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Preparation and characterization of inclusion complexes of a cationic β-cyclodextrin polymer with butylparaben or triclosan

L. Qian [a,b], Y. Guan [b], H. Xiao [a,b,*]

[a] State Key Lab of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China
[b] Department of Chemical Engineering, University of New Brunswick, Fredericton, N.B., Canada E3B 5A3

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Abstract

The preparation of a cationic β-cyclodextrin polymer (CPβCD) and its complexes with butylparaben and triclosan were reported in this paper. FT-IR and 2D ^{1}H–^{1}H gCOSY NMR spectra confirmed that the antibiotics could be included inside of the lipophilic cavities of CPβCD. The formation of complexation of CPβCD with the antibiotics significantly improved the water solubility. The solubility of the antibiotics linearly increased with the concentration of CPβCD, and the values of the association constant $K_{1:1}$ of the butylparapben/CPβCD and triclosan/CPβCD complexes were 3800 and 3082 M^{-1}, respectively. The results also suggested that it was easier for butylparaben, which had relative smaller molecular size, to form the complexes with CPβCD than triclosan. Due to the targeting effect after the complexation with CPβCD, the antimicrobial activity of butylparaben can be significantly improved. Meanwhile, this improvement effect was not obvious for triclosan.

Keywords: Cationic β-cyclodextrin polymer; Butylparaben; Triclosan; Inclusion complexes; Antimicrobial activity

1. Introduction

Cyclodextrins (CDs) are a series of cyclic oligosaccharides consisting of six to eight glucose units linked by $\alpha - 1, 4$ bonds. Among them, the β-form, consisting of seven glucose units, is the most important and widely used cyclodextrin. The cyclodextrin anatomy takes the form of a toroid or a hollow tapering cone. The internal hydrophobic cavities in the CDs facilitate the inclusion of a wide variety of guest molecules in aqueous solution (Szejtli, 1998).

Over the past decades, β-CD has been successfully applied in many industrial products, technologies and analytical methods. It is generally accepted that in aqueous solutions cyclodextrins form what is called "inclusion complexes" where water molecules located within the lipophilic central cavity are replaced by a lipophilic guest molecule (Loftsson and Duchene, 2007). Hedges (1998) used CDs as drug carriers to improve drug solubility, chemical stability, dissolution and bioavailability or decrease unfavorable side-effects. Mabuchi and Ngoa (2001) used CDs for food and flavors products. The CDs can also be applied in cosmetics (Buschmann and Schollmeyer, 2002), separation processes (Lu et al., 2002), environment protection (Baudin et al., 2000). However, the drawbacks of β-CD, such as poor water solubility (1.85 g/100 ml H_2O) and the relatively small size of the cavity (diameter, 7 Å), limit its applications. To overcome these problems, various CDs derivatives have been developed. Among them, the methylated CDs, hydroxyalkylated CDs and ionic CDs are the typical ones aiming at improving water solubility via disrupting the intermolecular hydrogen bonds between the secondary hydroxyl groups of parent CDs. Such hydrogen bonds are mainly responsible for their low aqueous solubility (Szejtli, 1998).

Cyclodextrin-based polymers are of interest due to their high solubility in water and capability to include hydrophobic compounds with relatively larger molecular size. The polymer

Abbreviations: 2D, two-dimensional; ATP, adenosine 5′-triphosphate; CC, choline chloride; CDs, cyclodextrins; CFU, colony-forming units; CPβCD, cationic β-cyclodextrin polymer; D_2O, deuterium oxide; EP, epichlorohydrin; FDA, Food and Drug Administration; FT-IR, Fourier transform infrared spectroscopy; GPC, gel permeation chromatography; ^{1}H–^{1}H gCOSY NMR, proton–proton gradient correlated spectroscopy nuclear magnetic resonance; LB, Lunia–Bertani; MIC, minimal inhibition concentration; PBS, phosphate aqueous saline; TAPPI, Technical Association of the Pulp and Paper Industry; UV, ultraviolet.

* Corresponding author. Tel.: +1 506 4533532; fax: +1 506 4533591.
E-mail address: hxiao@unb.ca (H. Xiao).

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effect on the formation of complexes with large substrates was caused by the cooperation of two adjacent β-CD moieties on a polymer chain and this conclusion is supported by an inclusion study using the model dimmers of β-CD (Harada et al., 1978, 1980). In our previous study (Li et al., 2004), a range of novel cationic β-CD polymers (CPβCD) were successfully synthesized and studied as drug carriers. It was found that CPβCD of high molecular weight and low cationic charge density exhibited good drug inclusion and dissolution abilities.

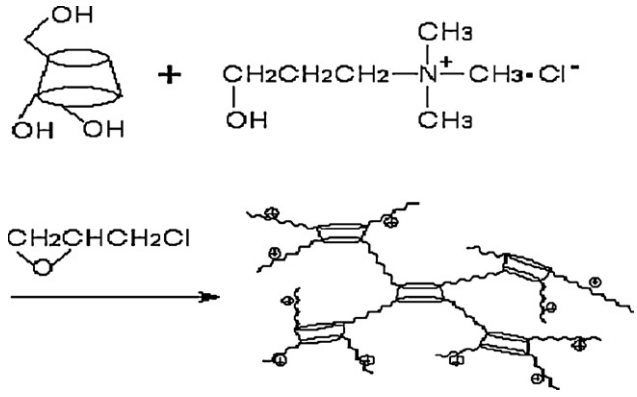
In this work, the CPβCD was used to form inclusion with antibiotics complexes and their structure and effects on antimicrobial activities were investigated. There were two antibiotics used in this work. One is triclosan (2,4,4′-trichloro 2′-hydroxydiphenyl ether), another is butylparaben (*p*-hydroxybenzoic butyl ester). They are non-ionic, white, odorless and tasteless crystalline powders. They can inhibit growth of a wide range of bacteria, yeast and molds. The antimicrobial mechanism of triclosan is to block the synthesis of lipids and inhibit the enzyme enoyl-acyl carrier protein reductase (Heath et al., 2000; McMurry et al., 1998), while the mechanism of butylparaben may be linked to the mitochondrial depolarization depletion of cellular ATP (adenosine 5′-triphosphate) through uncoupling of oxidative phosphorylation (Soni et al., 2001). Both of the antibiotics have very low toxicity and are FDA (Food and Drug Administration) approved. They have been widely used as disinfecting active ingredients in cosmetic, drug and food industry. A drawback which limits the application of these non-ionic compounds is their extremely low water solubility (only about 10–20 mg/l).

The key objective of this work was to prepare and characterize novel antibiotic inclusion compounds or complexes which could render cellulose products with antimicrobial property. Both triclosan and butylparaben have the right size and are suitable to be included in β-CD. By forming the complexes with cationic β-CD polymers, their water solubility can be significantly improved. Another advantage we choose CPβCD is that the cationic charge could help the adsorption or retention of the antibiotic complexes on negatively charged wood fibers.

2. Experimental

2.1. Materials

β-Cyclodextrin (β-CD), epichlorohydrin (EP) and choline chloride (CC) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Triclosan and butylparaben were obtained from Fluka Chemika (Oakville, ON, Canada). All reactants were used as received without further purification. The bleached sulfite pulps were supplied by Fraser Papers in Edmundston, New Brunswick, Canada. The received pulps were cleaned with distilled water for three times prior to being stored in a refrigerator at a high consistency (25.7 wt%), and used as stock suspensions. Deionized-distilled water was used throughout.



Scheme 1. Synthesis of CPβCD.

2.2. Preparation of cationic β-cyclodextrin polymer and complexes with antibiotics

The cationic β-cyclodextrin polymer (CPβCD) was synthesized by one-step condensation as described in our previous work (Scheme 1) (Li et al., 2004). In this work, the molar ratio of the reactants (β-CD/EP/CC) was fixed at 1/5/2. The specific procedure was as follows: 1 g of NaOH was dissolved in 20 ml of water, and then 5.675 g of β-CD were dissolved in the sodium hydroxide solution. The solution was magnetically stirred at room temperature for 24 h. Then, 1.396 g of CC was fed into the solution rapidly and 2.313 g of EP were added at a flow rate of about 0.1 ml/min. After the completion of EP feeding, the mixture was heated to 60 °C and kept stirring at 400 rpm for 6 h. The polymerization was stopped by neutralizing with an aqueous hydrochloride acid solution (1N). The solution obtained was dialyzed for 24 h with a dialysis membrane (Spectra/Por 6, molecular weight cut-off 1000) to remove unreacted EP and CC. The solution obtained was evaporated and the solid was pulverized to powder for further testing.

The triclosan/CPβCD or butylparaben/CPβCD complexes were prepared by adopting the procedures described by Arias et al. (1997) and Mura et al. (2002). Equimolecular of antibiotics and CPβCD were mixed together then manually ground using a mortar with a pestle for 10 min, in condition leading to the best yield and to the most stable complexes.

2.3. Characterization

Molecular structure was analyzed by Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (NMR). FT-IR spectra were obtained using a NEXUS 470 spectrophotometer [Nicolet Thermo Instruments (Canada) Inc.]. The samples were mixed with potassium bromide and pressed into small transparent disks, and scanned at frequencies from 400 to 4000 cm^{-1} and the number of scan times was 32. The resolution was set at 2 cm^{-1}.

All proton detection experiments including one-dimensional ^1H NMR (300 MHz) and two-dimensional (2D) ^1H–^1H homonuclear gradient correlated spectroscopy (gCOSY) NMR spectra of the samples in deuterium oxide (D_2O) were conducted using a Varian Unity 400 spectrometer operated at 25 °C.

To eliminate the interference of moisture, all the samples and NMR tubes were vacuum dried at 80 °C over 8 h before analysis. Molecular structure and composition were also investigated via C/N element analysis by a LECO CHN-600 elemental analyzer.

Gel permeation chromatography (GPC) (Pump: Waters 600E System Controller; Detector: Waters 410 Differential Refractometer) with Ultrahydrogel 250 and 500 columns was used to determine the molecular weights. The tests were carried out at 40 °C and the flow rate at 0.7 ml/min. A 0.05 M sodium sulfate aqueous solution was used as an eluent. Calibration was made using standard Pullulan samples from P-5 to P-1600 (i.e., molecular weights ranging from 5.8×10^3 to 1.66×10^6). β-Cyclodextrin ($M_w = 1135$) was also used as a standard sample for the calibration. Each sample solution (0.2 wt%) was filtered with a 0.45 μm Nylon Cameo filter-syringe prior to the test.

2.4. Phase-solubility studies

The solubility of cationic β-CD polymers was measured at 20 °C as follows: An adequate amount of sample was added to 5 ml of water. After equilibrium was reached, the remaining solid was filtrated off using a 0.45 μm Nylon Cameo filter-syringe. The filtrate was dried in an oven for sufficient period until a constant weight being reached. The solubility was estimated in terms of the weight of samples in the saturated solution and solution volume. Three repeats were conducted.

Solubility of antibiotics included in CPβCD was measured at 20 °C by adding excessive triclosan or butylparaben into 10 ml CPβCD solution of different concentration (0.5–10.0 wt%). The mixtures were shaken in a water bath shaker at 150 rpm for overnight. After the equilibration, an aliquot was withdrawn and filtered (pore size 0.45 μm). The concentration of triclosan or butylparaben was determined by measuring the ultraviolet (UV) absorbance (GENESYS^{TM} 10S Spectrophotometer, Thermo Electron Co.) of the saturated solutions at a wavelength of 282 or 256 nm and compared with the calibration curve.

2.5. Antimicrobial activities

The antimicrobial activities were tested against *E. coli* ATCC 11229.

The minimal inhibition concentration (MIC) of antimicrobial agents was measured using the serial dilution method. The antibiotics/CPβCD complexes were dissolved in water with the concentration from 0.10 to 2000 ppm.

Shaking flask method is a kind of quantitative test and used for the evaluation of the antimicrobial activity of textile products. The procedure is as follows: 0.10 g paper scraps and 5 ml bacterial culture (10^6 CFU/ml) were mixed and shaken at 200 rpm at 37 °C for a certain time, e.g. 1 h. After shaking, different dilutions (10^{-1}, 10^{-2}, ..., 10^{-6}) were made by successively adding 1 ml culture into 9 ml PBS (phosphate aqueous saline, pH 7.4). Then 0.1 ml of this culture was seeded on a layer of LB (Lunia–Bertani) agar in a Petri dish. The plates were put into an incubator at 37 °C for over 24 h. The number of colonies was counted and three repeats were carried out for each sample. The inhibition of cell growth can be quantified by comparing with the negative control:

$$\text{growth inhibition of cell (\%)} = \frac{A - B}{A} \times 100$$

where A and B are the number of the colonies detected from the control and treated samples, respectively.

Handsheets of the sulfite pulps adsorbed with antibiotics were prepared according to TAPPI Test Methods T205 for the antimicrobial tests. The content of antibiotics on the cellulose fibers was determined by measuring the difference of antibiotics concentration before and after adsorption using a UV spectrophotometer. A typical handsheet-making procedure was as follows: 24 g wood fibers (oven dried) in 2 l water were disintegrated in a standard disintegrator. The slurry was then further diluted to 8 l with water, leading to 0.3% fiber consistency. Four hundred millilitres of the 0.3% slurry were used for each handsheet to be formed. After two steps of pressing at 345 kPa (first for 5 min and second for 2 min), the handsheets with grammage of 60 g/m^2 were conditioned over 3 days at a temperature of 20 ± 2 °C and relative humidity of 50 ± 2%.

2.6. AFM analysis

Atomic force microscope (AFM) was used to investigate the morphology of *E. coli* prior to and after treated with antibiotics solutions. The samples were prepared by depositing a drop of bacteria suspension (10^3 CFU/ml) on a clean silicon wafer (Universitywafer, 66 N. St., South Boston) and semidried at room temperature. AFM measurements were performed using a Nanoscope IIIa from Veeco Instruments Inc., Santa Barbara, CA. The images were scanned in Multimode mode in air using a commercial silicon-tapping probe (NP-S20, Veeco Instruments) with a resonance frequency of about 273 kHz.

3. Results and discussion

3.1. Synthesis and characterization of CPβCD

The total conversion of reactants, determined by mass balance, was 46.21 wt%. Fig. 1 shows the molecular weight and distribution of synthesized CPβCD. Its molecular structure of was characterized by FT-IR and ^{1}H NMR (as shown in Figs. 2–4). There were 42.51 wt% of C and 0.76 wt% of N in the CPβCD according to the element analysis. Based on the results of element analysis and ^{1}H NMR, the content and conversion of CC can be further calculated. There were 7.52 wt% of CC in the final product and the conversion of CC was 23.57%—much lower than that of total reactants. Because the hydroxyl group ratio of β-CD to CC was 7:2 in the reaction system, more EP was consumed by β-CD. Therefore, the conversion of CC was relatively lower than the other reactants. The content of β-CD in the final products calculated as above is 83.17 wt%. Extending reaction time from 2 to 6 h generated the CD polymers with the molecular weight ($M_n = 3270$) higher than the one we synthesized previously (CPβCD 5_1.5, $M_n = 2320$) (Li et al., 2004). Meanwhile, the water solubility of new sample is 41.26 g/100 ml which is almost 22.30 times greater than that of unmodified β-

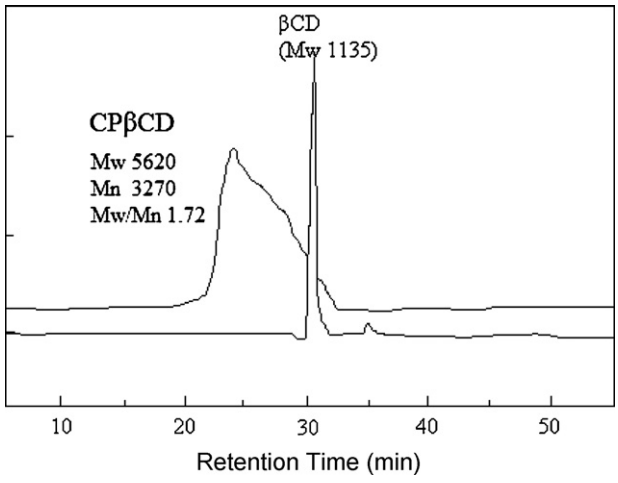


Fig. 1. GPC profiles of CPβCD and β-CD.

CD and similar to our previous report. However, it should be acknowledged that the molecular weight of CPβCD determined via GPC may be not an actual molecular weight. Because the standard samples (Pullulan and β-CD) for GPC calibration curve are non-ionic polymers, their hydrodynamic volume may differ from that of the cationic CPβCD of the same mass.

3.2. Antibiotics/CPβCD complexes

The formation of antibiotics/CPβCD complexes was confirmed by FT-IR spectra as shown in Fig. 2. As for CPβCD, the bands at 1023 and 1080 cm^{-1} are due to the coupled C–C and C–O stretching vibrations; and the band at 1154 cm^{-1} is attributed to the antisymmetric stretching vibration of the C–O–C glycosidic bridge. It is different from the spectra of

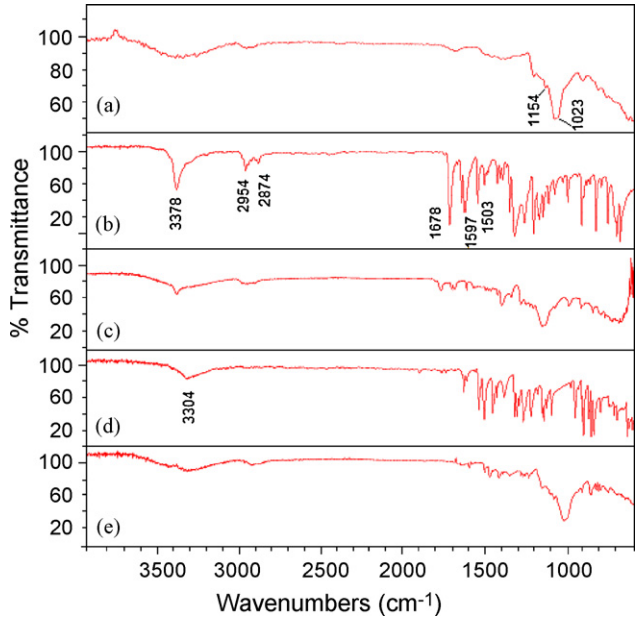


Fig. 2. FT-IR spectra in the region of 4000–500 cm^{-1}: (a) CPβCD; (b) butylparaben; (c) butylparaben/CPβCD complex (12.5 wt% of butylparaben); (d) triclosan; (e) triclosan/CPβCD complex (17.5 wt% of triclosan).

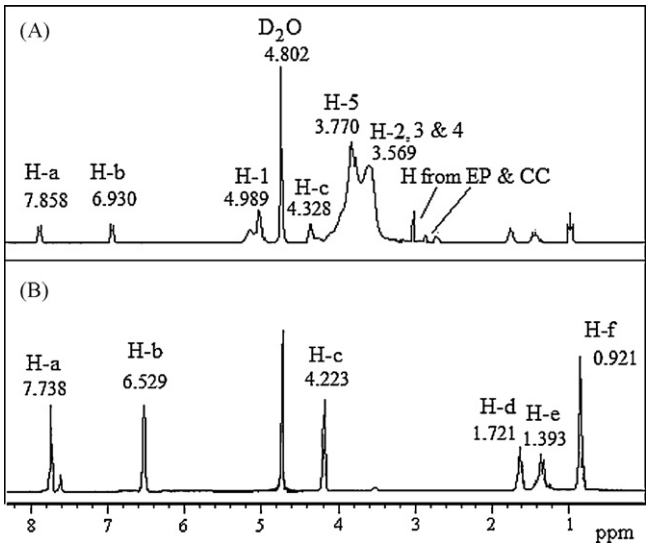


Fig. 3. ^{1}H NMR spectrum: (A) butylparaben/CPβCD complex (2.5 wt% of butylparaben) and (B) butylparaben.

antibiotics, which are characterized at 1597, 1503, 1470, 750 and 670 cm^{-1} (benzene rings), and 3330 cm^{-1} (phenol hydroxyl group). The main difference of FT-IR spectrum between triclosan and butylparaben occurs at 1678 cm^{-1} ($\nu_{C=O}$, carboxylic group in butylparaben), 2954 and 2874 cm^{-1} (ν_{C-H} methyl and methane groups in butylparaben), and the fingerprint peaks. As for the complexes, the attenuation of the antibiotics peaks can be obviously observed. These results confirmed the inclusion of the antibiotics in the cavities of CPβCD.

^{1}H NMR is another powerful tool to investigate the formation of the Antibiotics/CPβCD complexes. The chemical structures of the compounds are shown in Scheme 2. The ^{1}H NMR spectra

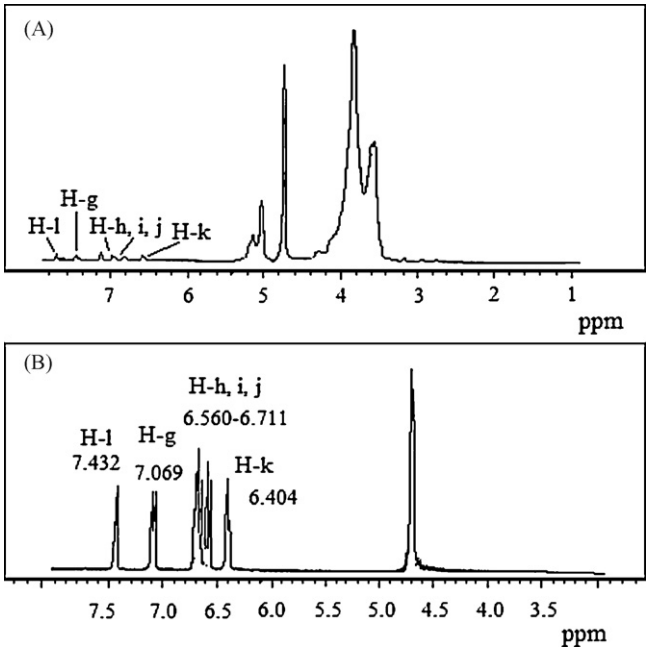
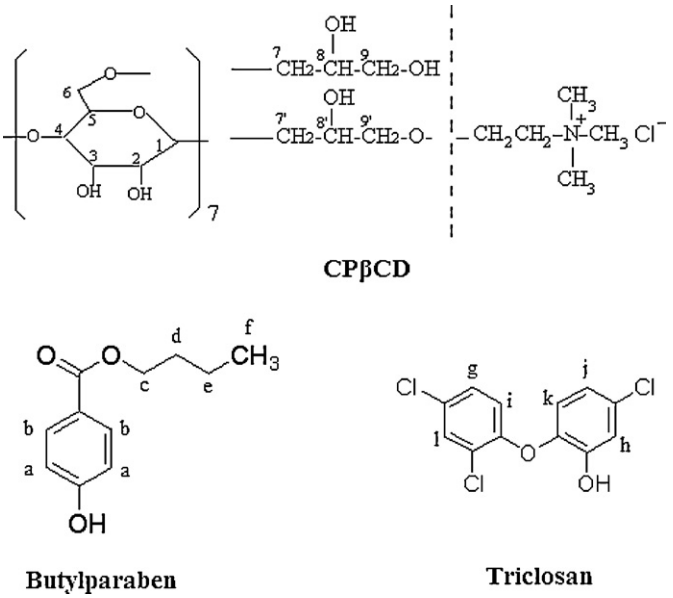


Fig. 4. ^{1}H NMR spectrum: (A) triclosann/CPβCD complex (2.5 wt% of triclosan) and (B) triclosan.



Scheme 2. The chemical structures of the polymer and antibiotics.

are shown in Figs. 3 and 4. The chemical shifts of the samples are listed in Table 1. It can be seen after inclusion, there were slightly downfield chemical shifts of the protons (H-1–H-5) in the CPβCD, while the chemical shifts of corresponding protons of butylparaben or triclosan increased. The results indicated that the environment of these protons was changed after the inclusion of antibiotics with the CPβCD.

To obtain further information of these complexes, two-dimensional (2D) NMR gradient spectroscopic technique (gCOSY) was employed. 2D NMR is highly suited for the characterization of formation of complexes because of the increased resolution offered by spreading chemical shift information in two spectral dimensions. The ^{1}H–^{1}H gCOSY spectra not only reveal the connectivity between neighboring protons in the compounds, but also display the interaction between the guest and the host molecules in the complexes. Figs. 5 and 6 present the 2D ^{1}H–^{1}H gCOSY spectra of butylparaben/CPβCD and triclosan/CPβCD, respectively. The cross-peaks in the spectra can be divided in two groups, originating from the neighborhood of the protons in the CPβCD and the interaction of the protons between the antibiotics and the CPβCD. The cross-peaks in the dashed boxes in Fig. 5 indicate a very close interaction of CPβCD (3.5–3.8 ppm, H-2–H-5) and butylparaben (0.9–1.6 ppm, H-d, e, f; 6.9–7.8 ppm, H-a, b.). Similar results can be found in Fig. 6, which demonstrate the interaction of CPβCD (3.5–3.8 ppm, H-2–H-5) and triclosan (6.9–7.7 ppm, all five aromatic protons). The results confirmed that both the hydrophobic benzyl rings and alkyl groups of the antibiotics were included in the cavities of CPβCD.

Table 1
^{1}H chemical shifts of the antibiotics, CPβCD and their complexes

Proton	CPβCD	Butylparaben	Butylparaben/CPβCD	Triclosan	Triclosan/CPβCD
H-1	5.115		4.898		5.005
H-2	3.676		3.569		3.567
H-3	3.561		3.552		3.532
H-4	3.553		3.489		3.521
H-5	3.808		3.770		3.789
H-a		7.738	7.858		
H-b		6.529	6.930		
H-c		4.223	4.328		
H-d		1.721	1.771		
H-e		1.393	1.413		
H-f		0.921	0.920		
H-g				7.069	7.107
H-h				6.711	6.952
H-i				6.648	6.924
H-j				6.560	6.788
H-k				6.404	6.561
H-l				7.432	7.694

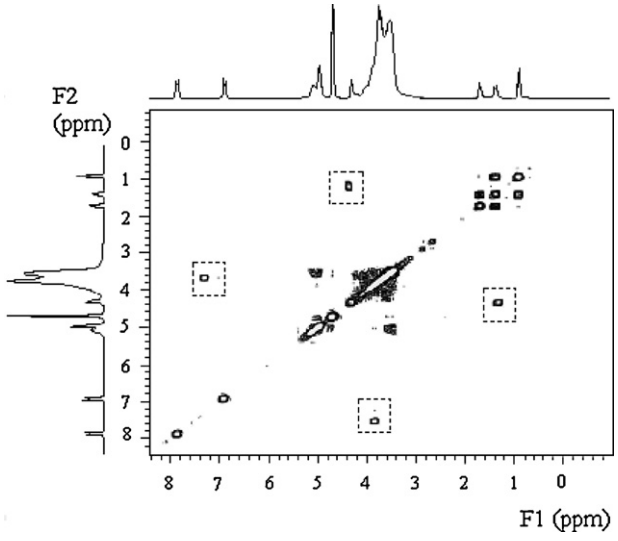


Fig. 5. 2D gCOSY spectrum of butylparaben/CPβCD (2.5 wt% of butylparaben) complex in D_2O at 25 °C.

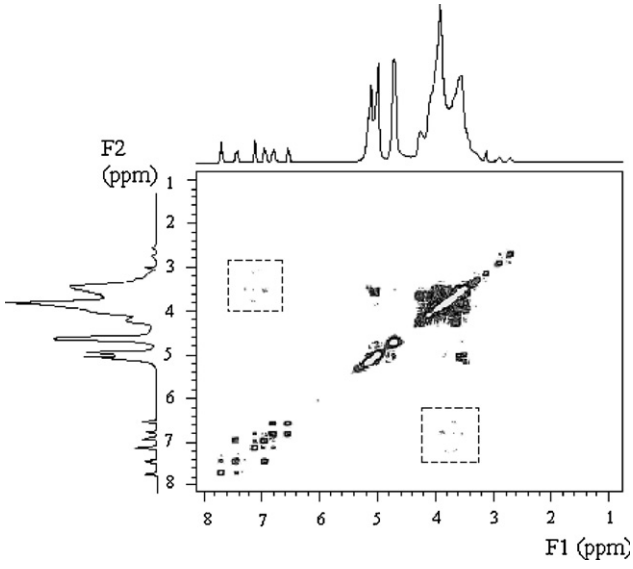


Fig. 6. 2D gCOSY spectrum of triclosan/CPβCD (2.5 wt% of triclosan) complex in D_2O at 25 °C.

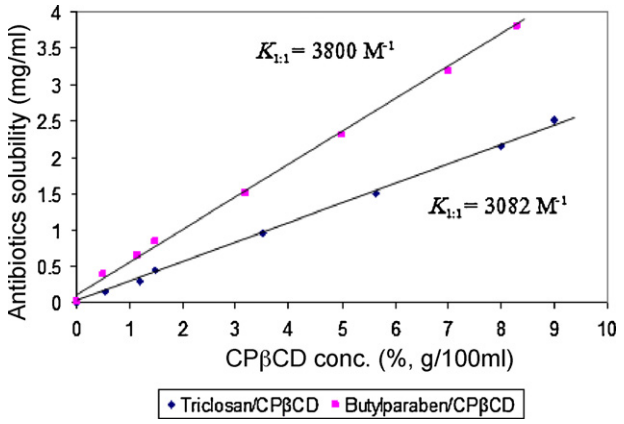


Fig. 7. Phase-solubility plots of butylparaben and triclosan in aqueous CPβCD solution at room temperature (20–22 °C).

3.3. Phase-solubility studies

The phase-solubility diagrams of butylparaben and triclosan in aqueous CPβCD solutions are shown in Fig. 7. As can be seen, the water solubility of butylparaben and triclosan is linear increased with the CPβCD concentration. Their extremely low water solubility (about 20 mg/l for butylparaben, and 10 mg/l for triclosan at 25 °C) can be significantly increased by inclusion with CPβCD. For example, at the CPβCD concentration of 8% (g/100 ml), the water solubility of butylparaben or triclosan was inceased to 7.1 g/l or 4.3 g/l—almost about 355 and 430 times higher than their respective original values.

The continuous variation method was used to determine the formation and stoichiometry of the inclusion complexes. The linear plots of Fig. 7 indicate that the formation of 1:1 complexes occurs in an extended range of concentration of CPβCD. Macomer's model (Bardelang et al., 2004, 2005), was employed to evaluate the association constant $K_{1:1}$ as following:

$$K_{1:1} = \frac{[HG]}{[H][G]}$$

whereas [G], [H] and [HG] were the molar concentration of guest, host and complex at equilibrium, respectively. In Fig. 7, the stability constant $K_{1:1}$ can be calculated from the slope S_t and intercept S_0 of the initial straight line portion of the diagram in terms of the equation (Higuchi and Connors, 1965):

$$K_{1:1} = \frac{S_t - S_0}{S_0 \times \{[CD]_t - (S_t - S_0)\}}$$

In the equation above, the molar concentration of β-CD repeat unit was used as the "host" instead of the entire macromolecules (CPβCD). The values of the association constant $K_{1:1}$ of the butylparapben/CPβCD and triclosan/CPβCD complexes are 3800 and 3082 M^{-1}, respectively (calculated according to the molecular weight of β-CD repeating unit and its content in the CPβCD as described previously). Because the molecular size of butylparaben (M_w = 194) is smaller than that of triclosan (M_w = 289.5), it is easier for butylparaben to enter the cavities of CPβCD and form inclusions than triclosan. The improvement of water solubility and $K_{1:1}$ of butylparaben is more obvious than that of triclosan.

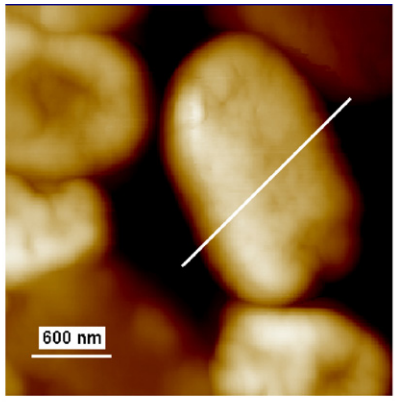
Table 2
MIC results against *E. coli*. ATCC 11229 (ppm)

Sample	MIC (ppm)
CPβCD	>2000
Butylparaben*	20.31
Triclosan*	0.24

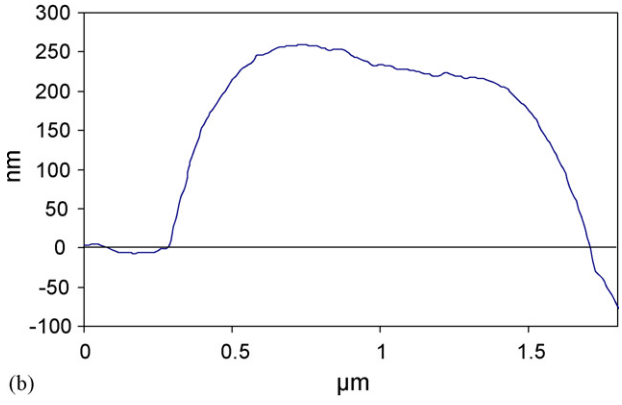
Note: the antibiotics were complexed with CPβCD prior to testing.

3.4. Antimicrobial activities

Both butylparaben and triclosan are effective antibiotics and have been extensively applied. The MIC results against *E. coli* of their complexes with CPβCD are listed in Table 2. The CPβCD was used as a control sample and its MIC was higher than 2000 ppm. It indicates that the antimicrobial activity of CPβCD itself is very weak and can be ignored. The results also confirm that triclosan is a much more effective antibiotic compound than butylparaben. The MIC value of triclosan (0.24 ppm) is according with the other's reports (0.13–0.39 ppm) (Singh, 2006; Li et al., 2002), whereas the MIC value of butylparaben (20.31 ppm) is much lower than the other's results (120–4000 ppm) (Pauli and Schilcher, 2004; Mizuba and Sheikh, 1987; Hasegawa et



(a)



(b)

Fig. 8. AFM images and section analysis of untreated *E. coli* (ATCC 11229). (a) AFM image of untreated *E. coli*. (b) Section analysis.

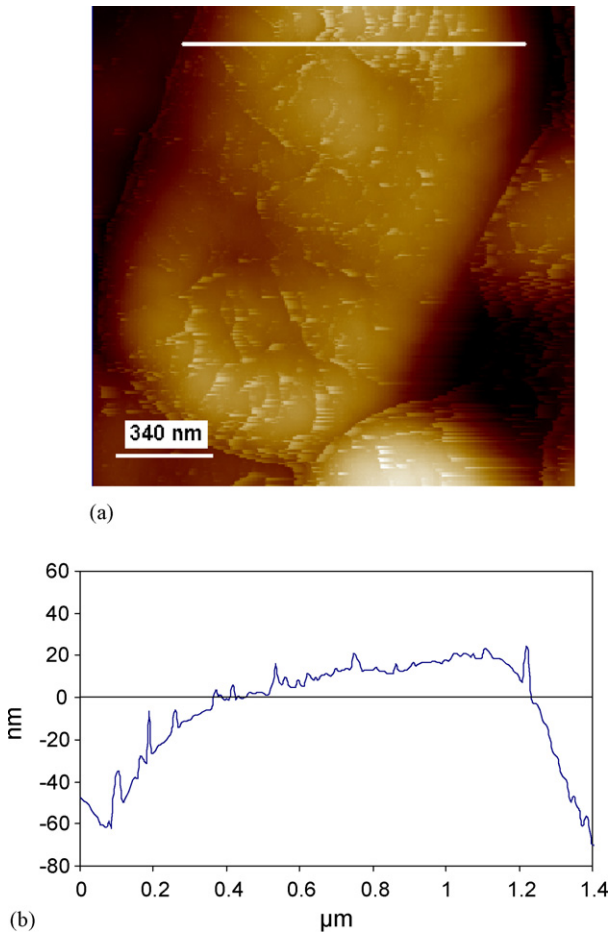


Fig. 9. AFM images and section analysis of triclosan/CPβCD complex solution treated *E. coli* (ATCC11229). (a) AFM image of triclosan/CPβCD complex treated *E. coli*. (b) Section analysis.

al., 2005). It indicates that the antimicrobial activity of butylparaben was significantly improved by included with CPβCD. Because the cell membrane is composed of protein and negatively charged phospholipids, the cationic antibiotics/CPβCD complexes can be effectively adsorbed onto the surface of bacterial membrane via electrostatic attraction. It suggests that the targeting abilities of the antibiotics can be endued by inclusion with CPβCD. This targeting effect can be further confirmed by the AFM images. The AFM images of *E. coli* treated with triclosan and butylparaben complexes are rather similar, and triclosan was selected as a representative. From Fig. 8, it can be seen that the surface of untreated *E. coli* is smooth, while there are many small particles on the surface of antibiotics treated *E. coli* (Fig. 9). From the section analysis (Fig. 9b), it can be found that the size of the particles ranges from 8 to 20 nm. These particles are supposed to be the dried aggregates of antibiotics/CPβCD complexes which retained on the surface of the cells. The antimicrobial mechanism of triclosan or butylparaben is to affect the metabolism of the microorganisms instead of causing the damage of the cell membrane. This AFM image is different from our previous work, in which the damage of the cell membrane can be clearly seen after treated with guanidine polymer solution (Guan et al., 2007). Therefore it

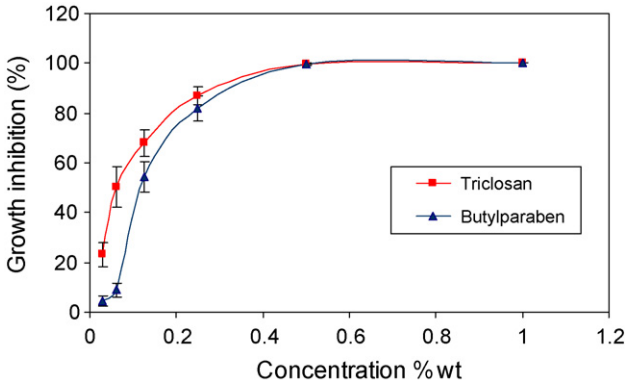


Fig. 10. Effect of antibiotics concentration (based on cellulose fibers) on the growth inhibition of *E. coli* ATCC 11229. Shaking time was fixed at 1 h. The number of colonies of blank control was 2.2×10^6 CFU/ml.

can be concluded that the particles are not the inner components leaked from the bacteria.

The retention of the complexes on the cell surface can explain the improvement of antimicrobial activity of butylparaben. As for triclosan, although the targeting effect still existed, there was no obvious improvement of its antimicrobial activity. The reason maybe due to the extremely low MIC value of original triclosan and the change on the antimicrobial activity became negligible.

In order to investigate the application of these antibiotics/CPβCD complexes on paper products, shaking flask method was employed to assess their antimicrobial activities. The effects of antibiotics concentration and contacting time are, respectively, shown in Figs. 10 and 11. The growth inhibition increased with the increasing of antibiotics concentration or contacting time. As discussed previously, triclosan has higher antimicrobial activity than bytulparaben. Therefore, the inhibition growth of triclosan is higher than that of butylparaben when their concentration is lower than 0.5% (Fig. 10). After that point, the growth inhibition reaches about 100% which indicates almost all the bacteria were killed. However, Fig. 11 shows the opposite trends when the effect of contacting time was investigated. Butylparaben exhibited faster inhibition effect than triclosan when contacting time was shorter than 10 min. After 10 min, the growth inhibition of both antibiotics reached 100%. Again, the molecular

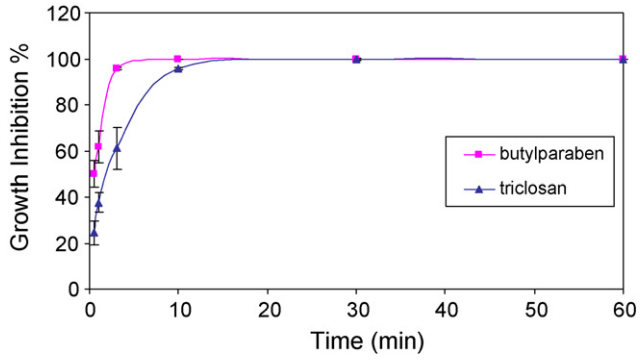


Fig. 11. Effect of contacting time on the growth inhibition of *E. coli* ATCC 11229. The concentration of antibiotics was 0.5% wt based on the cellulose fibers. The number of colonies of blank control was 2.2×10^6 CFU/ml.

size of butylparaben is smaller that that of triclosan, which facilitates butylparaben to enter the cavities of CPβCD and form the complexes. For the same reason, butylparaben is also easier to be released from the inclusions. Therefore, the butylparaben/CPβCD complexes could deactivate the bacteria in a relatively shorter time than triclosan/CPβCD complexes at appropriate concentrations.

4. Conclusions

In this paper, the preparation of two novel antimicrobial inclusion compounds, butylparaben/CPβCD and triclosan/CPβCD was reported. FT-IR and ^{1}H NMR techniques confirmed that the antibiotics could be included inside of the lipophilic cavities of CPβCD. The ^{1}H–^{1}H gCOSY NMR spectra provide further information of the formation of complexes.

The inclusion of complexation of CPβCD with the antibiotics significantly improved the water solubility. The solubility of the antibiotics linearly increased with the concentration of CPβCD, and the values of the association constant $K_{1:1}$ of the butylparapben/CPβCD and triclosan/CPβCD complexes were 3800 and 3082 M^{-1}, respectively. The results also suggested that it was easier for butylparaben, which had relatively smaller molecular size and less complicated steric structure, to form the complexes with CPβCD than triclosan.

Due to the targeting effect after the complexation with cationic CPβCD, the antimicrobial activity of butylparaben can be significantly improved. Meanwhile, this improvement effect was not obvious for triclosan. The excellent antimicrobial results indicate that these antibiotics/CPβCD complexes can be used in paper products and other applications.

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