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COMMUNICATION

Effect of Hydroxypropyl-β-cyclodextrin on the Solubility of an Antibacterial Isoxazolyl-naphthoquinone

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ABSTRACT

*The complexation of 2-hydroxy-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**I**) with a highly soluble cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD) was studied in aqueous media by solubility methods. **I** is an antibacterial and trypanocidal agent that is undergoing preclinical testing. Unfortunately, **I** exhibits low water solubility, and it is therefore difficult to prepare the solutions for biological tests. **I** inclusion took place with 1:1 stoichiometry. The stability constants of the **I** complexes calculated from the slope and the intercept of the phase solubility diagrams are larger in the less ionized form, whereas greater overall solubility is obtained in basic media.*

Key Words: *Complexation; Hydroxypropyl-β-cyclodextrin; Naphthoquinones; Solubility.*

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides known to form inclusion complexes with many lipophilic drugs, thus changing their physicochemical and biopharmaceutical properties. Complexation with cyclodextrins has been widely used to improve the solubility of poorly aqueous soluble drugs (1,2). As natural cyclodextrins show low aqueous solubility and toxic effects when used in parenteral applications, many efforts have been directed to the development of new cyclodextrin derivatives with better properties. Among the derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) is highly water soluble and has very low toxicity (3,4).

2-Hydroxy-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**I**) is an antibacterial and trypanocidal agent (5–8) that is undergoing preclinical testing. Unfortunately, **I** exhibits an extremely low solubility in aqueous solutions of about 1.70 μg/ml, which is below the desired concentration (5–50 mg/ml) needed for parenteral solution formulations to be used in early clinical studies.

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Therefore, it appears worthwhile to evaluate the feasibility of using HP-β-CD to improve the aqueous solubility of the naphthoquinone derivative **I**. For that purpose, we have analyzed the characteristics of the inclusion complexes formed and the effect of pH on the solubility.

EXPERIMENTAL

Materials

The synthesis and identification procedures for **I** have been described previously (9). HP-β-CD (MW 1326–1400; degree of molar substitution 7.0) was a gift from Cerestar USA, Incorporated (Hammond, IN). All other materials and solvents were analytical reagent grade.

Buffers

McIlvaine buffers (pH 3.00–8.00) were prepared as described in the literature (10). A KH_2PO_4/Na_2HPO_4 buffer was used at pH 7.40. The water used for the buffers was generated by a Millipore Milli-Q water purification system (Milton, MA).

Solubility Studies

Phase solubility studies were carried out as described by Higuchi and Connors (11). Excess amounts of **I** (≈5.00 mg) were added to the buffer solutions at different pH values, which varied from 3.50 to 8.00, and that contained different CD concentrations (from 0.0% to 53.0% w/v). The suspensions formed were sonicated in an ultrasonic bath for 1 hr and then placed in a 25.0° ± 0.1°C constant-temperature water bath. After equilibration up to 72 hr, an aliquot was filtered through 0.45-μm polyvinylidene difluoride membranes (Micron Separations, Inc., Sao Paulo, Brazil), the equilibrium pH of each solution was measured (Orion SA520 pH-meter, Boston, MA), suitably diluted, and analyzed by UV analysis. The data represent the mean value of two replicate analyses.

Analytical Methods

For the phase solubility profile, UV analysis was performed. **I** was analyzed using a Shimadzu UV 260 UV/Visible spectrophotometer. Standard curves were prepared using water as the solvent, and they were linear ($r > .9999$) over the range of concentrations of interest.

RESULTS AND DISCUSSION

One potential drug candidate derived from naphthoquinone was examined in this study. This derivative is relatively lipophilic, with a calculated log *P* value of 3.37 (12). Such high lipophilicity hinders the water solubility of the compound, which was determined to be $1.74\ 10^{-3}$ mg/ml.

The pH dependence of the complexation of the naphthoquinone derivative with HP-β-CD was investigated on the basis of solubility/pH profiles. Figure 1 shows the solubility profile of **I** in the absence and presence of 13.3% and 53.0% HP-β-CD. As can be seen, the solubility of the weak acid **I** underwent a considerable increase by the combined use of pH adjustment and complexation with HP-β-CD. An increase in solubility with increasing pH has been reported for other naphthoquinone derivatives (13). The degree of dissociation thus has a decisive influence on the complexability, and hence on the solubility, of ionic guest molecules. Owing to the pH dependence of the complexing abilities of HP-β-CD with ionic drug molecules, the amount solubilized can be optimized.

The phase solubility diagrams of **I** in aqueous HP-β-CD solutions and various pH values at 25°C are shown in Fig. 2. At pH values below 6.50, the curves obtained were of the A_L type; however, at pH values above 6.50, the solubility isotherms showed negative curvature and could be classified as those of the A_N type. The pK_a for **I** is 5.37 (14), that is, the drug is almost fully ionized at pH 6.00. The degree of ionization of the drug in solution conforms to the type of solubility curve obtained. The neutral drug gave an A_L diagram, while the ionized drug resulted in an A_N diagram. However, measurement of the pH values in the filtrates of all the solutions showed a little increase in the pH values of the acidic buffers, the

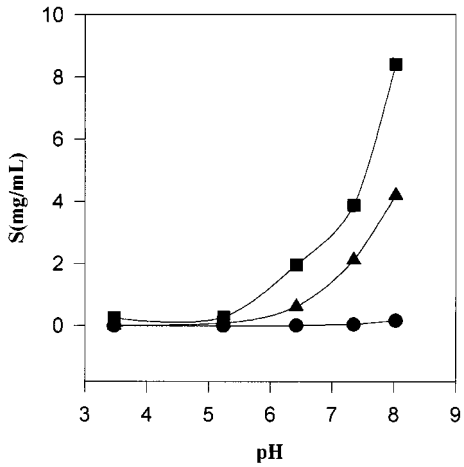

Figure 1. Solubility of **I** versus pH in aqueous solutions at different HP-β-CD concentrations. ●, 0.00%; ▲, 13.3%; ■, 53.3%.

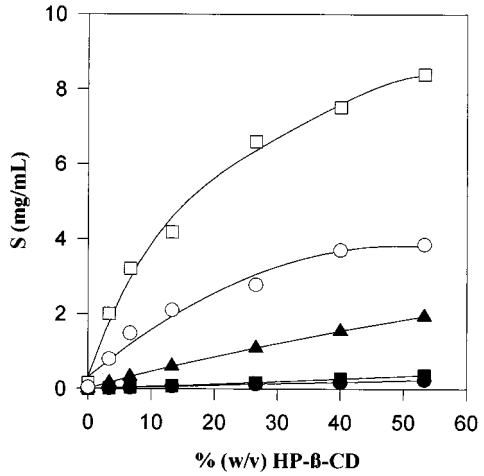

Figure 2. Phase solubility diagrams of **I** at 25°C at different pH values. ●, pH 3.52; ■, pH 5.11; ▲, pH 6.42; ○, pH 7.40; □, pH 8.03.

pH of the buffer (6.42) is not altered, and the basic buffers showed pH changes up to 0.9 units toward the acid side with the increase of the HP-β-CD percentage in solution. The **I** concentration noticed on the ordinate is the addition of the saturation solubility and that part complexed by the HP-β-CD. When a decrease takes place in the pH of the solution with increasing HP-β-CD concentration, it will be a decrease of the saturation concentration of the drug due to the opposing influence of the dissociation constant of the acid drug. Hence, the total amount of the naphthoquinone in solution cannot increase linearly. To

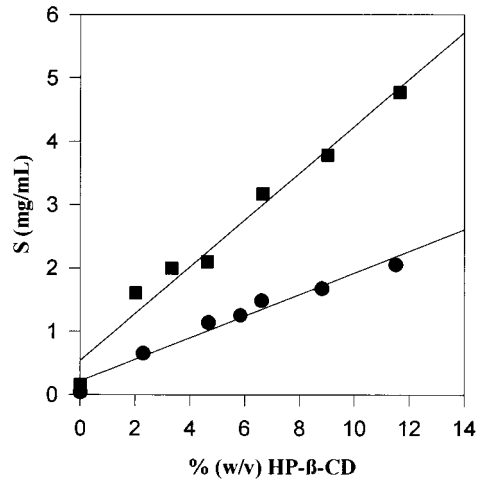

Figure 3. Phase solubility diagrams of **I** in basic buffers up to 13.3% HP-β-CD. ●, pH 7.35; ■, pH 8.03.

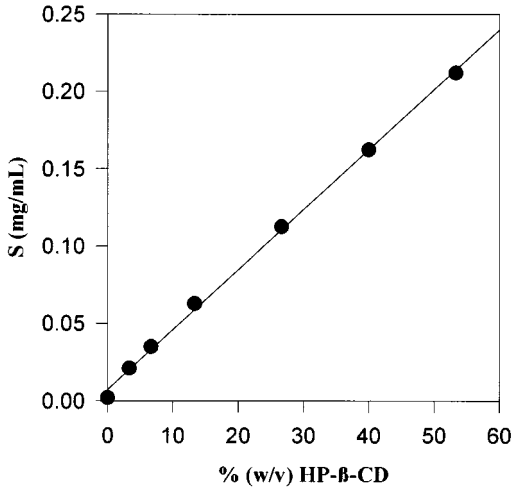

Figure 4. Phase solubility diagram of **I** in water.

corroborate if this A_N-type behavior is due to pH changes of the solution at high HP-β-CD concentration, we investigated the solubility of **I** in alkaline buffers at CD concentrations up to 13.3%. The results are shown in Fig. 3. The pH changes are very low, and the phase solubility behavior changes to the A_L type. In addition, the fact that the apparently exponential behavior of the isotherms is due to the pH effects when the buffer capacity has been exceeded is clearly demonstrated in Figs. 4 and 5. Strictly linear isotherms are obtained for **I** and HP-β-CD in pure water and in a more concentrated buffer solution (0.8 M) of pH 7.43.

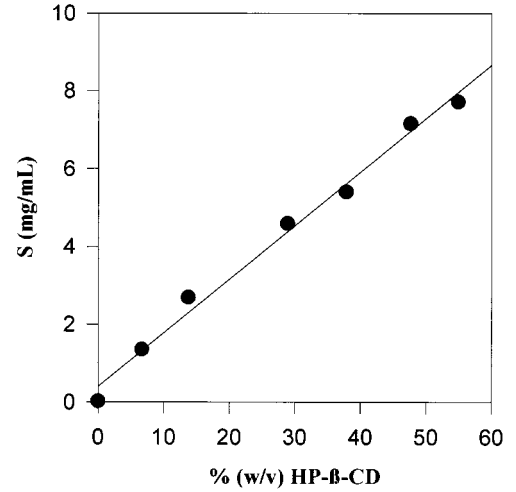

Figure 5. Phase solubility diagram of **I** in concentrated basic buffers.

Table 1

*Data of the Phase Solubility Isotherms of **I** with HP-β-CD at Different pH Values*

pH	S_0 (M)	$K_{1:1}$ (M^{-1})	Type of Curve
3.52[a]	1.9×10^{-6}	2.4×10^{3}	A_L
5.11[a]	3.2×10^{-6}	2.1×10^{3}	A_L
6.48[b]	6.5×10^{-6}	6.0×10^{2}	A_L
6.42[a]	2.7×10^{-5}	1.8×10^{3}	A_N
7.40[a]	1.5×10^{-4}	1.5×10^{3}	A_N
7.41[c]	2.5×10^{-4}	9.8×10^{2}	A_N
8.03[a]	5.8×10^{-4}	1.0×10^{3}	A_N

[a] Citrate/phosphate buffer.
[b] Water.
[c] Phosphate/phosphate buffer.

The intrinsic solubility, the type of diagram, and the apparent complex constants $K_{1:1}$ are summarized in Table 1. The stability constants $K_{1:1}$ of the **I** complexes calculated from the slope and the intercept of the phase solubility diagrams are dependent on the degree of ionization. The value of the constant between **I** and HP-β-CD at pH 3.52 is 2.4×10^3 M^{-1}, while that at pH 8.03 is 1.0×10^3 M^{-1}. These results are in agreement with the general observation that ionized species are weak complex agents as far as cyclodextrins are concerned (13,15–17).

The influence of the buffer ion on the stability constant of the complex formed was also observed. In Table 1, it is pointed out that $K_{1:1}$ was found to be highest in a citrate-phosphate buffer (1.5×10^3 M^{-1}) and was considerably reduced in systems containing only phosphate (9.8×10^2 M^{-1}).

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