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International Journal of Pharmaceutics 293 (2005) 165–170
international journal of pharmaceutics
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Preparation of molecularly imprinted cyclodextrin microspheres

Y. Egawa [a,*], Y. Shimura [b], Y. Nowatari [b], D. Aiba [b], K. Juni [b]

[a] *Pharmaceutical Research Laboratories, Nikken Chemicals Co. Ltd., 1-346 Kitabukuro-cho, Ohmiya-ku, Saitama-shi, Saitama, Japan*
[b] *Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado-shi, Saitama, Japan*

Received 21 November 2004; received in revised form 25 December 2004; accepted 26 December 2004

Abstract

Molecularly imprinted cyclodextrins (MI-CDs) are prepared by cross-linking CDs in the presence of a template molecule. The binding ability of MI-CDs to the template molecule is specific; therefore, MI-CDs will prove to be useful materials. In this study, we prepared microspheres of MI-CDs (MSs-MI-CD) in a dimethylsulfoxide/poly(dimethylsiloxane) (PDMS) emulsion, using cholesterol as the template molecule. MSs-MI-CD were prepared under various conditions and were evaluated with respect to their morphology, size, and binding ability. MSs-MI-CD prepared at 65 °C were in an aggregated form; however, we could prepare separated and uniform MSs-MI-CD at 95 °C. The viscosity of PDMS influenced the size of MSs-MI-CD. The mean particle diameters of MSs-MI-CD prepared with 50 and 1000 mm^2/s PDMS were 146 and 43 μm, respectively. The binding ability of MSs-MI-CD to cholesterol was higher than that of non-imprinted microspheres. Cholesterol imprinting also promoted the binding ability to other steroids; however, the increase in binding ability was most remarkable in the case of cholesterol, suggesting that we successfully introduced the cholesterol-specific binding ability into MSs-MI-CD. The novel MSs-MI-CD preparation method is useful and simple, and it will provide opportunities for further studies on the specific binding ability of MI-CDs.

Keywords: Cyclodextrin; Microsphere; Molecularly imprinted polymer; Molecular recognition; Cholesterol; Poly(dimethylsiloxane)

1. Introduction

Cyclodextrins (CDs) are doughnut-shaped cyclic oligosaccharides. They differ from one another in the number of glucopyranose units. Parent CDs contain six, seven, or eight glucopyranose units and are referred to as α-, β-, and γ-CD, respectively. They possess the ability to include guest molecules in their internal cavity. In order to utilize and effectively apply the inclusion ability of CDs, cross-linked CDs have been studied extensively (Suzuki et al., 2002). Although the parent CDs are soluble in water, highly cross-linked CD polymers are insoluble in any solvent. Insoluble CD polymers have been studied as adsorbents, stationary phases in chromatography, and pharmaceutical ingredients (Murai et al., 1997; Pariot et al., 2000, 2002). In

* Corresponding author. Tel.: +81 48 641 2388; fax: +81 48 641 5716.
E-mail address: moguinukuma@yahoo.co.jp (Y. Egawa).

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doi:10.1016/j.ijpharm.2004.12.020

such cases, it is preferable that the CD polymers are uniform and spherical. Spherical CD polymers enable easy handling and better reproducibility of the experimental data in comparison with irregularly shaped polymers.

Recently, molecularly imprinted CDs (MI-CDs) have been reported (Asanuma et al., 1997, 1998, 2000; Hishiya et al., 1999, 2002, 2003; Akiyama et al., 2002). MI-CDs were prepared by cross-linking β-CD with toluene 2,4-diisocyanate (TDI) in the presence of cholesterol as a template molecule. Since β-CDs were cross-linked when they were present as β-CD/cholesterol inclusion complexes, the MI-CDs have three-dimensional structures that bind cholesterol strongly and selectively. MI-CDs are referred to as artificial antibodies and will prove to be useful materials in several fields. However, pharmaceutical application of MI-CDs is difficult because MI-CDs ground with mortar and pestle are irregular in size and shape. In order to extend the application of MI-CDs, we attempted to prepare microspheres of molecularly imprinted cyclodextrin (MSs-MI-CD).

Spherical CD polymers prepared in water/oil emulsion have been previously reported (Murai et al., 1997; Pariot et al., 2000). The internal aqueous solution was highly alkaline, and this alkaline solution was employed to dissociate the hydroxyl group and to increase the solubility and reactivity of CDs. The alkaline internal medium was unsuitable for preparing spherical MSs-MI-CD because charged CDs could not form an inclusion complex. MI-CDs have been prepared in neutral dimethylsulfoxide (DMSO) to form stable CD inclusion complexes. Therefore, a solvent that could disperse DMSO was necessary for preparing MSs-MI-CD. However, since DMSO is amphiphilic, it was not easy to find a solvent that could be used as a dispersing medium. Furthermore, a dispersing medium that had high solvent power for hydrophobic molecules was inappropriate because there was a possibility of the hydrophobic template molecule dissolving in it. By focusing on these aspects, we discovered poly(dimethylsiloxane) (PDMS) to be a suitable dispersing medium for the preparation of MSs-MI-CD.

In this study, we prepared MSs-MI-CD in a DMSO/PDMS emulsion, using cholesterol as the template molecule. MSs-MI-CD were prepared under various conditions of reaction temperature and PDMS viscosity, and they were evaluated with respect to the morphology, size, and binding ability. Additionally, MSs-MI-CD were characterized by elemental analysis and IR spectroscopy. The binding ability of MSs-MI-CD was investigated by the adsorption of various steroids.

2. Materials and methods

2.1. Materials

PDMS of varying viscosities were purchased from Shin-Etsu Chemical Co. Ltd., Japan. Other reagents were purchased from Wako Pure Chemicals Industries Ltd., Japan. β-CDs and cholesterol were dried in vacuo for at least 16 h. DMSO and PDMS were treated with molecular sieves of 4 Å. Water was purified by WATER STILL® (WS-05, Sibata Scientific Technology Ltd., Japan).

2.2. Preparation of microspheres

In the standard procedure, β-CD (0.88 mmol) and cholesterol (0.30 mmol) were dissolved in dry DMSO (10 ml). Subsequently, PDMS (200 ml) was added and stirred with a magnetic stirrer at 800 rpm for 30 min at 95 °C. TDI (9.8 mmol) was added to the DMSO/PDMS emulsion and stirred for 2 h under the same conditions. After the contents of the flask were cooled, they were diluted with acetone and hexane. The resulting microspheres were collected by filtration and washed with hot water, tetrahydrofuran (THF), and hot ethanol. Variations were introduced in the standard procedure with respect to the reaction temperature and PDMS viscosity. Non-imprinted microspheres were prepared without cholesterol and were used as the controls.

In order to compare the binding abilities, MI-CDs were prepared by a previously reported method (Hishiya et al., 1999). β-CD (4.4 mmol) and cholesterol (1.5 mmol) were dissolved in dry DMSO (50 ml) at 65 °C. Subsequently, TDI (28 mmol) was added to the solution. After 2 h, a gel was formed, which was chopped into pieces, washed with acetone, and ground with a mortar and pestle. The polymers were washed with hot water, THF, and hot ethanol.

2.3. Characterization of microspheres

The morphology of microspheres was studied by scanning electron microscopy (Hitachi-S430, Hitachi

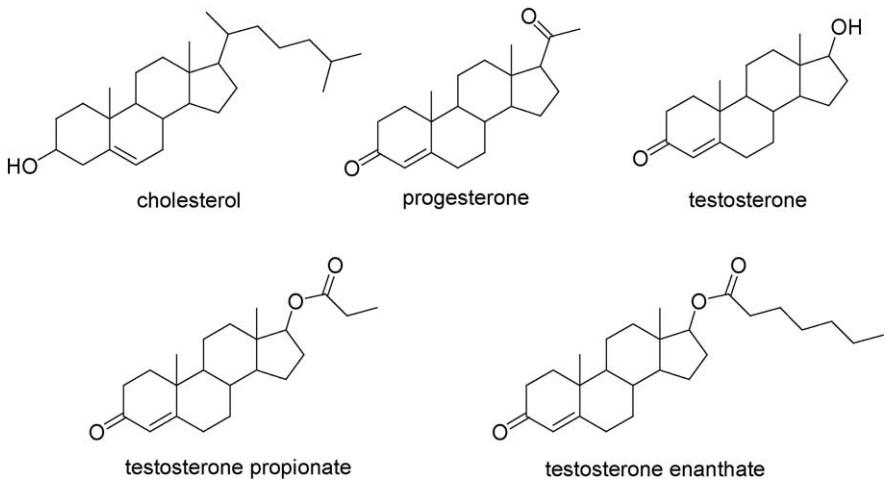

Fig. 1. Chemical structures of steroids used in this study.

Kyowa Engineering Co. Ltd., Japan). The particle size distribution was determined using a laser diffraction particle size analyzer (SALD-1100 (V2.1), Shimadzu Corporation, Japan). IR spectra were measured by the KBr method using a Fourier transform IR spectrometer (JASCO FT/IR-810, JASCO Corporation, Japan). Since only TDI contains nitrogen, the ratios of cross-linked residue to β-CD (TDI/β-CD) were evaluated using an elemental analyzer (CHN Corder MT-6, Yanako Analytical Instruments Corporation, Japan) (Asanuma et al., 1998).

2.4. Evaluation of binding ability

The binding ability of microspheres was evaluated using a previously reported method (Asanuma et al., 1998). In this study, cholesterol, progesterone, testosterone, testosterone propionate, and testosterone enanthate were used as the guest molecules (Fig. 1). Microspheres (1.0 g) were added to 11 ml of water/THF (5/6, v/v) mixture containing guest molecules (0.050 mmol) and stirred at 25 °C for 1 h. The resulting supernatant fluid was analyzed by reversed phase HPLC (LiChroCART 250-4, LiChrosphere 100 RP-18, Merck KgaA, Germany). Cholesterol was measured by UV detection at 216 nm, using an acetonitrile/2-propanol (4/1, v/v) mobile phase. Other guest molecules were detected at 254 nm. Acetonitrile/water (8/2, v/v) was used as the mobile phase for progesterone and testosterone whereas acetonitrile/water (9/1, v/v) was used in the case of testosterone propionate and testosterone enanthate. The binding ability was defined as the mole fraction of guest molecules adsorbed by the CD polymers with respect to the total amount of guest molecules.

3. Results and discussion

3.1. Influence of preparation condition on the morphology and size of microspheres

Previously, it was reported that MI-CDs were prepared at 65 °C (Hishiya et al., 1999). We also attempted to prepare MSs-MI-CD at 65 °C; however, the microspheres aggregated. Subsequently, we changed the reaction temperature from 65 to 95 °C. The increase in the reaction temperature prevented the aggregation of microspheres. MSs-MI-CD prepared at 95 °C were separated and spherical. The smallest mean diameter of MSs-MI-CD was obtained at 95 °C (Fig. 2). After fixing the reaction temperature at 95 °C, the viscosity of PDMS was changed. As the PDMS viscosity increased, the particle size distribution shifted to the smaller region (Fig. 3). Fig. 4 shows scanning electron photomicrographs of the MSs-MI-CD prepared with 50 or 1000 mm^2/s PDMS. The PDMS viscosity influenced the size of MSs-MI-CD. The mean particle diameters of MSs-MI-CD prepared with 50 and 1000 mm^2/s PDMS were 146 and 43 μm, respectively. These results show

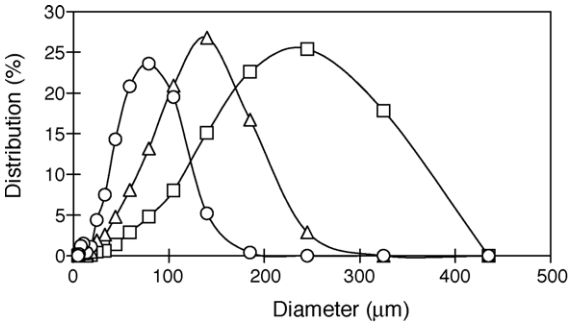

Fig. 2. Particle size distributions of MSs-MI-CD prepared at various temperatures with 100 mm^2/s PDMS: (□) 55 °C; (△) 75 °C; (◯) 95 °C.

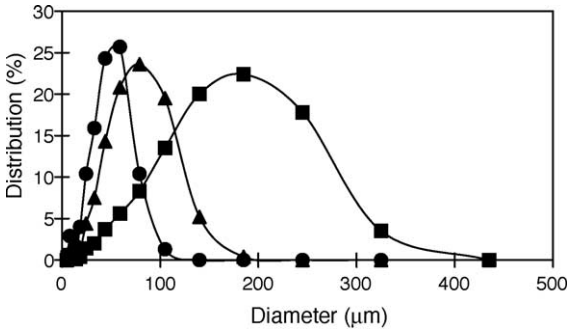

Fig. 3. Particle size distributions of MSs-MI-CD prepared with PDMS of varying viscosities at 95 °C: (■) 50 mm^2/s; (▲) 100 mm^2/s; (●) 1000 mm^2/s.

that we can control the particle diameter by selecting the PDMS viscosity.

3.2. FT-IR spectrum and elemental analysis

A urethane bond was detected at 1720 cm^{-1} in the FT-IR spectrum of MSs-MI-CD. Absorption of the hydroxyl group (3600–3000 cm^{-1}) was smaller than that of the parent β-CD. There was not difference in FT-IR spectrum between the MSs-MI-CD and non-imprinted microspheres. Using ^{13}C-CP/MAS NMR, Asanuma et al. confirmed that cholesterol was completely removed from MI-CDs by the washing procedure employed in their study (Asanuma et al., 1998). It was their consideration that the hydroxyl group of cholesterol would not react with the TDI during the polymerization because the hydroxyl group residues of β-CD were 60-fold excess to that of cholesterol and preferentially reacted with TDI.

The ratio of cross-linked residue to β-CD (TDI/β-CD) was calculated by elemental analysis. The elemental analysis results of MSs-MI-CD prepared with 100 mm^2/s PDMS at 95 °C were as follows: H, 5.66%; C, 53.63%; N, 9.51%. Based on these results, the TDI/β-CD value was 10/1. These ratios were not changed by altering the preparation conditions or by the use of the template molecule. In non-imprinted microspheres prepared with 100 mm^2/s PDMS at 95 °C,

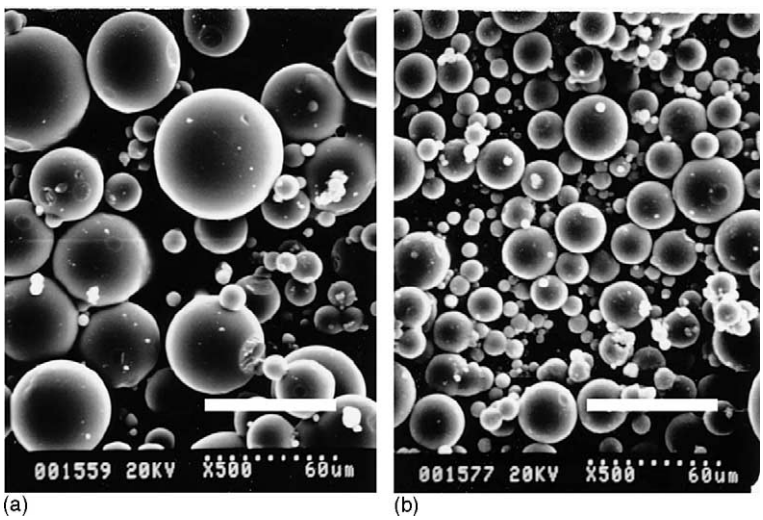

Fig. 4. Scanning electron photomicrographs of MSs-MI-CD: (a) MSs-MI-CD prepared with 50 mm^2/s PDMS at 95 °C; (b) MSs-MI-CD prepared with 1000 mm^2/s PDMS at 95 °C. Bar = 60 μm.

the TDI/β-CD value was also 10/1. Therefore, there was no difference in the β-CD contents between MSs-MI-CD and the non-imprinted microspheres.

3.3. Binding ability to template molecule

Table 1 shows the binding ability of CD polymers to cholesterol. Batches 1–6 comprised microspheres that were prepared with PDMS under various conditions. Batches 7 and 8 comprised CD polymers prepared without PDMS by the previously reported method (Hishiya et al., 1999). In all batches, the binding ability of MSs-MI-CD to cholesterol was higher than that of non-imprinted microspheres. It has been reported that the increase in binding ability of MI-CD to cholesterol is due to the specific three-dimensional structure (Hishiya et al., 2002). Cholesterol promoted the generation of CD dimer and trimer. It has been proposed that CDs are regularly aligned and cooperatively bound to long molecules such as cholesterol (Fig. 5) (Hishiya et al., 2002). The binding ability of MSs-MI-CD was equivalent to that of MI-CDs. These results indicate that cholesterol imprinting was successfully performed in the DMSO/PDMS emulsion.

In batches 1–6, there were no large differences in the binding ability to cholesterol, although these batches had greater differences in particle size distribution. It was suggested that the binding sites exist both on the surface of MSs-MI-CD and also within them.

3.4. Molecular recognition ability

Table 2 shows the binding ability to steroids. Microspheres were prepared with 100 mm^2/s PDMS at 95 °C. CD polymers prepared by the previously reported method are also listed (Hishiya et al., 1999). Cholesterol imprinting also promoted the binding ability to other steroids; however, the increase in the binding ability was most remarkable in the case of cholesterol. The adsorption of testosterone derivatives increased as the alkyl residue became longer. These results indicated that MSs-MI-CD recognized long alkyl residues at the 17-position of steroids, the structure of which is common to cholesterol and testosterone derivatives. The binding ability of MSs-MI-CD resembled that of MI-CDs. These results showed that we successfully introduced the cholesterol-specific binding ability of MI-CDs into the microspheres.

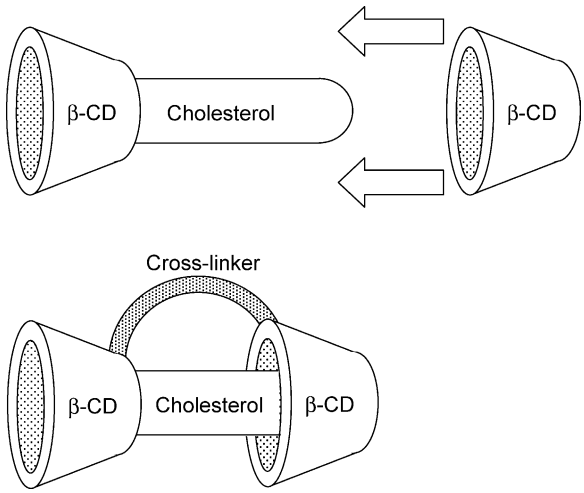

Fig. 5. Proposed mechanism for molecular imprinting of β-CD with cholesterol.

Table 1
Binding ability of CD polymers to cholesterol

Batch number	Viscosity of PDMS (mm^2/s)	Temperature (°C)	Binding ability (%)[a]	
			Imprinted	Non-imprinted
1	50	95	61	46
2	100	95	51	34
3	300	95	57	48
4	500	95	59	40
5	1000	95	63	40
6	100	65	52	33
7	–	65	60	16
8	–	95	58	16

Batches 1–6: microspheres prepared with PDMS; batches 7 and 8: CD polymers prepared without PDMS.
[a] The mole fraction of cholesterol adsorbed by the CD polymers with respect to the total amount of cholesterol.

Table 2
Binding ability of CD polymers to steroids

Guest	Binding ability (%)[a]			
	Microspheres[b]		Polymer[c]	
	Imprinted	Non-imprinted	Imprinted	Non-imprinted
Cholesterol	51	34	60	16
Progesterone	54	51	47	35
Testosterone	32	28	27	18
Testosterone propionate	48	43	43	27
Testosterone enanthate	59	52	55	30

[a] The mole fraction of the guest adsorbed by the CD polymers with respect to the total amount of guest.
[b] Microspheres prepared with 100 mm^2/s PDMS at 95 °C.
[c] CD polymers prepared without PDMS at 65 °C.

In conclusion, we could prepare MSs-MI-CD in the DMSO/PDMS emulsion system. The reaction temperatures were important for preparing separated MSs-MI-CD. The PDMS viscosity influenced the size of MSs-MI-CD. We could control the particle diameter by selecting the PDMS viscosity. The binding ability of MSs-MI-CD to cholesterol was higher than that of the non-imprinted microspheres. Cholesterol imprinting also promoted binding ability to other steroids; however, the increase in binding ability was most remarkable in the case of cholesterol, suggesting that we successfully introduced the cholesterol-specific binding ability into MSs-MI-CD. The novel MSs-MI-CD preparation method presented in this study is useful and simple, and it will provide opportunities for further studies on the specific binding ability of MI-CDs.

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