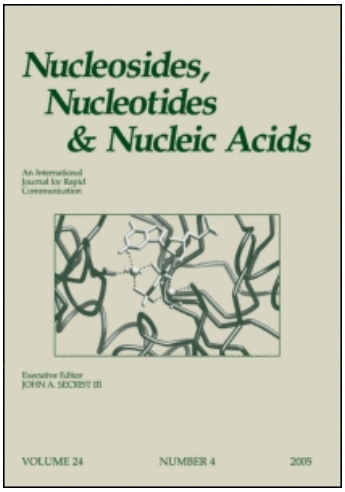

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3′-AZIDO-3′-DEOXY-5′-O-ISONICOTINOYLTHYMIDINE: A NOVEL ANTIRETROVIRAL ANALOG OF ZIDOVUDINE. III. SOLUBILITY STUDIES

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□ *The pH-solubility behavior and solubility of 3′-azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso), an antiretroviral derivative of zidovudine with important biological activity, was studied in water, ethanol, ethanol: water, and n-octanol. The N-pyridine pKa value was determined from its pH-solubility profile, which was in accordance with that of the experimental value of methyl isonicotinate. Also, the ethanol cosolvency in ethanol:water mixtures at 25° C was studied, and log-linear and nonlinear solubilization models were applied to the experimental solubility AZT-Iso data, which allowed us to predict its solubility in those solvent mixtures at a determined content of cosolvent.*

Keywords Water solubility; Cosolvency; Log-linear and nonlinear solubilization models

INTRODUCTION

The solubility of a drug molecule in water is of relevant importance in the process of drug discovery and development of pharmaceutical formulation and biopharmacy. Aqueous solubility has long been recognized as a key factor in controlling drug efficacy. Before an orally administered drug can become available to its receptor, it must first dissolve in the gastrointestinal fluid. Both the dissolution rate and the maximum amount of drug that can be dissolved are governed by the solubility of the drug in the medium;[1–3] therefore, the bases for reliable formulations development are the accurate determination of the solubility. Not surprisingly, a variety

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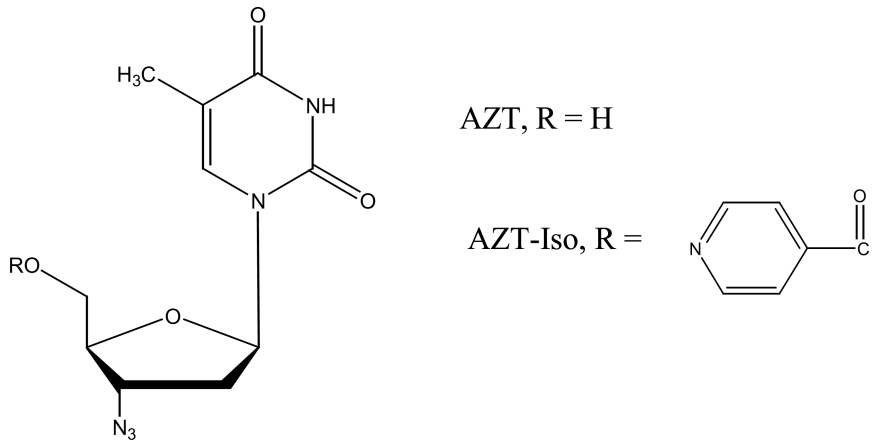

FIGURE 1 Chemical structure of 3′-azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso).

of solubilization techniques have been studied and widely used including direct determination, pH-adjustment, cosolvent addition, and phase-solubility diagram determinations.[2–7]

3′-Azido-3′-deoxy-5′-O-isonicotinoylthymidine, AZT-Iso (Figure 1) an analog of zidovudine, has demonstrated to exhibit important biological antiretroviral and antibacterial activity,[8,9] as well as suitable lipophilic, albumin binding, conformational, and stability properties.[8,10–13]

Taking into account the interesting biological and physicochemical properties of AZT-Iso, the purpose of the present work is to determine water solubility at different temperatures, as well as the solubility in ethanol, ethanol:water, and n-octanol solubility, and its pH-solubility profile. Furthermore, the ethanol cosolvency in ethanol:water mixtures was studied, and log-linear and nonlinear solubilization models were applied to the experimental solubility AZT-Iso data.

RESULTS AND DISCUSSION

Water, Ethanol, and n-Octanol Solubility

The traditional equilibrium method[3,7,14] to determine AZT-Iso solubility in water (Sw), ethanol (Seth), and n-octanol (Soct) at $25 \pm 0.1^{\circ}C$ was carried out (Sw = 0.6857 ± 0.0414, Seth = 14.9968 ± 0.0395, Soct = 2.3269 ± 0.0070 mg/mL), showing that ethanol is a better solvent than water and n-octanol. Thus, AZT-Iso is 6.5 times more soluble in ethanol than in n-octanol, and 22.4 times than in water. In addition, water and ethanol solubility at $37 \pm 0.1^{\circ}C$ were also determined (Sw = 1.0506 ± 0.0058, Seth = 23.6254 ± 0.0206 mg/mL).

Since pharmaceutical products may be subject to wide temperature variations at the stored conditions, the aqueous solubility of AZT-Iso was also analyzed at 8°C (Sw = 0.2901 ± 0.0221 mg/mL), showing that this compound is 1.53 times more soluble at 37°C than at 25°C, and 3.62 times more soluble than at 8°C, increasing its solubility in about 1.3 times each 10°C.

It was reported that the polarity and hydrophobicity of alcohols are important factors governing the solubility of drugs as well as the ability of the solvents to form hydrogen bonds with the heteroatoms in the drugs.[15] In this way, the solubility of AZT-Iso increases with the polarity of the alcohols (dielectric constant for ethanol: 24.30; n-octanol: 9.72);[15a] while it does not increase with their hydrophobicity (log Po/w ethanol: −0.31; [15b–d] log Po/w n-octanol: 2.737[15c,d]) since solubility was maximum in ethanol and decreased with an increase in the chain length of alcohol. The obtained results indicate that since AZT-Iso is some what polar (log Po/w = 0.826)[10] it will be more soluble in ethanol than octanol because ethanol is more polar than octanol. As the alkyl chain length in alcohols increases, their ability to form hydrogen bonds with the drug molecules decreases; hence, the solubility decreases.

The greater solubility of AZT-Iso in ethanol than in octanol suggests that the solubility is principally governed by the intermolecular interactions among the solvent molecules. This means that the energy involved in both, the creation of a hole in the solvent to accept the molecule solute and the solute molecule placed in the hole, is greater when the solvent is octanol than in ethanol.

As it can be seen, AZT-Iso exhibits poor water solubility because this drug, being predominantly a non-polar molecule, cannot effectively break into the lattice structure of water; hence water solubility is low. In view of this feature, the aqueous solubility could be enhanced significantly by using ethanol as the second solvent.

Experimental Aqueous Solubility Values from Different Methodologies

The intrinsic solubility of AZT-Iso at 25°C obtained by a direct determination in aqueous suspensions was found to be Sw = 0.6857 ± 0.0414 mg/mL (log Sw = −2.7348, as log molar aqueous solubility). The equilibrium solubility was achieved at approximately one hour and did not change for 24 h. This also suggests good stability of AZT-Iso at this temperature for 24 h, as we previously reported.[11] In addition, from the phase-solubility diagram,[2] the intrinsic solubility of AZT-Iso was Sw = 0.6530 ± 0.0067 mg/mL (log Sw = −2.7560).

The pH-dependence of solubility will have useful applications in the design of liquid and parenteral formulation of drugs. Thus, the aqueous

TABLE 1 pH-Solubility Profile of AZT-Iso at 25°C

Solubility (mg/mL)	pH
5.8190 ± 0.1626	1.68
2.6686 ± 0.1335	2.06
0.6017 ± 0.0340	4.20
0.7114 ± 0.0356	5.9
0.6857 ± 0.0414	6.6 (H_2O)
0.5705 ± 0.0286	6.7
0.5896 ± 0.0295	7.1
0.8420 ± 0.0421	8.0
0.8758 ± 0.0438	8.36

solubility of AZT-Iso as a function of pH between 1.68 and 8.36 was also determined by reporting the average of duplicate determinations (Table 1).

Under these pH conditions and over the time frame of these studies, AZT-Iso was also shown to be completely stable, as evidenced by the absence of degradation products by thin-layer chromatography (TLC). However, solubility over pH 8.36 was not determined due to instability of AZT-Iso at the studied conditions.

From Table 1 and the pH-solubility profile (Figure 2), the aqueous solubility of AZT-Iso at 25°C showed significant decreases with increases in the pH value, in accordance with Eq. (1) [3]

$$S_T = Sw(1 + Kb[H^+]/[Kw]) = Sw[1 + \text{antilog}(pKa - pH)] \quad (1)$$

where S_T is the buffer aqueous solubility, Sw is the intrinsic aqueous solubility, and Kb is the base dissociation constant of AZT-Iso.

One of the major factors responsible for dissolution of an organic compound is its ability to dissociate into ionic species, which in turn depends on the pH of the medium. As AZT-Iso contain ionizable groups, the percentage of drug ionized and thus the solubility increase with a decrease in the pH

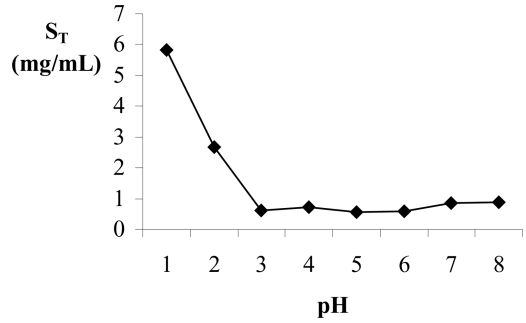

FIGURE 2 pH-Solubility profile of AZT-Iso at 25°C. S_T: is the buffer aqueous solubility.

TABLE 2 Experimental Log Molar Aqueous Solubility (Sw) Values of AZT-Iso Obtained by Different Methodologies

Methods	Log Sw
Direct determination in an aqueous suspension (traditional)	−2.7348
Phase-solubility diagram	−2.7560
pH-solubility profile	−2.7369

value. When the pH of AZT-Iso solution decreased from 4.20 to 1.68 (by about 2.5 units), the solubility increased about 9.7 times.

According to pH-solubility profile, the following considerations were taken into account: First, the weak base character of pyridine ring was confirmed since solubility increased as pH decreased (pH's 1–3), remaining approximately constant until pH 8.36. Secondly, the intrinsic aqueous solubility was estimated from the pH range of 3–8.36 yielding a Sw = 0.6830 ± 0.0461 mg/mL (log Sw = −2.7369), which is in accordance with those of aqueous suspension and phase-solubility diagram (Table 2).

Determination of pKa from Solubility Data

From Eq. (1), S_T vs $[H^+]$ was plotted (Figure 3), and the pKa of the N-pyridine group of AZT-Iso was calculated (pKa = 2.56), where the intercept is the intrinsic solubility (Sw) and the slope is the Sw/Ka value. This experimental value correlates well with the experimental one of methyl isonicotinate (pKa = 3.26), which has a methyl ester group on the *para*-position of pyridine ring.[16] This was a useful method for pKa determination since the potentiometric one does not have enough sensibility.

Solubilization by Cosolvent

Cosolvency, the addition of water miscible solvents to an aqueous system, is often considered at early stages due to its huge solubilization potential,

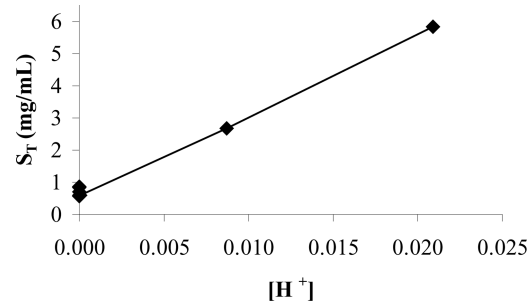

FIGURE 3 pKa of the N-pyridine group of AZT-Iso from solubility data. S_T: is the buffer aqueous solubility.

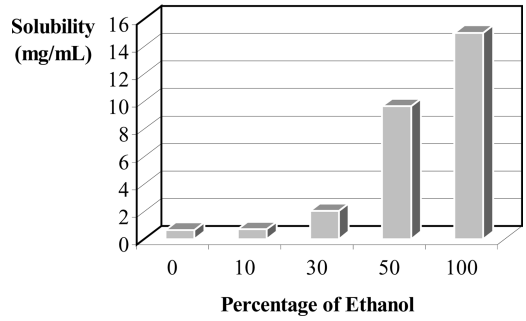

FIGURE 4 Solubilization by cosolvent (solubility in ethanol/water mixtures).

since it is one of the oldest and most effective methods used to prepare liquid dosage forms of poorly soluble drugs.[4,5,17–19]

Cosolvents form homogeneous solutions with water and act as new solvents with different polarities between that of water and the pure cosolvents. In this way, due to the major ethanol solubility of AZT-Iso in the assayed solvents, and taking into account that ethanol is a usual solvent in the pharmacy field, the influence of this solvent in a mixture of ethanol-water as the modifier change was studied. Figure 4 shows the experimental aqueous solubility of AZT-Iso versus the concentration of ethanol. As it can be seen, the drug solubility increases exponentially as the ethanol concentration increases.

Yalkowsky and Roseman[4] proposed the log-linear model (Eq. (2)), which describes the phenomenon of the exponential increase of the aqueous solubility for non-polar organic compound as the cosolvent concentration is increased,[4,5,17]

$$\text{Log Sm} = \log \text{Sw} + f\sigma \tag{2}$$

where f is the volume fraction of cosolvent in the solute-free solvent mixture; Sm, Sc, and Sw are the solubility of solute in water-cosolvent mixture, in pure cosolvent, and in pure water, respectively; and σ is the cosolvent solubilization power for a particular solute-cosolvent system, which value is experimentally obtained from the slope of a plot log Sm vs f. The importance of Eq. (2) is that of σ is not related to the crystalline structure of the solute; therefore, this predictive method does not require melting temperature and melting enthalpy energy values. Thus, the solubility data for AZT-Iso, employing ethanol as cosolvent, is shown in Table 3.

When the difference log Sm − log Sw of AZT-Iso (Eq. (2)) was plotted against f, a sigmoidal plot was obtained as shown in Figure 5.

The sigmoidal profile of Figure 5 could be indicating a non-ideality of the solvent mixture in the AZT-Iso solubilization. Taking into account that the equilibrium dissociation of a drug in a binary solvent mixture is controlled by solvent-solvent, solvent-solute, and solute-solute interactions, the deviation

TABLE 3 Solubility of AZT-Iso Using Ethanol as Cosolvent

Log Sm	Log Sm − log Sw [a]	Volume fraction of ethanol
—	0.0000	0.00
−2.7151	0.0278	0.10
−2.2596	0.4833	0.30
−1.5858	1.1571	0.50
−1.3949	1.3480	1.00

[a] Average of experimental values, log Sw = −2.7430.

from the log-linear model could be better controlled by water:ethanol interactions than for solute-solvent ones. It is in accordance with previously reported data, where σ estimation depends on the lipophilicity of the studied compound (log Po/w) in the water:cosolvent system, since σ is described as a microscopic partition coefficient if water and cosolvent act as two independent entities.[17]

The relationship between solubilization and ethanol content for AZT-Iso is linear between 10 and 50% of cosolvent. To explain the deviation at low cosolvent concentrations, Banerjee and Yalkowsky[20] proposed that this phenomenon is attributed to the structuring of water induced by low cosolvent concentration, describing this type of interaction as "hydrophobic hydration."

It would be expected that this phenomenon prevails at relatively low concentrations of cosolvent, where an excess of water molecules are present, since this phenomenon depends on the ability of water molecules to form cohesive bonds with other water molecules. Thus, the polar groups of cosolvent molecules lead to interactions with water of the hydrogen-bonding type. It has been suggested that low concentrations of relatively polar cosolvent, such as short-chain alcohols may enhance water structuring through hydrogen bonding interactions in addition to the hydrophobic effect.[17] At high cosolvent fraction, hydrogen bond also takes place losing the significant water structuring.

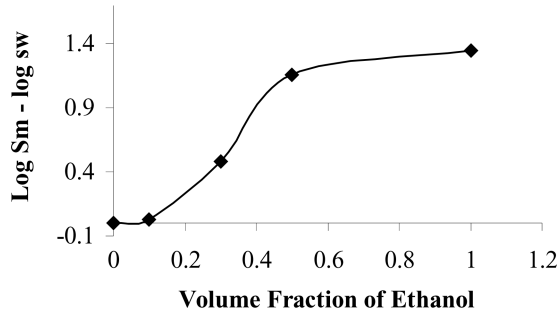

FIGURE 5 Solubility in water-ethanol systems.

In addition, when f leads to the unity, solubility of AZT-Iso does not increase as expected according to linear equation (Eq. (2)). On the contrary, the slope value (σ) is lower giving a subestimated solubility value in pure ethanol ($f = 1$) related to mixed one ($f < 1$).

On the other hand, Eq. (3) developed by Li and Yalkowsky[17] applying the log-linear model for water:ethanol systems shows the dependency of the water:ethanol solubility, with the log Po/w. Thus, the solubility of AZT-Iso in water:ethanol mixture at $f = 0.10$ and 25°C, using the average of experimental intrinsic solubility values, log Sw = −2.7430 and the log Po/w obtained from shake flask method (log P = 0.826),[10] gives a log Sm (calc) = −2.5503, showing increased values of 1.06-fold compared with the experimental one [log Sm (exp) = −2.7151].

$$\text{Log Sm} - \log \text{Sw} = f(1.274 + 0.791 \log \text{P}) \tag{3}$$

According to our results, the solubilization plots are linear near to $f = 0.5$ with a parabolic functions above this value. For this reason, Eq. (3) describes the log Sm − log Sw dependency with a slope of $\sigma_{0,5}$. In this way, it is possible to estimate the solubility of a drug in water-cosolvent mixtures using its experimental intrinsic water solubility (log Sw) and the log Po/w values, and thus to evaluate the predictive capacity of Eq. (3).

Millard et al.[19] have developed constants for the log-linear cosolvent models for the most common pharmaceutical cosolvents. The solubilization power (σ) of each cosolvent was determined for a large number of organic compounds from the slope of their log Sm vs. f plots. The linear relationship for σ is shown in Eq. (4)

$$\sigma = s \log \text{Po/w} + t \tag{4}$$

where s and t are cosolvent constants which are solute independent and Log Po/w is the partition coefficient of the solute of interest. The parameters s and t are the linear regression terms for slope and intercept respectively, obtained from data sets of solubilization power versus solute polarity for each cosolvent. Substitution of σ value from Eq. (4) into Eq. (2) leads to Eq. (5).

$$\log \text{Sm} = \log \text{Sw} + (s \log \text{Po/w} + t) f \tag{5}$$

In this way, knowing s and t for ethanol (0.93 and 0.40, respectively),[19] and log Sw and log Po/w[10] for AZT-Iso, it is possible to predict its total solubility in mixed ethanol-water systems, in terms of the properties of the pure components; without its experimental solubility data in cosolvents mixtures leading to great advantages related to cost and time.

As it can be seen from Eq. (5), a linear relationship is present between solubilization power (σ) and the partition coefficient in n-octanol:water, based

on the previous description of σ as a microscopic partition coefficient.[17] Although partition coefficients in different systems have linear relationship, Eq. (5) only describes linear behavior and it does not have good applicability to nonlinear behavior in solutes such as AZT-Iso.

In addition, Machatha et al.[21] have described Eq. (6), applying both linear and parabolic logarithm models,[22] for cosolvent solubilization.

$$\log \mathrm{Sm} = \log \mathrm{Sw} + (af)/(1 + bf + c\,f^2) \tag{6}$$

where the constant a is the initial slope with a value of $\sigma_{0,5}$; and those of b and c are a characteristic of the interaction solute-solvent changes produced by the cosolvent concentration increase, where b affects the maximum solute solubility, c affects the end slope, and f is near to the unity. When f is small, Eq. (6) leads to logarithm linear model described for Eq. (3). Equation (7) was obtained applying experimental data to Eq. (6).

$$\mathrm{Log\,Sm} = -2.7752 + (0.8374\,f)/(1 - 2.2025\,f + 1.8095\,f^2) \tag{7}$$
$$\mathrm{r} = 0.9990; \mathrm{r}^2 = 0.9979; \mathrm{F} = 158.74; \mathrm{n} = 5; \mathrm{s} = 0.0574$$

On the other hand, the experimental data were applied to the third-order polynomial model (Eq. (8)),[21] arising to Eq. (9)

$$\log \mathrm{Sm} = \log \mathrm{Sw} + a'f + b'f^2 + c'f^3 \tag{8}$$
$$\mathrm{Log\,Sm} = -2.7432 - 0.6136\,f + 9.7524\,f^2 - 7.7905\,f^3 \tag{9}$$
$$\mathrm{r} = 0.9999; \quad \mathrm{r}^2 = 0.9999; \quad \mathrm{F} = 2484222.80; \quad \mathrm{n} = 5; \quad \mathrm{s} = 0.0005$$

These last two models have the advantage to a major relationship with nonlinear systems, but the disadvantage of a little predictive capacity since opposite to partition coefficient model, it is necessary to determine each equation coefficient, which is specific for each compound.

Table 4 shows the obtained log Sm values using the five models described above to analyze the equation that gives the better correlation to predict the solubility in water-cosolvent mixtures.

From Table 4 it is possible to conclude that no major differences were observed in the log Sm values obtained from the experimental one ($\log \mathrm{Sm} = -2.7151$) and those calculated with Eqs. (2), (3), (5), (6), and (8). However, Eq. (2) presents the highest values of root mean square errors (RMSE) and average absolute error (AAE) (0.5765 and 0.3414, respectively), meaning that this equation has been associated to a high degree of uncertainty, since only a linear portion of the graph was employed (between 10 and 50% of ethanol).

TABLE 4 Comparison of Log Sm Values ($f = 0.10$), Obtained with Different Predictive Models

Eqs	Log Sw	Log Sm[d]	Δ[e]	RMSE[f]	AAE[g]
2	−3.0592	−2.7681	0.053	0.5765	0.3414
3[a]	−2.7430[b]	−2.5503	−0.1648	0.2857	0.2063
5[a]	−2.7430[b]	−2.6262	−0.0889	0.2780	0.1940
6	−2.7752[c]	−2.6702	−0.0449	0.0257	0.0192
8	−2.7432[c]	−2.7148	−0.0004	0.00021	0.00016

[a]Log Po/w = 0.826, obtained from the shake flask method.[10]

[b]Mean value obtained from traditional, phase-solubility diagram and pH-solubility profile methods.

[c]Value obtained from Eqs. (6) and (8), with a ΔLog Sw = −0.0322 and ΔLog Sw = −0.0002, respectively.

[d] $\text{Log Sm}_{exp} = -2.7151$.

[e] $(\text{Log Sm}_{exp} - \text{Log Sm}_{calc}) = \Delta$.

[f]RMSE = Root mean square errors.

[g]AAE = Average absolute error.

On the other hand, a significant difference was observed when water solubility (log Sw) represented by intercept was estimated from Eq. (2) (average of experimental values, log Sw = −2.7430). In this way, the polynomial method gave the most approximated values, and for this reason, Eq. (8) would be the best to describe the region where the percentage of ethanol is the lowest value, and the Sm is closer to the Sw value. This fact shows that the difference between the observed and experimental values for log Sm at 10% of ethanol is lower in cubic equation (Eq. (8)) than in the other ones. Taking into account that the major utility of the cosolvency in pharmacy is at low percentages of ethanol, the polynomial model is the most appropriate for predicting the solubility of AZT-Iso in a mixture of water:ethanol.

From the result obtained in this study, it is clear that although AZT-Iso have poor aqueous solubility, it could be significantly enhanced by using a pure ethanol and mixed water:ethanol, since ethanol is found to be good solvent for this drug, and it is generally considered safe for oral administration.[23–25]

EXPERIMENTAL SECTION

Materials

3′-Azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso) was prepared as previously described.[8] All other materials and solvents were of analytical reagent grade. Water reagent grade was generated by a Millipore Milli-Q water purification system. Buffer solutions consisted of suitable mixture of analytical grade chloride acid, potassium chloride, citric acid, sodium hydrogen phosphate, sodium bicarbonate, and sodium hydroxide. Ionic strengths of buffer were calculated and adjusted to 0.5 by the addition of potassium

chloride.[26] Thin-layer chromatography (TLC) (silica gel 60 F_{254}, 20 × 20) purchased from Merck (Darmstadt, Germany) was used.

Solubility Studies

Equilibrium solubility of AZT-Iso was determined in a constant temperature bath by adding an excess of the solid to 4 mL of aqueous buffer (or water or ethanol or n-octanol) in a series of 5 mL vials, under constant shaking. After equilibration, an aliquot was filtered through a 0.45-μm membrane filter, and then the concentration of drug was determined by ultraviolet absorption spectroscopy after appropriate dilution with the selected solvents (Shimadzu UV-160 A). We report the average of duplicate determinations.

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