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International Journal of Pharmaceutics 297 (2005) 213–222
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Cyclodextrin solubilization of the antibacterial agents triclosan and triclocarban: Formation of aggregates and higher-order complexes

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Received 17 September 2004; received in revised form 8 March 2005; accepted 5 April 2005

Abstract

It is well known that water-soluble cyclodextrins form inclusion complexes with many lipophilic water-insoluble drugs and that such complexation frequently enhances the aqueous solubility of drugs. It is also well known that various excipients, such as water-soluble polymers, organic acids and bases and metal ions can enhance the solubilizing effects of cyclodextrins. However, it is not clear how these excipients enhance the effects. The effects of cyclodextrins, 2-hydroxypropyl-β-cyclodextrin (HPβCD) and randomly methylated β-cyclodextrin (RMβCD) on the aqueous solubility of triclosan and triclocarban were investigated. The phase-solubility profiles were all of type A_P indicating formation of higher-order complexes or complex aggregates. Addition of lysine and other excipients enhanced the RMβCD solubilization of triclocarban. NMR spectroscopic studies, including 2D ROESY and 1D gROESY techniques, indicated that HPβCD and RMβCD, as well as their complexes, form aggregates of two to three cyclodextrin molecules. The critical concentration for the aggregate formation was determined to be 5.4% (w/v). Lysine, polyvinylpyrrolidone and magnesium ions formed non-inclusion complexes resulting in formation of multiple-component cyclodextrin complexes in aqueous solutions with triclocarban.

Keywords: Cyclodextrins; Inclusion complexes; Antibacterial agents

1. Introduction

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity in the center (Loftsson and Brewster, 1996). Cyclodextrins are able to form hydrophilic inclusion complexes with many lipophilic compounds. In aqueous solutions molecules bound within the inclusion complex are in a dynamic equilibrium with free molecules. Thus, cyclodextrins are able to enhance the aqueous solubility of many lipophilic drugs without changing their intrinsic ability to permeate lipophilic membranes

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doi:10.1016/j.ijpharm.2005.04.007

(Loftsson and Masson, 2001). This makes cyclodextrins attractive as enabling pharmaceutical excipients. In general, phase-solubility studies are used to evaluate the solubilizing effects of cyclodextrins on lipophilic water-insoluble drugs (Higuchi and Connors, 1965; Repta, 1985). Frequently, the stoichiometry of the complexes, and determination of the equilibrium constants involved, is obtained mathematically from the phase-solubility diagrams without any further verification. The phase-solubility method regards drug–cyclodextrin interactions as discrete phenomenon and ignores the possible interaction of the complexes with one another. It is becoming increasingly apparent that such assumptions may not be universally applicable or all encompassing (Loftsson et al., 2004). Specifically, there is a growing body of evidence that supports the important contribution of non-inclusion-based aspects for drug solubilization by cyclodextrins including surfactant-like effects and molecular aggregation (Loftsson et al., 2002).

It has been shown that addition of small amount of various additives, such as water-soluble polymers (Loftsson and Másson, 2004), organic acids and bases (Redenti et al., 2000, 2001; Gladys et al., 2003; Loftsson et al., 2003; Mura et al., 2003), and metal ions (Yamakawa and Nishimura, 2003), to aqueous complexation media can increase the cyclodextrin solubilization of drugs. It is thought that these additives enhance the cyclodextrin complexation of drugs by forming non-inclusion complexes with cyclodextrins and their complexes. For example, it has been shown that polymers form ternary complexes with drug/cyclodextrin complexes (Valero et al., 2003a,b). Furthermore, it has been shown that cyclodextrins and cyclodextrin complexes self-associate to form aggregates and that those aggregates can act as solubilizers themselves (Mele et al., 1998; González-Gaitano et al., 2002; Magnusdottir et al., 2002; Loftsson et al., 2004). There are some indications that the water-soluble polymers enhance the complexation efficiency by stabilizing these aggregates (Loftsson et al., 2003, 2004; Loftsson and Másson, 2004).

The purpose of the present study is to investigate formation of higher-order complexes and aggregates containing triclosan/cyclodextrin and triclocarban/cyclodextrin complexes, lysine, magnesium ions and polyvinylpyrrolidone.

2. Materials and methods

2.1. Materials

Triclosan (Irgasan DP 300 or cloxifenol) was purchased from Sigma (USA) and triclocarban (TCC or 3,4,4′-trichlorocarbanilide) was obtained from Colgate-Palmolive Co. (USA). β-Cyclodextrin (βCD) and randomly methylated β-cyclodextrin with degree of substitution 1.8 (RMβCD) were purchased from Wacker Chemie (Germany), 2-hydroxypropyl-β-cyclodextrin with molar substitution 0.6 (HPβCD) from Roquette (France) and polyvinylpyrrolidone 40,000 (PVP) from Sigma (USA). All other chemicals and solvents used in this study were commercial available products of analytical or special reagent grade.

2.2. Solubility studies

The solubility of triclosan and triclocarban was determined in aqueous complexation media containing 0–40% (w/v) HPβCD or 0–40% (w/v) RMβCD in pure water or aqueous 0.25% (w/v) PVP solutions or aqueous 4% (v/v) ammonia (pK_b 4.75), or aqueous 50 mM magnesium chloride ($MgCl_2$) solution, or 50 mM lysine solution, or 50 mM ascorbic acid solution, or mixture thereof. An excess amount of triclosan or triclocarban was added to the aqueous complexation media and the suspension formed heated in a sealed vial in ultrasonic bath (70 °C for 60 min). The suspension was heated to promote drug saturation of the aqueous complexation medium. After equilibration at room temperature (22–23 °C) overnight the vials were opened, small amount of solid triclosan or triclocarban added to each vial and the aqueous suspensions allowed to equilibrate in the resealed vial at room temperature under constant agitation for additional 6 days. This was done to promote precipitation. Preliminary experiments showed that 6 days are more than enough time to reach solubility equilibrium. The heating of the suspensions promotes complex formation through formation of supersaturated solutions and dehydration of dissolved cyclodextrin molecules. The chemical stability of the compounds was also monitored during heating and equilibration period and in all cases less than 1% degradation was observed. After equilibration the suspensions were filtered through 0.45 μm nylon membrane filters and the filtrate analyzed by HPLC.

Phase-solubility profiles were obtained by plotting the solubility of triclosan or triclocarban versus the cyclodextrin concentration. The pH of the aqueous complexation media was determined at room temperature at the end of the 7-day equilibration period (Corning pH meter 24, UK).

2.3. Quantitative determinations

Quantitative determinations of triclosan and triclocarban were performed on a high performance liquid chromatographic (HPLC) component system, consisting of ConstaMetric 3200 solvent delivery system operated at 1.5 ml/min, a SpectroMonitor 3200 UV–vis variable-wavelength detector operated at 283 nm, a Merck-Hitachi AS-2000A autosampler, Merck Hitachi D-2500 Chromato-Integrator and a Phenomex ODS 5 μm (150 mm × 4.6 mm) column. The mobile phase for determination of triclosan consisted of acetonitrile and water (74:26), and the retention time was 2.9 min. The mobile phase for determination of triclocarban consisted of acetonitrile and water (70:30), and the retention time was 2.6 min.

2.4. NMR studies

A stock solution of cyclodextrin (βCD, HPβCD or RMβCD) was prepared in D_2O. Sample solutions were prepared by diluting the stock solution with D_2O or solution of lysine, PVP or magnesium chloride or mixture thereof in D_2O. An excess amount of triclosan or triclocarban was added into a glass vial containing the complexation medium. The vials were capped tightly and sonicated for 1 h at 70 °C. The samples were allowed to equilibrate at room temperature for a few days. After equilibration the suspensions were filtered through 0.45 um nylon membrane filter and the filtrate analyzed by ^{1}H NMR and HPLC. ^{1}H NMR spectra were taken at 25 °C on a Varian INOVA 500 MHz NMR spectrometer, using 5 mm sample tube with coaxial insert. D_2O was used as a solvent; the TSP signal was used as an external reference for ^{1}H NMR. Chemical shifts were expressed in parts per million (ppm) relative to those of the TSP signal (0.000 ppm). The critical cyclodextrin concentration (cc) for aggregate formation and the aggregation number were estimated by methods of Ruso et al. (2000). Two-dimensional rotating-frame Overhauser (2D ROESY) and one-dimensional gradient-enhanced ROESY (GROESY) experiments were conducted to analyze intermolecular nuclear Overhauser effects (NOE) (Ikeda et al., 2004). Due to the acidity of the hydroxy groups, hydrogen bonding between lysine and cyclodextrin was studied in pure DMSO-d_6.

3. Results and discussion

Triclosan and triclocarban are antibacterial agents commonly found in household products such as toothpastes and antibacterial soaps. Triclosan is a weak acid with a pK_a value of 7.9, melting point of 54–57 °C and log $P_{octanol/water}$ of 4.8 (Moffat et al., 2004). Triclocarban is unionized at pH below 11, melting point of 255–256 °C and log $P_{octanol/water}$ of 4.9 (Moffat et al., 2004). Both compounds are lipophilic and water-insoluble but somewhat soluble in various organic solvents such as acetone and propylene glycol. The intrinsic solubility (S_0) of triclosan and triclocarban in pure water at room temperature has been determined to be about 1 μg/ml and less than 50 ng/ml, respectively (Loftsson et al., 2005).

3.1. Phase-solubility studies

The phase-solubility diagrams of triclosan in aqueous HPβCD and RMβCD solutions are shown in Fig. 1

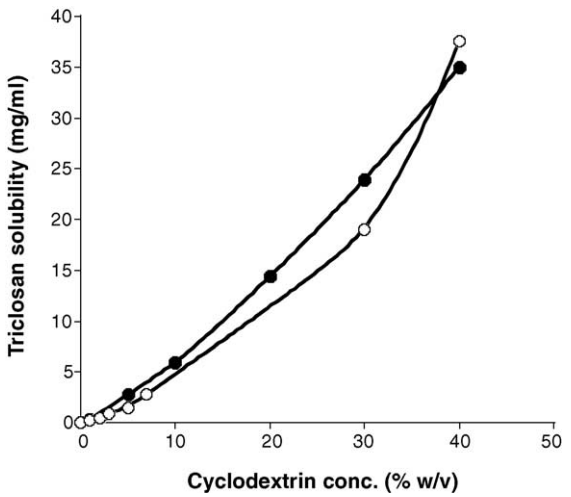

Fig. 1. Phase-solubility plots of triclosan in aqueous HPβCD solution (◯), and aqueous RMβCD solution (●), at room temperature (22–23 °C) and pH 5.58 ± 0.19 (mean ± standard deviation).

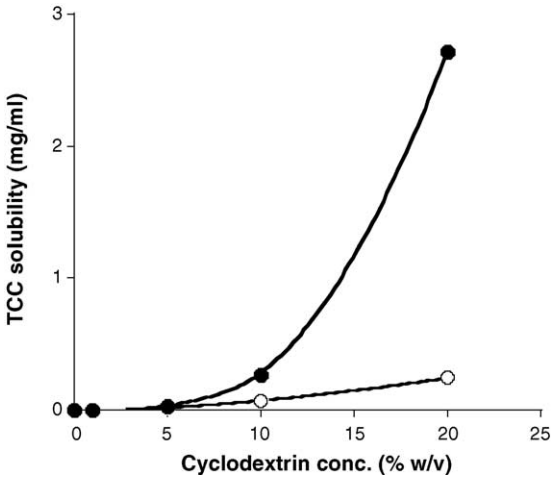

Fig. 2. Phase-solubility plot of triclocarban in aqueous HPβCD solution (◯) and aqueous RMβCD solution (●) at room temperature.

and those of triclocarban in Fig. 2. All the phase-solubility diagrams are of A_P type with a relatively sharp solubility increase when the cyclodextrin concentration was raised above about 5%. In general, A_P type phase-solubility diagrams are thought to indicate formation of complexes that are first order with respect to the guest (here triclosan or triclocarban) but second or higher order with respect to cyclodextrin (Higuchi and Connors, 1965). A_P type phase-solubility diagrams can, however, also indicate formation of complex aggregates that are capable of solubilizing addition amount of the guest through non-inclusion complexation or formation micelle-like structures (Loftsson et al., 2002, 2004).

Addition of small amount of polymers to the aqueous complexation media enhance the solubility of both triclosan and triclocarban, and both of the unionized and the ionized forms of the compounds (Loftsson et al., 1999, 2005). Similar results were obtained in aqueous HPβCD solutions where polymers, such as PVP, enhanced the solubilization but lysine had little or negative effect on triclosan solubility in the aqueous complexation media (Table 1). However, these same additives enhanced the cyclodextrin solubilization of triclocarban. The largest solubilization was obtained in aqueous solutions containing RMβCD and 0.25% PVP. Fig. 3 shows that addition of small amount of PVP and magnesium ions to the aqueous complexation media enhances somewhat the RMβCD solubilization of triclocarban. However, addition of ascorbic acid or lysine to the media results in significant solubilization enhancement. Addition of small amounts of these water-soluble compounds to the aqueous complexation media enhanced significantly the positive deviation of the A_P type phase-solubility diagrams. It is possible that these compounds enhance the solubilization through formation of non-inclusion complexes.

Table 1
Effect of HPβCD, PVP and lysine on the aqueous solubility of triclosan at room temperature (22–23 °C) and pH 5.60 ± 0.44 (mean ± standard deviation)

Composition of the aqueous complexation media			Triclosan solubility (mg/ml)
HPβCD (%, w/v)	PVP (% w/v)	Lysine (mM)	
0	0	0	0.00
0	0.25	0	0.00
0	0	50	0.00
10	0	0	5.93
10	0.25	0	6.51
10	0	50	3.76
10	0.25	50	3.67

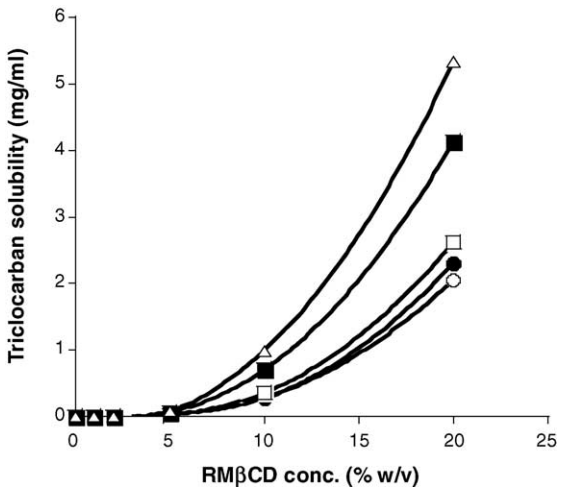

Fig. 3. The effect of PVP, magnesium ions (Mg^{2+}), ascorbic acid and lysine on the RMβCD solubilization of triclocarban at room temperature. Pure aqueous RMβCD solutions (◯), aqueous 0.25% (w/v) PVP solution (●), aqueous 0.25% (w/v) PVP solution containing 50 mM Mg^{2+} (□), aqueous 0.25% (w/v) PVP solution containing 50 mM Mg^{2+} and 50 mM ascorbic acid (■), and aqueous 0.25% (w/v) PVP solution containing 50 mM Mg^{2+} and 50 mM lysine (Δ).

3.2. Formation of aggregates

^{1}H NMR spectroscopy was used to obtain further information on cyclodextrin aggregate formation. Critical concentration and aggregation numbers were determined from chemical shift data. Plot of the chemical shift as a function of the inverse of the total cyclodextrin concentration show pronounced up-field shift upon aggregation (Fig. 4). Below the critical concentration (cc), no concentration dependence of the chemical shift was found. When the concentration exceeds the critical concentration the chemical shift changed linearly with the reciprocal of the total concentration. Critical concentrations were determined from the intersection of the linear portions of the plot at concentrations above and below the inflection region. Fig. 4 shows that cc of HPβCD is about 5.4% (w/v) and that the effects of PVP and/or triclosan on the cc value are insignificant. Similar results were obtained when HPβCD was replaced by RMβCD. Under all conditions tested HPβCD and RMβCD start to form aggregates at concentration of about 5.4% (w/v). This is in agreement with the phase-solubility diagrams (Figs. 1–3) where relatively sharp increase in the solubility is observed at cyclodextrin concentrations above about 5% (w/v).

The ^{1}H NMR spectra of triclosan in aqueous HPβCD solution are shown in Fig. 5. At HPβCD concentration of 30.8 mg/ml peaks Ha and Hb of triclosan broaden, indicating that motions of Ha and Hb are restricted, suggesting partial phenyl ring fits tight inside HPβCD cavity. At HPβCD concentration of 61.5 and 153.8 mg/ml Ha and Hb showed split pattern, one set of peaks suggests inclusion complex exists while the other set of peaks might indicate the presence of non-inclusion complex. Among six hydrogens of triclosan monitored for the peak broadening He broadens the most, that is from 4.41 Hz at lower HPβCD concentration (30.8 mg/ml) to 14.60 Hz at higher HPβCD concentration (up to 307.7 mg/ml, not shown) in the absence of polymer, the peak broadening effect was

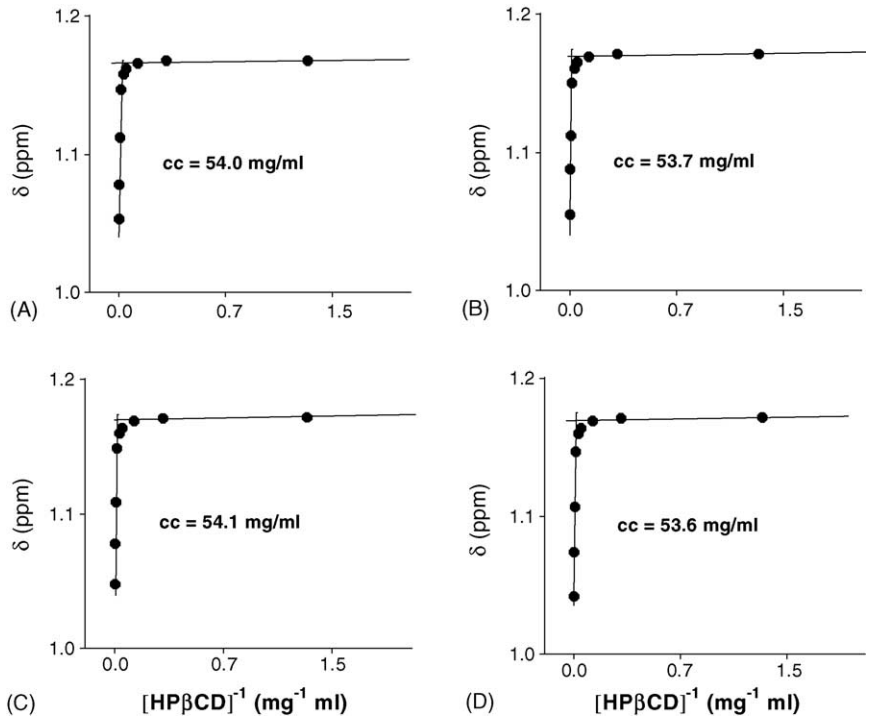

Fig. 4. The proton chemical shift of CH_3 in the $CH_3CH(OH)CH_2$— moiety of the HPβCD molecule, the pure HPβCD solution (A), HPβCD solution containing PVP (B), HPβCD solution saturated with triclosan (C), and HPβCD solution containing 0.25% (w/v) PVP and saturated with triclosan (D).

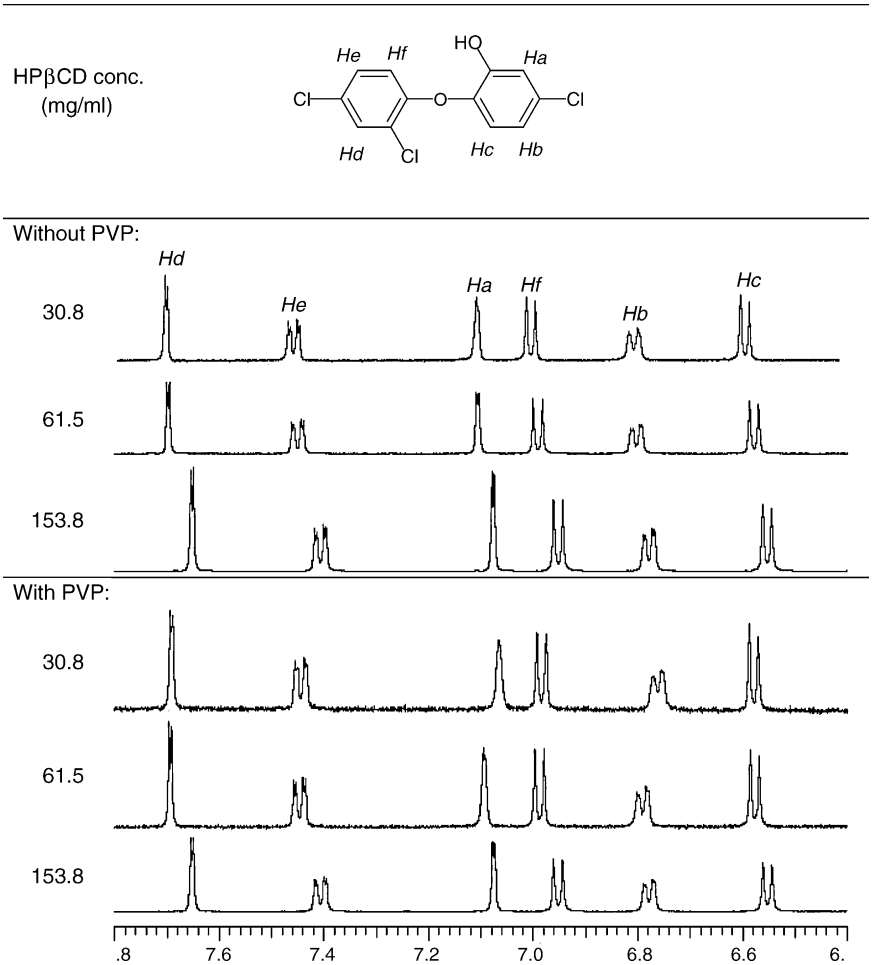

Fig. 5. ^{1}H NMR spectra (500 MHz) of aqueous HPβCD solutions saturated with triclosan and with or without 0.25% (w/v) PVP.

similar in the presence of PVP, or from 5.35 Hz at lower HPβCD concentration to 14.50 Hz at higher HPβCD concentration. Hb also shows significant broadening, and both Hc and Hf show some broadening, but the Hd peak hardly changed. These differential localized peak broadenings for different hydrogens suggested the existence of some individually distinct interaction between sections of triclosan and HPβCD. If He makes stronger interactions to allow slower chemical exchanges while others interact less, some characteristic differences in the degree of signal broadenings may be expected for each hydrogen atom.

The magnitude of differential peak broadening is inherently dependent on the molecular weight of the host present in the aqueous complexation media (Fejzo et al., 1999). Without polymer, the peak wide of the He peak is 14.60 Hz (3.3-fold increase) at higher HPβCD concentration compared with 4.41 Hz at lower HPβCD concentration. With PVP present, the peak wide of the He peak is 14.50 Hz (2.7-fold increase) at higher HPβCD concentration compared with 5.35 Hz at lower HPβCD concentration. The result suggests that formation of HPβCD aggregates at higher HPβCD concentration, which is in agreement with the chemical shift analysis and that PVP does not have a significant effect on formation of HPβCD aggregates. The HPβCD and RMβCD aggregation numbers were estimated from changes in the chemical shifts to be between 2 and 3

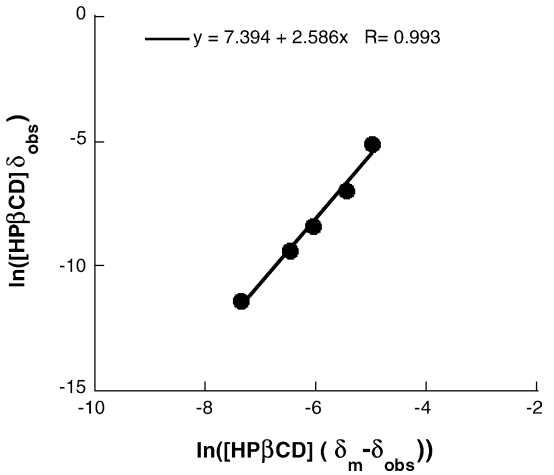

Fig. 6. NMR chemical shift data plotted according to method by Ruso et al. (2000) for pure HPβCD solutions. The aggregation number is 2.6.

in all the cyclodextrin solutions tested (Fig. 6 is a representative plot for determination of the aggregation number).

3.3. The lysine–cyclodextrin interaction

2D ROESY spectra are known to give no peaks or ghost peaks when the mixing time is inappropriately set. On the other hand, one-dimensional GROESY spectroscopy can probe NOE easily. GROESY was used to gain structural information of ternary complex among triclocarban, lysine and RMβCD (Fig. 7). The selective irradiation of H1 of triclocarban clearly gave NOE enhancement of H-3 of RMβCD, suggesting H1 of triclocarban is inside the cavity. However, the study does not distinguish between lysine competing with triclocarban for a space in the RMβCD cavity and lysine forming non-inclusion complex with RMβCD. Irradiation of Ha of lysine yielded strong peaks at 5.28 and 5.07, indicating the close interaction of Ha of lysine with H-1 of RMβCD. The irradiations of Hb and Hc gave NOE enhancement of the methyl hydrogens of RMβCD. Both H-1 and the methyl hydrogens are located outside the cavity and, thus, the results suggested that lysine is located outside the RMβCD cavity, with close interaction with H-1 and methyl groups. Furthermore, 2D ROESY technique was employed to identify the hydrogen bonding interaction between lysine and βCD, the parent of RMβCD in non-aqueous DMSO-d_6 (Fig. 8). The ROESY cross-peaks were observed between Ha of lysine and OH-2, OH-3, OH-6 of βCD, indicating interaction between Ha of lysine and hydroxyl at 2, 3, 6 positions of βCD. Overall, the above experiments clearly show that lysine forms a non-inclusion complex with RMβCD.

3.4. Higher-order complexes

A series of aqueous 20% (w/v) RMβCD solutions containing lysine, PVP, and/or $MgCl_2$ were prepared in D_2O. Excess amount of triclocarban was added to each sample and the samples were sonicated for 1 h, shaken overnight, filtered and analyzed by ^1H NMR. The chemical shifts of triclocarban and lysine are listed in Table 2. The proton shift changes of H1 of TCC suggested that lysine forms ternary complex with triclocarban and RMβCD (7.269–7.263 ppm), the addition of PVP to the ternary complex resulted very minor chemical shift change (7.263–7.264 ppm). But the addition of $MgCl_2$ has caused major shift changes to the quaternary complex (7.264–7.255 ppm), resulting the formation of a pentanary complex. Similar chemical shift changes were observed for H2 and H5 of triclocarban. The chemical shift changes were also

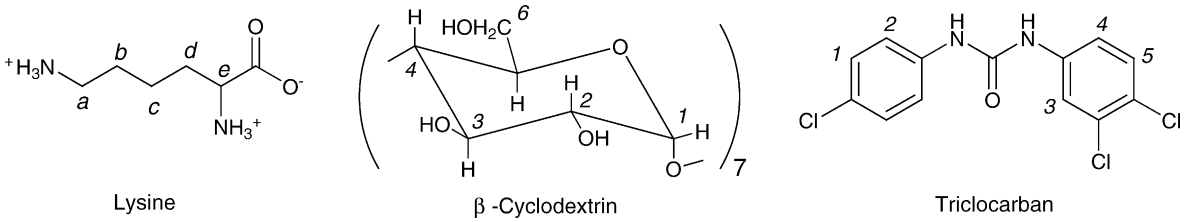

Fig. 7. Structure of the compounds tested and proton assignments.

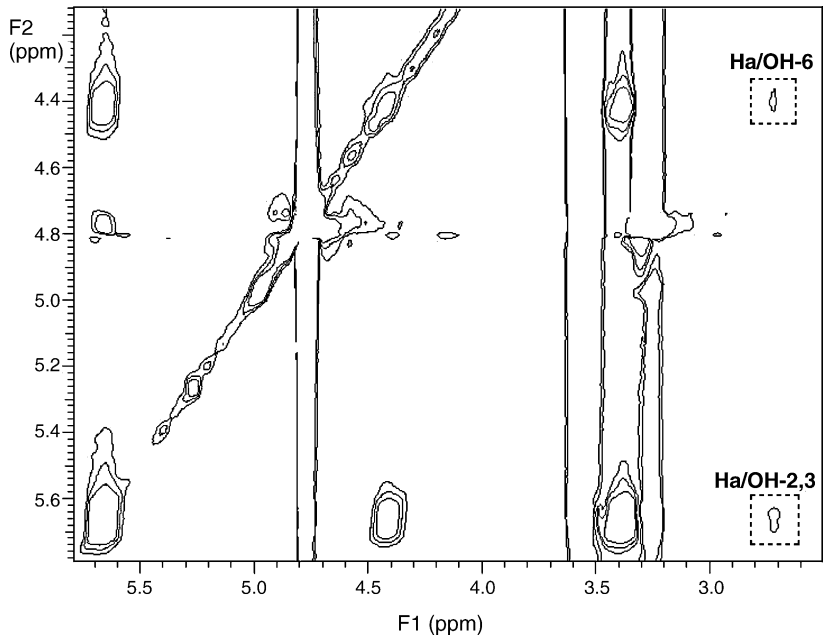

Fig. 8. Partial contour plot of 2D ROESY spectrum of lysine/βCD in DMSO-d_6 at 25 °C.

monitored for lysine. Lysine hydrogens experienced the chemical shift changes caused by the complex formation of binary, ternary, quaternary and penternary complexes.

2D ROESY studies were conducted to estimate the inclusion structure of triclocarban/RMβCD complex in the presence of lysine, polymer and metal ion. Fig. 9 shows a partial contour plot of 2D ROESY spectrum for the penternary complex. The cross-peaks (3.2–3.8 and 7.2–8.0 ppm) in the dashed box indicated a very close interaction of RMβCD (3.2–3.8 ppm, H-3 and H-5) and triclocarban (7.2–8.0 ppm, all five aromatic hydrogens) suggesting that both benzyl rings of triclocarban are include in the RMβCD cavity. No cross-peaks between RMβCD and lysine were observed, indicating that lysine is located outside the RMβCD cavity. This observation also agrees well with the GROESY studies of triclocarban, lysine, and RMβCD ternary complex.

Table 2
^{1}H chemical shifts of triclocarban and lysine

Proton	Lysine	Lysine, RMβCD	Triclocarban, RMβCD	Triclocarban, RMβCD, lysine	Triclocarban, RMβCD, lysine, PVP	Triclocarban, RMβCD, lysine, PVP, $MgCl_2$
H1			7.269	7.263	7.264	7.255
H2			7.553	7.547	7.548	7.538
H3			8.017	8.012	8.013	8.003
H4			7.486	7.480	7.481	7.471
H5			7.331	7.328	7.329	7.322
Ha	3.028	3.012		2.982	2.982	2.978
Hb	1.728	1.713		1.681	1.682	1.677
Hc	1.455					
Hd	1.913	1.897		1.865	1.866	1.860
He	3.757	3.741		3.706	3.706	3.702

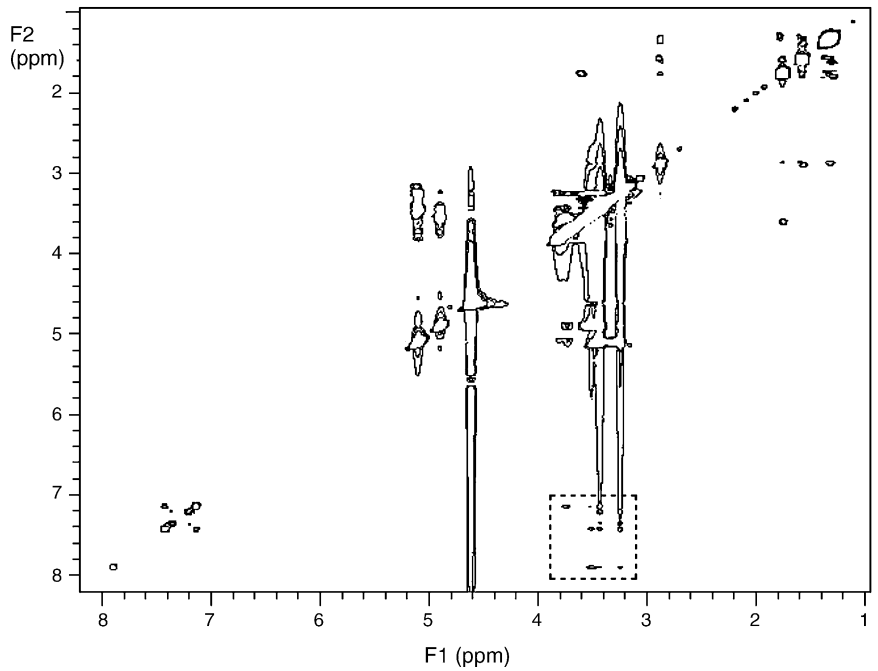

Fig. 9. Partial contour plot of 2D ROESY spectrum of triclocarban, RMβCD, lysine, PVP and $MgCl_2$ in D_2O at 25 °C.

4. Conclusions

This study indicates that both HPβCD and RMβCD form aggregates consisting of, on the average, two to three cyclodextrin molecules and other excipients, such as lysine and PVP, forming non-inclusion complexes with the cyclodextrin aggregates. The critical cyclodextrin concentration of the aggregate formation is about 5.4% (w/v). Furthermore, lysine is capable to enhance cyclodextrin solubilization of triclocarban by formation of non-inclusion complexes with the triclocarban/cyclodextrin complexes. Lysine, polyvinylpyrrolidone and magnesium ions formed non-inclusion complexes resulting in formation of ternary, quaternary and even pentenary complexes in aqueous solutions.

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