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AMINODIAZINE: A MOLECULAR COMPOUND OF SULFADIAZINE AND ETHYLENEDIAMINE

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ABSTRACT

Aminodiazine, a sulfadiazine-ethylenediamine solid compound was prepared. This compound possesses a markedly higher aqueous solubility compared to sulfadiazine. Equilibrium solubility and elemental analysis data indicate a 1:1 molecular interaction. Investigation of the structure of aminodiazine in the solid state suggests strong association of the sulfonamido group of sulfadiazine with one of the basic groups of ethylenediamine probably through ionic and hydrogen bonding.

From a practical standpoint, aminodiazine exhibits a much higher dissolution rate than sulfadiazine. In solution with a slight excess of ethylenediamine, aminodiazine proves compatible with four intravenous solutions over a study period of 8 hours. Further, the compound shows quantitatively the same antibacterial activity as sulfadiazine against test organisms.

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INTRODUCTION

Because of its wide margin of safety and high therapeutic index, sulfadiazine still retains its rank among the most widely used anti-infective agents. However, sulfadiazine is considered to present actual and potential bioavailability differences[1-3] owing to its extremely poor solubility (1 in 13000) and large therapeutic dose. Much work has been done in the area of formulation[4-6] to improve the availability of sulfadiazine from its dosage forms but it did not reach its final goals. Another approach is to increase the solubility of the drug through the formation of soluble products with basic compounds.

Aliphatic amines have been used successfully in the preparation of soluble molecular compounds of weakly acidic drugs such as sulfisoxazole[7] and theophylline[8]. Several attemts using various aliphatic amines have been recently made in order to improve the biopharmaceutical properties of acidic drugs[9-11]. In the present study, a soluble compound of sulfadiazine and ethylenediamine is prepared. The mode of interaction of these two species in solution and in the solid state is investigated. Further, the effects of such an interaction on the physicochemical properties of sulfadiazine and its antibacterial activity are assessed.

MATERIALS AND METHODS

Equilibrium Solubility Study- The apparent equilibrium solubility of sulfadiazine[a] as a function of ethylenediamine[b] concentration (0.0 - 0.5M) was determined. Ethylenediamine was boiled before use and cooled in a flask stoppered with a soda lime tube. An excess of

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[a] ACF Chemiefarma nv, Maarssen, Holland.

[b] E. Merck AG Darmstadt, F.R.G.

sulfadiazine was added to 20 ml of ethylenediamine solutions and equilibrated at 30 ± 1°C for 48 h. Samples were withdrawn through a 0.2 Um millipore filter and assayed spectrophotometrically for sulfadiazine at 254 nm.

The solubilities of sulfamethoxazole[c], sulfamethazine[c] and sulfaguanidine[c] were determined under similar conditions. The pH of all filtrates was recorded at the end of the experiment.

Preparation of Sulfadiazine-Ethylenediamine Molecular Compound- Equilibrium solubility data suggested that sulfadiazine forms a soluble compound with ethylenediamine in a molar ratio of 1:1. Consequently, 10 g (0.04 M) of sulfadiazine was added to 4 g of ethylenediamine (an amount slightly in excess of 0.04 M) with constant stirring. After complete solubility, the thick transparent solution was spread on a glass slab and dried under vacuum at 22°C. The dried product of interaction appeared as a white crystalline powder acquiring a yellow tint on exposure to light. The product was stored in a dark colored container.

IR Spectroscopy- The IR spectra of sulfadiazine, ethylenediamine and their product of interaction were made in Nujol using a Beckman IR 4210 spectrophotometer.

Dissolution Rate Study- The dissolution rate of sulfadiazine and sulfadiazine-ethylenediamine molecular compound was determined using the U.S.P. apparatus at 100 rpm and 37°C. Sulfadiazine (500 mg) or an equivalent amount of the compound (692 mg) were added to the dissolution medium consisting of 900 ml of either distilled water or 0.1N HCl.

Thermal Analysis- DTA and TGA curves were produced using a Heraeus Thermal Analyser DTA 500. Both experiments

[c]El-Nasr Pharmaceutical Chemicals Co., Egypt.

were carried out in static air at one atmospheric pressure using 5 mg (DTA) and 46 mg (TGA) of the sample. The temperature was elevated at a rate of 10°C/min in the range of 40-300°C. Alumina was used as a reference and the sensitivity was adjusted to 10 and 2 mV/cm for DTA and TGA respectively.

Compatibility with Some Intravenous Fluids- A test solution of sulfadiazine 250 mg/ml (1M) in 1.25M ethylenediamine solution was prepared according to the solubility diagram obtained in the present work (Fig. 1). This slightly higher concentration of ethylenediamine (1.25M instead of 1.0M) was used to ensure ready and complete solubility of the drug. Test dilutions of the sulfadiazine solution (1:10 to 1:100) were mixed with normal saline[d] (pH 6.0) 5% dextrose[d] (pH 4.2), Ringer's Solution U.S.P. (pH 5.3) and Lactated Ringer's Solution U.S.P. (pH 7.0). These dilutions were stored at 25°C for 8h. Samples were assayed for sulfadiazine content at different time intervals. The percentage concentration of the drug was calculated with reference to the initial concentration.

Comparative Antibacterial Activity- The antibacterial activity of the sulfadiazine-ethylenediamine molecular compound and its components was tested, using the agar diffusion method, against Staphylococcus aureus, Bacillus subtilis and Escherichia coli as test organisms.

RESULTS AND DISCUSSION

The effect of ethylenediamine on the solubility of sulfadiazine was studied in order to investigate the mode of interaction of these species in solution. Figure 1 shows an increase in the sulfadiazine solubility as a

[d]ADWIC Pharmaceutical Division, El-Nasr Pharmaceuticals Co., Egypt.

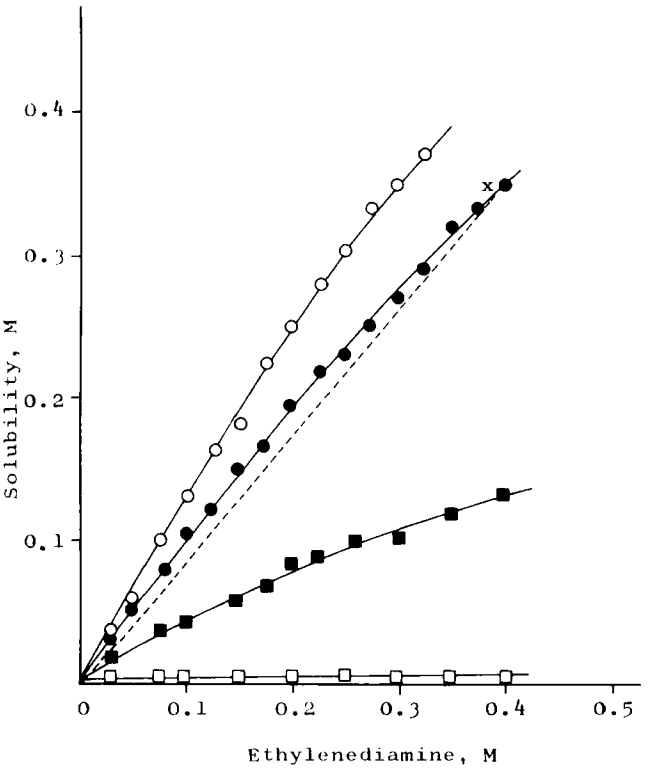


FIGURE 1

Effect of ethylenediamine on the equilibrium solubility of sulfamethoxazole (○), sulfadiazine (●), sulfamethazine (■) and sulfaguanidine (□).

function of ethylenediamine concentration. In order to gain more insight into the system under study, the solubilities of some structurally related sulfonamides were also determined as a function of ethylenediamine concentration. Consideration of the structural differences of these compounds and their pKa values[12] in relation to the solubility diagrams shown in Fig. 1 reveals the essential role of the acidic hydrogen of the sulfonamido group in the interaction with ethylenediamine. The relatively higher acidity of this hydrogen in sulfamethoxazole (pKa= 5.2) may account for the greater solubility of this compound compared to that of sulfadiazine (pKa=6.5) which is in

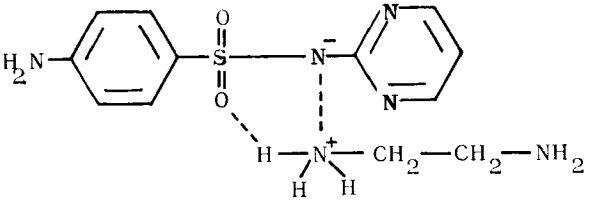
turn greater than that of sulfamethazine (pKa=7.4). On the other hand, the absence of a solubility enhancing effect in the case of sulfaguanidine (pKa=12.1) can be attributed to the lack of the acidic amido hydrogen[13].

However, in interpreting solubility data, the pH change brought about by the addition of ethylenediamine should be taken into account. The theoretical values of sulfadiazine solubility at the pH of the various ethylenediamine solutions used in the study were calculated using the equation:

$$pH = pKa + \log \left(\frac{S_t - S_o}{S_o}\right)$$

where S_t is the total solubility at a given pH and S_o is the solubility of unionized sulfadiazine. The larger experimental solubility values compared to those calculated theoretically (Fig. 2) tend to indicate a molecular interaction between sulfadiazine and ethylenediamine.

The linear portion of the solubility diagram of sulfadiazine (Fig. 2) suggests a 1:1 molecular compound with a relatively low stability constant (10.4). Accordingly, the following structure is proposed for the sulfadiazine-ethylenediamine molecular compound:



In this structure, ionic bonding is assumed to take part in the molecular interaction, the product being possibly stabilized by hydrogen bonding between the protonated amino group of ethylenediamine and the strongly electronegative sulfonyl oxygen of sulfadiazine.

To test this assumption, the sulfadiazine-ethylenediamine solid compound has been prepared according to the procedure described under materials and methods and

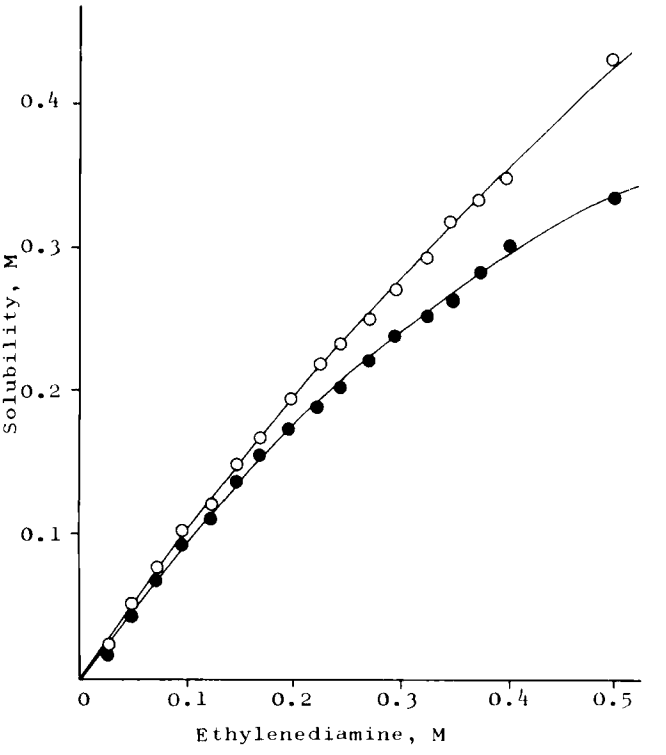


FIGURE 2

Experimental (○) and theoretical (●) solubilities of sulfadiazine as a function of ethylenediamine concentration.

subjected to elemental analysis[e]. Results of the analysis indicate that the compound is a combination of one mole of each of sulfadiazine and ethylenediamine accompanied by one mole of water of crystallization. Further, IR spectroscopy reveals essential differences in the spectra of the sulfadiazine-ethylenediamine compound and its components. Most important of these is the disappearance of the band at 3260 cm^{-1} assigned to the sreching vibration of -NH- of the sulfonamido group[13] from the spectrum

[e] Performed by the members of the Microanalytical Unit, Faculty of Sciences, University of Cairo, Cairo, Egypt.

of the molecular compound (Fig. 3a) as well as the shift of the band at 1160 attributed to the $-SO_2-$ group[14] to a lower frequency, 1115 cm^{-1} (Fig. 3b). This suggests the involvement of both the sulfonamido hydrogen and the $-SO_2-$ group of sulfadiazine in the interaction with ethylenediamine, thus, supporting the structure proposed.

The thermal decomposition of the sulfadiazine-ethylenediamine compound was monitored by differential thermal analysis (DTA) and thermogravimetric analysis (TGA). While the DTA curve of sulfadiazine shows a sharp endothermic peak at 256°C (Fig. 4) corresponding to its melting point, the DTA curve of the sulfadiazine-ethylenediamine molecular compound shows a number of endothermic peaks at 70, 90, 103, 113, 117 and 140°C. The peak at 70°C is attributed to a physical change since it is not accompanied by a loss in weight in the TGA curve (Fig. 4). The peaks over the temperature range of 90 to 117°C are associated by 5.48% loss in weight as detected by TGA, corresponding to the liberation of one mole of water (theoretical value 5.5%). The relatively high temperature range over which water is eliminated suggests strong binding of at least part of this water to the compound, probably through hydrogen bonding. The broad endothermic peak at 140°C is accompanied by 8.6% loss in weight in the TGA curve corresponding to only half a mole of ethylenediamine (theoretical value 18.3%). This can be possibly explained by assuming that the gain in thermal energy might result in a change in the molar ratio of the sulfadiazine-ethylenediamine compound from 1:1 to 2:1 with the release of half the amount of ethylenediamine. The relatively higher and wider temperature range over which ethylenediamine is eliminated from the compound under study compared to aminophylline (110-127°C)[15] reflects a probably stronger association of ethylenediamine and sulfadiazine. Accordingly, the sulfadiazine-ethylenediamine molecular compound has been provisionally assigned the name aminodiazine.

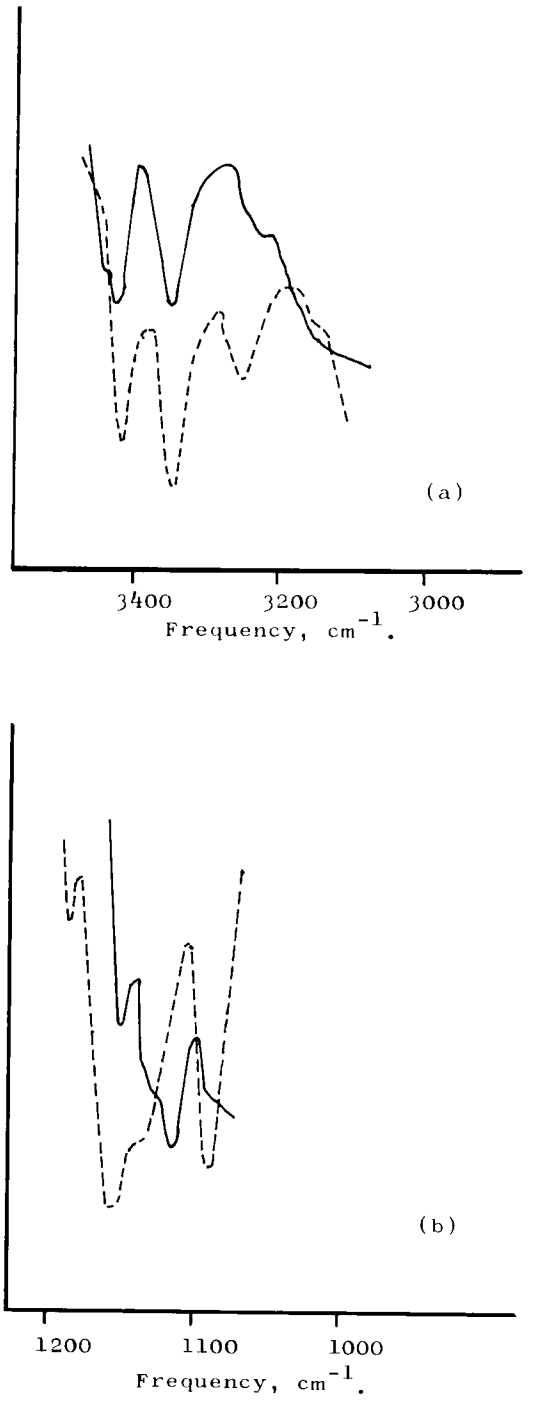


FIGURE 3

IR spectra of sulfadiazine (dotted lines) and sulfadiazine-ethylenediamine molecular compound (solid lines) in the regions of the streching vibration of -NH- (a) and $-SO_2-$ (b) of the sulfonamido group.

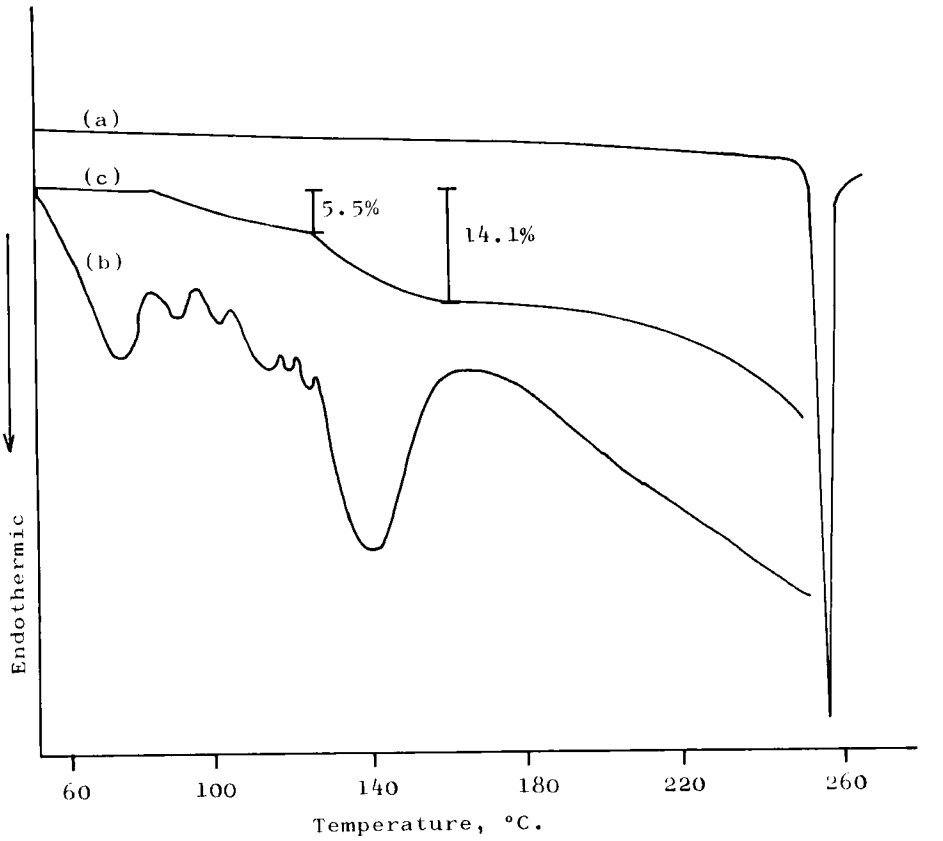


FIGURE 4

DTA curves of sulfadiazine (a) and sulfadiazine-ethylene-diamine molecular compound (b) and TGA curve of the compound (c).

From a practical standpoint, aminodiazine appears to have important implications in pharmaceutical formulation. Aminodiazine has an apparent aqueous solubility of 132.4 mg/ml at 30°C compared to sulfadiazine possessing an inherent solubility of 0.095 mg/ml at the same temperature. This seems most useful in achieving enhanced dissolution rates of sulfadiazine. Results of the dissolution rate study (Fig. 5) clearly illustrate the much faster dissolution rate of aminodiazine compared to sulfadiazine. The time for 50 % dissolution of amino-

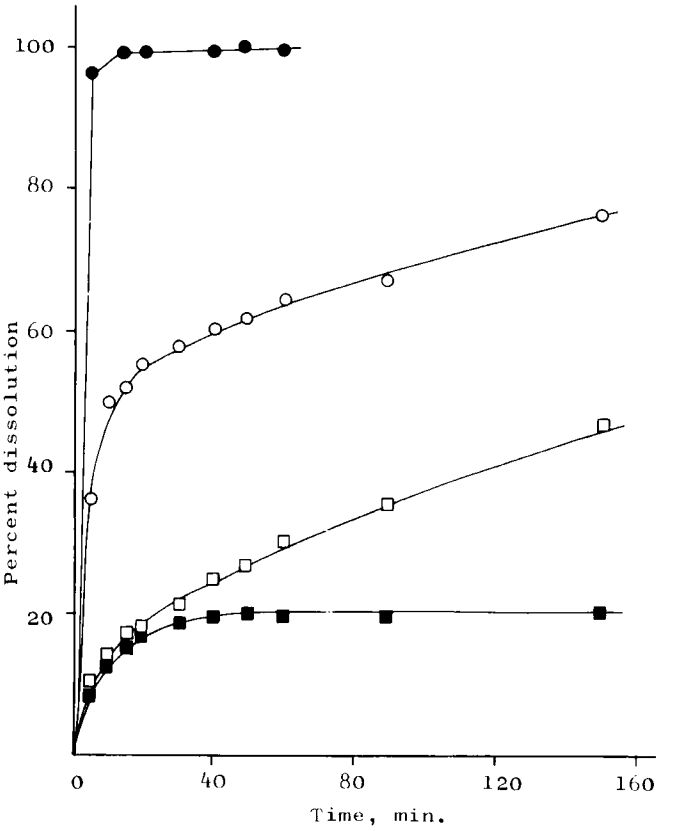


FIGURE 5

Dissolution rate of sulfadiazine and aminodiazine in water and 0.1N HCl
(■)sulfadiazine in water, (□)sulfadiazine in 0.1N HCl
(●)aminodiazine in water, (○)aminodiazine in 0.1N HCl

diazine being 3 min and 10 min in water and 0.1N HCl respectively.

Moreover, according to the solubility diagram in Fig. 1, a solution of sulfadiazine in ethylenediamine solution could be diluted with water without the formation of saturated solutions with respect to sulfadiazine. This is illustrated in Fig. 1 by diluting an arbitrary system x. Practically, such a solution could be diluted without the risk of precipitation. In the present work, we have tested the compatibility of a test sulfadiazine

TABLE 1a

Percent of Sulfadiazine 2h and 8h following Dilution with Normal Saline and 5% Dextrose

Dilution	Saline			5% Dextrose		
	pH	2h	8h	pH	2h	8h
1:10	9.2	100.1	100.0	8.9	100.0	100.1
1:25	9.2	100.0	99.9	8.7	100.2	100.2
1:50	9.1	99.7	99.8	8.5	99.7	100.0
1:100	9.0	100.0	100.3	8.3	100.3	100.1

TABLE 1b

Percent of Sulfadiazine 2h and 8h following Dilution with Ringer's and Lactated Ringer's Solutions

Dilution	Ringer's			Lactated Ringer's		
	pH	2h	8h	pH	2h	8h
1:10	9.1	99.9	100.1	9.2	100.0	100.0
1:25	9.0	100.1	100.2	9.0	100.3	100.3
1:50	8.9	100.2	99.8	8.8	99.8	100.2
1:100	8.7	100.3	100.1	8.6	100.2	100.2

solution 1M (250 mg/ml, the usual strength of sulfadiazine injection) in 1.25M ethylenediamine solution (97.6 mg/ml) as a solvent, upon dilution with four intravenous fluids, namely normal saline, 5% dextrose, Ringer's and Lactated Ringer's solutions. Results indicate that diluting the above solution from 1:10 to 1:100 results in no sign of physical incompatibility over a study period of 8h. Moreover, assay of sulfadiazine in the test dilutions shows no loss of potency. This could be advantageous if one considers that the addition of sulfadiazine sodium to polyionic intravenous solutions resulted in rapid and

TABLE 2

Comparative Antibacterial Activity of Sulfadiazine and Aminodiazine.

Solution	Inhibition Zone in mm		
	S.aureus	B.subtilis	E.coli
Sulfadiazine 5 mg/ml	44	34	35
Aminodiazine 6.92 mg/ml	45	34	35

marked precipitation of such a degree as to make intravenous administration of the medication prohibitive[16,17]. Sulfisoxazole diethanolamine was more compatible with polyionic intravenous solutions, particularly those of a relatively higher initial pH levels[16]. Compatibility of sulfadiazine-ethylenediamine solution with the intravenous fluids under study can be attributed to the relatively high pH of the test dilutions (Table 1a and b).

Further, the antibacterial activity of an aqueous aminodiazine solution 6.92 mg/ml containing the equivalent of 5 mg/ml of sulfadiazine was compared to that of a sulfadiazine solution 5 mg/ml. Results in Table 2 indicate that both solutions have quantitatively the same antibacterial activity against Staphylococcus aureus, Bacillus subtilis and Escherichia coli.

In conclusion, the considerably higher equilibrium solubility and dissolution rate of aminodiazine compared to sulfadiazine and the lack of precipitation when this compound is combined with some intravenous fluids provide great advantages regarding the formulation of sulfadiazine.

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