Drug Development and Industrial Pharmacy, 22(9&10), 1013-1019 (1996)

Enhancement of Thiacetazone Solubility by Isoniazid in Aqueous Solutions

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ABSTRACT

The aqueous solubility of thiacetazone was enhanced in the presence of different concentrations of isoniazid. The nature of this solubilization was investigated by measuring some physicochemical properties of isoniazid in water. Slight surface activity and conductance were observed. Osmotic pressure and partition coefficient measurements indicated that isoniazid dimerizes in aqueous solutions and were used to obtain and verify the dimerization constant. There was no evidence of higher oligomer formation in concentrations lower than 1.0 M. This excluded the possibility of solubilization by aggregate formation. Citric and glutaric acids in the presence of isoniazid were found to increase thiacetazone solubility as a function of the acid concentration and structure.

INTRODUCTION

The clinical treatment of primary tuberculosis is usually composed of multiple-drug therapy. This regimen choice is made to delay the emergence of organism resistance, to avoid toxicity by giving lower doses of more than one drug, and to achieve synergistic action against the infectious organisms. In current therapy, the most effective drug against tuberculosis is isoniazid (INH), which is usually administered in combination with other drugs (1). Formulating combinations usually involves optimizing of the drug release from their dosage forms to guard against physical incompatibility and inadequate bioavailability (2). Indeed, the bioavailability of isoniazid combined with rifampicin or with pyrazinamide showed no significant interaction between these drugs (3). In the INH–thiacetazone combined dosage form, INH is freely water soluble while thiacetazone has a very slight aqueous solubility (4). This hampers thiacetazone absorption from the alimentary canal. Consequently, methods to improve thiacetazone dissolution were attempted (5). These methods included surface hydrophilization using different carriers and ordered mixing techniques which were dependent on the particle size of thiacetazone and the physical properties of the matrix (6). The suggestion has been made that INH may promote thiacetazone solubility (6), and this prompted our investigation reported here.

EXPERIMENTAL

Materials

Isoniazid (172°C) was BP grade; thiacetazone (222°C) was Indian Pharmacopoeia grade. Solvents and reagents were of analytical grades. Distilled water was used. All chemicals used were obtained from the Jordanian Pharmaceutical Manufacturing Co. (Naur, Jordan).

Apparatus and Equipment

- Water bath shaker with temperature control ±0.5°C, 170 strokes per minute (GFL 1086, Germany)
- Ultraviolet (UV)–visible spectrophotometer (Beckman DU7 USA)
- Vapor pressure osmometer (Wescor, 5100 C, USA)
- Conductometer (Metrohm, 660, Switzerland)
- Melting point apparatus (Mettler, FP61, Switzerland)
- Dissolution apparatus (Hanson Research, SR8, USA)
- Tensiometer (Krüss, k8, Germany)

Dissolution Determination

The USP XXII dissolution method (apparatus 2) was adjusted at 50 rpm and 37°C. The dissolution medium volume was 500 ml of 0.1 M HCl. Physical mixtures were prepared by mixing thiacetazone and INH powders in a ratio of 1:2 (w/w) (150 mg:300 mg), respectively, using a mortar and pestle. This physical mixture was added to the dissolution medium and samples of 10 ml were withdrawn, filtered, and diluted with 0.1 M HCl. Sample contents were then determined from the first derivative spectral measurements at 343 nm.

Determination of Solubility Profiles

Different aqueous solutions of citric acid and glutaric acids having concentrations ranging from 0.0 to 1.5 M were made. Samples (25 ml) of these solutions were placed with excess amounts of thiacetazone (200 mg) in sealed glass flasks and were shaken until equilibrium was achieved (24 hr at 37°C). Filtered samples were diluted with 0.1 M HCl and their content was measured as in dissolution determinations. The effect of INH addition was tested by adding INH in concentrations 0.0% to 2.0% w/v, to 25-ml samples, each consisting of 0.1 M HCl, and 0.5, 1.0, and 1.5 M citric acid. Analysis followed as described for dissolution.

Determination of Partition Coefficient

Samples (25 ml of 6.4×10^{-3} to 7.3×10^{-3} M) from different aqueous solutions of INH were placed in sealed glass flasks. Water-saturated octanol (25 ml) was added to each flask and the containers were shaken at 30°C for 24 hr. Samples were then left to separate into two layers. The organic layers were subsequently separated, centrifuged, and diluted with octanol. The sample content was determined from the spectral absorbance at 261 nm.

Osmotic Pressure Measurements

A series of aqueous solutions of INH were made (0.0 to 1.0 M), and their osmotic pressure was measured using an osmometer calibrated with aqueous solutions provided by the osmometer manufacturer (100–1000 mOsmol/kg). The sample size was 10 µl. Each measurement was repeated twice.

Determination of Some Physical Measurements

Various concentrations of INH in water (0.0 to 1.0 M) were prepared. Their density, surface tension, and specific conductivities were measured at constant temperature.

RESULTS AND DISCUSSION

The dissolution profiles of physical mixtures of thiacetazone and INH powders illustrate the enhancement of the water solubility of the former by the latter. This profile was repeated using micronized thiacetazone powder, yielding similar results. There was no significant difference between the two experiments using different particle sizes, thus indicating that INH increases the solubility of thiacetazone in aqueous acidic solutions regardless of its particle size, as shown in Fig. 1.

The chemical structure of isoniazid resembles that of nicotinamide, which like other structurally related compounds, is known to self-associate in aqueous solutions (7). It is often believed that self-association of some species in aqueous solutions is the driving force behind the solubilization enhancement of water-insoluble drugs. Consequently, to investigate this mechanism, physical properties of INH in solutions were examined.

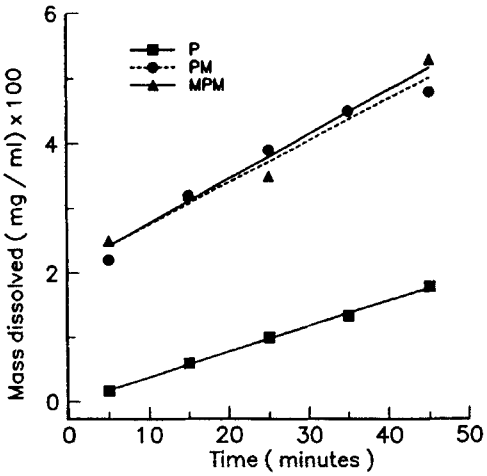

Figure 1. Dissolution profile of pure thiacetazone (P), its physical mixture powder (PM), and micronized physical mixture (MPM) with isoniazid at 30°C.

The surface tension of aqueous INH solutions was measured for various concentrations at 25°C. At 1.0 M concentration, the surface tension of isoniazid solution was only reduced by 5 $mN \cdot m^{-1}$ below that of water, showing that isoniazid displays slight surface activity. The specific conductivity of this solution was only 5 *s*/cm, indicating that isoniazid behaves as a non-electrolyte. Osmotic pressure measurements showed a linear increase with molar concentration to a point beyond which the slope slightly varies, as presented in Fig. 2, indicating possible solute–solute interaction. The partitioning of isoniazid between *n*-octanol and water, as illustrated in Fig. 3, shows positive curvature, which is indicative of possible association.

Self-association in the absence of dissociation of a molecular species (M) in water is usually described within the following framework:

$$M + M \overset{K_2}{\rightleftharpoons} M_2$$

$$M + M_2 \overset{K_3}{\rightleftharpoons} M_3$$

$$\vdots$$

$$M + M_{n-1} \overset{K_n}{\rightleftharpoons} M_n$$

where K_n is the partial association constant of the *n*-th oligomer, given by:

$$K_n = \frac{[M_n]}{[M][M_{n-1}]} \tag{1}$$

The overall association constant of the *n*-th oligomer β_n is given by:

$$\beta_n = \frac{[M_n]}{[M]^n} \tag{2}$$

which is equal to the product of individual formation constants leading to the *n*-th oligomer, i.e.

$$\beta_n = \prod_{\ell=1}^{n} K_\ell = K_1 \cdot K_2 \cdot K_3 \ldots K_n \tag{3}$$

with K_1 set equal to unity.

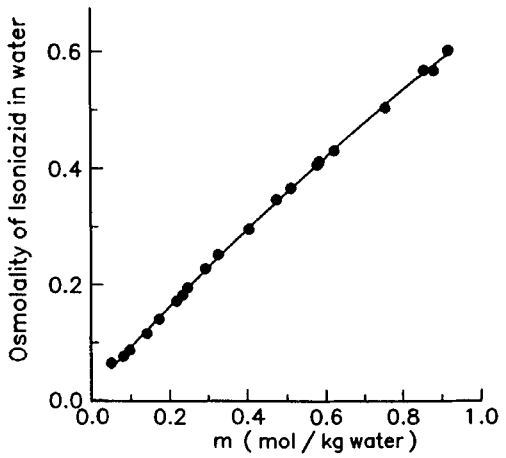

Figure 2. Osmolality of aqueous isoniazid solutions plotted against molality at 25°C.

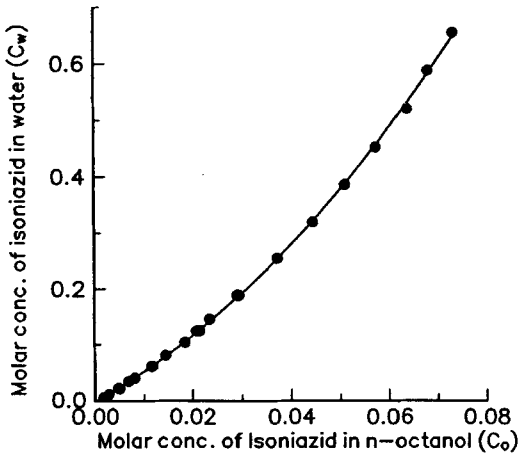

Figure 3. Partitioning of isoniazid between water and *n*-octanol at 30°C.

Having an aqueous solution of total analytical concentration in water, C_w, osmotic concentration C_{osm}, and monomeric species concentration C_m, the following relations are established:

$$C_w = \sum_{\ell=1}^{n} \cdot \beta_\ell \cdot C_m^\ell = C_m + 2\beta_2 \cdot C_m^2 + \ldots + n \cdot \beta_n \cdot C_m^n \quad (4)$$

$$C_w/C_m = \sum_{\ell=1}^{n} \beta_\ell \cdot C_m^{\ell-1} = 1 + 2\beta_2 \cdot C_m + \ldots + n\beta_n \cdot C_m^{n-1} \quad (5)$$

$$C_{osm} = \sum_{\ell=1} \beta_\ell \cdot C_m^\ell = C_m + \beta_2 \cdot C_m^2 + \ldots + \beta_n \cdot C_m^n \quad (6)$$

$$C_{osm}/C_m = \sum_{\ell=1}^{n} \beta \cdot C_m^{n-1} = 1 + \beta_2 \cdot C_m + \ldots + \beta_n \cdot C_m^{n-1} \quad (7)$$

The partition coefficient *PC* for INH equilibriated between water and *n*-octanol is given by:

$$PC = C_w/C_0 \quad (8)$$

where C_w is the aqueous INH concentrations and C_0 is the equilibrium molar concentration of INH in the organic phase (*n*-octanol).

The intercept of a plot of *PC* against C_0, at zero C_0, is essentially the partition coefficient of the monomeric species PC_m which, when multiplied by C_0 for each solution, yields the corresponding concentration of monomeric species in the aqueous phase; i.e.

$$C_m = PC_m \cdot C_0 \quad (9)$$

Equation (5) was used in this work to ascertain the occurrence of self-association of isoniazid in aqueous solutions. This was accomplished by fitting the experimental data to a polynomial in C_m through linear regression analysis using standard statistical procedures of error minimization (SPSSPC Software loaded onto an IBM-486 DX PC). The polynomial in C_m was truncated at an order *n* which produced the best possible fit with realistic positive coefficients, and beyond which negative coefficients began to appear and persist. Error limits on the association constants K_n were also estimated within reasonably established confidence limits (95%), with errors assumed to follow the standard *t* distribution.

A plot of *PC* against C_0 indicated an inflection point in the partition coefficient corresponding to an INH concentration in the organic phase of 6.5×10^{-3} M. Linear regression of the data below the inflection point produced an intercept corresponding to the partition coefficient of monomeric species $PC_m = 2.348$. Data beyond the inflection point yielded an intercept at zero C_0 concentration which, within experimental error, was twice that of PC_m. This may be explained as indicative of the dominance of dimeric species. The slopes of the two lines intercepting at the inflection point are irrelevant since they relate the variation of *PC* between two separate phases.

The plot of C_w/C_m against C_m demonstrated an inflection point at an aqueous isoniazid concentration of 1.5×10^{-2} M, with the intercept of the low-lying data estimated as unity at zero monomer concentration, while the intercept of the data beyond the inflection point was found equal to 2.00. This may indicate the onset of dimer formation beyond the inflection point. Fitting of the data beyond the inflection point after division by 2 against a polynomial in C_m according to Eq. (5) produced merely a linear relationship of the form:

$$C_w/C_m = (1.01 \pm 0.02) + (5.26 \pm 0.23) \cdot C_m \quad (10)$$

thus yielding the association constant for the dimeric species at 30°C:

$$\beta_2 = K_2 = 2.63 \pm 0.12 \text{ liters/mol} \quad (11)$$

From osmotic pressure measurements, in molal concentration units, Eqs. (5) and (7) may be cast in the following form:

$$m/m_1 - 1 = \sum_{\ell=2}^{n} \ell \cdot \beta_\ell \cdot m_1^{\ell-1} \quad (12)$$

$$m_{osm}/m_1 - 1 = \sum_{\ell=2}^{n} \beta_\ell \cdot m_1^{\ell-1} \quad (13)$$

where *m* is the analytical molal concentration of INH in water, m_{osm} is its measured osmolality, and m_1 is the concentration of monomeric species.

Following earlier analysis (8,9), the concentration of monomeric species was obtained by fitting the osmotic coefficient $\phi = m_{osm}/m$ to a polynomial in *m* according to:

$$\phi - 1 = \sum_{\ell=1}^{n} \alpha_\ell \cdot m^\ell \quad (14)$$

with the polynomial truncated at an order n which fits the data best, and thus yields the lowest standard errors of the coefficients α_ℓ (typically $3 \leq n \leq 5$). Once these coefficients are estimated, m_1 is obtained from the relationship:

$$m_1 = m \cdot \exp\left\{\sum_{\ell=1}^{n} \alpha_\ell \cdot m^\ell(\ell + 1)/\ell\right\} \quad (15)$$

For INH solutions at 25°C, those coefficients were estimated for a polynomial of order $n = 5$ representing the best possible fit with the following values:

$$\alpha_1 = -2.068,\ \alpha_2 = 8.146,\ \alpha_3 = -16.879,$$

$$\alpha_4 = 16.256,\ \alpha_5 = -5.831$$

Equation (15) was then used to evaluate m_1 for each solution with the results used in Eq. (13) to obtain the best fit for $[m_{osm}/m_1 - 1]$ against m_1, which yields positive overall formation constants β_ℓ, whose slope is:

$$\beta_2 = K_2 = 2.51 \pm 0.04 \text{ kg/mol} \quad (16)$$

It is apparent that INH self-associates to form dimers within the concentration range 0.0–1.0 M, and no evidence of higher oligomers has been established. Other procedures usually followed in data manipulations to estimate self-association constants, based on predetermined assumptions concerning the interrelationship between the constants, proved rather elusive. The isodesmic model, for example, presupposes that all partial association constants are equal ($K_2 = K_3 = \ldots = K_n$), and hence a plot of $(1 - \phi)/\phi^2$ against m yields the value of K for a polydisperse system with an infinite number of oligomers. The plot did actually yield a straight line, which demonstrates a linear relationship given by (9):

$$(1 - \phi)/\phi^2 = K \cdot m \quad (17)$$

with the best possible fit obtained as:

$$(1 - \phi)/\phi^2 = (0.16 \pm 0.03) + (0.74 \pm 0.05) \cdot m \quad (18)$$

This yielded an isodesmic association constant $K = 0.74 \pm 0.05$ kg/mol, which was far from the one obtained using rigorous analysis [Eq. (13)]. Moreover, the intercept thus obtained was not zero (0.16 ± 0.03) within experimental error, as it ought to be, and hence the isodesmic model is not applicable.

At this stage, assuming only dimer formation with $\alpha = (1 - \phi)$ representing the degree of dimerization, a plot of $(1 - \phi)/(2\phi - 1)^2$ or $\alpha/(1 - 2\alpha)^2$ against m should also yield a straight line whose intercept is zero and its slope equal to K_2. This plot showed excessive scattering due to error propagation in computing $\alpha/(1 - 2\alpha)^2$, and thus yielded inaccurate results. The value of K_2 obtained is 3.46 ± 0.34 kg/ml. A similar and frequently used plot is that of $(1 - \phi)/m$ or α/m against $(2\phi - 1)^2$ or $(1 - 2\alpha)^2$, respectively; these also showed a lot of scatter with nonzero intercept and yielded a different value of K_2 (2.74 ± 0.19 kg/mol).

Various results of manipulated data used to obtain the dimerization constant are given in Table 1. There was no evidence of multimer formation within the experi-

Table 1

Dimerization Constant of INH in Water Obtained Through Different Data Manipulations

Experimental Procedure (Plot Type)	K_2	T (°C)
Partition coefficient (C_w/C_m vs. C_m)	2.63 ± 0.12 liter/mol	30°C
Osmometry		
(m_{osm}/m_1–1) vs. m_1	2.51 ± 0.04 kg/mol	25°C
α/m vs. $(1 - 2\alpha)^2$	2.74 ± 0.19 kg/mol	25°C
$\alpha/(1 - 2\alpha)^2$ vs. m	3.45 ± 0.34 kg/mol	25°C
Isodesmic model		
$(\alpha/(1 - \alpha)^2$ vs. m	0.74 ± 0.05 kg/mol ($K_2 = K$)	25°C

mental error. The isodesmic model yields results which may cast doubts on all its premises. A more rigorous analysis provided a more reasonable value for K_2 = 2.63 ± 0.12 liter/mol at 30°C, and 2.51 ± 0.04 kg/mol at 25°C, as shown in Table 1.

The formation of dimers in INH aqueous solutions may explain various diversions from ideality observed in physicochemical measurements. The results obtained suggested that dimeric association cannot be the only responsible factor for solubilization of thiacetazone. Consequently, solubilities of thiacetazone in different solutions of INH containing HCl and citric acid were investigated and are presented in Fig. 4. The solubility was enhanced by both INH in solution and by a rise in citric acid concentration. Since the presence of HCl had no significant effect on solubility, it was suspected that the solubility enhancement is not an acidity function, but rather is acid structure dependent.

Figure 5 shows how the solubility is unaffected by pH variation for a mineral buffer of HCl–NaOH, yet it decreases with pH for a citric acid–NaOH buffer system. The decrease in the solubility of thiacetazone as the pH of the citric acid buffer increases suggests a more favorable interaction with the neutral citric acid molecule, through either hydrogen bonding or a reduction in the dielectric properties of the aqueous solutions.

To verify the buffer composition effect on solubilization, glutaric and citric acids were used in solubilizing thiacetazone, as shown in Fig. 6. The dramatic increase in thiacetazone solubility beyond 0.5 M concentration of glutaric acid invites attention. The increase in solubility of thiacetazone in the presence of isoniazid reported here may be a reflection of some mutually favorable interactions.

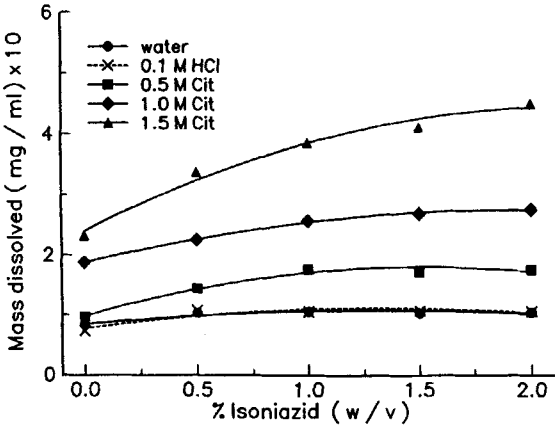

Figure 4. Solubility of thiacetazone as a function of isoniazid concentration in water, 0.1 M HCl; and in 0.5, 1.0, and 1.5 M citric acid (Cit) at 37°C.

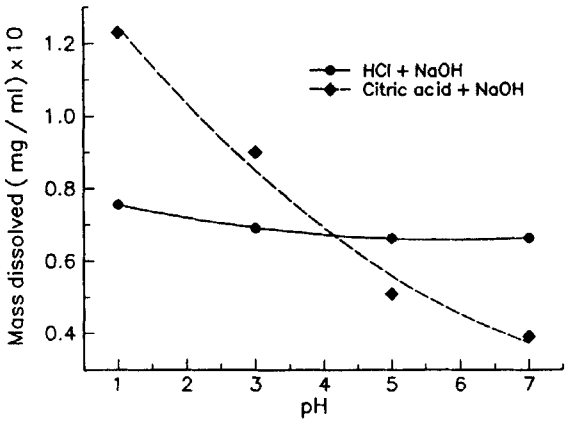

Figure 5. Solubility of thiacetazone as a function of the pH of aqueous mineral and organic acid buffer solutions at 37°C.

Attempts were made to use energy minimization schemes to explore the possible intermolecular forces acting in aqueous solutions (11). A molecular modeler was used to calculate the minimum energy involved in isoniazid–isoniazid dimerization and isoniazid–thiacetazone complexation. The calculated minimum energy for the former gave close values and was 558 kJ/mol and 588 kJ/mol for the latter. This calculated minimum energy is based on arrangement in vacuum. The introduction of water may favor the complexation between isoniazid and thiacetazone due to the hydrophobicity of the latter.

In conclusion, the extent of INH association in aqueous solutions does not justify solubilization by aggrega-

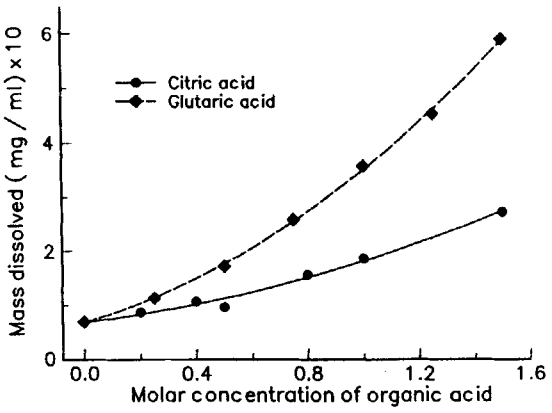

Figure 6. Solubility of thiacetazone as a function of citric and glutaric acid concentrations at 37°C.

tion; thus complex formation may be proposed for the solubility enhancement.

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