(10) C. V. Manion, D. W. Shoeman, and D. L. Azaroff, *J. Chromatogr.*, **101**, 169(1974).

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Nitrofurantoin Solubility in Aqueous Urea and Creatinine Solutions

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Abstract □ Experiments were carried out to determine the effect of urea and creatinine on the solubility of nitrofurantoin in water at different temperature and pH conditions. The addition of urea to aqueous media increased nitrofurantoin solubility up to a maximum concentration level and then decreased solubility at higher urea concentrations. The amount of urea needed to bring about maximum nitrofurantoin solubility was dependent on temperature and ranged between 1.75 and 2.50%. Spectral studies suggest a possible interaction between urea and nitrofurantoin molecules. Nitrofurantoin solubility increased with an increasing creatinine concentration ranging from 0.05 to 1.6%. Spectral studies indicate a strong interaction between creatinine and nitrofurantoin molecules in solution. The combined effect of urea and creatinine on the solubility of nitrofurantoin could account for the absence of crystalluria with this drug, even though unusually high concentrations in urine have been reported.

Keyphrases □ Nitrofurantoin—solubility in aqueous solutions, effect of urea and creatinine, varying temperature and pH □ Urea—effect on aqueous solubility of nitrofurantoin, varying temperature and pH □ Creatinine—effect on aqueous solubility of nitrofurantoin, varying temperature and pH □ Solubility—nitrofurantoin in aqueous solutions, effect of urea and creatinine, varying temperature and pH

Nitrofurantoin, 1-[(5-nitrofurfurylidene)amino]-hydantoin, is an antibacterial agent widely used to treat urinary tract infections. It is a weak acid (pKa 7.2) possessing relatively low aqueous solubility characteristics. Solubility at 37° was reported to be 190 mg/liter in distilled water (1) and 125 mg/liter in pH 4.8 water (2). Bates *et al.*[1] (3) reported the solubility of this drug in water at pH 1.12 and 7.20 to be 154 and 374 mg/liter, respectively.

Nitrofurantoin concentrations in urine ranged from 250 to 500 mg/liter after 200 mg was administered every 6 hr to an 80-kg patient (4). Urine concentrations of 200–400 mg/liter were reported (5), and maximum urine nitrofurantoin concentrations ranged from 158 to 372 mg/liter in nine normal individuals, each of whom received a 100-mg tablet of drug every 4 hr for four doses (6). Although unusually high concentrations of nitrofurantoin are found in the urine, no case of crystalluria associated with nitrofurantoin therapy has been reported. These observations suggest that substances normally present in urine might affect the aqueous solubility of nitrofurantoin.

Preliminary findings indicated that urea initially increased nitrofurantoin solubility at low urea concentrations and then decreased solubility at higher urea concentrations (7). In the present study, an attempt was made to ascertain the effect of urea and another urine component, creatinine, on nitrofurantoin solubility at various temperatures and pH conditions.

EXPERIMENTAL

Materials—Nitrofurantoin[2] was used as received without further purification. Nitromethane[3] was spectrophotometric grade; urea, methyl alcohol, citric acid, creatinine[4], hydrochloric acid, disodium phosphate, and potassium chloride were reagent grade. A high molecular weight quaternary ammonium hydroxide solution[5] was diluted with absolute methanol to 0.04 *M*.

Equipment—A rotating apparatus[6], capable of holding multiple samples, was immersed in a water bath kept at constant temperature by a controlled-temperature circulating pump[7]. The pH values of aqueous systems were measured using a pH meter[8].

Preparation of Solution—McIlvaine's buffer solutions (pH 3–7) (8) were prepared by mixing appropriate volumes of 0.1 *M* citric acid and 0.2 *M* disodium phosphate solutions in deionized water.

Stock solutions of 0.2 *M* potassium chloride and 0.2 *M* hydrochloric acid were prepared with deionized water. Appropriate portions of the two were mixed and diluted to make the hydrochloric acid buffer solutions (pH 1.2–2.0). A 0.1 *M* hydrochloric acid solution was used for the pH 1.12 medium.

Appropriate amounts of urea and creatinine were dissolved in deionized distilled water or in various pH buffer solutions to make urea solutions ranging from 0.25 to 10% and creatinine solutions ranging from 0.05 to 2.0%.

Solubility Studies—Excess nitrofurantoin (~50 mg) was added to 40 ml of the appropriate test solution in a screw-capped bottle of 45-ml capacity. The tightly closed container was placed in a water bath at various temperatures (24, 30, 37, and 45 ± 0.1°) and rotated

[1] T. R. Bates, School of Pharmacy, State University of New York at Buffalo, personal communication. A lower value was erroneously reported in Ref. 3.

[2] A pure sample (Lot. E3769) was supplied by Eaton Laboratories, Norwich, N.Y.

[3] Aldrich Chemical Co., Milwaukee, Wis.

[4] Nutritional Biochemicals Corp., Cleveland, Ohio.

[5] Hyamine, Sigma Chemical Co., St. Louis, Mo.

[6] Menold, Lester, Pa.

[7] Haake E52, Berlin-Lichterfeid, West Germany.

[8] Photovolt-digicord, W. H. Curtin & Co.

Table I—Solubilities and Enthalpy Values of Nitrofurantoin in Distilled Water

Temperature	Solubility, mg/liter	Mole Fraction × 10^6	Enthalpy (ΔH)[a], kcal/mole
24°	79.5	6.01	18.70
30°	113.4	8.57	4.60
37°	174.1	13.16	4.37
45°	251.2	18.99	2.95

[a]Enthalpy values were calculated using the tangent of the van't Hoff plot at different temperatures.

for at least 24 hr. Preliminary experiments indicated that equilibrium was established within 12–18 hr.

After equilibrium, the test solutions were subjected to filtration[9] (0.45-μm pore size). The filtrate was diluted with deionized distilled water to make a solution of proper concentration (40–100 mg/liter) for spectrophotometric assay. At least five experimental runs were carried out for each test medium.

Assay Procedure—The assay procedure was adapted from Conklin and Hollifield (9). A mixture of 4.0 ml of 0.1 *N* hydrochloric acid, 1.0 ml of sample solution, and 10.0 ml of nitromethane was placed in a separator and shaken for 2 min. The mixture was allowed to separate into two clear layers, and an 8.0-ml portion of the nitromethane layer (bottom layer) was removed and centrifuged for 5 min. Then a 4.0-ml portion of this nitromethane solution and 0.5 ml of 0.04 *M* quaternary ammonium hydroxide methanolic solution were thoroughly mixed in a test tube, and the absorbance of the resulting solution was measured at 400 nm[10].

Nitromethane, which was treated with 1.0 ml of distilled water, 4.0 ml of 0.1 *N* hydrochloric acid, and 0.5 ml of quaternary ammonium hydroxide methanolic solution, was used as the blank. The absorbance readings were converted to concentration values (milligrams per liter) using a standard Beer–Lambert plot.

Spectral Studies—Difference spectroscopy (tandem technique) (10) was used to study the possible interactions between nitrofurantoin and urea and between nitrofurantoin and creatinine. Identical amounts of nitrofurantoin solution (20 mg/liter) and blanks were placed in both the reference and sample compartments, and the baseline was determined using a recording spectrophotometer[10]. Appropriate buffer solutions were used as blanks in both compartments.

A 10–50-μl amount of 30% urea stock solution containing 20 mg/liter of nitrofurantoin was added[11] to the nitrofurantoin solution in the sample compartment. An equal amount of plain 30% urea solution was added to the blank in the reference compartment. The spectrum was scanned and recorded between 500 and 230 nm. Additional amounts of stock solution were added, and the spectra were recorded. The same procedure was used for a creatinine–nitrofurantoin system in which a 5% creatinine stock solution was added to the nitrofurantoin solution (20 mg/liter).

RESULTS AND DISCUSSION

Solubility in Water and Buffer Media—The present study reports the solubility of nitrofurantoin in distilled water over a range of temperatures. These data served as a means of explaining the thermodynamic properties of nitrofurantoin in aqueous solutions. As shown in Table I, the solubilities of this drug increased at higher temperatures (endothermic), and the heat of solution (ΔH) varied at different temperatures. The variation in the enthalpy of the systems may be due to the formation of intermolecular interaction between the drug molecules.

A plot of the logarithm of the solute mole fraction against the reciprocal of the absolute temperature (van't Hoff plot) was found to be curvilinear. This graphic representation is frequently employed to determine the presence of molecular association in solution, and curvature of this plot suggests the possible occurrence of molecular interactions. Hildebrand and Scott (11) suggested the use of the van't Hoff plot for this purpose. In addition, when the logarithm of the solute mole fraction was plotted against the logarithm of the absolute temperature, the line was linear. This type of plot also has been used to predict the effects of temperature on the solubility of various compounds (12).

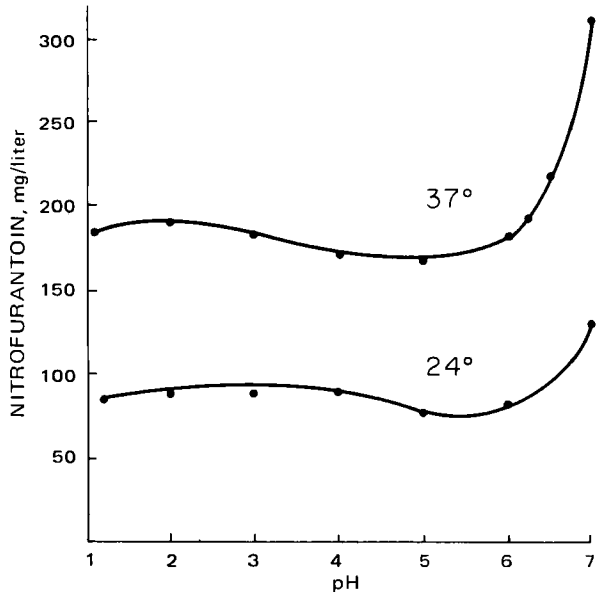

Figure 1—*Effect of pH on nitrofurantoin solubility in water.*

The solubility of nitrofurantoin in water was determined in this study at various hydronium-ion concentrations. Figure 1 depicts the effect of pH on the solubility of this drug. A large increase in solubility, starting at pH 6, was due to ionization of the drug (pKa 7.2) in the solution. This figure also shows that the solubility of nitrofurantoin was slightly higher at pH 2–3 than at about pH 5. This phenomenon might be explained by electron dislocalization of the molecule at the lower pH values, resulting in higher solubilities. Electron dislocalization in the nitrofurantoin molecule is possible due to the existence of three nitrogen atoms, each containing unshared electron pairs and conjugated double bonds.

The determined solubilities of nitrofurantoin are generally in good agreement with previously reported values. Paul *et al.* (6) found the nitrofurantoin solubility in distilled water to be 190 mg/liter at 37°; the value in the current study was 174 mg/liter. They also determined the solubility in pH 5 buffer to be 155 mg/liter. This value is in fair agreement with the value of 168 mg/liter reported in this study. The solubility value obtained in this study in 0.1 *N* hydrochloric acid (pH 1.12) at 37° (177 mg/liter) was slightly higher than the value of 154 mg/liter recently reported[1] (3).

Effect of Urea on Nitrofurantoin Solubility—Figures 2–5 summarize the data concerning the effect of urea on nitrofurantoin solubility in water and buffer. The addition of urea to aqueous media first increased nitrofurantoin solubility up to a maximum level and then decreased solubility at higher urea concentrations. After the maximum solubility was reached at an optimal urea concentration, nitrofurantoin solubility abruptly decreased to a level considerably lower than its solubility in distilled water.

Figure 2 illustrates this unusual solubility behavior of nitrofurantoin in water as a function of urea concentrations. At all temperatures studied, similar solubility curves were obtained. However, the amounts of urea needed for the maximum solubility of nitrofurantoin varied at different temperatures, ranging from 1.75 to 2.5% between 24 and 45°.

Figure 3 shows a linear correlation between the concentration of urea that produced maximum nitrofurantoin solubility in water and temperatures of the system. The slope of the line could be used for the prediction of urea concentrations exhibiting the maximum solubilizing effect at different temperatures. Although the pH of 5% urea in water was around 7.8, the solubility of nitrofurantoin did not increase in these solutions; in fact, the solubility of nitrofurantoin in 5% urea was much lower than in distilled water at 24, 30, and 37°.

When the temperature of the system was kept constant, the pH of the solution did not seem to affect the optimal urea concentrations at which the maximum solubility of nitrofurantoin was obtained.

[9] Millipore.
[10] Cary 118 spectrophotometer.
[11] Hamilton microliter syringe.

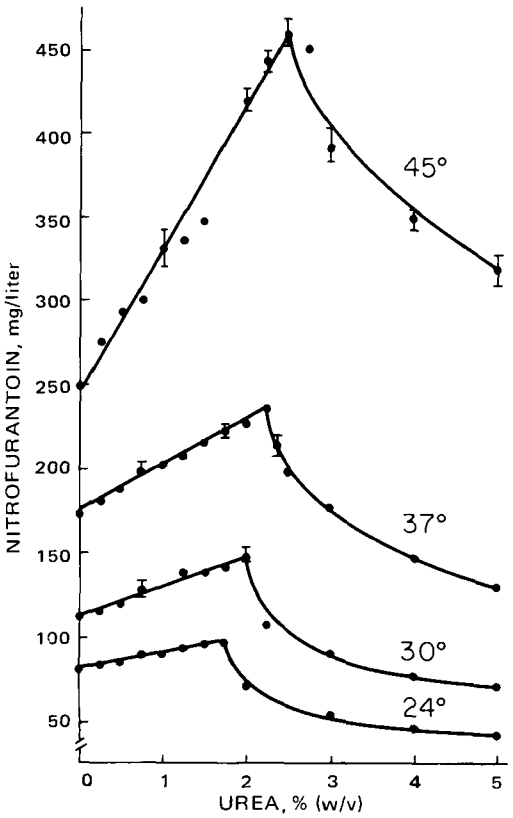

Figure 2—*Nitrofurantoin solubility in aqueous urea solutions at different temperatures.*

Figures 4 and 5 show the effects of hydronium-ion concentration on the amount of urea exhibiting the maximum solubilizing effect. The concentrations of urea needed for the maximum nitrofurantoin solubility were equal in different pH solutions used. The addition of urea to the buffer solutions did not significantly change the pH of the systems.

The findings that urea did increase the aqueous solubility of nitrofurantoin up to an optimum level might explain the elevated solubilities of this drug in urine frequently reported (5, 6). Concentrations of urea in the urine are normally in the neighborhood of 2% (13, 14).

The effect of urea on the aqueous solubility of organic compounds has been widely studied. Urea has been shown to increase the water solubility of chloramphenicol (15, 16) and acetaminophen (17) and to decrease the solubility of sulfathiazole (18). Mukerjee and Ghosh (19) reported the solubility of methylene blue to be 30 times higher in 10 *M* urea solution than in water. In a recent investigation, 1–5 *M* urea increased the water solubility of methyl *p*-hydroxybenzoate (20).

The effects of urea and thiourea on the solubility of benzoic and salicylic acids also were studied (21, 22), and it was inferred that the interaction of urea with salicylic acid was relatively complex but that

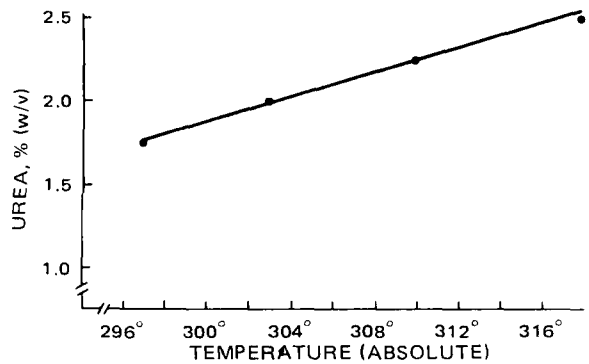

Figure 3—*Effect of temperature on the concentration of urea that provides maximum nitrofurantoin solubility in water.*

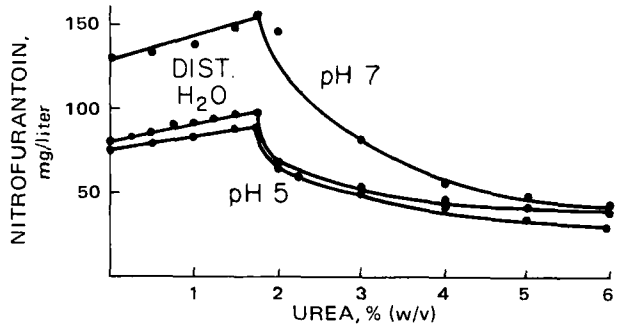

Figure 4—*Effect of pH on nitrofurantoin solubility in urea solutions at 24°.*

increases in the solubility of these compounds were due to formation of soluble complexes. Feldman and Gibaldi (23), however, concluded that the enhanced solubility of benzoic and salicylic acids in urea and alkylurea solutions did not involve complexation. They proposed that urea and alkylurea solubilized benzoic and salicylic acids by "breaking up" water clusters surrounding the nonpolar molecule, increasing the entropy of the system, and producing a "driving" force for the solubilization.

Thus, the literature reports indicate that urea exhibits two distinctly opposite effects as to its ability to change the solubility of various organic compounds in aqueous solutions, and there are also contradictory reports as to the mechanism of the urea effect on the solubility of various compounds. In studying the effect of urea on hydrocarbon solubility, Wetlaufer *et al.* (24) suggested several mechanisms to explain the increased solubility of hydrocarbons in aqueous urea solutions: (*a*) the solute is solely dissolved by the urea, (*b*) urea alters the structure of water so as to enhance solvation of the solute by water molecules, and (*c*) solute molecules are solvated both by urea and water molecules. These proposed mechanisms, however, usually have been associated with increased solubility of compounds at relatively high urea concentrations (1–5 *M*). There is no conclusive evidence to support any one mechanism whereby urea changes solubilities.

In the present study, because of the unusual behavior of the system (first an increase followed by a considerable decrease in nitrofurantoin solubility), a single explanation of the phenomenon is most difficult. A similar phenomenon was reported for the effect of urea on the solubility of tetracycline in water (25). The increased solubility of tetracycline was attributed in part to the formation of a nonclathrate complex; the complex continued to form up to a point (maximum

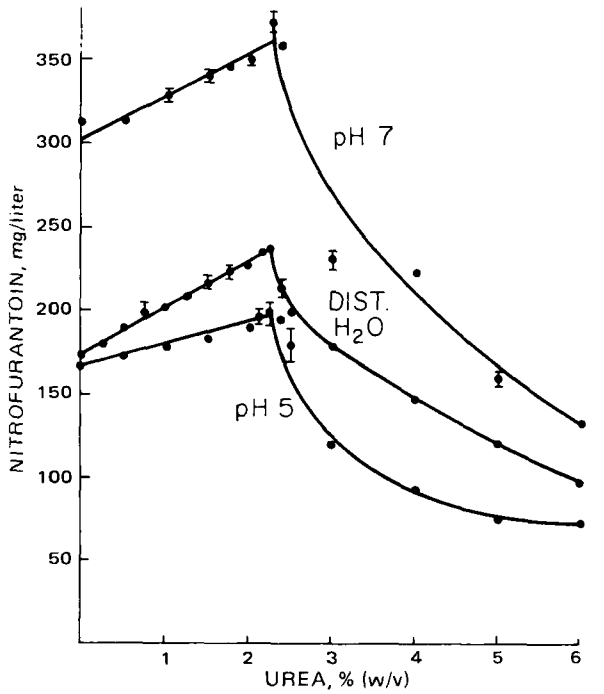

Figure 5—*Effect of pH on nitrofurantoin in urea solutions at 37°.*

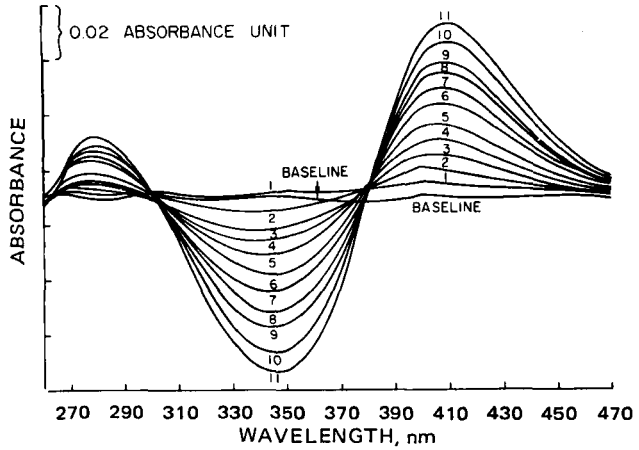

Figure 6—*Difference spectra of nitrofurantoin–urea in pH 7 buffer solution. Key [percent (w/v) urea in solution]: 1, 0.15; 2, 1.08; 3, 2.09; 4, 3.03; 5, 3.91; 6, 5.0; 7, 6.0; 8, 6.91; 9, 7.78; 10, 9.31; and 11, 10.65. All solutions contained the identical concentration of nitrofurantoin (20 mg/liter).*

solubility attained in 2.25 *M* urea at 35°), which can be considered the solubility limit of the complex. Higuchi and Ikeda (26) also found a similar solubility behavior with digoxin in aqueous hydroquinone solutions.

To examine the mechanism(s) of the urea effect on the solubility of nitrofurantoin, spectral properties of the system were studied. Analysis of the spectra (Fig. 6) suggests some type of interaction between nitrofurantoin and urea molecules. In practice, it has been generally recognized that many experimental problems associated with detecting and measuring spectral changes are greatly simplified by using difference spectral techniques (10). Instead of comparing the spectrum of nitrofurantoin with the spectrum of the mixture of nitrofurantoin and urea, the difference spectra of these substances were obtained and compared (Fig. 6). A distinctive new maximum band appeared at 410 nm when urea was added to the nitrofurantoin solution. The normal maximum band for nitrofurantoin in the visible range at pH 7 was at 375 nm. This large spectral shift (35 nm) is a strong indication of molecular interaction between urea and nitrofurantoin molecules. There was also an appreciable band at 345 nm (normally 310 for nitrofurantoin at pH 7). These spectral changes usually indicate conformational changes occurring in and/or near the main chromophore of the drug.

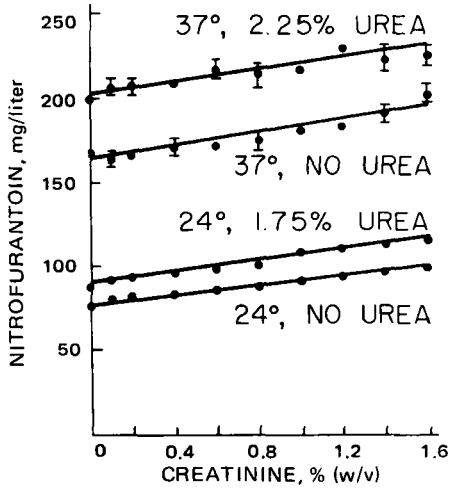

Figure 8—*Effect of creatinine on nitrofurantoin solubility in pH 5 aqueous medium (with and without urea) at 24 and 37°.*

Although no attempts were made in this study to quantitate the stoichiometry of the urea–nitrofurantoin interaction, preliminary spectral studies do indicate that the intensities of the spectral changes are directly proportional to the urea concentrations in the system. In addition, the presence of isosbestic points at 380 and 300 nm in the difference spectra (Fig. 6) suggests that the nature of the interaction between nitrofurantoin and urea molecules is of a specific type rather than a nonspecific interaction. The proposed intermolecular complexation may explain the increased solubility of nitrofurantoin at low urea concentrations.

On the other hand, the abrupt decrease in nitrofurantoin solubility when urea concentrations reach 1.75–2.5% at different temperatures might be explained by a salting-out effect. It is possible that when urea is added in an excessive amount, nitrofurantoin or its complex would

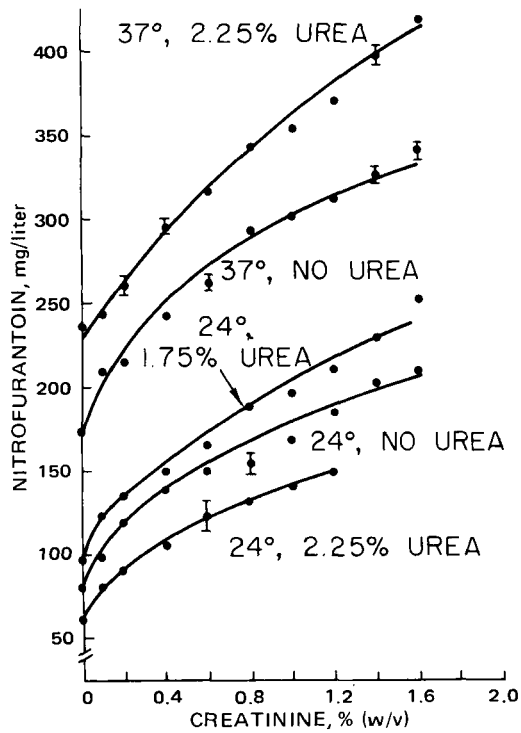

Figure 7—*Effect of creatinine on nitrofurantoin solubility in distilled water (with and without urea) at 24 and 37°.*

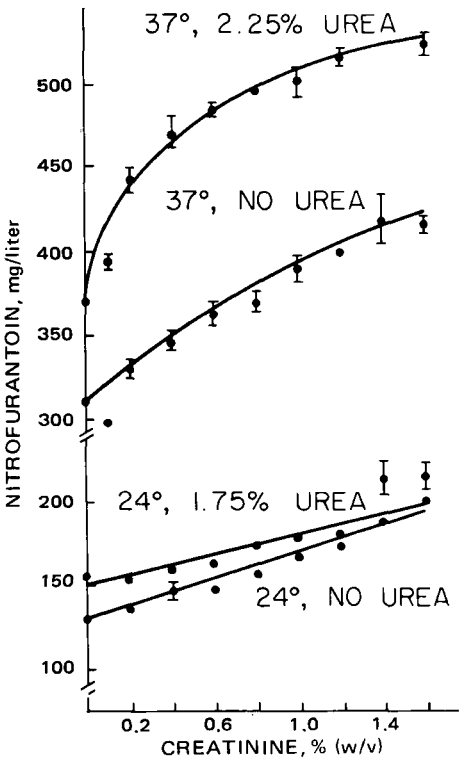

Figure 9—*Effect of creatinine on nitrofurantoin solubility in pH 7 aqueous medium (with and without urea) at 24 and 37°.*

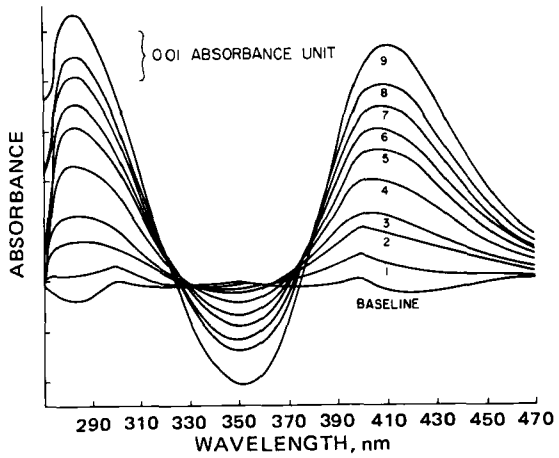

Figure 10—*Difference spectra of nitrofurantoin–creatinine in pH 7 buffer solution. Key [for percent (w/v) creatinine in solution]: 1, 0.122; 2, 0.238; 3, 0.349; 4, 0.555; 5, 0.746; 6, 0.918; 7, 1.078; 8, 1.226; and 9, 1.49. All solutions contained the identical concentration of nitrofurantoin (20 mg/liter).*

be precipitated. The breakdown of water structure might account for the increase in solubility at lower urea concentrations; however, disruption of the water structure probably would not occur at the low urea concentrations used in this study. The paradoxical behavior of nitrofurantoin in urea solutions makes it difficult to propose an unequivocal mechanism for the unusual solubility phenomenon.

Effect of Creatinine on Nitrofurantoin Solubility—The effect of creatinine on the aqueous solubility of nitrofurantoin at 24 and 37° is summarized in Figs. 7–9. As shown in these figures, the nitrofurantoin solubility was increased significantly in the presence of creatinine, even in relatively low creatinine concentrations (0.05–1.6%). The increases in the solubility of nitrofurantoin in 1.6% creatinine solution compared to distilled water at 24 and 37° were from 80 to 190 mg/liter and from 170 to 330 mg/liter, respectively. However, the general solubilizing effect of creatinine did not seem to be affected by temperature, pH, or addition of urea to the system.

On the other hand, creatinine enhanced the maximum solubility of nitrofurantoin in urea solutions. When creatinine was added to the solutions containing the optimal urea concentrations that exhibited the maximum solubility effect (2.25% at 37° and 1.75% at 24°), the maximum nitrofurantoin solubility was further increased. The solubility of nitrofurantoin was also increased by creatinine at a urea concentration (2.25% at 24°) that reduced nitrofurantoin solubility (Fig. 7). These findings lend support to the idea that the lack of crystalluria with nitrofurantoin, a compound of limited water solubility, is due to the combined solubilizing effect of urea and creatinine. The additional solubilizing effect of creatinine would offset the solubility-decreasing effect of urea which might occur at an unusually high urea concentration (higher than 2.0%) in the urine.

The spectra for nitrofurantoin–creatinine solutions (Fig. 10) indicate a possible interaction between the two compounds. There were two strong positive bands at 283 and 410 nm and a negative band at 352 nm. These spectral shifts were similar to those for nitrofurantoin–urea interactions and suggest a steric effect in the chromophore group. The presence of isosbestic points at 375 and 325 nm indicates that the interaction is of a specific type.

REFERENCES

(1) H. E. Paul and M. F. Paul, in "Experimental Chemotherapy," vol. II, R. J. Schnitzer and F. Hawking, Eds., Academic, New York, N.Y., 1964, p. 334.

(2) M. F. Paul, R. C. Bender, and E. G. Nohle, *Am. J. Physiol.*, **197**, 580(1959).

(3) T. R. Bates, J. M. Young, C. M. Wu, and H. A. Rosenberg, *J. Pharm. Sci.*, **63**, 643(1974).

(4) W. A. Richards, E. Riss, E. H. Kass, and M. Finland, *Arch. Intern. Med.*, **96**, 437(1955).

(5) E. H. Beutner, J. J. Petronio, H. E. Lind, H. M. Trafton, and M. Correia-Branco, "Antibiotics Annual 1954–1955," H. Welch and F. Marti-Ibanez, Eds., Medical Encyclopedia Inc., New York, N.Y., 1955, p. 988.

(6) M. F. Paul, C. Harrington, R. C. Bender, E. Nohle, and M. J. Bryson, *Proc. Soc. Exp. Biol. Med.*, **125**, 941(1967).

(7) D. E. Cadwallader, H. W. Jun, and L.-K. Chen, *J. Pharm. Sci.*, **64**, 886(1975).

(8) T. C. McIlvaine, *J. Biol. Chem.*, **49**, 183(1921).

(9) J. D. Conklin and R. D. Hollifield, *Clin. Chem.*, **11**, 925 (1965).

(10) T. T. Herskovits, in "Methods in Enzymology," vol. XI, C. H. W. Hirs, Ed., Academic, New York, N.Y., 1967, pp. 748–775.

(11) J. H. Hildebrand and R. L. Scott, "The Solubility of Nonelectrolytes," 3rd ed., Reinhold, New York, N.Y., 1964, pp. 273–285.

(12) J. H. Hildebrand, *J. Chem. Phys.*, **20**, 190(1952).

(13) H. A. Harper, "Review of Physiological Chemistry," 14th ed., Lange Medical, Los Altos, Calif., 1973, p. 398.

(14) A. White, P. H. Handler, and E. L. Smith, "Principles of Biochemistry," 3rd ed., McGraw-Hill, New York, N.Y., 1964, p. 737.

(15) K. Sekiguchi, N. Obi, and Y. Ueda, *Chem. Pharm. Bull.*, **12**, 134(1964).

(16) A. H. Goldberg, M. Gibaldi, J. L. Kanig, and M. Mayersohn, *J. Pharm. Sci.*, **55**, 581(1966).

(17) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *ibid.*, **55**, 482(1966).

(18) K. Sekiguchi and N. Obi, *Chem. Pharm. Bull.*, **9**, 866(1961).

(19) P. Mukerjee and A. K. Ghosh, *J. Phys. Chem.*, **67**, 193(1963).

(20) C. McDonald and E. Lindstrom, *J. Pharm. Pharmacol.*, **26**, 39(1974).

(21) S. Bolton, *J. Pharm. Sci.*, **52**, 1071(1963).

(22) D. M. Altwein, J. N. Delgado, and F. P. Cosgrove, *ibid.*, **54**, 603(1965).

(23) S. Feldman and M. Gibaldi, *ibid.*, **56**, 370(1967).

(24) D. B. Wetlaufer, S. K. Malik, L. Stoller, and R. L. Coffin, *J. Am. Chem. Soc.*, **86**, 509(1964).

(25) V. Naggar, N. A. Daabis, and M. M. Motawi, *Pharmazie*, **29**, 122(1974).

(26) T. Higuchi and M. Ikeda, *J. Pharm. Sci.*, **63**, 809(1974).

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