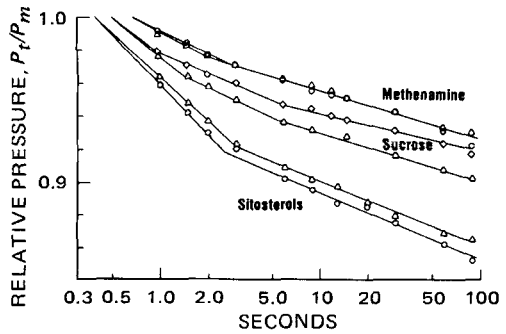

Figure 21—*Relative punch pressure* versus *log time. Zero time corresponds to the start of stress application. Circles and diamonds are data from an in air measurement; triangles are data from an* in vacuo *measurement. Only material with the slowest rate of relaxation fractured upon rapid decompression in the die. Maximum pressures (in* $N/m^2 \times 10^{-7}$*) were: methenamine, air 8.34 and* in vacuo *8.41; sucrose, air 6.96 and* in vacuo *6.52; and sitosterols, air 4.62 and* in vacuo *4.07.*

The time-dependent nature of plastic flow or stress relaxation also must be considered and may account for differences in the properties of tablets produced on various machines or at various machine settings.

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Solubility Studies of Silver Sulfadiazine

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Abstract □ The solubility of silver sulfadiazine as a function of pH was determined in nitric acid–potassium nitrate buffer for pH 2–3 and in 2-(*N*-morpholino)ethanesulfonic acid buffer for pH 6–7. As the salt of a weak organic acid, silver sulfadiazine exhibits the anticipated increase in solubility with an increasing hydrogen-ion concentration. Measurement of the silver-ion concentration was carried out using a silver-ion selective electrode. The methods of known subtraction and known addition were utilized to measure the total concentration of the silver ion in solution. Evidence was obtained to indicate that the salt is completely ionized in aqueous solution.

Keyphrases □ Silver sulfadiazine—solubility as a function of pH, potentiometric study □ Solubility—silver sulfadiazine as a function of pH, potentiometric study □ pH—effect on solubility of silver sulfadiazine, potentiometric study □ Potentiometry—study of solubility of silver sulfadiazine as a function of pH □ Anti-infectives, topical—silver sulfadiazine, solubility as a function of pH, potentiometric study

Silver sulfadiazine, a substance with extremely low water solubility, was reported to be particularly efficacious as a topical antibacterial agent for the control of *Pseudomonas* infection in burns (1). When applied locally to burned skin, silver sulfadiazine is claimed to offer definite therapeutic advantages over other similar chemotherapeutic agents used to treat infection. Unlike other drugs that diffuse rapidly or deplete chloride ions from body fluids, silver sulfadiazine remains in the wound exudate for a prolonged effect and appears to enhance conditions favorable for epithelial regeneration.

BACKGROUND

The mode of antibacterial action is different than that of sulfonamides, because the drug is not antagonized *in vitro* by aminobenzoic acid. In binding studies using radioactive silver sulfadiazine prepared from radioactive ^{110}Ag- and ^{35}S-tracers, the silver ion was found to bind with the *Pseudomonas* cells. No cellular binding of sulfadiazine was detected (2, 3).

The binding of silver to bacterial DNA was proposed as important for inhibiting microbial growth. Silver displaces the hydrogen bonds between adjacent nitrogens of the purines (adenine or guanine) and pyrimidines (thymine and cytosine) in the DNA molecule. The nitrogen–silver bonds, once formed, appear to be stronger than the nitrogen–hydrogen bonds; therefore, bacteria having this silver–nucleic acid complex presumably

do not replicate (4). In another report, silver sulfadiazine was said to bind to cell membranes rather than to interact with cellular DNA (5).

The mechanism of action of silver sulfadiazine is incompletely defined, and the exact role of the sulfadiazine moiety remains unclear. One proposal suggests that sulfadiazine localizes the action of the drug to the microbial cells (2). The very slow reaction of silver sulfadiazine with endogenous chloride ions is interesting when enough silver ions are apparently present to produce a strong antibacterial effect, but silver chloride reportedly does not precipitate in tissue fluids. The dissociation of silver sulfadiazine during inhibition of bacterial growth was described (3) in contrast to an earlier report that silver sulfadiazine does not ionize (6). Additional studies to determine the solubility and ionization properties of silver sulfadiazine are necessary for a complete understanding of its mechanism of action.

The crystallization and structure of silver sulfadiazine were recently reported, and the drug was characterized as a salt of a weak acid (7). A study using X-ray diffraction confirmed the assigned structure (8). The current report is concerned with the behavior of silver sulfadiazine in aqueous solutions in the presence of electrolytes and buffers. The objectives of this study were to investigate the ionization and solubility of silver sulfadiazine using recently developed potentiometric methods and to relate these properties with those of the silver salts of other N^1-substituted sulfonamides.

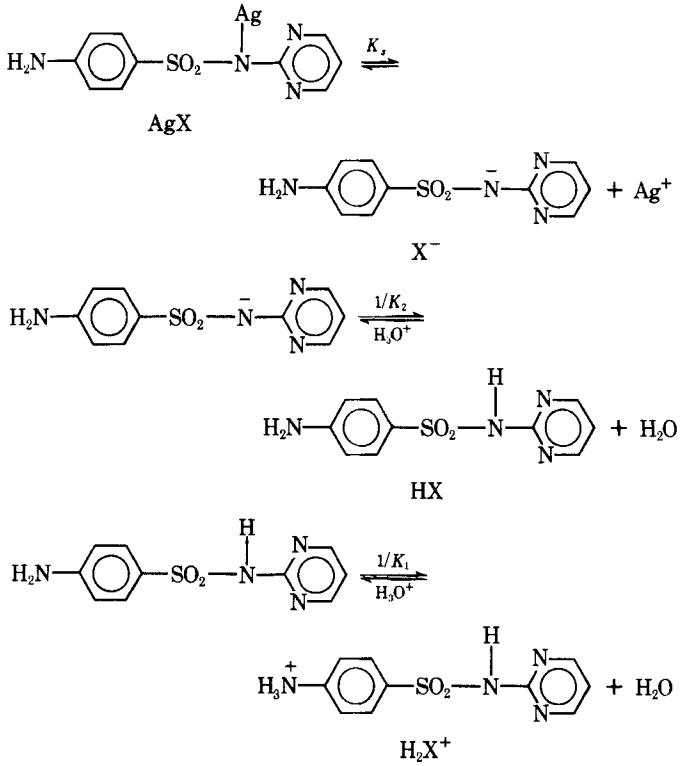
EXPERIMENTAL

Equipment—Potentiometric measurements were made using a pH meter[1], accurate to ±0.1 mv or ±0.001 pH unit, in a thermostated bath regulated at 25 ± 0.1°. The silver-ion concentration was measured with a silver-ion selective electrode[2]. The hydronium-ion concentration was measured with a triple-purpose pH electrode[3]. A double-junction silver chloride reference electrode[4] with a filling solution of 10% KNO_3 was used to eliminate the possible precipitation of silver chloride in the sample solutions.

Reagents—All reagents were of analytical grade, unless otherwise stated. Water with a specific conductivity of $1–10 \times 10^{-7}$ ohm^{-1} cm^{-1} was employed. Silver nitrate[5] and sodium sulfadiazine[5] were obtained commercially. Silver sulfadiazine was prepared by the method of Braun and Towle (9) and recrystallized as previously described (7). The 2-(*N*-morpholino)ethanesulfonic acid buffer[6] was prepared by titration of the acid with a standard sodium hydroxide solution.

Preparation of Equilibrium Mixtures—Silver sulfadiazine was screened through a size 80 standard screen[7]. Paraffin-coated vials with film-covered[8] rubber closures held in place by aluminum bands were utilized. Mixtures of 100 mg of silver sulfadiazine, 25 ml of nitric acid buffer, or 25 ml of 0.05 *M* 2-(*N*-morpholino)ethanesulfonic acid buffer were prepared, adjusted to an ionic strength of 0.1 *M* with potassium nitrate, and placed in the coated vials. For the measurement of total silver, 27 ml of buffer solution was used. The vials were rotated end-over-end in the thermostated bath until equilibrium solubility was obtained.

Measurement of Silver—After filtration of the equilibrated mixtures, the solutions were analyzed at 25 ± 0.1° in paraffin-coated beakers for the silver-ion concentration using a silver-ion selective electrode[2] standardized at 25 ± 0.1° and an ionic strength of 0.1 *M*. The electrode displayed a Nerstian response throughout the concentration range of 1×10^{-2}–1.5×10^{-6} *M* for the silver ion. The pH was measured with a pH electrode[3] standardized using standard buffers meeting National Bureau of Standards requirements (10). The total silver concentration was determined by the method of known subtraction (11, 12) in the nitric acid-buffered solution, to which a sufficient amount of a standard solution of potassium iodide was added to precipitate approximately one-half of the free silver ion. In the 2-(*N*-morpholino)ethanesulfonic acid-buffered solutions, the total silver concentration was determined by the method of known addition (12); the added reagent was a standard solution of silver nitrate representing a 100-fold increase in the free silver ion present in the sample solutions.



Scheme I

RESULTS AND DISCUSSION

In aqueous solutions, silver sulfadiazine would be expected to exhibit the general properties of the salt of a weak acid. The measurement of these properties, however, presents an analytical problem due to its extremely low water solubility. The recent development of ion selective electrode technology along with two new techniques for measuring total ion concentrations suggested the application of a potentiometric method for the analysis of silver sulfadiazine. In distilled water, the solubility of silver sulfadiazine is below the limits of analysis by any potentiometric method. However, in dilute acid, enough silver ions are ionized to give a concentration well within the sensitivity of a silver-ion specific electrode. Hydrolysis of the silver ion at near neutral or alkaline pH limits the study of silver sulfadiazine solubility to pH values less than 7.

The tendency of the silver ion to complex with various molecules and other ions reduced the number of possible buffering agents. Of the common inorganic salts of strong electrolytes, only nitrate and perchlorate ions remain in solution in the presence of silver ions. Nitric acid–potassium nitrate buffers were employed over a limited pH range. A suitable buffer for silver ions in the pH 6–7 range has not been identified. The sulfonic acid buffer system chosen exhibits minimal complexation with the silver ion (13). The use of conditional solubility product constants permits limited conclusions on solubility at this pH range.

Saturated solutions of silver sulfadiazine were analyzed for free and total silver concentrations by the analytical techniques of known addition (12) and known subtraction (11, 12). These methods were necessary due to the high probability of complexation of the silver with the sulfonic acid buffer as well as the uncertainty of the ionization properties of silver sulfadiazine. With nitrate buffers, the known subtraction method was utilized to determine the extent of ionization of the silver ion from the sulfadiazine. With this method, a complexing agent that will completely precipitate about one-half of the ion measured is added to the sample and the observed change in potential is used to calculate the original total ion concentration. By measuring the activity of free ion by a direct measurement using a calibration curve, it is possible to calculate the amount undissociated.

Due to the complexation of silver with the sulfonic acid buffer, the known addition method was used wherein a known solution of the ion being measured is added to the sample of interest. The original ion concentration is then calculated from the observed increase in potential. The usefulness of these methods depends on how well the system obeys the inherent assumptions.

The equilibria of the saturated solutions depicted by Scheme I may

[1] Corning digital 112 research pH meter, Corning Scientific Instruments, Medfield, Mass.
[2] No. 94-16, Orion Research, Cambridge, Mass.
[3] Corning Scientific Instruments, Medfield, Mass.
[4] No. 90-02, Orion Research, Cambridge, Mass.
[5] Marion Laboratories, Kansas City, Mo.
[6] United States Biochemical Corp., Cleveland, Ohio.
[7] W. S. Tyler Co., Minton, Ohio.
[8] Parafilm, American Can Co., Neenah, Wis.

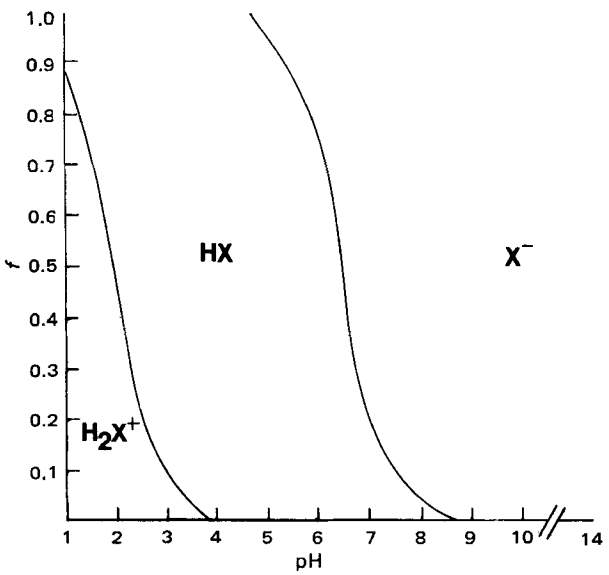


Figure 1—*Distribution diagram: fraction of sulfadiazine present as some particular form, f, as a function of pH; $pK_1 = 2.09$, and $pK_2 = 6.45$ (14).*

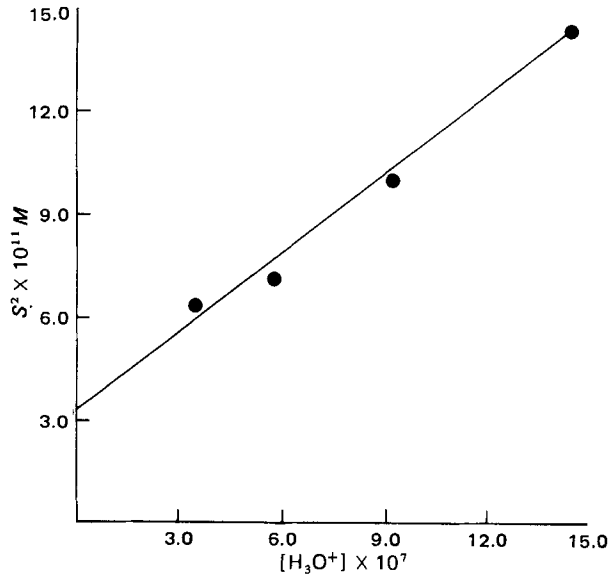


Figure 3—*Equilibrium values of* S^2 versus $[H_3O^+]$ *in 0.05* M *2-(N-morpholino)ethanesulfonic acid buffer at 0.1* M *ionic strength and 25 ± 0.1°.*

be described by:

$$K_s = [Ag^+][X^-] \quad \text{(Eq. 1)}$$

$$\frac{1}{K_2} = \frac{[HX]}{[H_3O^+][X^-]} \quad \text{(Eq. 2)}$$

$$\frac{1}{K_1} = \frac{[H_2X^+]}{[HX][H_3O^+]} \quad \text{(Eq. 3)}$$

$$S = [Ag^+] \quad \text{(Eq. 4)}$$

$$S = [H_2X^+] + [HX] + [X^-] \quad \text{(Eq. 5)}$$

where S is the total molar solubility of silver sulfadiazine. Substituting Eqs. 1–4 into Eq. 5 gives an equation that describes the solubility of silver sulfadiazine in terms of only the silver- and hydronium-ion concentrations of the saturated solution:

$$S^2 = [Ag^+]^2 = \frac{[H_3O^+]^2K_s}{K_1K_2} + \frac{[H_3O^+]K_s}{K_2} + K_s \quad \text{(Eq. 6)}$$

where K_s is the solubility product constant, and K_1 and K_2 are the ionization constants of the N^4- and N^1-hydrogens, respectively.

Equation 6 may be simplified by using the limiting conditions of low pH (2–3) and high pH (6–7). These approximations were derived by using the theoretical distribution diagram for sulfadiazine (Fig. 1), noting which species of sulfadiazine were not present in the pH range of interest, and neglecting this term in the mass balance (Eq. 5).

At the low pH range, Eq. 6 may be approximated to give:

$$S^2 = [Ag^+]^2 = \frac{[H_3O^+]^2K_s}{K_1K_2} + \frac{[H_3O^+]K_s}{K_2} \quad \text{(Eq. 7)}$$

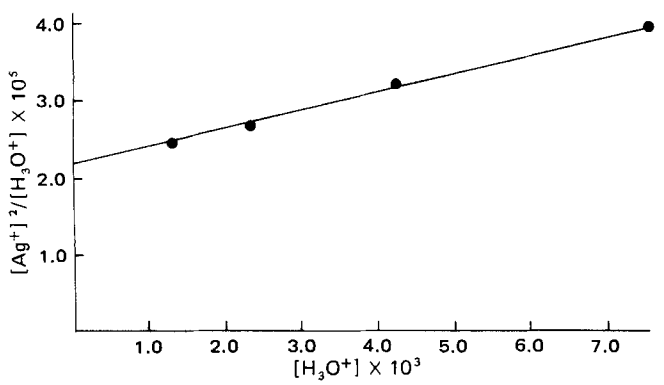


Figure 2—*Equilibrium values of* $[Ag^+]^2/[H_3O^+]$ versus $[H_3O^+]$ *at 0.1* M *ionic strength and 25 ± 0.1°.*

by neglecting the X^- species in Eq. 5. This equation may be linearized by dividing both sides by the hydronium-ion concentration, giving Eq. 8:

$$\frac{[Ag^+]^2}{[H_3O^+]} = \frac{[H_3O^+]K_s}{K_1K_2} + \frac{K_s}{K_2} \quad \text{(Eq. 8)}$$

When $[Ag^+]^2/[H_3O^+]$ is plotted against $[H_3O^+]$, a linear relationship should exist with an intercept of K_s/K_2 and a slope of K_s/K_2 $(1/K_1)$. From the slope and intercept, K_1 may be experimentally determined under the given conditions.

In the pH range of 6–7, Eq. 6 may be approximated to give:

$$S^2 = [Ag^+]^2 = \frac{[H_3O^+]K_s}{K_2} + K_s \quad \text{(Eq. 9)}$$

where the H_2X^+ species in Eq. 5 has been neglected. The application of Eq. 9 to the experimental data in the presence of the 2-(*N*-morpholino)-ethanesulfonic acid buffer does not completely represent the system due to apparent complexation of the free silver ion with the buffer, giving an increased value of the solubility, S, and subsequently a higher value for K_s, the solubility product constant.

A suitable modification of Eq. 9 to include this complexation may be made by using a conditional solubility product constant, K_s', defined by:

$$K_s' = [Ag^+]'[X^-] \quad \text{(Eq. 10)}$$

where $[Ag^+]'$ represents the concentration of all silver in solution irrespective of the form in which the silver ion may be present. Equation 9 may now be rewritten as:

$$S^2 = \frac{[H_3O^+]K_s'}{K_2} + K_s' \quad \text{(Eq. 11)}$$

Table I—Comparison of Total Molar Solubility, *S*, of Silver Sulfadiazine Determined by the Method of Known Subtraction with the Molar Concentration of the Silver Ion Determined by Direct Potentiometry on Identical Samples at 25 ± 0.1°, Ionic Strength 0.1 *M*, in Nitric Acid Buffers

	pH 2.128		pH 3.851	
	$S \times 10^5$	$[Ag^+] \times 10^5$	$S \times 10^5$	$[Ag^+] \times 10^5$
	59.18	59.11	6.690	6.455
	58.06	57.97	6.690	6.517
	59.35	59.34	6.434	6.517
	58.53	58.42	6.768	6.475
	59.19	60.00	6.586	6.375
	57.30	57.97	6.612	6.455
Mean	58.60	58.80	6.466	6.629

Table II—Calculation of Solubility Product of Silver Sulfadiazine Using Eq. 13 at 25 ± 0.1° and Ionic Strength 0.1 *M*

pH	f_0[a]	$[Ag^+]^2$	K_s
2.122	2.688×10^{-5}	2.980×10^{-7}	8.04×10^{-12}
2.373	6.024×10^{-5}	1.352×10^{-7}	8.16×10^{-12}
2.630	1.279×10^{-4}	6.165×10^{-8}	7.90×10^{-12}
2.891	2.583×10^{-4}	3.139×10^{-8}	8.14×10^{-12}
Mean	$8.06 \times 10^{-12} \pm 0.12$		

[a] $K_1 = 8.59 \times 10^{-3}$, and $K_2 = 3.82 \times 10^{-7}$.

where S is the total molar solubility, and K_2 is defined by Eq. 2. A plot of S^2 *versus* $[H_3O^+]$ would be expected to be linear with an intercept of K_s' and a slope of K_s'/K_2, from which K_2 may be experimentally determined.

Figure 2 is a linear plot of the equilibrium values of $[Ag^+]^2/[H_3O^+]$ against the equilibrium $[H_3O^+]$ value, having a slope of 2.459×10^{-3} and an intercept of 2.112×10^{-5}. From the slope and intercept, pK_1 was found to be 2.07 ± 0.06 at an ionic strength of 0.1 *M*. This value is in agreement with a literature value of 2.09 (14). As shown in Fig. 3, a plot of S^2 *versus* $[H_3O^+]$ at equilibrium is linear with an intercept of 3.05×10^{-11} and a slope of 7.98×10^{-5}. From the slope and intercept, pK_2 was evaluated experimentally to be 6.42 ± 0.04, in agreement with a previously reported value of 6.45 (14).

The possibility of intact silver sulfadiazine in solution in a pH range of 2.1–3.8 was investigated by comparison of the total molar solubility, S, as determined by the method of known subtraction, with the molar concentration of free silver ion, $[Ag^+]$, by direct potentiometric analysis on identical samples under the given experimental conditions. By the use of one-way analysis of variance, the means displayed in Table I are not statistically different at the 5% level. Therefore, in the pH 2–3 range,

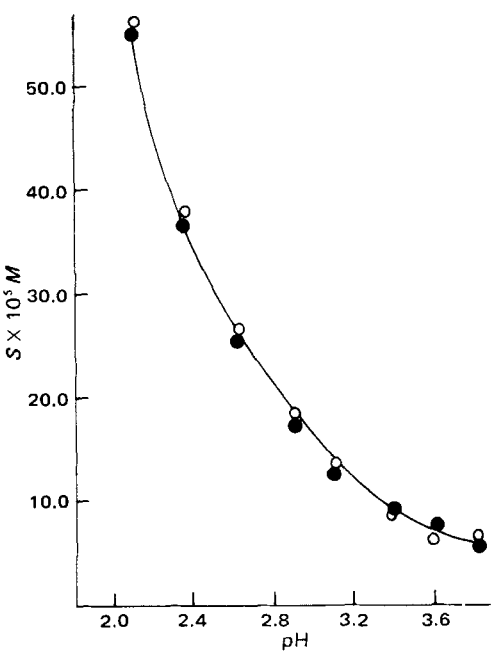


Figure 4—*Molar solubility (S) of silver sulfadiazine* versus *pH at 0.1 M ionic strength and 25 ± 0.1°. Key: ●, experimental values; and ○, calculated from Eq. 7 and the equilibrium constants determined in this study.*

the solubility, S, of silver sulfadiazine is given by Eqs. 4 and 5. The solubility product of silver sulfadiazine is given by Eq. 1. By substituting Eqs. 2 and 3 into Eq. 5, an expression may be derived giving the ratio, f_0, of the sulfadiazine anion to the total solubility, where:

$$f_0 = \frac{[X^-]}{S} = \left(1 + \frac{[H_3O^+]}{K_2} + \frac{[H_3O^+]^2}{K_1K_2}\right)^{-1} \quad \text{(Eq. 12)}$$

Combining this expression with Eq. 1 gives:

$$K_s = f_0S^2 \quad \text{(Eq. 13)}$$

Application of this equation to the experimental data at the low pH range is given in Table II, where the mean solubility product constant for silver sulfadiazine was $8.06 \pm 0.12 \times 10^{-12}$.

A plot of the molar solubility, S, of silver sulfadiazine against pH over the pH range studied is displayed in Fig. 4. This plot at pH 2–3 compares very closely with the distribution of the sulfadiazine cation over the same pH range. Thus, the solubility of silver sulfadiazine is directly a function of sulfadiazine ionization.

The solubility would be expected to be nearly pH independent in the pH 4–5 range where the predominant sulfadiazine species is the neutral molecule. For pH 6–7, the solubility is estimated by Eq. 9. At pH 6, the calculated solubility, S, was 5.40×10^{-6} *M*; at pH 7, it was 3.19×10^{-6} *M*. Where the predominant sulfadiazine species is the sulfadiazine anion, the limiting value of the solubility would be expected to be the square root of the solubility product constant, $\sqrt{K_s} = 2.83 \times 10^{-6}$ *M*.

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