Modulating drug loading and release profile of β-cyclodextrin polymers by means of cross-linked degree

WANG Qi-fang[1], LI San-ming[1*], ZHANG Yu-yang[2], ZHANG Hong[2]

(*1. College of Pharmacy, 2. School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China*)

Abstract: The purpose of the present study is to use β-cyclodextrin polymers (β-CDP) with different cross-linked degree (CLD) to form inclusion complexes with ibuprofen and examine the effects of structural and compositional factors of β-CDP on its drug loading and release behaviors. A series of β-CDP with different CLD were synthesized and characterized by Fourier Transform Infrared Spectroscopy (FT-IR) and ^{13}C NMR spectrum. The β-CDP was systemically characterized for the relation between the CLD of β-CDP and the drug loading and release as well. The results of FT-IR and ^{13}C NMR showed that similar peak-shaped vibration of β-CDP and β-CD implies that the polymer keeps the original characteristic structure of β-CD. The CLD of the β-CDP played a critical role in the drug loading and release, increasing the CLD resulted in reduction of drug loading, but increase in drug release.

Key words: β-cyclodextrin; β-cyclodextrin polymer; cross-linked degree; drug loading; drug release

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通过交联度控制 β-环糊精聚合物对药物的载入与释放

王齐放[1], 李三鸣[1*], 张予阳[2], 张 弘[2]

(沈阳药科大学 1. 药学院, 2. 生命科学与生物制药学院, 辽宁 沈阳 110016)

摘要: 本研究使用具有不同交联度 (CLD) 的 β-环糊精聚合物 (β-CDP) 为载体与布洛芬形成包合物, 考察 β-CDP 结构和组成对药物载入和释放的影响。通过 FT-IR 和 ^{13}C NMR 确定 β-CDP 结构特征。结果表明在 β-CDP 中仍保留 β-CD 的原有结构特征。β-CDP 的 CLD 是决定药物载入及释放的重要因素, 增加 β-CDP 的 CLD 会使药物的载入量减少, 释放量增大。

关键词: β-环糊精; β-环糊精聚合物; 交联度; 药物载入; 药物释放

β-Cyclodextrin (β-CD) is a torus-shaped cyclic oligosaccharide consisting of seven glucose units linked with a 1, 4-glucosidic bond. Its molecular structure is very special for the outer hydrophilic surface and the hydrophobic cavity with a certain size. The internal hydrophobic cavity in the β-CD facilitates the inclusion of guest molecules[1−3]. Based on this point, β-CD has been used successfully as drug carriers to improve drug solubility, chemical stability, dissolution and bio-availability[4−9]. However, the low water solubility and toxicity of parent β-CD limit their further application in pharmaceutical formulations[10−13]. Recently, the research indicated that many β-cyclodextrin polymers (β-CDP) which have been prepared by cross linking the β-CD with a crosslinking agent retained their original structures and their inclusion abilities[14−17]. Due to the stereo network by many branch points formed in the polymerization process, β-CDP offer numerous advantages including better mechanical strength, good

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*Corresponding author Tel: 86-24-23986258, Fax: 86-24-23986293,
E-mail: li_sanming@sina.com or
oshwang@sina.com

stability and chemical controllability[18, 19]. It has been reported that when β-CDP were used as a drug carrier, their inclusion function was better than β-CD and its derivatives[20−23]. As a compositional measurement for polymers, the cross-linked degree (CLD) might be a key parameter to exhibit the microstructure and macroproperty of the polymer. However, the role of CLD of β-CDP has not been well characterized in its structure, density of the net, content of β-CD monomer, chemistry selectivity, *etc*. We hypothesize that the CLD in β-CDP may play a critical role in the drug loading and drug release. In this paper, we focused primarily on using β-CDP with different CLD as carriers to form inclusion complexes with model drug ibuprofen. The effects of structural and compositional factors of β-CDP on drug loading and releasing behaviors were then investigated.

Materials and methods

Materials β-CD was purchased from Tianjin Bodi Chemical Industry Company (China), and was purified before use. Epichlorohydrine (EPI) was purchased from Tianjin Yuanhang Chemical Industry Company (China). Dialysis bag (M_w cutoff: 3 500) was obtained from Shanghai Green Bird Science & Technology Development Co., Ltd. Other chemicals were purchased from Liaoning Chemical Industry Company (China) and were of analytical grade.

Preparation of the β-CDP As listed in Table 1, β-CD was added into 35% NaOH solution, and dissolved completely, then EPI was added into the solution, of which the temperature was kept at 35 ℃ under stirring. The reaction was stopped after 3.5 h by addition of acetone. After decantation, acetone was removed. The product was neutralized with 6 mol·L^{-1} HCl, followed by removing NaCl using dialysis method. The solution was evaporated and the white precipitate was washed three times using a large amount of acetone, the resulted β-CDP was isolated by filtration and dried in the vacuum[24−26].

Table 1 Constituent feed composition for synthesis of β-cyclodextin polymers (β-CDP). [a]35% (*w*/*w*) NaOH in de-ionized water; EPI: Epichlorohydrine

Composition	β-CDP$_1$	β-CDP$_2$	β-CDP$_3$	β-CDP$_4$	β-CDP$_5$
β-CD /g	11.35	11.35	11.35	11.35	11.35
EPI /mL	3.93	4.71	5.50	6.28	7.06
35% NaOH[a] /mL	10	10	10	10	10
De-ionized water/mL	5	5	5	5	5

Instrument analysis FT-IR spectroscopy measurement was performed on a Swiss Bruker-IFS 55 spectrometer, using the KBr method, and scanned in the wavelength region from 400 to 4 000 cm^{-1}. ^{13}C NMR spectrum was recorded on a Swiss Bruker ARX-300 spectrometer, at room temperature using D_2O as solvent.

Measurement of the cross-linked degree The cross-linked degree (CLD) was defined as the number of EPI molecule linking on each β-CD molecule. Firstly, the content of β-CD in polymer (%, *w*/*w*) was determined using the phenol-sulphuric acid reaction for carbohydrates as previously reported[26]. Based on the method described by reference[27], the CLD can be calculated according to Eq. (1).

$$\mathrm{CLD} = \frac{(1-b-\mathrm{CD}\%)\cdot M_{b-\mathrm{CD}}}{b-\mathrm{CD}\%\cdot M_{\mathrm{EPI}}} \quad (1)$$

Where $M_{\beta\text{-CD}}$ and M_{EPI} are the molecular weight of β-CD and epichlorohydrine, respectively.

Measurement of the molecular weight of β-CDP The molecular weight of β-CDP was determined by viscometry (Ostwald viscosimeter 1 mm×140 mm) in water thermostated at 25 ℃, 0.5% − 3.0% (*w*/*v* in de-ionized water) samples was prepared. The intrinsic viscosity [η] of β-CDP was calculated by Eq. (2)

$$[h] = \lim_{c\to 0}\left(\frac{h-h_0}{h_0 c}\right) \quad (2)$$

Where η_0, η and c are the solvent viscosity, solution viscosity, and concentration of β-CDP, respectively. [η] was determined by extrapolating the linear portion of the reduced viscosity *vs* concentration plot to the zero concentration. From the value of [η], the viscosity average molecular weight, $\overline{M}_v$, was calculated using the Mark Houwink-Sakurada (MHS) equation (3).

$$[\eta] = k\,(\overline{M}_v)^{\alpha} \quad (3)$$

The values of the MHS parameters, k and α, were taken as 1.3×10^{-2} and 0.76, respectively, from the literature[28].

Drug loading Ibuprofen (IBU) used as a model drug was dissolved with a small amount of alcohol, then slowly added into β-CDP aqueous solution at 70 ℃ under stirring for 30 min. The sample was cooled to the room temperature and continued stirring for 6 h. After being refrigerated for 48 h, the precipitation was filtered. The IBU in the filtrate was collected and measured by HPLC. The HPLC system consisted of a pump (LC-10AD, Shimadzu), an UV-V is spectrophotometric detector (SPD-10AVP, Shimadzu) operating at a wavelength of 225 nm and a reverse-phase column

(C_{18}, 15 cm × 4.6 mm). The mobile phase, acetonitrile/water (65 ∶ 35), was pumped at a flow rate of 1.0 $mL \cdot min^{-1}$.

Drug release Fifty milligram of sample (drug loaded β-CDP) was sealed in a dialysis bag, which was followed by incubating in 250 mL of release medium (pH 7.4 phosphate buffer solution under mechanical shaking at 60 $r \cdot min^{-1}$) at 37 ℃. Five millimeters of the release medium was collected at predetermined time intervals, and then filtered through a 0.45 μm membrane filter. Same volume of fresh medium was added. The amount of IBU released into the removed solution was analyzed by HPLC method. The data were expressed as means of triplicate. The recovery was 98.02% with RSD of 1.5%.

Results and discussion

1 Preparation of the β-CDP

Preparation of β-CDP was achieved by the reaction of β-CD with EPI in an alkaline medium by a two step procedure. The possible reactions are presented in Figure 1. First the β-CD was stirred with an excess of NaOH in order to form alcoholate sites. Then, EPI was added to the suspension obtained. Hydroxyl groups can react with one reactive group of the EPI. The side chain obtained can further react, the epoxyde ring can react with another hydroxyl group of a second β-CD molecule, resulting in a glyceryl bridge connecting two β-CDs.

The property of the obtained β-CDP depends on many factors such as the molar ratio of β-CD to EPI, the concentration of NaOH and the reaction temperature, and so on. Hydrosoluble polymer was obtained with the molar ratio of β-CD to EPI lower than 1 ∶ 10, while water insoluble polymer was formed as the molar ratio was higher than 1 ∶ 10[24, 25]. In this study, in order to obtain several β-CDP having adequate CLD, the molar ratios of β-CD to EPI were adjusted to 1 ∶ 4.5, 1 ∶ 6, 1 ∶ 7.5, 1 ∶ 9 and 1 ∶ 10, the reaction being carried out in 35% NaOH at 35 ℃. The properties of β-CDP obtained are summarized in Table 2.

Table 2 Characteristic parameters of β-CDP. $n = 3$, $\bar{x} \pm s$. [a]β-CD contents were determined by colorimetric method. [b]Molecular weights were determined by viscometry. CLD: Cross-linked degree

Polymer	CLD	β-CD content[a]/%	M_v[b]×10^3
β-CDP_1	3.3	85.56 ± 0.33	9.36 ± 1.05
β-CDP_2	4.1	82.67 ± 0.04	10.63 ± 1.31
β-CDP_3	4.9	79.97 ± 0.26	12.81 ± 0.13
β-CDP_4	6.3	75.65 ± 0.41	13.70 ± 0.77
β-CDP_5	7.3	72.83 ± 0.19	13.84 ± 0.63

As can be seen from Table 2, a series of polymers was prepared with different CLD and M_v, and a slight increase of both CLD and M_v is observed increasing the molar ratios of β-CD to EPI (as noted from Table 1). Moreover, β-CD content in the polymer is influenced by β-CD to EPI initial ratio, while β-CD content in the polymer decreased for higher initial β-CD to EPI ratio. For reasons discussed, initially the β-CD reacts with EPI molecules to produce β-CDP of low molecular weight, which contain a maximum of the β-CD, then, both M_v and CLD of β-CDP increase, EPI displays a tendency to homoploymerize and to increase the size of the arms. The consequence allows a higher probability of oligomer linkage together. The proposed structure is probably a hyperbranched structure made of short chain and long chain branches, and loop of β-CD molecular segments.

2 IR and NMR analysis

The structures of β-CDP were characterized used β-CD as monitored by FT-IR. As shown in Figure 2 (a), the characteristic bands of O–H appear strongly at nearly 3 381 cm^{-1} ($\nu_{s(O-H)}$: O–H of stretching) and 1 649 cm^{-1} (O–H of plane bending), and the characteristic bands of β-CD in the β-CDP are observed at 1 028–1 157 cm^{-1} ($\nu_{s(C-O)}$: C–O and C–O–C stretching)[29]. Compared with β-CD in the Figure 2 (b), the corresponding

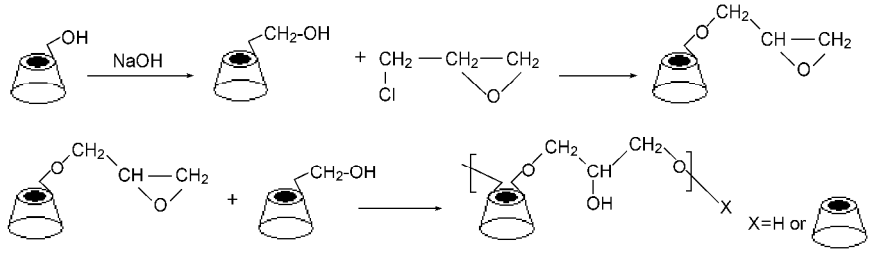

Figure 1 Schematic mechanism of formation of β-CDP

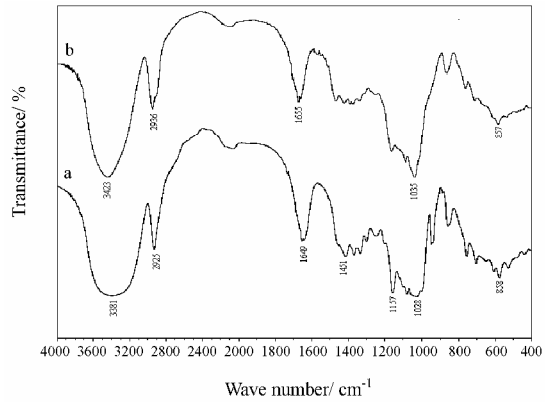

Figure 2　The FT-IR spectra of *β*-CDP (a) and *β*-CD (b)

characteristic peaks of the *β*-CDP undergo blue shifts, which are 42 cm^{-1} for the O–H stretching vibrations, 6 cm^{-1} for the plane bending of O–H and 7 cm^{-1} for the *β*-CD rings stretching, indicating that *β*-CD rings are indeed bond to one or more electric-donor groups such as the glycerol tail.

^{13}C NMR spectrum analysis provides a very sensitive method for determining the substitution site. The spectra of *β*-CDP and *β*-CD are shown in Figure 3. As shown in Figure 3 (b), the signal assignments of *β*-CD were 96.7 (C-1), 70.3 (C-2), 70.8(C-3), 76.6 (C-4), 69.5 (C-5) and 62.4 (C-6), respectively. Except for the signals of *β*-CD denoted in Figure 3 (a), several new signals appear at 67.8 (C-7), 66.7 (C-8), and 61.8 (C-9), respectively, which confirmed the combination of EPI onto *β*-CD. Resonances C-2', and C-3' are the result of C-2 and C-3 substitution. These substitutions produce a downfield chemical shift (～5) of the carbon involved in the substitution, whereas a small up field shift is observed for the carbon (C-1'). Resonance C-6' is the result of substitution taking place at C-6. A downfield chemical shift about 8 is produced. Resonance C-9 is due to the terminal carbon of the 2-hydroxyproyl ether segment. Other substituent carbon atoms have chemical shift corresponding to the peak ⓐ and ⓑ of Figure 3 (a). The assignments C-8' and C-9' show the existence of the glycidyl group which can be responsible for gelation of the polymer. The present results permit us to propose a mechanism of polycondensation. We can conclude that the final structure depends on the position of the OH groups of *β*-CD molecules where the substitutions occur.

3　Drug loading and drug release

IBU was selected as the model drug and carried by *β*-CDP_1−*β*-CDP_5. The drug loading and release were examined. As show in Figure 4, the IBU loaded in *β*-CDP_1−*β*-CDP_5 was 13.66%, 11.14%, 8.32%, 7.35% and 5.56%, respectively, and the cumulative release of IBU at 37 ℃ for 24 h from *β*-CDP_1−*β*-CDP_5 was 79.36%, 83.40%, 86.00%, 87.80% and 90.16%, respectively.

It is quite interesting that CLD of the *β*-CDP strongly influenced the IBU loading and release. As noted from Figure 4, increasing the CLD resulted in decrease in drug loading and increase in the drug release. The reason may be due to different network

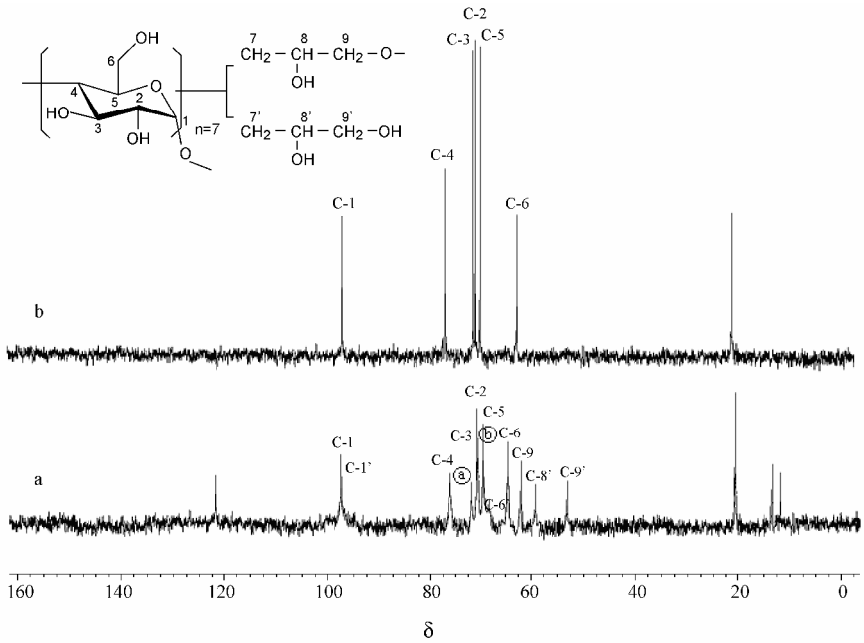

Figure 3　^{13}C spectra of *β*-CDP (a) and *β*-CD (b) in D_2O.　ⓐ = C-2', C-3';　ⓑ = C-7, C-7', C-8

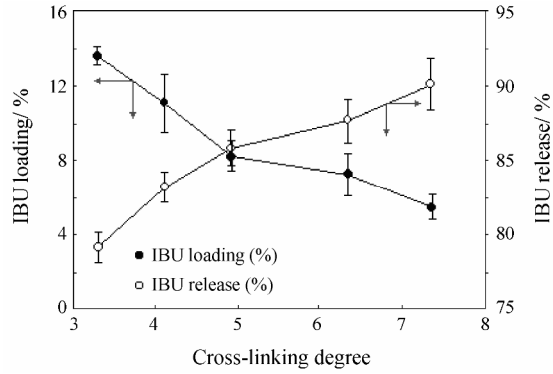

Figure 4 Effect of cross-linked degree on ibuprofen (IBU) loading and release. The release was in phosphate buffer solution (pH 7.4) at 37 ℃ for 24 h

densities of β-CDP, and contents of β-CD conjugated. The β-CDP with low CLD had a loose density of network and high content of β-CD, which led to a strong interaction between the drug and hydrophobic cavity of β-CD thus increased the drug loading. The strong interaction may also cause decrease of drug release. On the other hand, the β-CDP with higher CLD had a low content of β-CD and many grafted EPI chains, so that the network became tight and the channel became small, which increased obstruction to the drug entry, as a result, the drug loading decreased. In this case, most of drug may stay in the network skeleton through weakly acting force between the drug and the β-CDP, which makes the drug easy to diffuse or release, resulting in a higher drug releasing[30−32].

In order to further investigate the influences of CLD on drug release of β-CDP_1, β-CDP_3 and β-CDP_5, the drug release from the polymers was determined at the scheduled time. It can be seen from the curves in Figure 5 that drug release followed the order of β-CDP_5 > β-CDP_3> β-CDP_1 for 12 h.

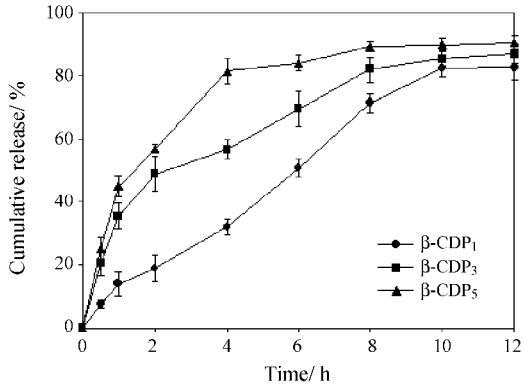

Figure 5 The cumulative release of IBU from β-CDP_1, β-CDP_3 and β-CDP_5 in phosphate buffer solution (pH 7.4) at 37 ℃

As mentioned previously, the release mechanism of IBU from the β-CDP is rather complex. Hereby, as shown in Figure 5, the β-CDP_1 (low CLD) based on higher β-CD content and strong interaction between water insoluble IBU and hydrophobic cavity of β-CD, released the loaded IBU over a period of 10 h; the β-CDP_3 (intermediate CLD) released most of the IBU within 8 h; whereas the β-CDP_5 (high CLD) releasing most of the IBU within 4 h. This is certainly related to structures of β-CDP. As we have known, they have the same guest (IBU) in β-CDP_1, β-CDP_3, β-CDP_5 and thereby the host (β-CDP) structure plays a significant role in IBU release.

Conclusion

β-CDP has two advantages to be used as a drug carrier, the one is that the structural composition itself can be modified by selecting a suitable CLD, the other is that drug loading and release can be controlled by the CLD. Based on this, five β-CDP_s with different CLD were synthesized and the drug loading and release were examined used IBU as model drug. It is found that the CLD plays a significant role in the drug loading and release. The higher the CLD, the lower the drug loading and the larger drug release, and the lower the CLD, the higher the drug loading and the smaller drug release were observed. Therefore, selecting a β-CDP with appropriate CLD is quite necessary for designing and developing novel controlled drug carriers.

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