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Preparation of molecularly imprinted cyclodextrin microspheres

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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

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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