Interaction of Metronidazole with Antibiotics Containing the 2-Aminothiazole Moiety

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The mechanism of possible incompatibility between commercially available metronidazole parenteral solutions and the injectable aztreonam leading to the development of pink color in their intravenous admixtures was studied. It was demonstrated that nitrite ions may be produced in metronidazole solutions at the time of preparation or during storage by the effects of temperature and light. Under acidic pH conditions of admixtures the aminothiazole moiety of aztreonam was diazotized by the nitrite ion contributed by metronidazole solutions. The diazotized molecule, in turn, reacted with another aztreonam molecule by diazo-coupling. The resultant pink-colored product was isolated by chromatography and its structure was determined by mass spectral and NMR analyses. Other β-lactam antibiotics containing the 2-aminothiazole moiety also react in acidic media in a similar manner.

KEY WORDS: aztreonam; metronidazole; 2-aminothiazole antibiotics; nitrite ion formation; drug-drug interaction; color formation.

INTRODUCTION

Parenteral solutions of metronidazole, an antiprotozoal and antibacterial agent used in the treatment of trichomoniasis, amoebiasis, anaerobic bacterial infectious diseases, etc. (1), are commonly admixed with other antibiotics in infusion bags prior to intravenous administration. A potential for drug–drug interaction exists in such a dosage delivery depending on the nature of administered drugs. An incompatibility between commercially available injectable solutions of metronidazole and aztreonam leading to the development of a pink color was reported by Bell et al. (2). Although the potency loss of both drugs in the mixtures was insignificant, the authors recommended, on the basis of the color reaction, that if metronidazole and aztreonam are to be used together, they should be administered separately. This incompatibility between metronidazole and aztreonam has also been recorded in the *Handbook on Injectable Drugs* published by the American Society of Hospital Pharmacists (3). The objective of the present study was to determine the mechanism underlying the pink color-forming reaction between metronidazole and aztreonam. Metronidazole has been reported to liberate trace amounts of nitrite ions (4,5). Since aztreonam, which contains a 2-aminothiazole functionality, could diazotize in the presence of nitrite ions and undergo a diazo-coupling reaction with another aztreonam molecule, the specific goal of the study was to investigate the role of nitrite ions in the formation of color.

MATERIALS AND METHODS

Materials

Metronidazole, lot no. 97F-0147, was obtained from Sigma Chemical Co. (St. Louis, MO) and aztreonam, lot no. 93868-960, was synthesized by the Bristol-Myers Squibb co. (Princeton, NJ). The following commercial preparations of metronidazole, ceftazidime, ceftriaxone, and ceftizoxime were purchased from a retail pharmacy: metronidazole solutions (Flagyl i.v. RTU, lot 8P-114, Searle; metronidazole injection, lot 31-704-DM-01, Abbott), metronidazole i.v. pouch (Metro i.v., lot D5353-52, Kendall McGaw Laboratories), metronidazole hydrochloride lyophilized (Flagyl i.v., lot 9F-210, Searle), ceftazidime sodium (Fortaz, lot Z20478NN, Glaxo), ceftriaxone sodium (Rocephin, lot 0132, Roche), and ceftizoxime sodium (Cefizox, lot 168C15, Smith Kline Beecham). The lyophilized aztreonam injectable (Azactam, lot OH29787) was manufactured by Bristol-Myers Squibb Co., New York.

Spectral Analysis of Metronidazole–Aztreonam Mixtures

To 10 ml of a 1 mg/ml solution of aztreonam powder or lyophilized injectable in 0.1 *M* acetic acid, different volumes (0.1 to 1.0 ml) of a commercially available metronidazole injectable (5 mg/ml) were added. The mixture was allowed to stand for 1 hr at 5°C and the visible spectrum was recorded (Perkin–Elmer spectrophotometer, model Lambda 5) between 400- and 600-nm wavelengths after suitable dilution with water. Because of the acidic nature of aztreonam, the spectra of metronidazole–aztreonam solutions in the absence of acetic acid were similar to those in acidified media. However, acetic acid was used during quantitative analysis of the color formed for the purpose of standardizing the system.

Spectral Analysis of Sodium Nitrite–Aztreonam Mixtures

Solutions of $NaNO_2$ in the concentration range of 0.005 to 0.1 m*M* (corresponding to 0.35 to 6.9 ppm) were prepared in 0.1 *M* acetic acid with an excess of aztreonam (5 m*M* or 2.2 mg/ml). The solutions were then allowed to stand for 1 hr at 5°C for the completion of the development of pink color, and the absorbances at 500 nm of suitably diluted solutions were recorded. The observed linear relationship of nitrite ion concentration versus absorbance was used to determine the nitrite content of various metronidazole solutions by similar color reactions with aztreonam. The pink color was detectable when the nitrite ion concentration in the reaction mixture was as low as 0.2 ppm; however, the lowest concentration of nitrite ion used in the standard plot was 0.35 ppm and the quantitation was made above this concentration.

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In the above experiment a direct relationship between the color development and the nitrite ion content was observed when the concentration of aztreonam was much in excess of the nitrite ion concentration. In a separate experiment the molar ratios of $NaNO_2$ to aztreonam were varied from 0.1 to 25 for determining the effect of reactant ratio on the intensity of color developed.

Ion-Chromatographic Determination of Nitrite Ion Concentration

An ion chromatographic system (Waters) with IC-Pak anion-exchange column, 4.6 mm × 5.0 cm, and a conductivity detector were used for the HPLC analysis of nitrite ions. A lithium gluconate/borate buffer (pH 7) at a flow rate of 1.2 ml/min was used as the mobile phase. Standard solutions of $NaNO_2$ in water in the concentration range of 0.001 to 0.1 mg/ml (1 to 100 ppm) were injected. A linear plot of detector response versus nitrite ion concentration was obtained. This standard plot was used for the determination of nitrite ion concentrations in metronidazole-solutions.

Stability Study of Metronidazole in Aqueous Solutions

A 5 mg/ml solution of metronidazole in water was filled into 5-ml clear glass vials (2 ml/vial). The vials were then stored at different temperatures ranging between 5 and 80°C, in the absence of light. Some samples were exposed to the light intensity of 400 ft-candles at 33°C for up to 8 weeks. Samples were also autoclaved at 120°C for 30 min.

Isolation of Pink Product

The pink color-forming compound produced in aztreonam–metronidazole or aztreonam–sodium nitrite mixtures was isolated by HPLC using a Waters 710B auto injector equipped with a Perkin–Elmer gradient pump (Model 410) and a Waters C-18 μ-Bondapak column (7.8 mm × 30 cm). In the 60-min run time, a 1:4 mixture of methanol and 0.05 *M* KH_2PO_4 aqueous buffer was used for the first 30 min, and a 9:1 methanol–water mixture was used for the remaining 30 min. The detector was set at 254 nm. Pink fractions eluting at ~45 min were collected from several injections, pooled, and freeze-dried.

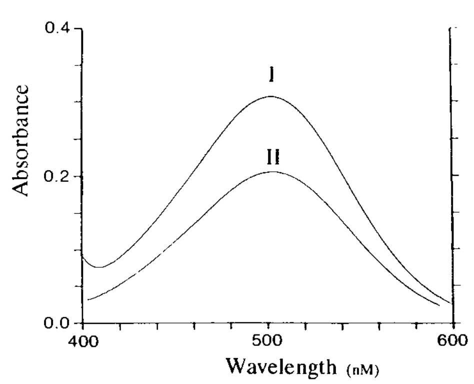


Fig. 1. Absorption spectra of the mixtures (I) aztreonam (0.545 mg/ml) with degraded metronidazole solution (0.0625 mg/ml) and (II) aztreonam (0.545 mg/ml) with sodium nitrite (0.8 ppm) in water.

Table I. Nitrite Ion Concentrations in Metronidazole Solution (5 mg/ml) Stored at 80°C as Determined by Ion Chromatography and by Reaction with Aztreonam

Time (days)	Nitrite ion concentration (ppm of $NaNO_2$): Ion chromatography	Nitrite ion concentration (ppm of $NaNO_2$): Aztreonam reaction
0	ND[a]	ND
1	5.8	5.6
3	16.6	15.4
5	24.3	24.4
7	25.3	27.7
10	26.5	28.8

[a] None detected.

Mass Spectral and NMR Analyses

Mass spectra were obtained on a double-focusing magnetic sector mass spectrometer (Model ZAB-1F, VG Analytical) by fast atom bombardment (FAB) using a 4- to 8-kV xenon source. Proton spectra were recorded in D_2O solution using a JOEL, GSX-500, spectrometer.

RESULTS AND DISCUSSIONS

Relationship of Color Development with Nitrite Ion Formation

Visible absorption spectra of the mixtures of an aztreonam solution in water with a commercially available injectable solution of metronidazole and with a $NaNO_2$ solution are shown in Fig. 1. Preliminary experiments have shown that color developed in the admixtures under acidic pH conditions only. Aztreonam provided the acidic environment of the aqueous solutions used in Fig. 1. In this figure, the spectra of both mixtures were similar, with maxima at 500 nm, indicating that the development of color in an aztreonam–

Table II. Nitrite Ion Concentrations in Metronidazole Solution (5 mg/ml) Stored Under Different Accelerated Storage Conditions as Determined by Reaction with Aztreonam

Time (weeks)	Nitrite ion concentration (ppm of $NaNO_2$): 33°C	Nitrite ion concentration (ppm of $NaNO_2$): 33°C/400 ft-candle light	Nitrite ion concentration (ppm of $NaNO_2$): 50°C
1	<0.5	0.6	2.0
2	<0.5	0.9	3.5
3	0.7	2.7	4.9
4	0.8	4.2	6.6
5	0.9	4.2	7.1
6	1.1	7.4	9.3
7	1.7	8.4	9.6
8	1.9	8.9	11.1

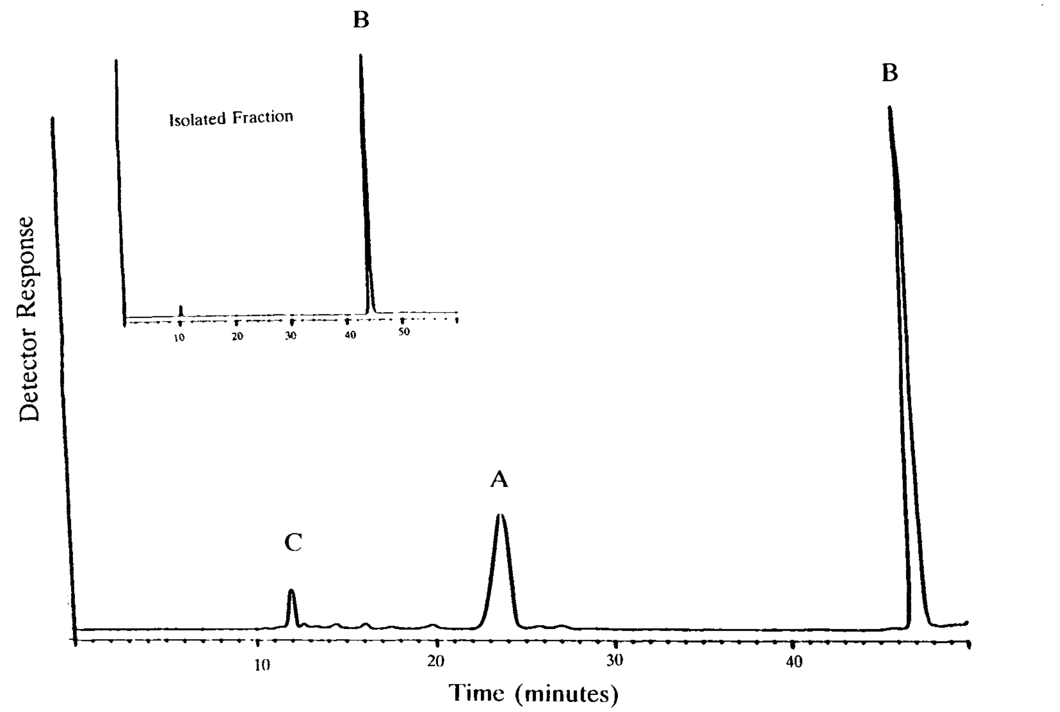


Fig. 2. HPLC chromatograms of the aztreonam–sodium nitrite reaction mixture. The chromatogram of the isolated pink-colored product is given in the inset. A, unreacted aztreonam; B, pink-colored diazo product; C, unidentified degradation product.

metronidazole mixture is related to the presence of nitrite ions.

Samples from three batches of metronidazole injectable solution and one lyophilized powder procured from a local pharmacy were analyzed by ion chromatography for their nitrite ion contents and also admixed separately with an aztreonam solution for the spectral analysis of any color produced. The metronidazole solutions had nitrite ion concentrations ranging from 0.055 to 0.338 m*M* (3.8 to 23.3 ppm expressed as $NaNO_2$ equivalents) and produced proportionately different intensities of color with spectra similar to those in Fig. 1. The commercially available lyophilized metronidazole did not contain any significant nitrite ion as analyzed by ion chromatography, and there was no color development when reacted with aztreonam. The highest concentration of nitrite ion detected in any commercially available

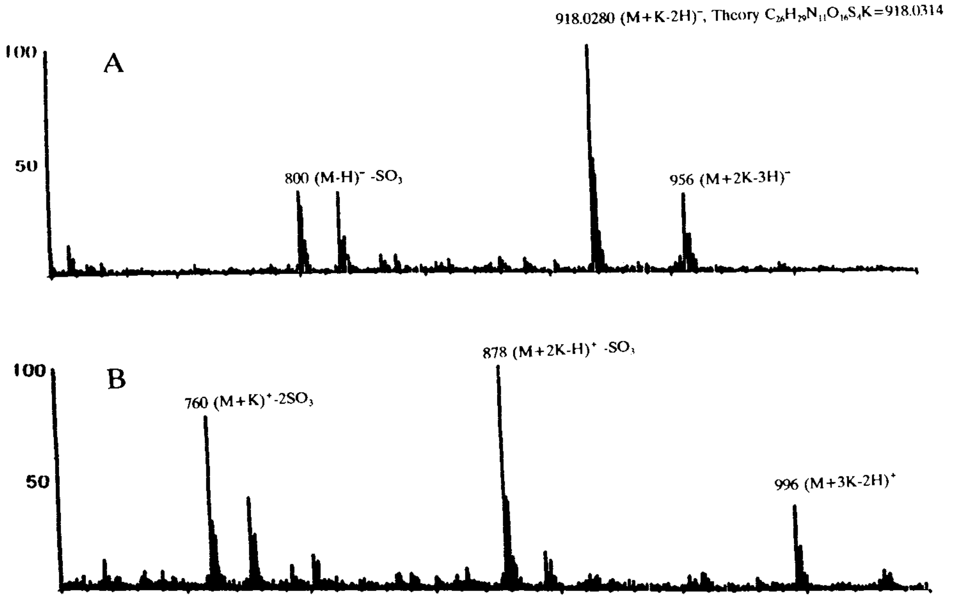


Fig. 3. Fast atom bombardment (FAB) negative ion (A) and positive ion (B) spectra of the pink-colored product.

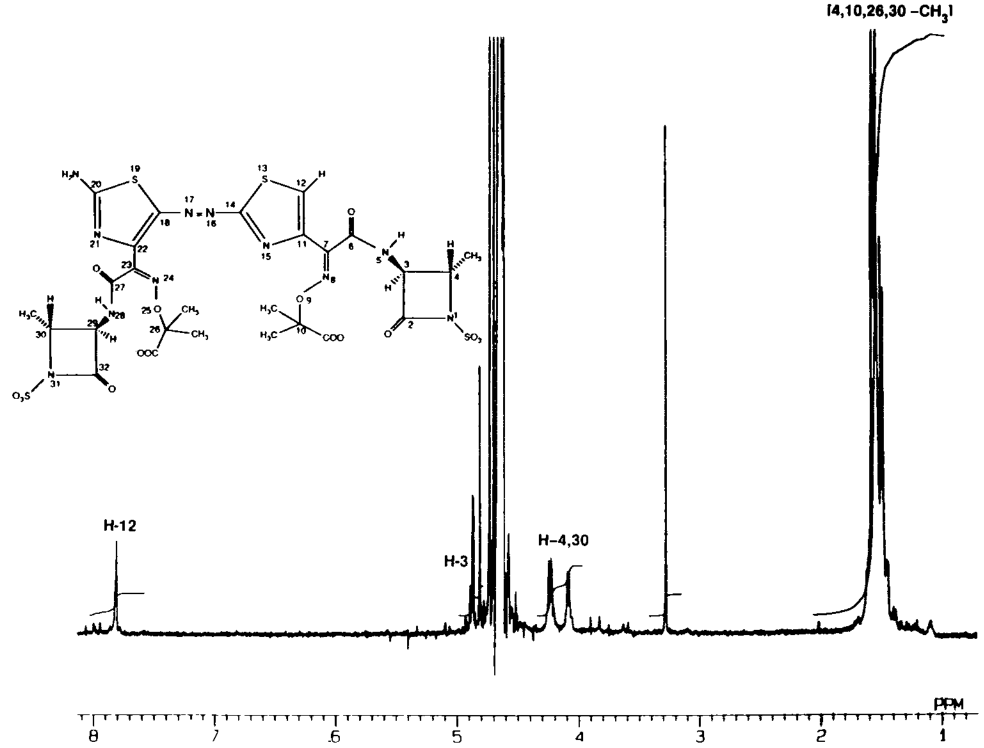


Fig. 4. The ^{1}H-NMR spectrum of the pink colored product in D_2O at 400 MHz.

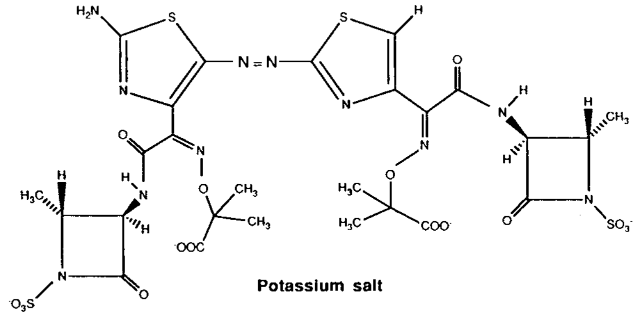
metronidazole solutions (5 mg/ml) was 23 ppm, which corresponds to approximately 1% degradation of metronidazole.

Effect of Storage Conditions and Autoclaving on Nitrite Ion Formation

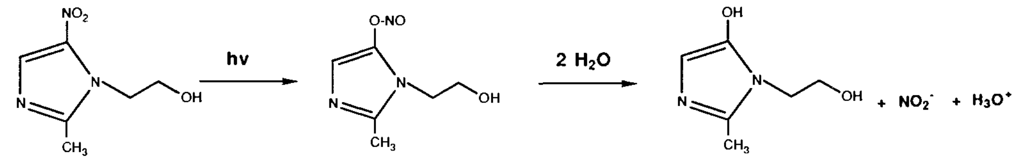
A metronidazole solution (5 mg/ml) stored at 80°C was periodically analyzed using ion chromatography for the determination of nitrite ion concentration. The samples were also analyzed by reacting with an aztreonam solution in 0.1 *M* acetic acid and determining the absorbance at 500 nm. As shown in Table I, the nitrite ion concentration in the solution progressively increased with time. Since a good agreement between the concentrations of nitrite ions determined by the two methods was observed (Table I), the samples stored under other storage conditions were analyzed for their nitrite contents by the photometric method only. The results presented in Table II demonstrate that nitrite ions are produced in a metronidazole solution by the effects of both temperature and light. Similarly, autoclaving also resulted in the formation of nitrite ions in solution. While there was no nitrite ion present in the initial solution, the concentration of nitrite ion in the autoclaved solution was 0.080 m*M* (5.5 ppm), with no significant difference from vial to vial. These data suggest that the method of manufacturing and the subsequent storage conditions will influence the extent of nitrite ion formation in metronidazole solutions. This may explain the variation in nitrite ion contents of different commercially available metronidazole products.

Isolation and Characterization of Pink Product

The pink product was isolated using the HPLC method; the chromatograms of the aztreonam–sodium nitrite reaction mixture and the isolated pink-colored product are given in Fig. 2. The mass spectrum and the NMR spectrum of the product are shown in Figs. 3 and 4, respectively. Since potassium phosphate was used in the mobile phase of HPLC, the isolate was a potassium salt. The negative FAB mass spectrum of the isolate (Fig. 3) showed an $(M + K - 2H)^-$ ion at m/z = 918. Accurate mass measurement yielded m/z = 918.0280, corresponding to an empirical formula of $C_{26}H_{29}N_{11}O_{16}S_4K$ (theory, 918.0314). The other major fragments were desulfonated $(M - H)^-$ at m/z = 800 and $(M + 2K - 3H)^-$ at m/z = 956. In the positive mass spectrum, ions were detected at m/z = 878 for $(M + 3K - 2H)^+ - SO_3$, m/z = 996 for $(M + 3K - 2H)^+$, and m/z = 760 for $(M + K)^+ - 2SO_3$. Based on the mass spectral data a molecular weight of 881 appears correct and the molecular formula of $C_{26}H_{31}O_{16}N_{11}S_4$ is in agreement with the diazo-coupled dimer of aztreonam.



Scheme I



Scheme II

NMR spectroscopy provided further evidence that a diazo-coupled dimer of aztreonam was found (Fig. 4). The H spectrum was qualitatively similar to that of aztreonam, with signals for methyl, methine, and heteroaromatic proton. Notably, the single thiazole proton in the azo dimer was shifted to 7.8 ppm, probably as a result of the deshielding effect of the azo linkage. In the spectrum of aztreonam, it occurs at 6.8 ppm. A pair of double quartets (J = 3.0 and 6.2 H_z), integrating to one proton each at 4.3 and 4.1 ppm, is assignable to the respective methine protons of the two monobactam rings. The signal due to the other ring proton occurs as a doublet at 4.9 ppm (J = 3.0 H_z), whereas the similar signal due to the second ring is obscured by the large HDO solvent peak. The vicinal coupling constant of 3.0 H_z confirms the *trans* stereochemistry of the monobactam ring protons. The methyl protons occur in the region 1.4–1.5 ppm, integrating 18 protons due to six methyl groups in the proposed structure. The proposed structure of the compound based on above considerations is shown in Scheme I.

Mechanism of Reactions

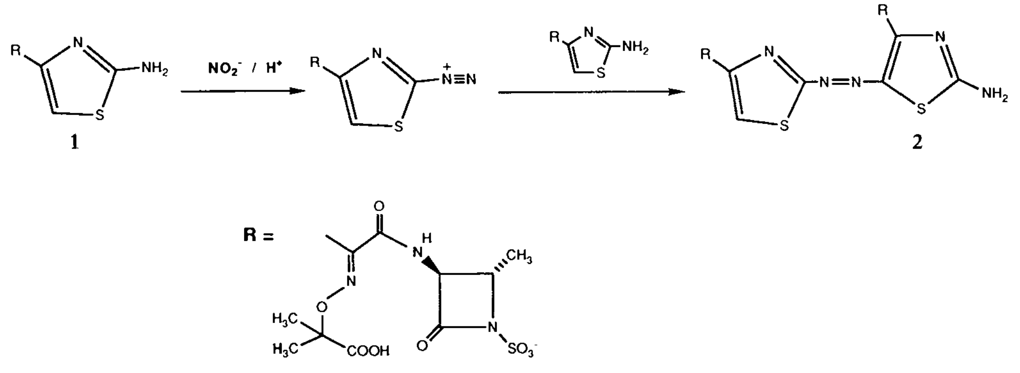
Photodissociation of nitro compounds to form nitrite ions, possibly mediated by the formation of free radicals, has been reported in the literature (6). For metronidazole, Theuer (4,5) reported the hydrolytic reaction shown in Scheme II, which is accelerated by light and temperature. In the present study the exposure to temperature and light also resulted in the formation of nitrite ions in metronidazole solutions. The resultant nitrous acid in an aqueous solution of metronidazole can react with aromatic and heteroaromatic amines. Therefore, the addition of aztreonam (compound 1 in Scheme III) to degraded metronidazole results in the diazotization of the 2-aminothiazole moiety, which can further undergo a diazo-coupling reaction to form azo dye. It has been reported that the aminothiazole group reacts exclusively at the 5-position when this position is unsubstituted (7). As a result, only one diazo-coupled product between two aztreonam molecules (compound 2 in Scheme III) is feasible. The structure of compound 2 is in agreement with the structure of the pink product which was proposed earlier based on mass spectral and NMR results.

An injectable aztreonam solution has a pH of ~5, which is favorable for a diazotization reaction. In a separate experiment, the molar ratio of nitrite ion to aztreonam was varied by the controlled addition of sodium nitrite at ~5°C to a known concentration of aztreonam in 0.1 *M* acetic acid. The maximum intensity of color was observed at a nitrite ion-to-aztreonam molar ratio of ~1:2 (Fig. 5). This is consistent with the stoichiometry of diazo-coupling reaction shown in Scheme II, wherein one mole of aztreonam is diazotized by one mole of nitrous acid and then condensed with another mole of aztreonam to form the colored diazo compound.

Under controlled conditions of 5°C and pH 2.5 to 5, other β-lactam antibiotics such as ceftizoxime, ceftazidime, and ceftriaxone, which contain 2-aminothiazole groups, exhibited pink coloration with sodium nitrite as well as with degraded metronidazole solutions. This shows that the diazo-coupling reaction depicted by Scheme III is not unique to aztreonam. Other compounds containing the 2-aminothiazole moiety may undergo such a reaction. There is, however, no visible incompatibility of ceftizoxime, ceftazidime, and ceftriaxone with metronidazole, during normal usage, because unlike aztreonam, the pH values of their injectable solutions are >6, where the diazo-coupling reaction is not favored.

CONCLUSIONS

The development of pink color observed in the intravenous admixture of metronidazole and aztreonam is a result of the formation of trace amounts of nitrite ions in a metronidazole solution. The nitrite ions are formed by the effects of temperature and light. The reaction of nitrite ion with



Scheme III

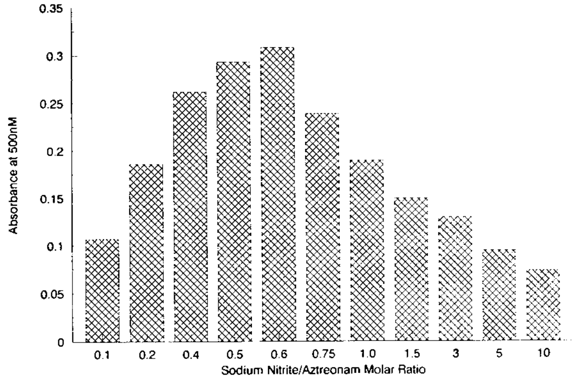


Fig. 5. Effect of the molar ratio of sodium nitrite to aztreonam on the development of pink color in solution in 0.1 *M* acetic acid.

aztreonam is so sensitive that the pink coloration may be visible even when the degradation of metronidazole is minimal (<1%). The nitrite ion diazotizes the aztreonam molecule under acidic pH conditions (<6), which then undergoes a diazo-coupling reaction with another aztreonam molecule, producing the pink-colored product. Such a reaction is also feasible with other antibiotics containing the aminothiazole functionality.

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