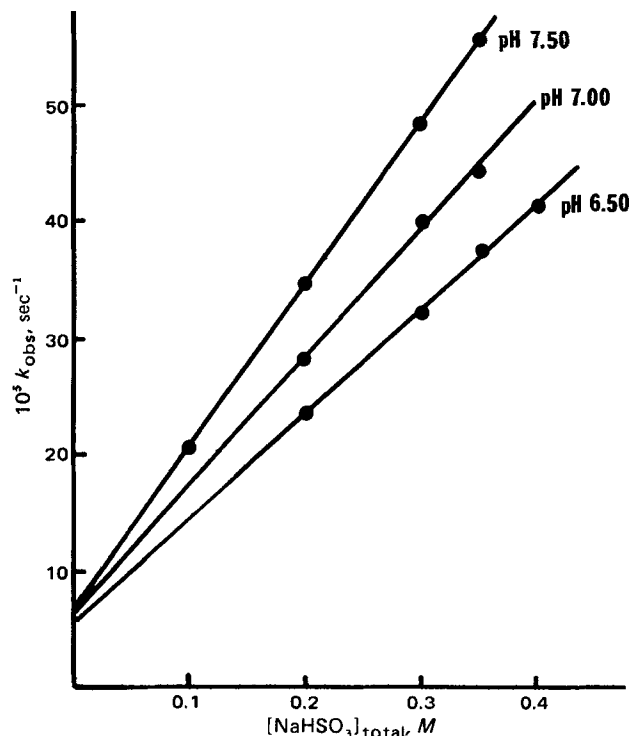


Figure 1—Plot of first-order rate constants for the hydrolysis of aspirin as a function of total sodium bisulfite concentration.

varied from 0.1 to 0.4 M. The final concentration of I was 0.0001 M in all cases. Compound I was added to suppress heavy metal-catalyzed oxidation of bisulfite. The volume of this mixture was adjusted to approximately 90 ml with nitrogen-saturated water.

The pH of this mixture was adjusted to the desired value under a nitrogen atmosphere. The mixture was then brought to volume, and final pH adjustments were made at 40° under nitrogen. Aliquots of 1 ml of the aspirin stock solution (acetonitrile) were added to this mixture and stirred. Samples were removed as a function of time, and the absorbance was measured immediately at 296 nm. A nitrogen atmosphere was maintained at all times. The pH of the reaction mixtures was measured at the end of the reaction. If the pH changed more than 0.05 unit during the reaction, the data were discarded.

Pseudo-first-order rate constants were determined from plots of  $A_\infty - A_t$  versus time, where  $A_\infty$  is the absorbance at 296 nm at the end of the reaction and  $A_t$  is the absorbance at a given time  $t$ . The actual rate constants were evaluated by an exponential least-squares fit<sup>4</sup>. Only reactions that gave a correlation coefficient greater than 0.995 were used in the final calculations.

## RESULTS AND DISCUSSION

The pKa for the dissociation of bisulfite to sulfite was 6.36 ( $K_a = 4.29 \times 10^{-7} M$ ) at 40° and an ionic strength of 1.0 M. This value was used to calculate ionic strength adjustments for the kinetic analysis and in the calculation of the second-order rate constants. The increase in the hydrolysis rate with increasing pH and increasing total bisulfite concentration suggested that both bisulfite and sulfite catalyses play an important role.

<sup>4</sup> Wang 600 programmable calculator.

<sup>1</sup> Beckman.

<sup>2</sup> Gilford model 240 spectrophotometer.

<sup>3</sup> Haake F2 and Sargent ST. thermometers carried an American Society of Testing Materials certificate of calibration.

**Table I—Linear Regression Analysis Data for Plots of  $k_{\text{obs}}$  versus  $[\text{NaHSO}_3]_{\text{total}}$** 

pH	Slope, liter mole <sup>-1</sup> sec <sup>-1</sup>	Intercept, sec <sup>-1</sup>	Correlation Coefficient	SE of Estimate ( $S_{xy}$ )
6.5	$9.09 \times 10^{-4}$	$5.30 \times 10^{-5}$	0.998	$3.87 \times 10^{-6}$
7.0	$11.08 \times 10^{-4}$	$6.26 \times 10^{-5}$	0.995	$7.09 \times 10^{-6}$
7.5	$13.98 \times 10^{-4}$	$6.59 \times 10^{-5}$	0.999	$1.48 \times 10^{-6}$

The rate equation that describes catalysis by both species is:

$$k_{\text{obs}} = k_0 + k_1[\text{HSO}_3^-] + k_2[\text{SO}_3^{2-}] \quad (\text{Eq. 1})$$

where  $k_0$  is the rate constant for the uncatalyzed hydrolysis,  $k_1$  is the rate constant for the bisulfite-catalyzed hydrolysis, and  $k_2$  is the rate constant for the sulfite-catalyzed hydrolysis. This equation can be written as:

$$k_{\text{obs}} = k_0 + (k_1 F_{\text{HSO}_3^-} + k_2 F_{\text{SO}_3^{2-}})[\text{NaHSO}_3]_{\text{total}} \quad (\text{Eq. 2})$$

where  $F_{\text{HSO}_3^-}$  is the fraction of the total concentration that exists as the bisulfite ion and  $F_{\text{SO}_3^{2-}}$  is the fraction that exists as the sulfite ion. In solving the appropriate simultaneous equations derived from the data, the value for  $k_1$  sometimes appears as a negative number. Furthermore, the values for  $k_2$  differ by as much as 25%. These findings suggest that the bisulfite ion does not play a significant role in the catalysis of aspirin hydrolysis.

The rate equation that describes only sulfite catalysis is:

$$k_{\text{obs}} = k_1 + k_2[\text{SO}_3^{2-}] \quad (\text{Eq. 3})$$

where  $k_1$  is the rate constant for uncatalyzed aspirin hydrolysis and  $k_2$  is the rate constant for sulfite-catalyzed aspirin hydrolysis. This equation can be written as a function of total bisulfite concentration:

$$k_{\text{obs}} = k_1 + k_2 F_{\text{SO}_3^{2-}}[\text{NaHSO}_3]_{\text{total}} \quad (\text{Eq. 4})$$

The values for  $F_{\text{SO}_3^{2-}}$  are 0.576 (pH 6.50), 0.88 (pH 7.00), and 0.931 (pH 7.50). Therefore, the slope of a plot of  $k_{\text{obs}}$  versus total bisulfite concentration, at a given pH, is equal to the fraction existing as the sulfite ion

**Table II—Rate Constants at 40°,  $\mu = 1.0$** 

pH	$10^5 k_1, \text{sec}^{-1}$	$10^3 k_2, \text{liter mole}^{-1} \text{sec}^{-1}$
6.5	5.30	1.57
7.0	6.26	1.36
7.5	6.59	1.50

**Table III—Second-Order Rate Constant for Aspirin Hydrolysis by Different Species at 40°**

Species	$k \times 10^5 M^{-1} \text{min}^{-1}$
Sulfite	8800.0
Acetate	7.4
Phosphate	69.0
Carbonate	291.0

times the second-order rate constant,  $k_2$ . The intercept of this plot corresponds to the rate constant for the uncatalyzed reaction,  $k_1$ . The slopes and intercepts for the lines shown in Fig. 1 are given in Table I. The rate constants derived from these data are presented in Table II. The average values for the rate constants are:  $k_1, 6.05 \times 10^{-5} \text{sec}^{-1}$  ( $SD \pm 6.70 \times 10^{-6}$ ); and  $k_2, 1.48 \times 10^{-3} \text{liter mole}^{-1} \text{sec}^{-1}$  ( $SD \pm 1.07 \times 10^{-4}$ ).

To compare the catalytic effect of sulfite to other catalytic species commonly employed in pharmaceutical formulations, the data of Fersht and Kirby (7) for the hydrolysis of aspirin catalyzed by acetate, phosphate, and carbonate have been included in Table III. As can be seen from these data, sulfite is 1200 times more effective than acetate and 120 times more effective than phosphate.

The significance of these findings with regard to the mechanism of aspirin hydrolysis as well as the catalytic effect of sulfite on the hydrolysis of other esters employed in pharmaceutical products is being studied in this laboratory. In conclusion, these results suggest that the possibility of sulfite-catalyzed ester hydrolysis should be considered carefully in formulations.

## REFERENCES

- (1) C. M. Suter, "The Organic Chemistry of Sulfur," Wiley, New York, N. Y., 1944, pp. 98, 101, 126.
- (2) M. S. Kharasch, E. M. May, and F. B. Mayo, *J. Org. Chem.*, **3**, 175 (1938).
- (3) T. Higuchi and L. Schroeter, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 535 (1959).
- (4) T. Higuchi and L. Schroeter, *J. Am. Chem. Soc.*, **82**, 1904 (1960).
- (5) G. C. Rork and I. H. Pitman, *ibid.*, **97**, 5559 (1975).
- (6) R. G. Bates, *J. Res. Natl. Bur. Stand.*, **66A**, 179 (1962).
- (7) A. R. Fersht and A. J. Kirby, *J. Am. Chem. Soc.*, **89**, 4857 (1967).

## ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1976, from the College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Accepted for publication February 28, 1977.

The authors thank Mr. Kenneth Pearce and Dr. Hassan Abdine for assistance.

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