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Solubilization of naphthoquinones by complexation with hydroxypropyl-β-cyclodextrin

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Abstract

The effects of the hydroxypropyl-β-cyclodextrin (HPCD) on the solubility of 2-hydroxy-*N*-(5-methyl-3-isoxazolyl)-1,4-naphthoquinone-4-imine (**I**) were investigated. **I** is an experimental drug for the treatment of cancer which exhibits low water solubility and it is therefore difficult to prepare the solutions for biological tests. The presence of an ionizable hydroxyl moiety ($pK_a = 5.80$) increases the solubility via pH adjustment, but only a solubility of 0.124 mg/ml was obtained at pH 8.00. **I** was found to form inclusion complexes in either its neutral or its anionic form with HPCD. Although the stability constant of the **I** complex is larger in the neutral form, a greater overall solubility is obtained when **I** is in its ionized form. A 270-fold solubility enhancement is possible by using a combined approach of pH adjustment and complexation with HPCD. © 1997 Elsevier Science B.V.

Keywords: Charge effects; Hydroxypropyl-β-cyclodextrin; Inclusion complexation; Isoxazolylnaphthoquinoneimine; 1,2-Naphthoquinone; Solubility study

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α-CD), seven (β-CD) or eight (γ-CD), α-1,4-linked glucopyranose units, with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity in the centre. CDs are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or some part of it, into the cavity (Loftsson et al., 1991; Szejtli, 1994). Such molecular encapsulation will affect many of the physicochemical properties of the drugs, such as their aqueous solubility and stability. No covalent bonds are formed or broken during the drug–CD complex formation, and under physiological conditions the complex is readily dissociated and the free drug molecules are in a rapid equilibrium with the drug molecules bound within the CD cavity. The alkylated and

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hydroxyalkylated CDs appear to be more suitable for the formulation of solutions of poorly soluble drugs than the non-substituted parent CD, because of their increased aqueous solubility, lack of toxicity and ability to alter the phase solubility behaviour in favour of isotherms of the A-type (Müller and Brauns, 1985; Backensfeld et al., 1988).

The 2-hydroxy-*N*-(5-methyl-3-isoxazolyl)-1,4-naphthoquinone-4-imine (**I**) depicted in Fig. 1 is an experimental agent under consideration for antibacterial and anticancer testings. A difficulty encountered in the preparation of the solutions for biological tests lies in its extremely low solubility in aqueous solutions, at least three to four orders of magnitude below the desired concentration (2 mg/ml). Although the drug is very lipophilic (log $P = 5.86$) (Longhi et al., 1996), solubilization with various cosolvent systems proved inadequate.

The aim of this work was to increase the aqueous solubility of **I** with hydroxypropyl-β-cyclodextrin. In this study, the influence of the pH on the solubility-increasing effect of the CD was considered.

2. Materials and methods

2.1. Materials

The synthesis and identification procedures for **I** have been described previously (Fernández et al., 1982). HP-β-CD (MW = 1326–1400; degree of molar substitution, 7.0) was a gift from American Maize-Products Co. (Hammond, IN). All other materials and solvents were of analytical reagent grade.

2.2. Buffers

McIlvaine buffers (pH 3.00–6.50) were prepared according to literature (Elving et al., 1956); KH_2PO_4/Na_2HPO_4 buffers were used at pH above 6.50. The water used for the buffer was generated by a Millipore Milli-Q Water purification system.

2.3. Solubility studies

Phase solubility studies were carried out as described by Higuchi and Connors (1965). Excess amounts of **I** (≈ 5.00 mg) were added to the solutions with different pH values which varied from 3.50 to 8.00, containing various concentrations of CD ranging from 0.0 to 53.0% (w/v). The suspensions formed were sonicated in an ultrasonic bath for 1 h and placed in a 25.0 ± 0.1°C constant-temperature water bath. After equilibration up to 72 h, an aliquot was filtered through a 0.45 μm membrane filter (Micron Separations, USA), the equilibrium pH of each solution was measured (ORION SA520 pH-meter), suitably diluted, and analyzed by UV.

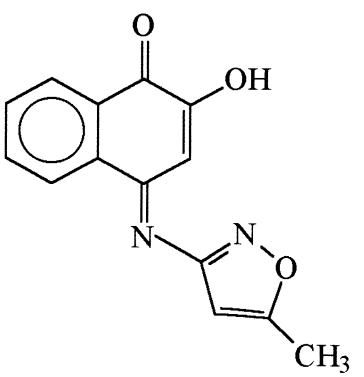
2.4. Analytical methods

The quantitative determinations of **I** were made spectrophotometrically (Shimadzu UV 260 UV/visible spectrophotometer).

3. Results and discussion

3.1. Solubility in various cosolvent systems

Preliminary solubility studies employing a variety of classical cosolvent systems were done in an attempt to identify systems providing a solubility



I

Fig. 1. Structure of 2-hydroxy-*N*-(5-methyl-3-isoxazolyl)-1,4-naphthoquinone-4-imine (**I**).

of at least 2 mg/ml. These solubility data are listed in Table 1. Compound **I** is very lipophilic, with an oil/water partition coefficient of 5.86 (Longhi et al., 1996). However, the highest concentration achievable in a cosolvent system was found to be of only 2.30 mg/ml. Relatively high percentages of dimethyl sulfoxide (DMSO) were required to reach this solubility, and therefore such systems would be physiologically unacceptable for parenteral solutions.

3.2. pH–Solubility behaviour

Fig. 2 illustrates the relationship between pH and **I** solubility at 25°C. A significant increase in solubility was obtained with pH adjustment. However, the higher solubility attained was only 0.124 mg/ml, which is below the desired concentration of 2 mg/ml. The increase in solubility with increasing pH is consistent with Eq. (1), which assumes that the solid phase is the free acid over the entire pH region examined and that ionization of the molecule occurs within this region.

$$S = S_0(1 + K_a/[H^+]) \quad (1)$$

In Eq. (1), S_0 denotes the intrinsic solubility of the substrate and K_a is the dissociation constant.

The solid line in Fig. 2 represents the best fit of the data by nonlinear least-squares regression analysis. This analysis yielded estimates for the intrinsic solubility of 1.42×10^{-1} μg/ml and a pK_a of 5.80, which is approximately the same as that previously obtained by using the spectrophotometric method (Casarosa et al., 1996).

3.3. Solubility by pH adjustment and complexation with hydroxypropyl-β-cyclodextrin (HPCD)

The phase solubility behaviour of **I** with aqueous HPCD was investigated at 25.0 ± 0.1°C. Fig. 3 shows the phase solubility diagrams recorded for pH values of 5.20, 7.35 and 8.00.

The solubility of **I** increased in an approximately linear manner as a function of the HPCD concentration. This would lead to the conclusion that soluble complexes having 1:1 stoichiometry were formed with HPCD, i.e. A_L-type solubility

Table 1
Solubility of **I** in various solvent systems at 25°C

Solvent	Solubility (mg/ml)	*n*
Dimethylsulfoxide (DMSO)		
40% DMSO/60% buffer pH 8.0	1.301	4
50% DMSO/50% buffer pH 8.0	2.113	3
60% DMSO/40% buffer pH 8.0	2.272	3
40% DMSO/60% buffer pH 6.0	0.079	4
50% DMSO/50% buffer ph 6.0	0.358	3
60% DMSO/40% buffer pH 6.0	0.391	3
40% DMSO/60% buffer pH 3.8	0.024	4
50% DMSO/50% buffer pH 3.8	0.040	4
60% DMSO/40% buffer pH 3.8	0.069	4
Ethanol (ETOL)		
40% ETOL/60% buffer pH 8.0	0.293	3
50% ETOL/50% buffer pH 8.0	0.404	3
60% ETOL/60% buffer pH 8.0	0.772	3
40% ETOL/60% buffer pH 6.0	0.115	3
50% ETOL/50% buffer pH 6.0	0.183	3
60% ETOL/40% buffer pH 6.0	0.897	3
40% ETOL/60% buffer pH 3.8	0.017	4
50% ETOL/50% buffer pH 3.8	0.040	4
60% ETOL/40% buffer pH 3.8	0.067	3
Propylene glycol (PG)		
40% PG/60% buffer pH 8.0	0.118	4
50% PG/50% buffer pH 8.0	0.212	3
60% PG/40% buffer pH 8.0	0.245	3
40% PG/60% buffer pH 6.0	0.032	4
50% PG/50% buffer pH 6.0	0.057	3
60% PG/40% buffer pH 6.0	0.083	3
40% PG/60% buffer pH 3.8	0.008	4
50% PG/50% buffer pH 3.8	0.012	3
60% PG/40% buffer pH 3.8	0.024	4
Glycerin (GL)		
40% GL/60% buffer pH 8.0	0.065	3
50% GL/50% buffer pH 8.0	0.078	3
60% GL/40% buffer pH 8.0	0.100	3
40% GL/60% buffer pH 6.0	0.018	3
50% GL/50% buffer pH 6.0	0.021	4
60% GL/40% buffer pH 6.0	0.047	3
40% GL/60% buffer pH 3.8	0.006	4
50% GL/50% buffer pH 3.8	0.007	4
60% GL/40% buffer pH 3.8	0.008	4

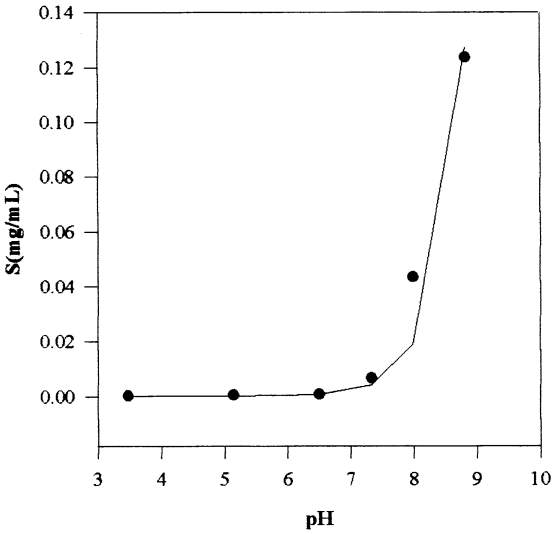


Fig. 2. pH–Solubility profile for **I**.

diagrams according to Higuchi and Connors (1965).

In aqueous solutions, CD complexation of ionized drug molecules can result in much larger total drug solubilization (Müller and Albers, 1992; Pedersen et al., 1993; Tinwalla et al., 1993; Johnson et al., 1994; Okimoto et al., 1996).

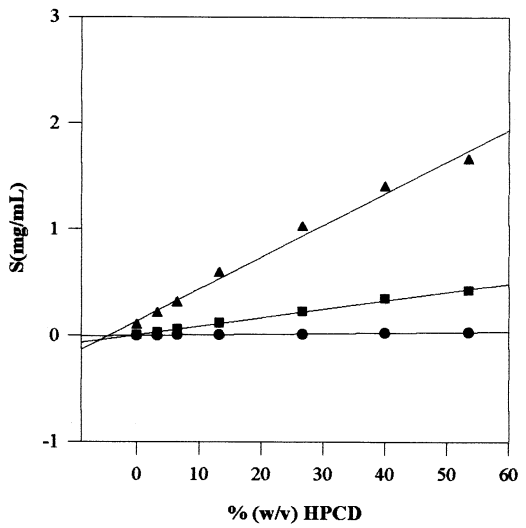


Fig. 3. Phase solubility diagram of **I** at 25°C at different pH values. Key: (●) pH 5.20; (■) pH 7.35; (▲) pH 8.00.

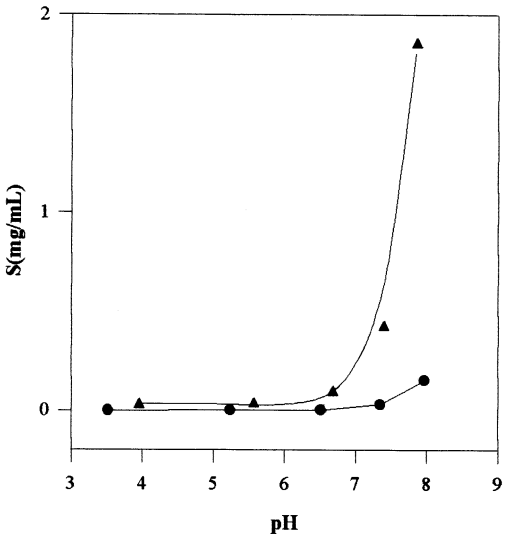
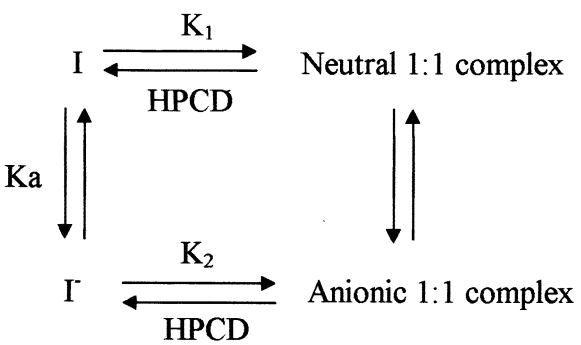


Fig. 4. Solubility of **I** vs. pH in aqueous solutions at different HPCD concentrations. Key: (●) 3.33% HPCD; (▲) 53.3% HPCD.

Fig. 4 displays a plot of **I** solubility versus pH in the presence of 3.30 and 53.3% HPCD. 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 HPCD. A 270-fold increase in solubility was achieved at pH 8.00 and using 53.3% HPCD. However, the higher solubilization obtained was only 1.90 mg/ml.

A mathematical model for 1:1 interaction between the acidic agent **I** and the HPCD can be defined for Scheme 1. This model considers the



Scheme 1. Scheme of interaction between **I** and HPCD.

Table 2
Parameters describing the solubility of **I** in aqueous solutions of HPCD and varying pH

Parameter	Estimate	S.D.
S_0	5.5×10^{-7} M	1.6×10^{-8}
K_a	2.7×10^{-6}	2.2×10^{-7}
K_1	399 M^{-1}	12
K_2	112 M^{-1}	9

ionization of **I** and the fact that both the neutral and the anionic species form 1:1 complexes with HPCD with different binding constants.

In this scheme, K_1 is the binding constant for the neutral compound (**I**) with the HPCD; K_2 is the binding constant for the anion ($\mathbf{I}^-$) with the HPCD; and K_a is the dissociation constant of **I**.

When solid **I** (neutral form) is in excess, the total solubility, S, is given by the following equation:

$$S = [\mathbf{I}] + [\mathbf{I}^-] + [\text{neutral complex}] + [\text{anionic complex}] \quad (2)$$

where

$$[\mathbf{I}] = \text{intrinsic solubility of the acid, } S_0 \text{ in Eq. (1)} \quad (3)$$

$$[\mathbf{I}^-] = \frac{S_0 K_a}{[H^+]} \quad (4)$$

$$[\text{neutral complex}] = K_1 S_0 [\text{HPCD}]_{ef} \quad (5)$$

$$[\text{anionic complex}] = K_2[\mathbf{I}^-][\text{HPCD}]_{ef} = \frac{K_2 S_0 K_a [\text{HPCD}]_{ef}}{[H^+]} \quad (6)$$

$$[\text{HPCD}]_{ef} = \frac{[\text{HPCD}]_{total}}{(1 + K_1 S_0 + K_2 K_a S_0/[H^+])} \quad (7)$$

Eq. (2), which describes the solubility of **I** over a wide range of pH values and concentrations of HPCD complexing agent, was fitted by nonlinear least-squares regression analysis to the solubility data, to obtain the complexation constants and their errors listed in Table 2. The values of S_0 and K_a can be determined from fits to Eq. (1). It is obvious that the magnitude of the stability constant is dependent on the degree of ionization of **I**.

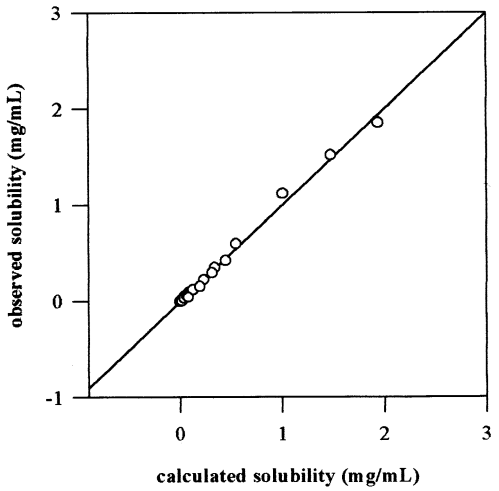


Fig. 5. Calculated vs. observed **I** solubility in aqueous solutions varying in HPCD concentration and pH.

The stability constant, K, of the complexes is 3.5-fold larger in the unionized form.

In Fig. 5, the solubilities calculated according to Eq. (2) are displayed versus the observed results. The good linearity (slope = 1.001 and r^2 = 0.995) prove that the model defined by Eq. (2) closely describes the combined effect of pH adjustment and HPCD concentration on the solubility of **I**.

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