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RESEARCH PAPER

The Influence of β-Cyclodextrin on the Solubility of Chlorthalidone and Its Enantiomers

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ABSTRACT

The solubility of chlorthalidone in 16 solvent systems was determined in the absence and presence of different amounts of β-cyclodextrin (β-CD). Chlorthalidone (CT) was shown to be more soluble in hydrophilic organic solvents, with the highest solubility in ethylacetate (EtOAc) saturated with water. The solubility of CT in water, butanol, octanol, and dichloromethane (DCM) was enhanced by the addition of β-cyclodextrin. The enantioselective partitioning of CT between water and EtOAc, DCM, butan-1-ol, butan-2-ol, and octan-1-ol was determined in the presence of β-CD at pH 5, 7, and 9. According to the results, both the solubility and partitioning properties of CT are affected by β-CD in aqueous solution. It was also shown that the solubility of the individual enantiomers differs in the presence of β-CD.

INTRODUCTION

Solubility profiles of drugs play an important role both in the formulation of drugs in dosage forms (1) and in the development of resolution methods such as liquid membrane separations. This study was performed to identify solvents for potential use in a chiral liquid membrane system, containing β-cyclodextrin (β-CD) as the chiral carrier, for the separation of chlorthalidone (CT) enantiomers.

β-Cyclodextrin is a cyclic oligoglucose molecule containing seven D-(+)-glucose units, linked through (1–4)-bonds, forming a truncated cone with both sides open in aqueous media (2). The interior of the cone is rather

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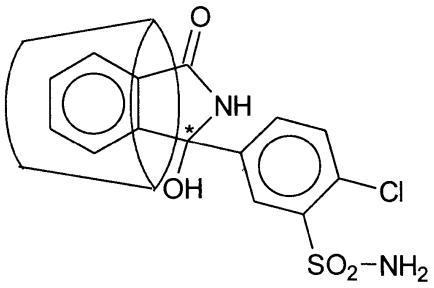


Figure 1. Proposed inclusion complex between chlorthalidone and β-cyclodextrin.

hydrophobic and can host a hydrophobic guest molecule such as CT, a well-known diuretic (Fig. 1). β-Cyclodextrin has been used widely for chiral resolution of amino acids and certain drugs (3,4).

Due to the relatively high water solubility of β-CD and the low water solubility of CT, the formation of an inclusion complex between β-CD and CT has the potential of increasing the water solubility of relatively hydrophobic compounds such as CT. Therefore, it may also have a pronounced effect on the partitioning properties of CT, and if the enantioselective properties of β-CD are taken into account, enantioselective partitioning of CT from an organic phase into an aqueous phase containing β-CD is conceivable. This simulates the transport of CT from an organic feed phase into an aqueous membrane phase, as found in a bulk liquid membrane (BLM) system.

In a BLM system, a relatively thick layer of liquid, which may contain specific carrier molecules, is used to separate two liquid phases (also referred to as the external phases) with which it is immiscible (5). The BLM can be used for separation processes, in which case the following terminology (6) is used:

- *feed*: the liquid phase rich in solute (CT) that supplies the membrane with solute molecules;
- *membrane phase*: also referred to as the *internal phase*; the liquid phase containing the carrier through which mass transfer occurs (from the feed to the strip) under the influence of certain driving forces;
- *strip*: the receiving phase that extracts the desired solute from the membrane and from which the product can be extracted;
- *external phases*: the feed and strip phases.

The objective of this study was to identify solvents for the construction of a BLM system for the resolution of CT enantiomers.

EXPERIMENTAL

Materials

Racemic CT was donated by Lennon Medicines (Port Elizabeth, South Africa). β-Cyclodextrin and organic solvents were purchased from Saarchem (Muldersdrift, South Africa). Buffer materials were purchased from Merck (Midrand, South Africa). All chemicals were analytical grade and were used as received.

Solubility

Five organic solvents (butan-1-ol, butan-2-ol, dichloromethane [DCM], ethylacetate [EtOAc], and octan-1-ol) and water (double distilled and deionized) were evaluated. For each of the organic solvents, three single-phase experiments were conducted, involving the solvent as is, the solvent saturated with water, and water saturated with the solvent.

The organic solvents were saturated with water and vice versa by shaking equal amounts of the solvent and water; after this, the phases were allowed 24 hr to separate. These phases were then kept in separate containers.

A volume of 25 ml of solvent was poured into a glass container. An excess of CT powder was added, and the container was stoppered to prevent evaporation of the solvent. The solution was stirred magnetically at a constant temperature of 20°C for 24 hr (7–9). Samples (in triplicate) were taken until no further increase in CT concentration was observed. Different amounts of β-CD, calculated according to the solubility of CT found in the solvent, were added, and the mixture was stirred for an additional 24 hr. The molar ratios of β-CD to CT added were 1:10, 1:1, 2:1, and 5:1.

Again, samples were taken in triplicate until no further change in CT concentration was observed. Prior to analysis, suspended particles were removed from the samples by filtration.

Partitioning

A volume of 40 ml of water at a specified pH, saturated with EtOAc or octan-1-ol (see Solubility section for the saturation procedure), was saturated with β-CD. Simultaneously, 30 ml of the specified organic solvent

(EtOAc or octan-1-ol, saturated with water at the specified pH) was saturated with CT. The aqueous phase was poured into a glass container with subsequent careful addition of the organic phase. The container containing both phases was stoppered to prevent solvent evaporation, and the solution was stirred magnetically for 24 hr at 20°C.

Tests were conducted at pH 5 (0.1% triethylammonium acetate/acetic acid), pH 7 (no buffer), and pH 9 (borate buffer) (10).

Regularly, samples were taken in triplicate from the aqueous phase until no further increase in CT concentration was observed. Prior to analysis, suspended particles were removed from the samples by filtration.

Analysis

High-performance liquid chromatography (HPLC) was performed using a Waters LC Module 1™ chromatograph equipped with a Waters 600 E Powerline™ pump, a Waters 715 UltraWISP™ automatic injector with a 200-µl loop, and a Waters 486 variable-wavelength ultraviolet detector (254 nm). Nonchiral HPLC was performed using

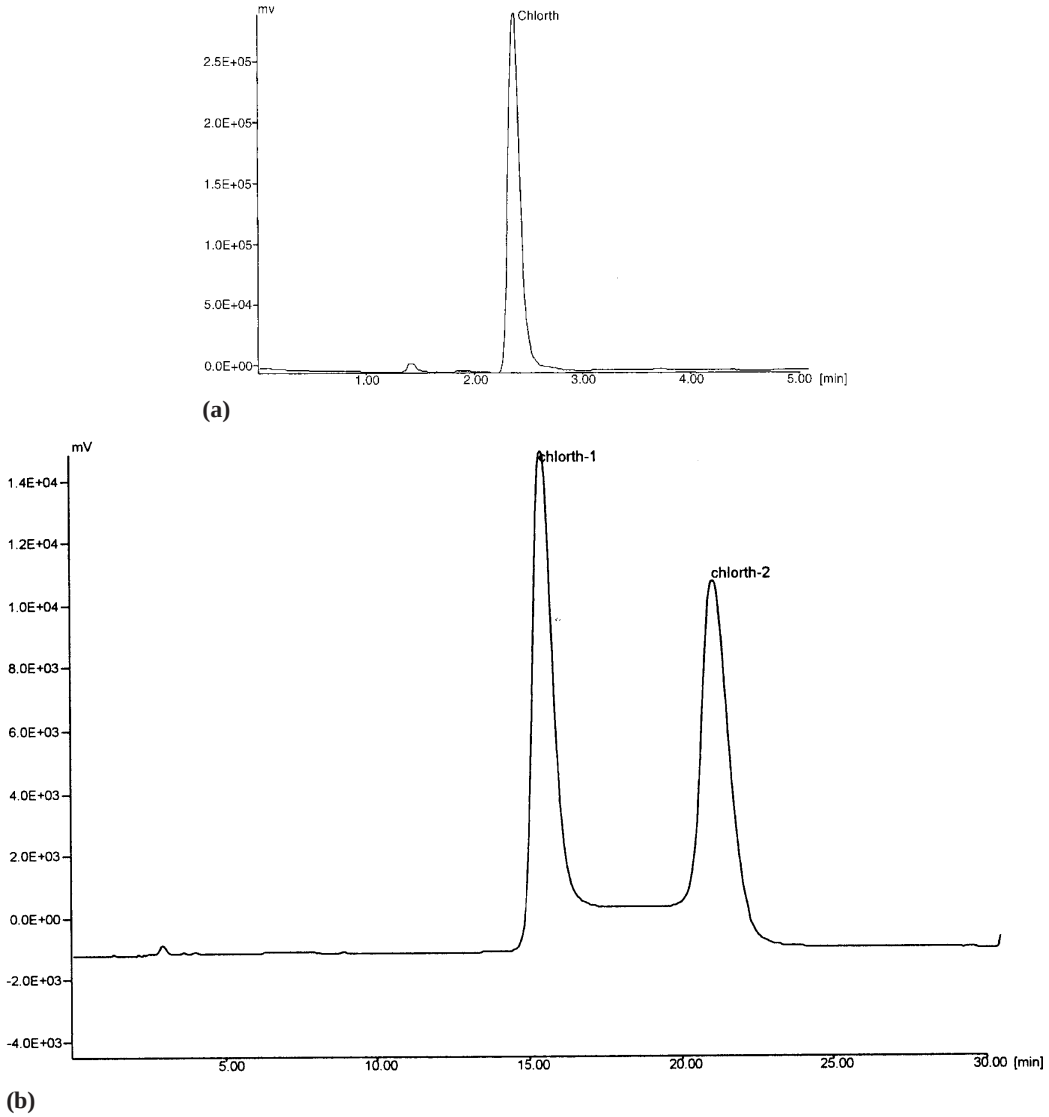


Figure 2. (a) HPLC chromatogram of chlorthalidone on LiChrospher column (nonchiral analysis); (b) HPLC chromatogram of chlorthalidone on ChiraDex column (chiral analysis).

Table 1

Chlorthalidone Enantiomer Concentrations ($mol.dm^{-3} \times 10^{-3}$) in Aqueous Phases Saturated with β-Cyclodextrin After 24 Hours (Standard Deviation ≤ 0.004)

Solvent	Solubility of	pH 5	pH 7	pH 9
Water saturated with octan-1-ol	Enantiomer 1	0.151	0.165	0.237
	Enantiomer 2	0.161	0.175	0.240
	Total (1+2)	0.312	0.340	0.477
Water saturated with EtOAc	Enantiomer 1	0.682	0.692	1.187
	Enantiomer 2	0.707	0.711	1.200
	Total (1+2)	1.389	1.403	2.387

a LiChrospher™ guard column (4 mm × 4 mm, 5-μm C_{18} packing) and a LiChrospher™ analytical column (24.4 cm × 4 mm, 10-μm C_{18}) supplied by Merck (Midrand, South Africa). Analysis of solutes was accomplished at ambient temperature using a mobile phase consisting of acetonitrile/water (70:30 v/v) at a constant flow rate of 1.0 ml/min (Fig. 2a). Chiral HPLC was performed using a ChiraDex™ guard column (4 mm × 4 mm; 5-μm β-CD packing) and a ChiraDex™ analytical column (24.4 cm × 4 mm, 5-μm β-CD) supplied by Merck. Analysis of solutes was accomplished at ambient temperature using a mobile phase consisting of methanol/0.1% triethylammonium acetate pH 3.7 (15:85, v/v) at a constant flow rate of 1.0 ml/min (Fig. 2b). Each sample was analyzed three times. (The solubility values in Table 1, therefore, in each case are the average of nine measurements.)

RESULTS AND DISCUSSION

Chlorthalidone Solubility

As shown in Figs. 3a–3c, increasing β-CD concentration had different effects on the solubility of CT in different solvents; Fig. 3a shows the results for the effect on pure solvents, Fig. 3b the effect on solvents saturated with water, and Fig. 3c the effect on water saturated with the organic solvents.

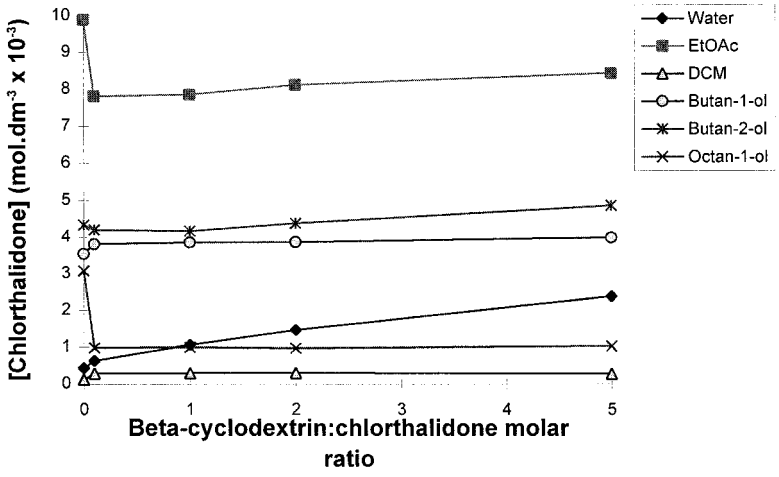
Figure 3a shows that β-CD improves the solubility of CT in water, and that the solubility of CT in water increases linearly with the β-CD concentration. The presence and concentration of β-CD has little or no effect on the solubility of CT in butan-1-ol, butan-2-ol, and DCM, but causes a decrease in CT solubility in both octan-1-ol and EtOAc. These decreases in solubility could be ascribed to the fact that β-CD is not soluble in either octan-1-ol or EtOAc: When added to the CT/solvent system, the β-CD precipitates and removes CT from the solvent due to complexation, causing a decrease in CT concentration. This indicates that β-CD might extract CT from an organic solvent (in which it is more soluble) into an aqueous phase in which CT is less soluble, but in which β-CD is soluble. This also means that octan-1-ol and EtOAc could have potential application in a liquid-liquid extraction technique, for example, a BLM system.

When comparing Fig. 3a with Fig. 3b, it is clear that the presence of water in an organic solvent increases the solubility of CT in the solvent, except for DCM (water) and octan-1-ol (water). The CT showed a marked increase in solubility, especially in butan-1-ol (water) and butan-2-ol (water). This increase is probably the result of the solubility of β-CD in the large amounts of water in these solvents. The presence of water in a solvent therefore increases the solubility of β-CD in the solvent, which in turn increases the solubility of CT. Since octan-1-ol accommodates little or no water, no β-CD dissolved, and consequently the solubility of CT remained unchanged in this solvent.

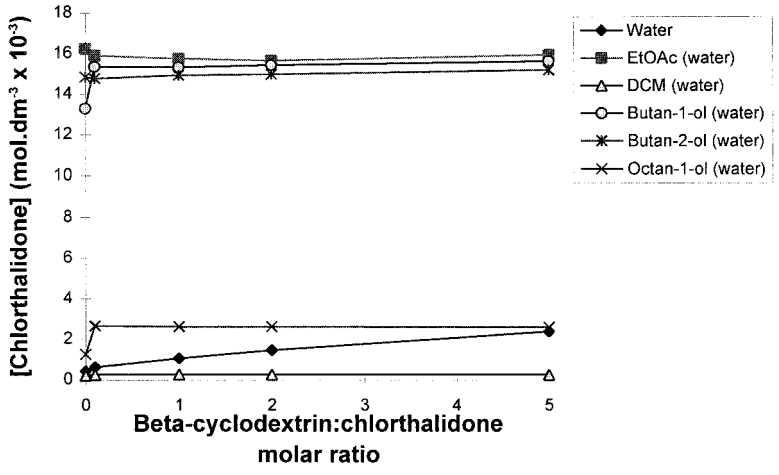
Figure 3c shows that the presence of an organic solvent in water causes an increase in the solubility of CT in such a phase when no β-CD is present. The addition of β-CD caused a further increase in CT solubility in these solvents, except for water saturated with either of the two butanols.

The main intent of these experiments was to identify solvents to be used in a BLM system. In such a membrane system, the substrate (CT) should not be soluble in the membrane phase except in the presence of a carrier (β-CD), which in turn should be soluble in the membrane phase only and not in the external phases.

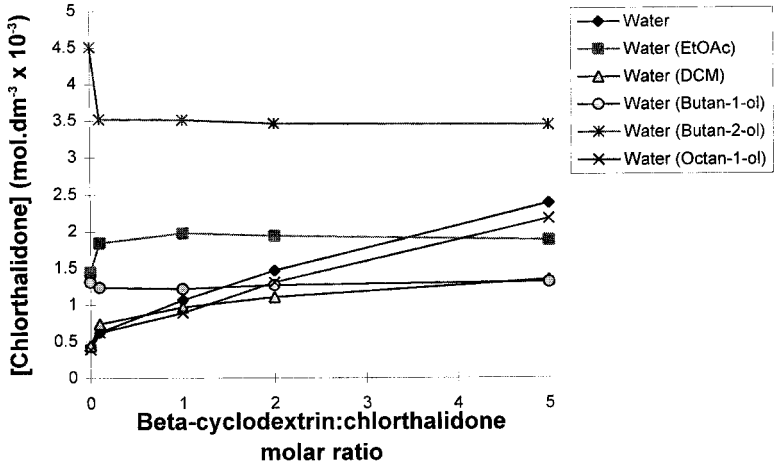
From this, two solvent systems have been identified for possible use in a liquid membrane system, namely, EtOAc (water)/water (EtOAc) and octan-1-ol (water)/water (octan-1-ol). When EtOAc and octan-1-ol are used as external phases, Fig. 3a shows that β-CD removes CT



(a)



(b)



(c)

Figure 3. (a) The solubility of chlorthalidone in organic solvents (standard deviation ≤ 0.02); (b) the solubility of chlorthalidone in organic solvents saturated with water (standard deviation ≤ 0.02); (c) the solubility of chlorthalidone in water saturated with organic solvents (standard deviation ≤ 0.02).

from the external (i.e., feed phase) into the aqueous membrane, while Fig. 3c shows that subsequently the CT concentration increases in the aqueous membrane phase [water (EtOAc) and water (octan-1-ol)] as the β-CD concentration increases. As a result, β-CD effectively removes CT from the organic (feed) phase into the aqueous membrane phase, making the two above-mentioned solvent combinations ideal for further applications. Partitioning experiments were therefore only conducted for these solvent systems.

Enantioselective Chlorthalidone Partitioning

Since CT is amphoteric, the pH of the aqueous phase should have an influence on the partitioning of CT and its enantiomers. The maximum CT enantiomer concentrations reached in the different aqueous phases are shown in Table 1.

The pK_a of the CT molecule (9.36) (11) indicates that CT should be more soluble at low pH, which was not the case: In both systems, the solubility of both enantiomers surprisingly increased with an increase in pH. This increase in solubility at alkaline pH could possibly be due to the presence of organic solvent in the aqueous phase and also as a result of the affinity of β-CD to form inclusion complexes with hydrophobic molecules (in this case, the un-ionized form of CT at alkaline pH). It was also observed that the higher the solubility of CT in the solvent was, the lower was the enantioselectivity.

Enantioselectivity in both systems was low since measurements were taken after 24 hr, that is, after equilibrium was reached in the aqueous phase (from which all samples were taken). Higher enantioselectivity can be expected during the earlier stages of partitioning.

CONCLUSION

In this study, the influence of β-CD on the solubility of CT in aqueous and some organic phases has been demonstrated. β-Cyclodextrin also affects the partitioning of CT between organic and aqueous solvents containing β-CD, and it was shown that β-CD discriminates between the enantiomers of CT in aqueous solution (saturated with an organic phase), causing one enantiomer to diffuse from the organic phase into the aqueous phase (in which it is less soluble) more rapidly. These events can be ascribed to the formation of inclusion complexes between β-CD and CT, which is the main requirement for chiral recognition (7,12–16). The study, therefore, has also shown the suitability of these solvent systems for the development of a novel technique for the resolution of CT enantiomers.

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