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RESEARCH PAPER

Cyclodextrin Solubilization of ETH-615, a Zwitterionic Drug

Thorsteinn Loftsson* and Dorte Seir Petersen

Department of Pharmacy, University of Iceland, P.O. Box 7210, IS-127 Reykjavik, Iceland

ABSTRACT

Therapeutic usefulness of many zwitterionic drugs is hampered by their very low aqueous solubility. The purpose of this work was to investigate the effects of cyclodextrins on the solubility of the zwitterionic drug ETH-615, the role that charge might play in the cyclodextrin complexation, and the influence of polymers and ion-pairing agents on the cyclodextrin solubilization. The effects of five different β-cyclodextrin derivatives were evaluated, i.e., the anionic β-cyclodextrin sulfobutyl ether sodium salt and carboxymethyl-β-cyclodextrin sodium salt, the uncharged 2-hydroxypropyl-β-cyclodextrin and randomly methylated β-cyclodextrin, and the cationic 2-hydroxy-3-trimethyl-ammoniopropyl-β-cyclodextrin. The uncharged cyclodextrins had much larger solubilizing effect on ETH-615 than the charged ones. However, due to the highly polar zwitterionic structure of ETH-615 the stability constants of its cyclodextrin complexes were several orders of magnitude smaller than those commonly observed for uncharged lipophilic compounds. Cyclodextrin solubilization of ETH-615 was enhanced by water-soluble polymers and ion-pairing agents.

INTRODUCTION

ETH-615 is N-substituted quinolylmethoxyphenylamine which has been shown to be a potent inhibitor of leukotriene biosynthesis with anti-inflammatory and immunodulating activity (1). Unfortunately, its therapeutic application is hampered by its insolubility in water at physiological pH. ETH-615 is an amphoteric drug which forms a zwitterion, i.e., the molecule carries one cation and one anion, at pH between approximately 5 and 9. Although zwitterionic drugs are highly polar, their aqueous solubility is usually very limited at the intermediate pH values. It is possible to enhance the aqueous solubility of a zwitterionic drug by masking one

*To whom correspondence should be addressed.

of the ionizable groups through prodrug formation (2). This allows the remaining ion to be fully effective and, at the same time, the prodrug formation can lower the crystal lattice energy of the solid drug, both of which result in enhanced aqueous solubility. Ampicillin, for example, forms a zwitterion and its solubility has been reported to be about 1 g in 170 ml of water. On the other hand, the solubility of its prodrug pivampicillin, where the anion is masked by a pivaloyloxymethylester moiety, is 1 g in 2 ml of water (3). It is also possible to enhance aqueous solubility of zwitterionic drug, such as tetracyclines, through salt formations. As in the case of prodrugs, salt formation can enhance the apparent solubility of a parent drug by lowering crystal lattice energy (4). However, the situation is more complex than it appears since salts are fully dissociated in aqueous solutions and the aqueous solubility of a zwitterionic drug is pH dependent. Salt formation can therefore lead to faster dissolution of the solid drug without affecting the drug's intrinsic solubility at some given pH.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity in the center. Cyclodextrins are capable of solubilizing many water-insoluble drugs by taking up the whole drug molecule, or some lipophilic part of it, into the cavity (5). The drug molecules inside the cavity are in equilibrium with free molecules out in the solution. However, when an aqueous cyclodextrin solution is saturated with some water-insoluble drug the fraction of free drug is generally very low, i.e., most of the drug molecules in the solution will be physically bound to cyclodextrin molecules. In a sense solubilization through complexation can be regarded as an intermediate between the prodrug method and the salt method. No covalent bonds are formed or broken during the complexation but still it results in physicochemical changes that go beyond the solid state. Thus, solubilization of zwitterionic drugs through cyclodextrin complexation could be an interesting alternative to the two previously mentioned methods. The purpose of this present investigation was to study the effects of cyclodextrins on the aqueous solubility of the zwitterionic ETH-615, and how introduction of charge to the cyclodextrin molecule and addition of polymers and ion-pair agents can affect the solubilization. Also, the effects of an eye-drop formulation on the cyclodextrin solubilization was evaluated.

MATERIALS

ETH-615 was kindly donated by Leo Pharmaceutical Products (Ballerup, Denmark). 2-Hydroxypropyl-β-cyclodextrin DS 0.2 (Encapsin®) (HPβCD) was purchased from American Maize-Products Company (Hammond, IN). β-Cyclodextrin sulfobutyl ether sodium salt (CDSBE) was a gift from CyDex (Kansas City, KS). 2-Hydroxy-3-trimethyl-ammoniopropyl-β-cyclodextrin DS 0.5 (HTMAPCD), carboxymethyl-β-cyclodextrin sodium salt DS 0.6 (CMβCD), and randomly methylated β-cyclodextrin DS 0.6 (MβCD) were a gift fom Wacker-Chemie (Munich, Germany). Hexadimethrine bromide (HDMB) was purchased from Sigma Chemical Co. (St. Louis, MO), and hydroxypropylmethylcellulose 4000 (HPMC) and polyvinyl pyrrolidone MW: 40,000 (PVP) were from Mecobenzon (Copenhagen, Denmark). Benzalkonium chloride was purchased from Sigma, and choline chloride and disodium edetate (Titriplex® III) (EDTA) were from Merck (Darmstadt, Germany). All other chemicals used were commercially available products of special reagent grade.

METHODS

Stability Evaluation

The stability of ETH-615 was evaluated in aqueous buffer solutions containing 10% (w/v) HPβCD at 70°C. Methanolic stock solution (10 μl) was added to 1.5 ml of the aqueous degradation medium which had previously been heated to 70°C, resulting in initial ETH-614 concentration of 1 μg/ml. Changes in drug concentration in the degradation medium were monitored by a high-performance liquid chromatography (HPLC) method and the pH of the medium was determined at the end of each experiment with a pH meter (Corning 240, UK) standardized at 70°C.

Solubility Determinations

An excess amount of ETH 615 was added to aqueous buffer solution (see below) containing various amounts of the different β-cyclodextrins (βCD) with or without a polymer. The suspension formed was heated in an autoclave in a sealed container to 120°C for 20 min, then cooled to room temperature (22–23°C). A small amount of solid drug was then added to the container to promote precipitation. Then the suspension was allowed to equilibrate for at least 3 days at room temperature, protected from light. After equilibration was attained, an aliquot of the suspension was filtered through a 0.45-μm membrane filter (Nylon Acrodisc® from Gelman, Northampton, UK), diluted with the HPLC mobile phase or pure methanol in water, and analyzed by HPLC. Each solution was analyzed three times and the

results are presented as the mean ± standard deviation (SD). The pH values given were determined after the equilibration period at room temperature.

The effect of pH on the stability constant (K_c) of the drug–cyclodextrin complex was determined. The solubility of ETH-615 was determined as described above in aqueous solutions containing from 0 to 10% (w/v) cyclodextrin. The aqueous solutions were as follows: 0.1 M hydrochloric acid (pH about 1.2), 0.08 M acetic acid (pH about 3.2), water (pH about 6.6), 0.01 M phosphate buffer (pH about 6.8), 0.10 M phosphate buffer (pH about 7.5), and 6.2 M ammonium chloride buffer (pH about 10.0). The exact pH of each solution was determined at the end of the equilibration period. Differences in pH were corrected by drawing the pH–solubility profiles at each cyclodextrin concentration and determining the solubilities of ETH-615 from these profiles at selected pH values. The values obtained were used to draw the phase-solubility diagrams, all of which were linear. Finally, K_c was calculated from the equation

$$K_C = \frac{\text{Slope}}{S_O(1 - \text{Slope})}$$

where K_c is the stability constant of the ETH-615 · cyclodextrin (1:1) complex, slope is the calculated slope of the linear phase-solubility diagram, and S_O is the solubility of ETH-615 obtained from the pH–solubility profile when no cyclodextrin was present.

Analytical Procedure

The quantitative determination of ETH-615 was performed on an HPLC component system consisting of a ConstaMetric 3200 solvent delivery system operated at 1.20 ml/min, a Merck-Hitachi AS4000 autosampler (Merck, Darmstadt, Germany), a Beckman Ultrasphere ODS 5 μm (4.6 × 150 mm) column (Beckman Instruments, Fullerton, CA), a Spectro Monitor 3200 UV/VIS variable-wavelength detector operated at 240 nm, and a Merck-Hitachi D-2500 Chromato-Integrator. The mobile phase consisted of acetonitrile and aqueous 0.1 M $(NH_4)_2HPO_4$ solution (1:1) and the retention time of ETH-615 was 2.8 min.

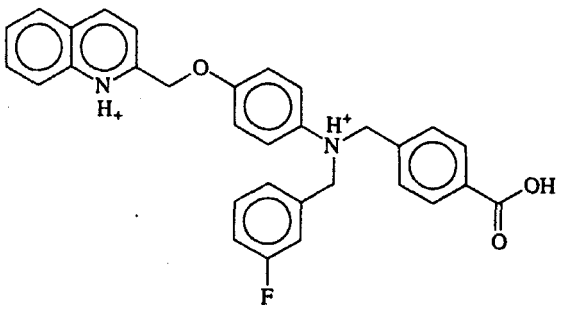
RESULTS AND DISCUSSION

Stability Studies

The chemical stability of ETH-615 was evaluated in aqueous buffer solutions containing 10% (w/v) HPβCD from pH 1.2 to 9.7. The ETH-615 concentration was monitored in the 70°C solutions for up to 6 hr. No degradation could be detected during those 6 hr at pH from 2.7 to 9.7, but some (although insignificant) degradation could be detected at pH 1.2. ETH-615 was stable in aqueous cyclodextrin solutions, at pH above 4, during heating in an autoclave (120°C for 20 min).

Solubility in Water and Aqueous Cyclodextrin Solutions

ETH-615 can exist in four different ionization forms (Fig. 1) and each of these four forms has different physicochemical properties, such as aqueous solubility and ability to form cyclodextrin complexes. The following pK_a values have been estimated in aqueous ethanolic solutions: $pK_{a_1} = 2.6$, $pK_{a_2} = 5.5$, and $pK_{a_3} = 10.6$ (personal communications, Leo Pharmaceuticals). The pK_{a_1} must be assigned to the carboxylic acid group. The acidity of the carboxylic acid group is greater than that of benzoic acid (pK_a 4.2) due to the electron attracting cation group in para position. pK_{a_2} and pK_{a_3} are ascribed to the protonated quinoline nitrogen and the protonated trialkylammonium group, respectively. The protonated quinoline itself has a pK_a value of 4.8. Thus, ETH-615 is a zwitterion at pH between pK_{a_2} and pK_{a_3}. At room temperature the solubility of ETH-615 in pure aqueous buffer solutions was determined to be about 70 μg/ml at pH 1.3, about 1 μg/ml at pH between 3 and 7, and about 1 mg/ml at pH 8. The pH–solubility profiles obtained in aqueous cyclodextrin solutions show that the solubility of ETH-615 is very low at pH below 7, but then it increases with increasing pH (Fig. 2).



H_3E^{2+}

$$H_3E^{2+} \overset{pKa_1}{\rightleftharpoons} H_2E^{2+} \cdot \overset{pKa_2}{\rightleftharpoons} HE^{+} \cdot \overset{pKa_3}{\rightleftharpoons} E^{-}$$

Figure 1. Ionization of ETH-615.

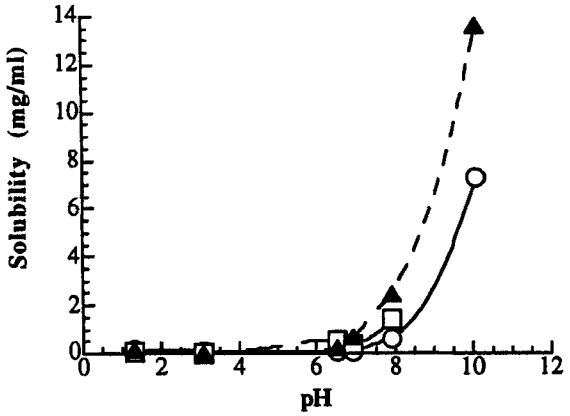


Figure 2. The effect of pH on the solubility of ETH-615 in 10% (w/v) cyclodextrin solutions at room temperature (22–23°C): HPβCD (▲), CDSBE (□) and HTMAPCD (○). No polymer was present in the aqueous complexation media.

The effects of five different βCD derivatives on the aqueous solubility of the zwitterionic ETH-615 were determined (Table 1). Two of the derivatives carried an anion (CMβCD and CDSBE), two were uncharged (HPβCD and MβCD), and one carried a cation (HTMAPCD). The uncharged cyclodextrins had much larger solubilizing effect than the positively charged cyclodextrin or negatively charged cyclodextrins (Table 1). MβCD had somewhat larger effect than HPβCD, increasing the aqueous solubility to 2.24 mg/ml when no polymer was present in the complexation medium, which is about 2000-fold solubility enhancement. There are several examples of enhanced complexation when the drug molecule and the cyclodextrin molecules are oppositely charged (6,7). However, in this case of zwitterionic drug the charged βCD derivatives were shown to be less effective than the uncharged ones. The stability constant of the selected ETH-615 · cyclodextrin complexes are shown in Table 2. At pH 5, where ETH-615 mainly exists as a zwitterion, the stability constants of the complexes are extremely low but they were somewhat larger at pH 10, where the drug is mainly in the anionic form. The highly polar zwitterionic drug appears to have very little tendency to enter the rather lipophilic βCD cavity. The anionic form of the drug is less polar and, thus, has somewhat greater ability to enter the cavity. In general, the stability constants obtained for the βCD complexes of the zwitterionic ETH-615 are several orders of magnitude smaller than those of simple lipophilic compounds such as steroids (8).

The Effect of Polymers

It has been shown that addition of water-soluble polymers, such as PVP, HPMC, and carboxymethylcellulose (CMC), to aqueous cyclodextrin solutions can significantly enhance the solubilizing effect of the cyclodextrins (9,10). The effect of three different polymers was investigated, i.e., the uncharged PVP and HPMC, and the cationic HDMB. Preliminary studies showed that the anionic CMC did not enhance the cyclodextrin solubilization of the zwitterionic ETH-615. Addition of 0.25% HDMB to the complexation media resulted in significant solubilization enhancement (Table 1). The largest enhancement was observed with the anionic cyclodextrins, i.e., CMβCD and CDSBE, but still the two uncharged cyclodextrins, i.e., MβCD and HPβCD, were the best solubilizers. Previously we have shown that HPβCD is nontoxic when it is given topically to the eye (11,12). Thus, the solubilizing effects HPβCD and polymers were studied further. The effect of increasing HDMB concentration on the HPβCD solubilization of ETH-615 is shown in Table 3. Even at very low concentrations this cationic polymer resulted in significant solubilization enhancement with a maximum

Table 1

Solubility of ETH-615 in Aqueous 0.01M Phosphate Buffer Solution Containing 10% (w/v) Cyclodextrin With or Without 0.25% (w/v) HDMB at Room Temperature (22–23°C); pH = 6.99 ± 0.22 (SD)

Cyclodextrin	Solubility (mg/ml, Mean ± SD)		Solubility Ratio
	No Polymer	With Polymer	
CMβCD	0.065 ± 0.002	0.278 ± 0.004	4.3
CDSBE	0.304 ± 0.004	0.795 ± 0.021	2.6
HPβCD	1.86 ± 0.10	2.88 ± 0.12	1.6
MβCD	2.24 ± 0.02	4.05 ± 0.04	1.8
HTMAPCD	1.00 ± 0.00	1.37 ± 0.01	1.4

Table 2

Effect of pH on the Stability Constant (K_c) of ETH-615–Cyclodextrin Complex Assuming 1:1 Complex Formation at Room Temperature; No Polymer Was Present in the Aqueous Complexation Media

Cyclodextrin	pH	$K_c(M^{-1})$
CDSBE	5.0	0.67
	10	25
HPβCD	5.0	0.17
	10	30
HTMAPCD	5.0	0.23
	10	9.5

enhancement observed at 0.5% (w/v) concentration. However, this enhancement was somewhat less than what could be obtained by comparable concentrations of the uncharged polymers (Table 4). Previous studies had indicated that optimum polymer concentration for co-complex formation is about 0.25% for PVP and 0.10% for HPMC. At these concentrations addition of PVP resulted in further 1.6-fold solubilization enhancement but addition of HPMC resulted in almost two-fold enhancement (Table 4). In the presence of HPMC the solubility of ETH-615 was 3.8 mg/ml in the 10% (w/v) HPβCD solution, which is almost 4000-fold solubility enhancement compared to the solubility in pure water (about 1 μg/ml).

The Effect of Choline and an Eye-Drop Formulation

It is also possible to enhance cyclodextrin complexation of ionic drugs by masking their charge through ion-pair formation. For example, for ETH-615 it was possible to enhance the HPβCD solubilization by addition of an ion-pairing agent, such as choline chloride or benzalkonium chloride, to the complexation medium,

Table 3

Effect of Increasing HDMB Concentration on the Solubility of ETH-615 in Aqueous 0.01 M Phosphate Buffer Solution Containing 10% (w/v) HPβCD at pH 6.89 ± 0.09 (SD) and Room Temperature

HDMB Conc. (% w/v)	Solubility (mg/ml, Mean ± SD)	Ratio
0.00	1.86 ± 0.10	1.0
0.03	2.65 ± 0.10	1.4
0.05	2.39 ± 0.05	1.3
0.10	2.37 ± 0.03	1.3
0.25	2.80 ± 0.12	1.5
0.50	3.41 ± 0.05	1.8

Table 4

Effect of Various Polymers and Choline Chloride on the Solubilization of ETH-615 in Aqueous 0.0.01 M Phosphate Buffer Solution Containing 10% (w/v) HPβCD at pH 6.77 ± 0.02 (SD) and Room Temperature

Polymer	Solubility (mg/ml, Mean ± SD)	Ratio
No polymer	1.95 ± 0.10	1.0
0.25% (w/v) PVP	3.18 ± 0.11	1.6
0.10% (w/v) HPMC	3.80 ± 0.09	1.9
0.30% (w/v) Choline chloride	3.04 ± 0.01	1.6
0.30% (w/v) Choline chloride and 0.10% (w/v) HPMC	3.33 ± 0.13	1.7
Eye-drop solution[a]	2.32 ± 0.14	1.2

[a]Eye drop solution: aqueous solution containing 10% (w/v) HPβCD, 0.10% (w/v) HPMC, 0.05% (w/v) EDTA, 0.02% (w/v) benzalkonium chloride, and sufficient sodium chloride to obtain isotonic solution. The osmolality of the solution was determined to be 277 ± 7 mOsm.

but HPMC had less effect in the presence of choline chloride than when choline chloride was not present. The eye-drop formulation tested contained both 0.02% of the positively charged benzalkonium chloride and 0.10% HPMC, in addition to 10% HPβCD, but the solubility of ETH-615 in this solution was much less than in comparable solution of HPβCD and HPMC in water (Table 4). Thus, it appears that the positively charged ion-pairing agents tested do not have an additive or synergistic effect on the HPβCD–HPMC solubilization of ETH-615.

CONCLUSIONS

ETH-615 is in a zwitterionic form at physiological pH with an aqueous solubility of only about 1 μg/ml at room temperature. Due to its highly polar zwitterionic structure the drug has little tendency to form inclusion complexes with βCDs, the stability constants of its complexes being several orders of magnitude smaller than those of uncharged lipophilic compounds. However, it is possible to enhance the complexation by addition of water-soluble polymers to the complexation media, i.e., formation of ETH-615 · cyclodextrin · polymer ternary complexes. The solubility of ETH-615 in aqueous 10% (w/v) HPβCD solution containing 0.10% (w/v) HPMC at room temperature (about 23°C) was determined to be 3.8 mg/ml.

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