The Mechanism of the Decomposition of a Bronchodilator, *S*-Nitroso-N-acetyl-*D,L*-penicillamine (SNAP), by a Bronchoconstrictor, Aqueous Sulfite: Detection of the N-Nitrosohydroxylamine-N-sulfonate ion

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Received April 17 2002

The mechanism of the decomposition of a bronchodilator, *S*-nitroso-N-acetyl-*D,L*-penicillamine (SNAP) by a bronchoconstrictor, aqueous sulfite, has been investigated in detail. The decomposition was studied using a conventional spectrophotometer at 336 nm over the ranges: $0.010 \leq [S^{IV}]_T \leq 0.045$ mol dm^{-3}, $3.96 \leq$ pH ≤ 6.80 and $15.0 \leq \theta \leq 30.0$ °C, $0.60 \leq I \leq 1.00$ mol dm^{-3}, and at ionic strength 1.00 mol dm^{-3} (NaCl). The rate of reaction is dependent on the total sulfite concentration and pH in a complex manner, *i.e.*, $k_{obs} = k_1K_2[S^{IV}]_T/([H^+] + K_2)$. At 25.0 °C, the second order rate constant, k_1, was determined as 12.5 ± 0.15 mol^{-1} dm^3 s^{-1}. $\Delta H^{\neq} = +32 \pm 3$ kJ mol^{-1} and $\Delta S^{\neq} = -138 \pm 13$ J mol^{-1}K^{-1}. The N-nitrosohydroxylamine-N-sulfonate ion was detected as an intermediate before the formation of any of the by-products, namely, *N*-acetyl-*D,L*-penicillamine. The effect of concentration of aqueous copper(II) ions on this reaction was also examined at pH 4.75, but there was no dependence on [Cu^{2+}]. In addition, the pK_a of SNAP was determined as 3.51 ± 0.06 at 25.4 °C [$I = 1.0$ mol dm^{-3} (NaCl)].

Key Words : *S*-Nitrosothiols, Bronchoconstrictor, Bronchodilator, Aqueous sulfite, *S*-Nitroso-N-acetyl-*D,L*-penicillamine

Introduction

S-nitrosothiols (RSNOs), products of *S*-nitrosation of thiols, are important nitric oxide (NO) donors that show similar biological properties to NO, particularly in the vasodilation of veins and arteries, along with inhibition of platelet aggregation.[1,2] RSNOs are believed to play an important role in storing, transporting, and releasing NO under physiological conditions.[1-5] Depending on the conditions present, the S-N bond can be cleaved homolytically or heterolytically to form the nitrosonium cation (NO^+), the nitroxyl anion (NO^-) or "free" nitric oxide (NO) gas.[6,7]

In 1990 it was reported that *S*-nitrosoproteins, which are formed readily under physiological conditions can possess endothelium-derived relaxing factor-like effects.[8] Two years later Stamler *et al.*[9,10] reported that nitrosated thiol groups on proteins produced a vasodilator, which was more stable than NO itself. During that period there was a belief that NO was stored in smooth muscle cells as an *S*-nitrocompound,[9] but it was not clear how the thiol groups were nitrosated as NO itself is not a nitrosating agent. Some examples of such *S*-nitrosoproteins were derived from serum albumin and reduced glutathione, which were believed to act as NO carriers in the serum and cytosol, respectively.[11]

Four years ago it was reported by Gaston *et al.*,[12] that human airways were known to contain RSNOs which had a broad range of bioactivities, including bronchodilation, receptor-mediated neurotransmission, and host defence, and that their synthesis or breakdown would be regulated.[12] Now airways concentrations of both NO and RSNOs are believed to reflect inducible (inflammatory) nitric oxide synthase expression, which was increased in asthmatic patients.[13] Gaston *et al.* also reported that asthmatic respiratory failure was paradoxically associated with low airway concentrations of RSNOs, where this observation raised the possibility that depletion of bronchodilators (RSNOs) may have contributed to the pathophysiology of severe airflow obstruction.[12] Gaston *et al.*[12] found this as surprising since severe asthma was classically characterised by an excess of brochoconstricting and inflammatory mediators and not by a bronchodilator deficiency. Based on their findings, they speculated that low concentrations of airways RSNOs might have represented a distinct metabolic consequence of asthmatic inflammation.[12]

Sulfur(IV)-containing compounds such as sodium metabisulfite, sodium bisulfite, and sulfur dioxide, are very popular preservatives for drugs, foods, wines, and fruit juices.[14-18] However, it has been reported that these compounds can cause bronchoconstriction,[14,16-18] while leading to asthma attacks. This is clearly an antagonistic effect, which is opposite to the findings of Gaston *et al.*[12]

Along with these interesting findings, there were reports of the reaction involving nitric oxide and aqueous sulfite.[19-22]

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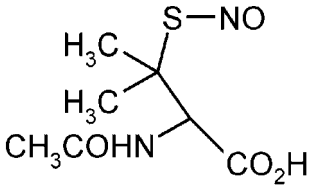
One such case was reported by Littlejohn *et al.*,[23] who studied the reaction of NO with bisulfite and sulfite anions in aqueous solution. Littlejohn *et al.* detected and identified the N-nitrosohydroxylamine-N-sulfonate ion as the only product from such a reaction, with this ion having a strong ultraviolet absorption at 258 nm.[23] Seven years ago, Harvey and Nelsestuen had reported[14] that the reaction between NO and *S*-nitrosobovine serum albumin, but the results were not conclusive due to the fact that a metal ion chelator was not used in the aqueous system and due to the fact that ionic strength was not controlled. More recently, studies on the reactivity of sulfur nucleophiles with some RSNOs were reported by Munro and Williams,[24] who carried out a very partial study of the reaction between aqueous sulfite and several RSNOs.

This detailed work completes the partial study carried out by Munro and Williams,[24] but using only *S*-nitroso-N-acetyl-*D,L*-penicillamine (SNAP) [I], while controlling the ionic strength. In our study, variations in temperature (in order to determine the activation parameters) and ionic strength are studied for the very first time.



I

This is also the very first report where the N-nitroso-hydroxylamine-N-sulfonate ion is detected in aqueous solution from the reaction between any *S*-nitrosothiol and aqueous sulfite. In addition, the pK_a of SNAP was determined for the very first time, and the effect of aqueous copper(II) ions on the decomposition reaction was also investigated. This work complements findings of the effects of aqueous sulfite-containing species and RSNOs species on asthma patients through the use of kinetics studies in aqueous media.

Experimental Section

Materials. All reagents used were of analytical grade (BDH or Sigma-Aldrich Chemicals Company). Ultra pure water, obtained by deionising distilled water using a Houseman Ionmiser, model 3C water system, was used for all preparative work and to make up solutions for all physical measurements. To prevent the decomposition of SNAP by trace metal ions particularly that of aqueous Cu^{2+}, disodium dihydrogenethylenediaminetetra-acetic acid dihydrate ($Na_2H_2EDTA{\cdot}2H_2O$) was used as a chelating agent. Solid sodium metabisulfite, $Na_2S_2O_5$, was used as a source of aqueous sulfite (S^{IV}); this salt is very stable in the solid form but hydrates rapidly and completely when dissolved in water to yield aqueous sulfite.[25]

Preparation of *S*-nitroso-N-acetyl-*D,L*-penicillamine (SNAP). *S*-nitroso-N-acetyl-*D,L*-penicillamine was prepared as by Fields and Ravichandran:[26] Sodium nitrite (3.59 g, 52 mmol) in deionized water (52 cm^3) was added to N-acetyl-*D,L*-penicillamine (5 g, 26 mmol), which was dissolved in a solution consisting of methanol (52 cm^3), HCl (52 cm^3, 1 mol dm^{-3}) and H_2SO_4 (5.2 cm^3, 18 mol dm^{-3}). This addition was carried out over a two-minute period with vigorous stirring at 24.0 °C. The reddish-green mixture was allowed to stand for an additional 20 minutes, after which SNAP precipitated. The product was collected by filtration under suction. The crystals, which were green with red reflections, were washed with water and air-dried. Yield = 5.55 g (96%).

UV-visible: λ_{max} 336 nm (H_2O), ε = 857 mol^{-1} dm^3 cm^{-1} [literature:[27] λ_{max} = 350 nm (MeOH), ε = 500 mol^{-1} dm^3 cm^{-1}]. Infrared stretching frequencies: ν (N-O) = 1488 cm^{-1}, ν (C-S) = 666 and 691 cm^{-1} [literature:[28] ν (N-O) 1488-1530 cm^{-1}, ν (C-S) 600-730 cm^{-1}].

Kinetic measurements. All kinetic measurements and UV-visible spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer, which was thermostatted by a Fisher Scientific refrigerant water bath. Both SNAP and aqueous sulfite solutions were buffered and placed in the syringes of Hitech Scientific rapid mix accessory fitted with an umbilical cuvette. $Na_2H_2EDTA{\cdot}2H_2O$ was added to the SNAP solution in order to sequester any Cu^{2+} ions or any heavy metals ions present in the aqueous solution. Mixing was done manually and all reactions were followed at 336 nm, where there is a maximum absorption change in going from reactants to products. A stock sodium chloride solution, which was used in maintaining the ionic strength, was standardized by an ion-exchange method using Dowex 50 W-X8 (50 mesh, H^+ form) resin before any dilution. Total ionic strength in all reactions was maintained at 1.00 mol dm^{-3} (NaCl), with the exception of the experiment whereby the ionic strength was varied. Pseudo-first order conditions (sulfite concentration was at least 10-fold excess of SNAP) were maintained for all reactions. The recorded pseudo-first order rate constants (k_{obs}) are an average of at least three kinetic runs with a standard deviation of ± 5%. A Specfit Global Analysis software was used to fit all absorbance-time traces in order to calculate the k_{obs} values.

Citric acid/HPO_4^{2-} buffer system was used to control the pH during all reactions. An Orion Research EA 920 expandable ion analyser fitted with a Cole Parmer combination electrode, was used to measure the pH of each solution.

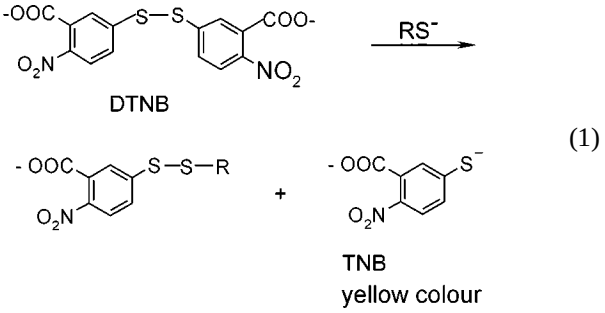
Determination of the pK_a for *S*-nitroso-N-acetyl-*D,L*-penicillamine (SNAP). A solution of SNAP (1×10^{-3} mol dm^{-3}) in deionised water at I = 1.0 mol dm^{-3} (NaCl), was titrated against NaOH [1×10^{-2} mol dm^{-3} also at I = 1.0 mol dm^{-3} (NaCl)], at 25.4 °C. After each addition of a known volume of NaOH, the mixture was stirred and the pH of the solution was measured. The respective data is shown in Table 1. The pK_a was determined as 3.51 ± 0.06 using calculations as for $[(H_3N)_5CoOH_2](ClO_4)_3$, which is a monobasic acid.[29] This value is quite similar within experimental errors

Table 1. The results obtained for the determination of pK_a of SNAP at 25.4 °C. [SNAP] = 1×10^{-3} mol dm^{-3}, [NaOH] = 1×10^{-2} mol dm^{-3}, and I = 1.0 mol dm^{-3} (NaCl)

Volume of NaOH added/cm^3	pH	Volume of NaOH added/cm^3	pH
0.5	3.30	5.7	4.05
0.7	3.33	6.3	4.32
0.9	3.35	6.8	4.57
1.4	3.42	7.2	6.03
1.9	3.52	7.7	8.87
2.4	3.63	8.3	9.11
2.9	3.76	8.6	9.34
3.4	3.83	9.1	9.61
4.0	3.84	9.6	9.79
4.8	3.96	10.1	9.91
5.1	4.06	10.6	10.04

to the calculated pK_a value of 3.13, which was determined using the software, pK_{calc}, available from CompuDrug International, 705 Grandview Drive, South San Francisco, CA 94080, U.S.A.

Stoichiometry. The stoichiometry was obtained with the use of the Ellman's reagent. 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) or Ellman's reagent,[30] as it is also known, was used in quantifying the amount of "free" thiol (RSH) present at the end of the reaction. DTNB reacts with "free" thiols to produce a mixed disulfide and the thiolate anion, 5-thio-2-nitrobenzoate (TNB), as shown in equation (1). TNB is quantified by its very strong visible absorbance[30,31] at 412 nm (ε_{412} = 13,600 mol^{-1} dm^3 cm^{-1}).



(1)

Results

Nature of the reaction. On mixing the reactants, a decrease in the absorbance at 336 nm was observed over the 3.96 ≤ pH ≤ 6.80 range, where there was a colour change from green to colourless as the reaction proceeded. A repetitive scan over the wavelength range is shown for the reaction in Figure 1. Preliminary studies of the reaction indicate that there is a uniphasic reaction at 336 nm under pseudo-first order conditions. Figure 2 shows the spectral change in going from the reactant (SNAP) to its final decomposed products exactly one day after the reaction had been carried out. Immediately at the end of a kinetic run at pH 4.51, the N-nitrosohydroxylamine-N-sulfonate ion was detected as shown in Figure 3. At 256 nm, the molar extinction coefficient of the species was calculated as 3839 mol^{-1} dm^3 cm^{-1}.

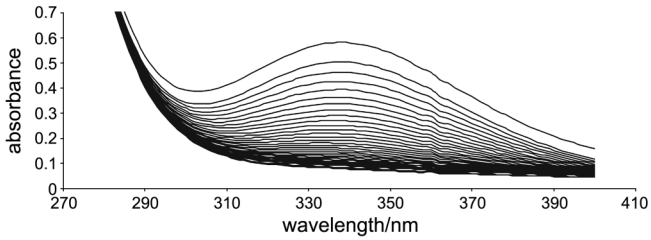


Figure 1. Repetitive scan for the decomposition of SNAP by aqueous sulfite at 15.0 °C. [SNAP] = 5×10^{-4} mol dm^{-3}, $[S^{IV}]_T$ = 0.05 mol dm^{-3}, I = 1.0 mol dm^{-3} (NaCl), pH = 5.05 (buffer = Citric acid/HPO_4^{2-}), cycle time = 5 seconds.

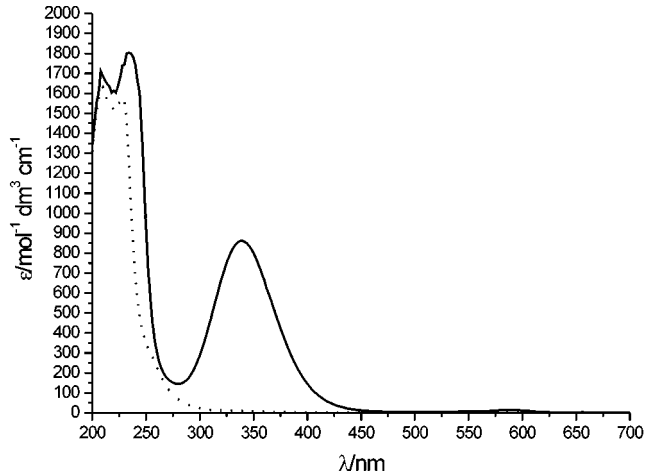


Figure 2. The spectral change in going from the reactant [SNAP with no aqueous sulfite] (–) to its decomposed products (…). [SNAP] = 1.51×10^{-3} mol dm^{-3}, $[S^{IV}]_T$ = 0.02 mol dm^{-3}, [H_2EDTA^{2-}] = 2×10^{-3} mol dm^{-3}, pH = 4.42. Buffer = citric acid/HPO_4^{2-}.

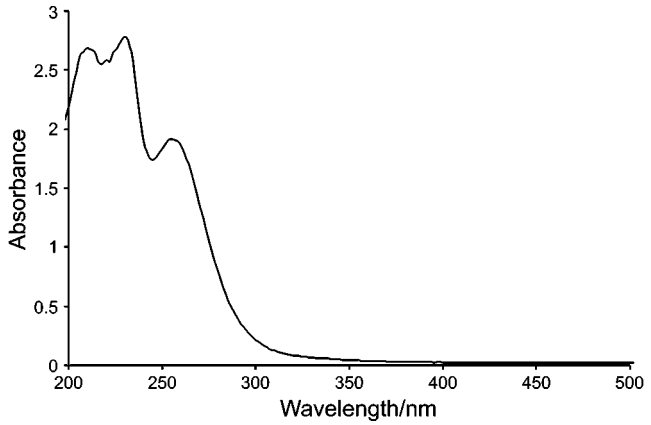


Figure 3. UV/Visible spectrum of the N-nitrosohydroxylamine-N-sulfonate ion at pH 4.51. [SNAP] = 5×10^{-4} mol dm^{-3}, $[S^{IV}]$ = 0.10 mol dm^{-3}, [Na_2H_2EDTA] = 1×10^{-3} mol dm^{-3}, I_{Total} = 1.0 mol dm^{-3} (NaCl) at 20.0 °C.

Stoichiometry of the reaction. Studies using the Ellman's reagent under the conditions of [SNAP] = 20.0×10^{-6} mol dm^{-3}, $[S^{IV}]_T$ = 5×10^{-3} mol dm^{-3}, and [DTNB] = 25.2×10^{-6}

mol dm^{-3} [I = 1.0 mol dm^{-3} (NaCl)] resulted in the concentration of TNB detected as $(19.3 \pm 0.50) \times 10^{-6}$ mol dm^{-3}. This verified that one mole of "free" thiol (RSH) is formed for every mole of SNAP consumed. So from a ratio of a 0.97 : 1 (S^{IV} : SNAP), a stoichiometry of 1 : 1 [S^{IV} : SNAP] is proven.

Sulfite, ionic strength, temperature, and pH dependence of the reaction. Kinetic runs were carried out over the $0.010 \leq [S^{IV}]_T \leq 0.045$ mol dm^{-3}, $3.96 \leq$ pH ≤ 6.80, and $15.0 \leq q \leq 30.0$, $0.60 \leq I \leq 1.00$ mol dm^{-3} ranges, and at ionic strength 1.00 mol dm^{-3} (NaCl). All the kinetic runs showed very good first order behaviour. The pseudo-first order rate constants (k_{obs}) for the reaction at the various $[S^{IV}]_T$, ionic strength, and pH are shown in Tables 2-4. A plot of k_{obs} versus $[S^{IV}]_T$ is shown in Figure 4. There is a zero intercept, which implies that the rate is first order with respect to $[S^{IV}]_T$. k_{obs} was found to increase with an increase in pH, justifying that the reaction is definitely pH dependent as shown in Table 4.

Effect of aqueous copper(II) ions. Here kinetic runs were carried out over the $3 \leq [Cu^{2+}] \leq 9$ μmol dm^{-3} range at 25.0 °C. The k_{obs} values for the variation in the concentration of aqueous Cu^{2+} ions are shown in Table 5. A plot of k_{obs} versus $[Cu^{2+}]$ is shown in Figure 5, where there is no change in the rate of reaction. This implies that the rate of reaction is zero order with respect to the concentration of aqueous Cu^{2+} ions.

Table 2. Pseudo First Order Rate Constants for the Decomposition of SNAP by Aqueous Sulfite. Variation in $[S^{IV}]_T$: [SNAP] = 5×10^{-4} mol dm^{-3}, [Na_2H_2EDTA] = 1.0×10^{-3} mol dm^{-3}, pH = 6.80, λ = 336 nm, I = 1.0 mol dm^{-3} (NaCl), θ = 15.0 °C , buffer = Citric acid/HPO_4^{2-}

$[S^{IV}]_T$/mol dm^{-3}	$10^2 k_{obs}$/s^{-1}
0.01	3.90
0.015	6.00
0.02	8.06
0.025	9.98
0.03	12.6
0.045	18.0

Table 3. Pseudo First Order Rate Constants for the Decomposition of SNAP by Aqueous Sulfite. Variation in Ionic Strength: λ = 336 nm, θ = 30.0 °C, $[S^{IV}]_T = 5.0 \times 10^{-3}$ mol dm^{-3}, [SNAP] = 5×10^{-4} mol dm^{-3}, [Na_2H_2EDTA] = 1.0×10^{-3} mol dm^{-3}, pH = 3.96, buffer = Citric acid/HPO_4^{2-}

I/mol dm^{-3}	$10^3 k_{obs}$/s^{-1}
0.6	5.63
0.7	6.10
0.8	6.22
0.9	6.71
1.0	6.84

Table 4. Pseudo First Order Rate Constants for the Decomposition of SNAP by Aqueous Sulfite. Variation in pH and temperature: [SNAP] = 5×10^{-4} mol dm^{-3}, $[S^{IV}]_T$ = 0.05 mol dm^{-3}, λ = 336 nm, [Na_2H_2EDTA] = 1.0×10^{-3} mol dm^{-3}, I = 1.0 mol dm^{-3} (NaCl), buffer = Citric acid/HPO_4^{2-}

15.0 °C		20.0 °C		25.0 °C		30.0 °C	
pH	$10^2 k_{obs}$/s^{-1}	pH	$10^2 k_{obs}$/s^{-1}	pH	$10^2 k_{obs}$/s^{-1}	pH	$10^2 k_{obs}$/s^{-1}
4.18	0.44	4.51	1.08	4.41	1.26	4.56	1.93
4.44	0.66	4.79	1.76	4.63	1.83	4.77	2.91
4.63	0.93	4.87	2.38	4.72	2.21	4.88	3.44
4.75	1.14	5.10	3.62	4.90	2.44	5.19	6.75
5.10	2.18	5.19	3.81	5.02	3.38	5.37	9.71
5.17	2.68	5.42	5.97	5.17	5.20	5.76	16.3
5.61	6.24	5.75	11.0	5.62	12.2	5.84	18.6
5.70	6.92	5.88	12.7	5.76	13.8	5.95	26.9
5.76	7.70	5.98	15.2	6.02	20.4	6.17	27.0
6.35	20.0	6.15	18.7	6.35	33.2	6.45	42.9
6.51	21.4	6.42	27.4	6.52	38.8	6.61	46.6
–	–	6.56	31.7	–	–	–	–

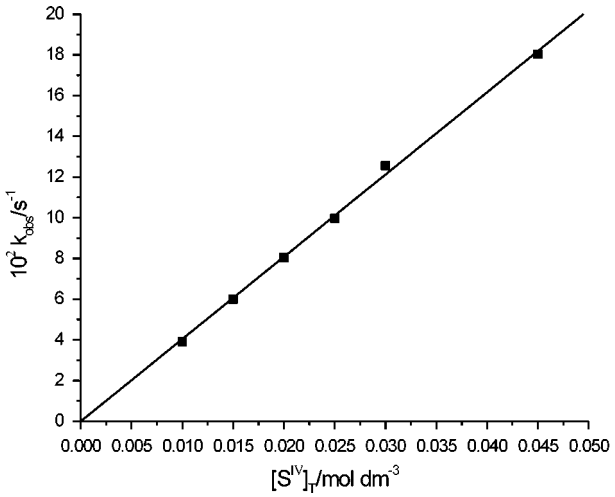


Figure 4. A plot of k_{obs} *versus* $[S^{IV}]_T$ for the decomposition of SNAP by aqueous sulfite at 15.0 °C.

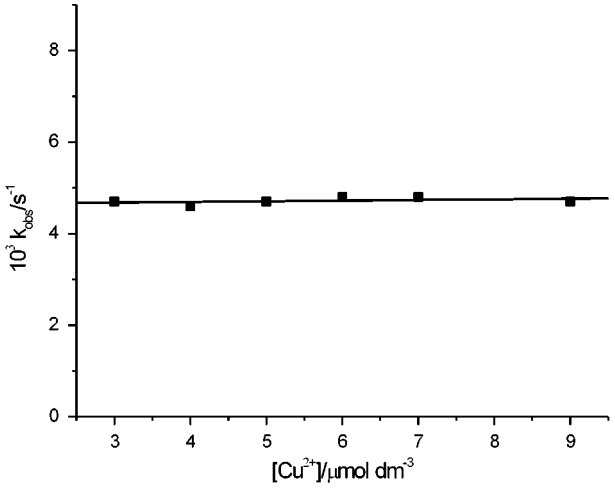


Figure 5. A plot of k_{obs} *versus* $[Cu^{2+}]$ for the decomposition of SNAP by aqueous sulfite in the presence of aqueous Cu^{2+} ions at 25.0 °C. [SNAP] = 5×10^{-4} mol dm^{-3}, pH = 4.75, $[S^{IV}]_T = 1.25 \times 10^{-2}$ mol dm^{-3}, I = 1.0 mol dm^{-3} (NaCl), buffer = citric acid/HPO_4^{2-}.

Discussion

The stoichiometry is summarised in the following equations:

$$2RSNO + 2HSO_3^- \rightarrow RSSR + 2HSO_3(NO)^- \quad (2)$$

then

$$2HSO_3(NO)^- \rightarrow HSO_3^- + SO_3(NO)_2^{2-} + H^+ \quad (3)$$

followed by:

$$SO_3(NO)_2^{2-} \rightarrow N_2O + SO_4^{2-} \quad (4)$$

Overall

$$2RSNO + 2HSO_3^- \rightarrow N_2O + SO_4^{2-} + RSSR + H^+ + HSO_3^- \quad (5)$$

The disulfide, RSSR, is now reduced by the S^{IV} species (HSO_3^-) as shown below:

$$H_2O + 2H^+ + 2HSO_3^- + RSSR \rightarrow 2RSH + 2SO_4^{2-} + 3H^+ \quad (6)$$

The detection of the N-nitrosohydroxylamine-N-sulfonate ion at pH 4.51 highlights the stoichiometry shown above. We believe that a precursor to its formation, namely, $HSO_3(NO)^-$,[33] is formed, which then dimerises to form the N-nitrosohydroxylamine-N-sulfonate (NHAS) ion, $SO_3(NO)_2^{2-}$, as shown in in equation (3). Due to the fact that the NHAS ion is unstable in acidic media,[23,33] and from our value of ε_{256} =3839 mol^{-1} dm^3 cm^{-1} at pH 4.51, which is less than the literature value of 7140 mol^{-1} dm^3 cm^{-1} at 258 nm,[33] it is believed that NHAS could have decomposed to form nitrous oxide gas and the sulfate anion over our pH range of 3.96 to 6.80. Equations (5) and (6) are supported by the spectral change in Figure 2, which lacks an absorbance at 258 nm (the λ_{max} for the NHAS ion) a day after the reaction had been carried out; and also from the quantification of N-acetyl-*D,L*-penicillamine by the Ellmans reageant.

It is well known that for species of like charges, the rate of reaction increases with an increase in ionic strength.[34] However, with unlike species, there is an increase in the rate of reaction as the ionic strength decreases.[34] For uncharged species, the rate of reaction is unaffected by the ionic strength. Over the pH range of 3.96 to 6.80 the main existing sulfite species are HSO_3^- and SO_3^{2-} ions (see Figure 6); while the undissociated form of SNAP (HA) and the dissociated from (A^-) exist in equilibrium, but since the pK_a of SNAP is 3.51, the dissociated species, A^-, is the reactive species in the reaction over the pH range. The A^- species exists as > 60% as the predominant dissociated form. Therefore, we would expect A^- and HSO_3^- to be the main reacting species at pH 3.96, where each species has a -1 charge. In Table 3, there is an increase in the rate of reaction as the ionic strength is increased at pH 3.96 due to the fact that species of like charges are reacting. By applying the Brönsted-Bjerrum equation:[35]

$$\log k_{obs} = \log k_{obs}^o + 2Z_1Z_2AI^{½}/(1 + I^{½}) \quad (7)$$

where k_{obs}^o is the rate constant at infinite dilution, Z_1 and Z_2, are the ionic charges, and A is the Debye Hückel constant (A = 0.5141 $mol^{-1/2}$ $dm^{3/2}$ at 30.0 °C).[35] A plot of log k_{obs} versus $I^{1/2}/(1+I^{1/2})$ is linear as shown in Figure 7, from which the slope = $2Z_1Z_2A$ = +1.33 ± 0.13 and the intercept=log k_{obs}^o = –2.83 ± 0.06. From the intercept, k_{obs}^o is calculated as (1.50 ± 0.03) × 10^{-3} s^{-1}. A slope of +1.33 represents the product of the charges on the reactive species, that is, Z_1 = -1

Table 5. Variation in concentration of copper(II) ions for the decomposition of SNAP by aqueous sulfite in the presence of Cu^{2+} ions at 25.0 °C. [SNAP] = 5 × 10^{-4} mol dm^{-3}, pH = 4.75, $[S^{IV}]_T$ = 1.25 × 10^{-2} mol dm^{-3}, I = 1.0 mol dm^{-3} (NaCl), buffer = citric acid/ HPO_4^{2-}

$[Cu^{2+}]/\mu M$	10^3 k_{obs}/s^{-1}
3	4.66
4	4.63
5	4.68
6	4.77
7	4.79
9	4.74

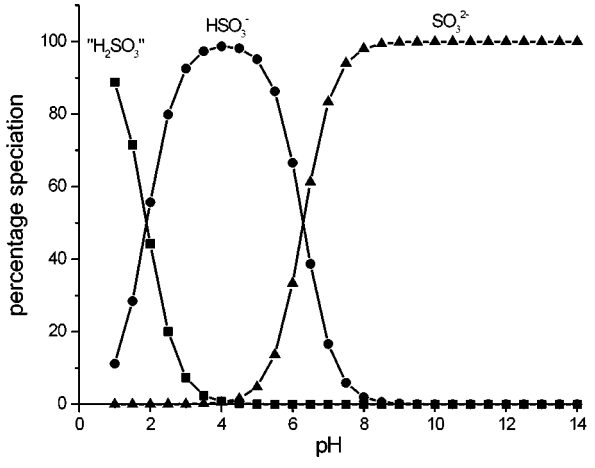


Figure 6. A plot showing the percent speciation of "H_2SO_3", HSO_3^- and SO_3^{2-}, with variation in pH.

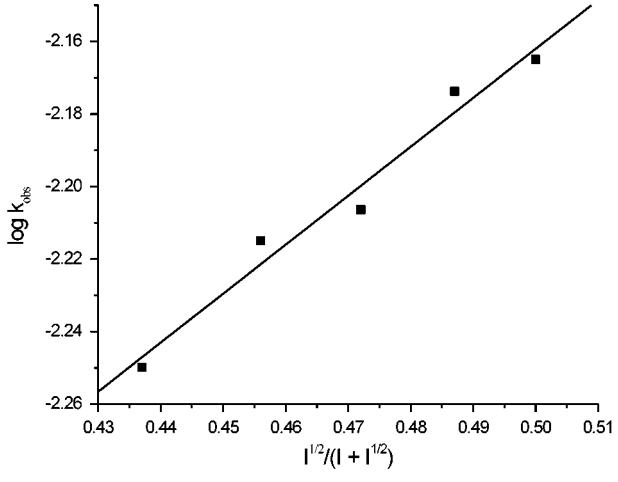
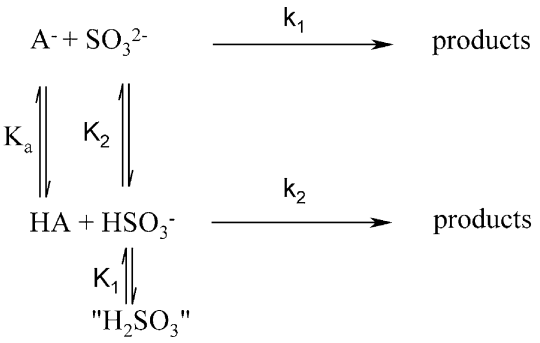


Figure 7. A plot of log k_{obs} *versus* $I^{1/2}/(1+I^{1/2})$ for the decomposition of SNAP by aqueous sulfite at 30.0 °C.



Scheme 1

and $Z_2 = -1$. From these results, one can deduce that the reactive species at pH = 3.96 are A^- and HSO_3^-.

An alternative treatment for the ionic strength dependence on k_{obs} would be to apply the full Brönsted-Bjerrum equation (where α is the distance of closest approach of the reacting ions;[35] for aqueous solutions at 30 °C, B = 3.30 $dm^{3/2}$ $mol^{-1/2}$ nm^{-1}). The best fit for the data in Table 3 was obtained from non-linear regression analysis by using a Statgraphics programme while floating log $k_{obs}°$ and α until the best fit was obtained. It was found that $\alpha = 0.30 \pm 0.03$ nm and $k_{obs}° = (1.50 \pm 0.03) \times 10^{-3}$ s^{-1}. The values obtained for α is taken to be the sum of the radii of the two reacting species (A^- and HSO_3^-).

The observed pseudo-first order rate constants, k_{obs}, increase with an increase in pH (Table 4), and this observation may be interpreted in terms of the reactive species *via* the proposed mechanism in Scheme 1, where $K_1 = 1.26 \times 10^{-2}$ mol dm^{-3} and $K_2 = 5.01 \times 10^{-7}$ mol dm^{-3} at 25.0 °C.[36] Based on the increase of k_{obs} with pH it can be deduced that SO_3^{2-} is the more reactive species in the decomposition reaction; so k_1 is the main contributing path over the pH range. The proposed mechanism given in Scheme 1 leads to the expression:

$$k_{obs} = \frac{k_1 K_2 [S^{IV}]_T}{[H^+] + K_2} \quad (8)$$

where HA is the undissociated form of SNAP, A^- the dissociated form of SNAP, and $[S^{IV}]_T$ is the total sulfite concentration.

The second-order rate constant, k_1, was determined by non-linear regressional analysis using Statgraphics 4.0 software. The second-order rate constant for each temperature, along with the corresponding enthalpy and entropy of activation, which were calculated using the Eyring's equation[37] are shown in Table 6. The second-order rate constant determined at 25.0 °C was 12.5 ± 0.15 dm^3 mol^{-1} s^{-1}, which can be compared with the value of 7.6 ± 0.1 dm^3 mol^{-1} s^{-1}, which was determined by Munro and Williams.[24] This value is slightly higher than that determined by Munro and Williams[24] even though they did not control the ionic strength in their study involving SNAP. The k_1 value of 620 dm^3 mol^{-1} s^{-1}, which was obtained by Littlejohn *et al.* for the reaction[23] between NO and SO_3^{2-} over the pH range of 4 to 10.5, is ~50 times our value of 12.5 ± 0.15 dm^3 mol^{-1} s^{-1}.

Table 6. Rate and Activation Parameters for the Decomposition of SNAP by Aqueous Sulfite.

θ/°C	k_1/mol^{-1} dm^3 s^{-1}
15.0	7.16 ± 0.10
20.0	9.61 ± 0.11
25.0	12.5 ± 0.15
30.0	14.3 ± 0.40
$\Delta H_1^{\neq}$ = + 32.3 ± 3 kJ mol^{-1}	
$\Delta S_1^{\neq}$ = -116 ± 8 J mol^{-1} K^{-1}	

The very small enthalpy of activation, $\Delta H_1^{\neq} = 32.3 \pm 3$ kJ mol^{-1} (Table 6), indicates the relative ease in which the reaction occurs, while the entropy of activation ($\Delta S_1^{\neq}$ = -116 ± 8 J mol^{-1} K^{-1}), which is very negative, is indicative of a highly ordered transition state. From the Arrhenius equation,[23] a preexponential factor of $A = (1.09 \pm 0.09) \times 10^7$ mol^{-1} dm^3 s^{-1} and an activation energy of $E_a = 34 \pm 3$ kJ mol^{-1} were obtained. The activation energy is 10 kJ mol^{-1} less than the value of 44 kJ mol^{-1}, which was obtained by Littlejohn *et al.* for the reaction between NO and SO_3^{2-}; while our preexponential factor $(1.09 \pm 0.09) \times 10^7$ mol^{-1} dm^3 s^{-1} is less than their value of 3.2×10^{10} mol^{-1} dm^3 s^{-1}. This lower value is due to steric hindrance as SNAP is a bigger molecule than NO when either of the two molecules is reacted with SO_3^{2-}.

From $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$ at 298.15 K, $\Delta G^{\neq}$ is calculated as +67 kJ mol^{-1}, which indicates that the reaction is not thermodynamically favourable, but kinetically favourable. As the reaction is kinetically controlled, where aqueous sulfite significantly reduces the concentration of SNAP, it is possible that there can be an antagonistic effect with reference to bronchodilation in asthma patients when RSNOs are present in their airways. It has been reported that bronchoconstriction and bronchospasm occur with a greater frequency after inhalation of sulfur dioxide rather than after oral ingestion of sulfites in both asthmatics and non-asthmatic individuals.[38-41] There are reports that inhalation of bronchodilators which contain sulfur(IV) species results in bronchospasm and anaphylaxis in asthmatic individuals.[42,43] We believe that under physiological conditions the concentration of airways *S*-nitrosothiols would be significantly reduced at a faster rate by aqueous sulfite based on our findings, where the rate of reaction increases as physiological pHs are approached. These findings would complement the findings of Gaston *et al.*,[12] who reported that airway *S*-nitrosothiols concentrations of asthmatic children were substantially lower than those of normal children (65 nmol dm^{-3} versus 502 nmol dm^{-3}). Clearly some physiological studies must be carried out so as to supplement our kinetic results.

The studies involving the effect of aqueous Cu^{2+} ions have proven to be quite interesting as it is known that the decompostion of RSNOs is mediated by aqueous Cu^+ ions,[44,45] which are generated by the reduction of aqueous Cu^{2+} by thiolate anions in aqueous solution as shown by equations

(9) and (10):

$$2Cu^{2+} + 2RS^{-} \rightarrow 2Cu^{+} + RSSR \quad (9)$$

$$Cu^{+} + RSNO \rightarrow Cu^{2+} + RS^{-} + NO \quad (10)$$

In equation (10) aqueous Cu^{2+} is generated in a catalytic process. The fact that the presence of aqueous Cu^{2+} ions along with an excess amount of sulfur(IV) species in the form of HSO_3^- and/or SO_3^{2-} did not show any dependence on the concentration of Cu^{2+} (see Figure 5), is quite interesting. There are two reports where sulfur(IV) species can react with aqueous Cu^{2+} to form a Chevreul's salt [a mixed valence copper sulfite, $Cu_3(SO_3)_2 \cdot 2H_2O$].[46,47] Aqueous sulfite can reduce Cu^{2+} to Cu^{+} ions is in highly basic media, as proven by Al-Ajlouni and Gould,[48] who reported that the predominant copper(II)-containing species is $Cu(OH)_4^{2-}$. Over the pH range of 3.96 to 6.80 the percentage of $Cu(OH)_4^{2-}$ would be zero; so this explains why the rate of reaction is zero order with respect to the concentration of aqueous Cu^{2+} ions. This explains why there is no production of "free" aqueous Cu^{+}, which can cause the decomposition of SNAP.

In conclusion, SNAP is decomposed by aqueous sulfite in a kinetically favourable process, with the rate of reaction increasing with an increase in pH.

Acknowledgements. We thank the laboratory technicians, whose assistance made the completion of this work possible. S.C.M wishes to thank the Board of Graduate Studies, University of the West Indies, Cave Hill Campus, Barbados, for a Postgraduate Award.

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