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Sustained release applications of a fluoroalkyl ester-functionalized amphiphilic cyclodextrin by inclusion complex formation with water-soluble drugs in supercritical carbon dioxide

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ABSTRACT

An amphiphilic γ-cyclodextrin, selectively functionalized with perfluorobutanoyl group, octakis(6-*O*-perfluorobutanoyl)-γ-cyclodextrin (γ-CyD-F), was investigated as a potential sustained release carrier for hydrophilic drugs, taking molsidomine (MOL) as a model drug. Supercritical carbon dioxide, an environmentally benign solvent, was used for the preparation of MOL/γ-CyD-F inclusion complexes. The molecular encapsulation of MOL by the amphiphilic cyclodextrin was confirmed by differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD) studies. Additionally, ^{1}H NMR spectroscopy was used to investigate the inclusion mode of drug with the γ-CyD-F. The *in-vitro* release of MOL from the peanut oil suspensions into aqueous phase was found to be significantly retarded by the complexation with γ-CyD-F, mainly due to the hydrophobic properties associated with the γ-CyD-F.

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1. Introduction

There is an increasing interest in optimizing the efficiency of drug activity through the use of rationally designed drug carrier materials. Cyclodextrins (CyDs) are well known candidates for a role, modifying physical, chemical, and biological properties of drug molecules through their unique property of inclusion complex formation with a variety of drugs [1]. In general, hydrophilic CyDs are employed to enhance the dissolution rate of poorly water-soluble drugs for immediate release applications, whereas hydrophobic CyDs are used as sustained release carriers. However, as the potential use of CyDs in biological system often requires amphiphilic properties, several modifications have been made on CyDs with the aim of providing versatile carriers and delivery systems for hydrophilic and lipophilic drugs [2]. Amphiphilic CyDs can be obtained by the introduction of long alkyl or fluoroalkyl chains at primary face and/or secondary face of the CyDs, and they have been shown to form monolayers at the air–water interface and micelles or bilayer vesicles in water [3–6].

Fluorinated organic compounds have attracted much attention owing to their potential importance in industrial as well as in biomedical research. Because of the unique properties conferred by the fluorinated chains to molecules, several fluorine containing organic compounds have been reported for their promising pharmaceutical application such as oxygen delivery (liquid ventilation) and temporary blood substitutes and they are currently being investigated in phase-III clinical trials for the treatment of the diseases like respiratory distress syndrome [7]. At present more than two hundred fluorinated pharmaceuticals are available in the market and others are appearing [8]. Recently, fluorine containing β-cyclodextrins were prepared as a new class of amphiphilic carriers and they exhibited amphiphilic behavior at the air–water interface [3]. Nanocapsules and nanospheres of amphiphilic perfluoro-β-cyclodextrins and perfluoroalkylthio-β-cyclodextrins were also prepared and investigated for their potential role as oxygen carriers [9]. Additionally, fluorine containing cyclodextrins and their inclusion complexes were recently developed as novel drug carriers. They have been successfully tested for the *in-vivo* site-specific delivery of various fluorinated drugs including trifluridine and flutamide, a non-steroidal fluorinated nitrophenyl propamide used for its anti-androgen effects in prostate cancer chemotherapy [10].

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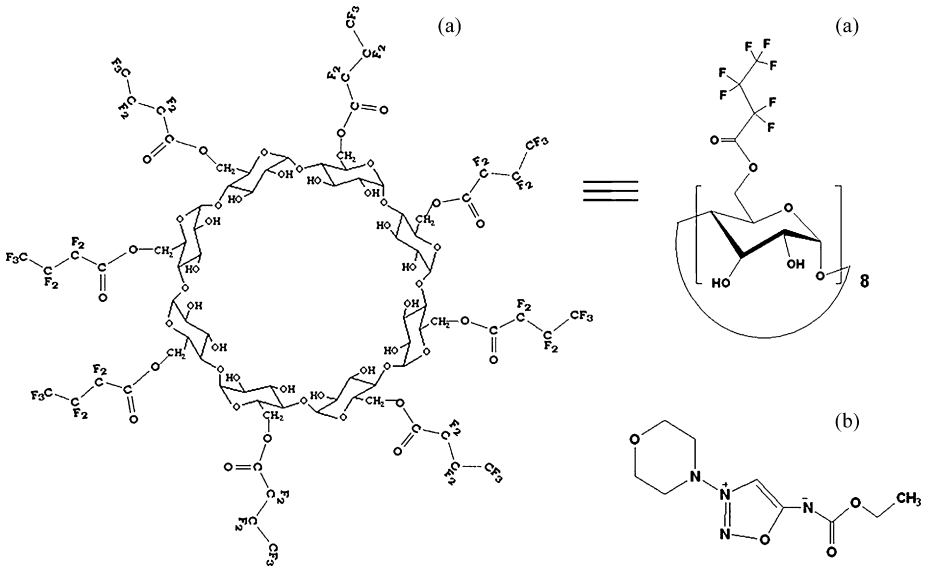

Fig. 1. Molecular structures of (a) γ-CyD-F and (b) MOL.

In general, several preparation methods in solution are available to prepare CyD–drug inclusion complexes. However, many of these methods are time consuming and need multistage processing, involving the evaporation of a large volume of liquid solvents, which are often found in the inclusion complex as harmful residues. Herein, we report a simple and organic solvent-free method to molecularly encapsulate water-soluble drugs by an amphiphilic cyclodextrin, octakis(6-*O*-perfluorobutanoyl)-γ-cyclodextrin (γ-CyD-F), using an environmentally benign solvent, supercritical carbon dioxide ($scCO_2$). Apart from the key advantages such as the lack of toxic solvent residues and the ease of product recovery, CO_2 is inexpensive, inert, non-flammable, and has recently been explored as a promising medium for protein extraction and bioconversion, polymer synthesis, material processing, and also in the particle engineering [11–14].

Molsidomine (MOL) or N-(ethoxycarbonyl)-3-(4-morpholinyl)sydnone imine), a prodrug, was selected as a model drug in this study. MOL is a peripheral nitrovasodilator and particularly useful for the treatment of angina pectoris [15]. The drug is freely soluble in water (0.25 g/dL^{-1} at 25 °C) and the duration of antihypertensive action after single oral dosing is only 2.1–2.7 h which necessitates the clinical use of 38–75 mg to be taken at 3–4 times a day [16]. Due to its effectiveness and intensive use as a drug of choice, several controlled release methods of MOL have been reported in the past [17,18]. The combination of inclusion properties of the CyDs and the useful amphiphilic properties of fluorocarbon chains are expected to give molecules possessing novel physical, chemical and biological properties compared to their hydrocarbon analogs. Thus, it was anticipated that the γ-CyD-F could give thermodynamically stable inclusion complexes with hydrophilic drugs such as MOL, whereas the hydrophobic properties conferred by the fluorinated chains could be exploited as a tool to retard the release rate of highly water-soluble drugs [19].

In this study, the inclusion complex formation of γ-CyD-F with MOL was investigated using $scCO_2$ as a solvent. The complexes were further studied with the aid of differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), and ^{1}H NMR spectroscopic studies. Additionally, the potential use of γ-CyD-F as a drug carrier material was demonstrated by *in-vitro* controlled release of MOL from γ-CyD-F complexes.

2. Materials and methods

2.1. Materials

MOL was purchased from Aldrich and used as received. γ-CyD-F was synthesized by selective functionalization at C-6 position of the all glucose units of CyD by esterification with heptafluorobutyric acid [20]. Complete procedure for synthesis and characterization of γ-CyD-F was described in our previous report. Molecular structures of γ-CyD-F and MOL are shown in Fig. 1.

2.2. Preparation of MOL and γ-CyD-F inclusion complexes in $scCO_2$

The experimental apparatus used in the preparation of inclusion complexes in $scCO_2$ is shown in Fig. 2. It consists of a 10 cc high-pressure stainless steel reactor equipped with sapphire quartz window and a high-pressure syringe pump (ISCO model 260D series) for pressurizing carbon dioxide. Heating was provided by a water bath and the temperature was measured with a thermocouple (Doric Trendicator 400A). Teflon-coated magnetic stir bar was used to mix the cell contents. In a typical inclusion experiment, equimolar mixtures of MOL and γ-CyD-F were placed in the high-pressure reactor, and carbon dioxide was charged into the cell using the syringe pump until the pressure reached up to 34.5 MPa at 45 °C. After 6 h of stirring, the CO_2 was slowly vented off, and the powders were collected. The physical mixtures of MOL and γ-CyD-F were prepared by mixing both solids at 1:1 molar ratio (8 mg of MOL and 100 mg of γ-CyD-F) at room temperature.

2.3. Characterization of inclusion complexes

The inclusion complexes were investigated with the aid of differential scanning calorimetry (DSC-60 Shimadzu, Japan) and powder X-ray diffractometry (XRD Philips X'Pert-MPD, Japan). ^{1}H NMR spectra were recorded using a JNM-ECP 400 (JEOL) spectrometer, with DMSO-d_6 as a solvent (internal reference δ_H = 2.5 ppm).

2.4. In-vitro drug release studies

The *in-vitro* release rate of MOL from an oily suspension of drug-γ-CyD-F complex was measured according to the paddle method of

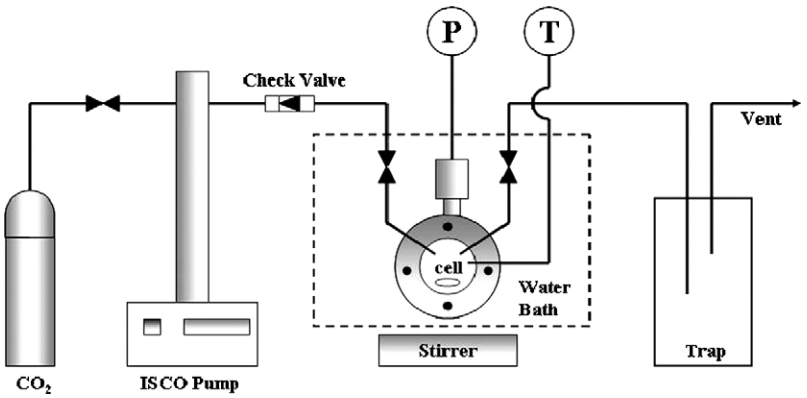

Fig. 2. Experimental set up for the preparation MOL/γ-CyD-F inclusion complexes by $scCO_2$ process.

dissolution test described in Japanese Pharmacopoeia XIV. MOL or its 1:1 molar complex of γ-CyD-F (equivalent to 5 mg of MOL) was suspended in 10 mL peanut oil which was used as a vehicle for sustained release preparations. The oil suspension was further added with 50 mL water and the mixture was kept at 37 °C with a stirring of 60 rpm. Sample solutions (0.5 mL) were drawn with a cotton plug and diluted with water. The release rate of MOL from the oily phase into water was assayed by spectrophotometrically (Lambda 40 UV–vis spectrometer, PerkinElmer) at a wavelength of 313 nm where linearity of the concentration versus absorption relation was ascertained, as reported previously [21].

3. Results and discussion

3.1. Preparation and characterization of MOL/γ-CyD-F inclusion complexes

Recently, we have shown that the amphiphilic cyclodextrin, functionalized at the 6-position of the all glucose units of CyD with perfluoroalkanoate moieties, can be prepared by a facile one-pot synthesis [20]. Prior to the preparation of inclusion complexes in $scCO_2$, the solubility of γ-CyD-F in $scCO_2$ was studied by cloud point method. It is well known that fluorinated compounds are highly soluble in $scCO_2$. Thus, given the fluorophilicity of CO_2, the γ-CyD-F which contains eight heptafluoropropyl chains ($-CF_2-CF_2-CF_3$) was found to be highly soluble in $scCO_2$. For example, about 1 wt% of γ-CyD-F (with respect to $scCO_2$) was known to be soluble above 17.9 MPa at 40 °C [22]. Moreover, the fluoroalkyl ester groups substituted on the primary face of the cyclodextrin did not hamper the formation of complexes as it was evident from the formation of predominant inclusion complexes with organic molecules in both organic media and densified carbon dioxide, demonstrated previously [19,20,22].

To investigate the host–guest interaction between the MOL and γ-CyD-F, the complexes were primarily subjected to DSC and XRD which are standard tools generally used to confirm the inclusion phenomena. Results from the DSC and XRD studies of the pure MOL, γ-CyD-F, physical mixtures of drug-γ-CyD-F, and the inclusion complex are shown in Fig. 3. Powder X-ray diffractogram shows sharp diffraction peaks for MOL indicating the high crystallinity of the drug, whereas the γ-CyD-F is an amorphous compound. While the presence of drug crystalline peaks is clearly evident in the physical mixture of MOL and γ-CyD-F, there were no detectable peaks in the inclusion compound, indicating the drug is no longer present as a crystalline material. These results substantiate the formation of an amorphous complex between the drug and γ-CyD-F [20,22]. Similarly, in DSC thermogram, free MOL exhibits an endothermic peak at 140 °C corresponding to its melting point. While a peak observed in the same range for the physical mixture consists of equimolar amount of drug and γ-CyD-F, inclusion compound prepared with the similar composition did not show any peak (see Fig. 3). It is well known that the disappearance or shifting of endo- or exothermic peaks is an evidence for the encapsulation of host species by the cyclodextrins at the molecular level [20–23].

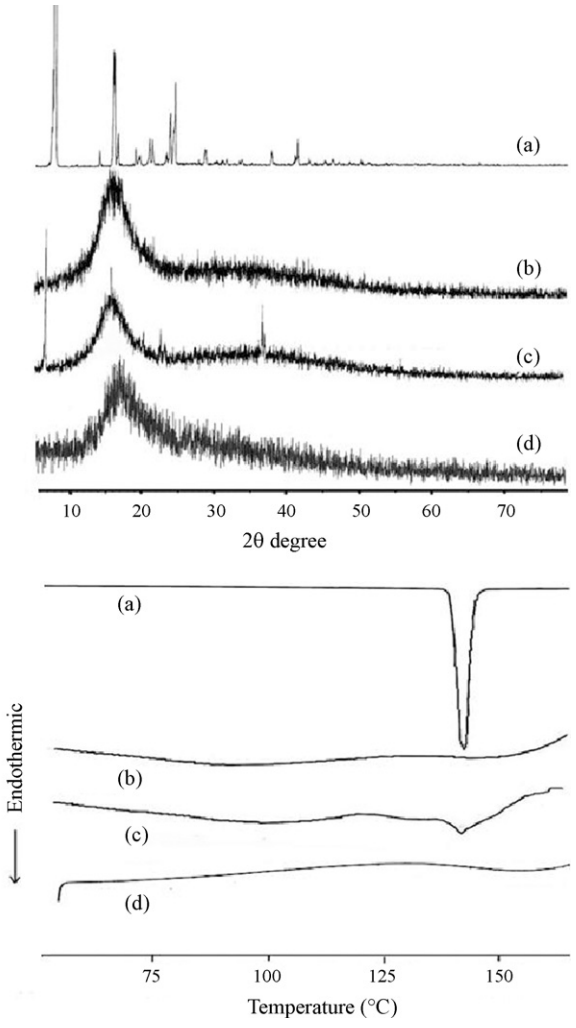

Fig. 3. XRD patterns and DSC thermograms of (a) MOL, (b) γ-CyD-F, (c) physical mixture, and (d) inclusion complex of MOL and γ-CyD-F.

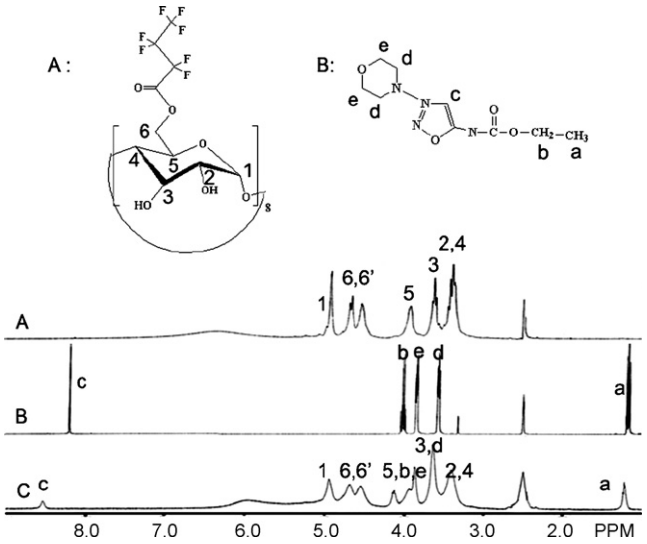

Fig. 4. ^{1}H NMR spectra of (A) γ-CD-F, (B) MOL, and (C) the drug-γ-CD-F inclusion complex in DMSO-d_6.

3.2. ^{1}H NMR studies of MOL/γ-CyD-F inclusion complexes

The ^{1}H NMR spectra gave further insight into the formation of stable complex between the MOL and the hydrophobically modified γ-CyD. It is well known that when drugs and cyclodextrins form inclusion complexes, H-bonding and non-specific forces change in both drug and the carrier molecules, giving rise to changes in the chemical shifts of nuclear magnetic resonances [25]. Thus, the presence of equimolar amount of γ-CyD-F is expected to cause detectable complexation shifts of MOL protons. Similarly, the addition of MOL could give rise to a considerable displacement in ^{1}H-chemical shifts of γ-CyD-F. The ^{1}H NMR spectra of bulk γ-CyD-F, pure MOL and, the inclusion complex in DMSO-d_6 are shown in Fig. 4. The NMR peaks of the fluorinated ester-functionalized γ-CyD and MOL were assigned as described previously [20,24]. As can be seen in Fig. 4, the NMR spectra of the inclusion complex clearly show the presence of both γ-CyD-F and MOL in the complex. Moreover, the peaks of the inclusion complex slightly become broader with considerable chemical shifts as compared with their bulk compounds. This guest induced chemical shifts and broadening of NMR signals are generally considered as an evidence for the formation of host–guest inclusion complex. It is also known that the chemical shift displacement values can provide information about the inclusion mode of a host–guest complex [25]. Table 1 shows the chemical shift displacements of MOL and γ-CyD-F after complexation. The chemical shifts were calculated as $\Delta\delta = \delta_{mix} - \delta_{free}$, where δ_{mix} is the chemical shift measured in the inclusion complex and δ_{free} is the chemical shift of the pure compound. From $\Delta\delta$ values obtained from NMR spectra, it is clear that the presence of γ-CyD-F very much affected the side chain ethyl protons of MOL and its H-c proton, as the complexation shifts of 0.124, 0.186, and 0.557 ppm were, respectively, measured. Therefore, the ethyl group of the drug should be involved in the complexation with the γ-CyD-F. Significantly, minor effects were detected on the morpholine proton (H-e) (see Table 1). The analysis of the variations undergone by cyclodextrin protons as a consequence of the presence of MOL strongly suggests complexation involving an inclusion into the cavity of the host, as the external protons H-1, H-2 and H-4 are slightly affected, whereas the internal H-5 protons located on the internal large rim show comparatively higher complexation shift of 0.1812 ppm. Though it was difficult to measure the chemical shifts of internal H-3 protons due to superimposition between some cyclodextrin resonances and H-d morpholine protons, the large complexation shifts observed in internal H-5 protons suggest that the inclusion occurs from the large diameter rim of the cyclodextrin. Considering the steric factors imposed by fluorinated chains present in the external 6-position, the morpholine ring could be likely to avoid entering from the larger rim of the γ-CyD-F. This argument was supported by the very low chemical shift value obtained for the H-6 protons present in the external large rim of γ-CyD-F compare to its H-5 internal protons. This guest induced chemical shift displacement again confirms that the efficient encapsulation of MOL into the γ-CyD-F cavity at the molecular level. To obtain additional evidence for the inclusion phenomena, two-dimensional ROESY-NMR studies were also attempted to determine the inclusion structure of the MOL/γ-CyD-F complexes. Unfortunately, MOL/γ-CyD-F system gave no distinct cross-peaks between the host and guest molecules, which may be due to the large cavity size of γ-CyD-F [25].

Table 1
^{1}H NMR chemical shift displacements of γ-CyD-F and MOL inclusion complexes.

Chemical shift displacement ($\Delta\delta$ (ppm)), $\Delta\delta = \delta_{mix} - \delta_{free}$			
MOL protons	γ-CyD-F/MOL	γ-CyD-F protons	γ-CyD-F/MOL
H-a	0.124	H-1	−0.0032
H-b	0.186	H-2 and H-4	−0.0263
H-c	0.557	H-3	–
H-d	–	H-5	0.1812
H-e	0.014	H-6	0.0023

3.3. In-vitro drug release studies

The *in-vitro* release rate of MOL from γ-CyD-F complexes was studied by paddle method of dissolution test using peanut oil as the vehicle for the sustained release formulations. MOL, its 1:1 molar complex of γ-CyD-F (equivalent to 5 mg of MOL), and the physical mixtures were separately dispersed 10 mL peanut oil, and the suspensions were used for the dissolution studies. The release rate of MOL from the oily phase into water was assayed by UV spectroscopy at a wavelength of 313 nm where linearity of the concentration versus absorption relation was ascertained. Fig. 5 shows the *in-vitro* release profiles of MOL from the oily suspension containing the drug and γ-CyD-F inclusion complex into the aqueous phase. The dissolution rate of pure MOL itself from the

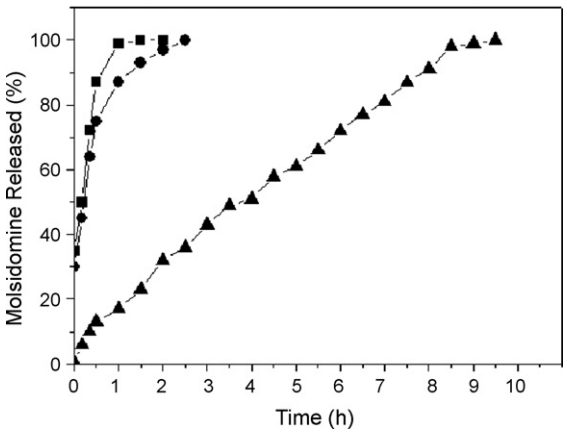

Fig. 5. *In-vitro* release profiles of MOL from the oily suspensions containing the drug and its γ-CyD-F complex to water at 37 °C. (■) MOL alone; (●) physical mixture; (▲) γ-CyD-F/drug complex.

suspension was very fast due to the high aqueous solubility of MOL. Similarly, the solubility of MOL from the physical mixture was also found to be almost similar to the dissolution rate of bulk MOL. In contrast, it is apparent that the interfacial transfer of MOL was significantly retarded by complexation with γ-CyD-F confirming the efficiency of the amphiphilic γ-CyD-F as a slow release drug carrier. The released percentage of drug from the amphiphilic γ-cyclodextrin after 3 h was about 45% and slowly reached to the maximum at 8.5 h under the present experimental conditions. The release rate of MOL from γ-CyD-F/oil suspensions to water was found be almost linear with the time. Although it has been reported that viscous oils can be used as sustained release vehicles for hydrophobic substances, the immediate release of MOL from the oily suspension into aqueous solution suggests that peanut oil works merely as a drug carrier in the case of a simple suspension of hydrophilic MOL in peanut oil [26]. The release of drug from a vehicle is known to be influenced by various factors including drug–vehicle interactions, solubility, partition coefficient, and the particle size of drug in the vehicle [27]. MOL was practically insoluble in peanut oil and its solubility was not increased by complexation with γ-CyD-F. In addition, the release rate of MOL from the inclusion complex in the absence of the release vehicle (peanut oil) was found to be similar to the release rate of MOL from inclusion complex/peanut oil suspensions. Therefore the extended duration of the drug release could be explained by the slow dissolution of the inclusion complex at oil/water interface, owing to the poor aqueous solubility of the amphiphilic γ-CyD-F [20]. Though MOL was used as a model drug in this study, the inclusion phenomena of the fluorinated amphiphilic cyclodextrins can also be used for the encapsulation of a variety of other water-soluble drugs such as captopril, an active inhibitor of angiotensin-converting enzyme, and non-steroidal anti-inflammatory drugs such as ibuprofen and ketoprofen etc. These results show that the selective side chain modification of CyDs at the smaller rim (primary face) using fluoroalkyl groups leads to not only the useful amphiphilic CyDs but also the possibility for encapsulating highly water-soluble drugs, which makes them novel cyclodextrin sustained release carriers having different physio-chemical properties.

4. Conclusions

The potential use of an amphiphilic cyclodextrin, γ-CyD-F as a controlled release carrier for water-soluble drugs was investigated by the complexation with MOL. The drug/γ-CyD-F inclusion complexes were prepared in $scCO_2$ and confirmed by DSC, XRD and ^1H NMR analyses. The investigation of ^1H NMR chemical shift displacements clearly indicated that the side chain ethyl group of MOL was included into the cavity of the γ-CyD-F through the larger rim of the cyclodextrin. The *in-vitro* drug release studies confirmed that the release rate of MOL from the peanut oil suspensions was markedly retarded by the complexation with γ-CyD-F, mainly due to the poor water solubility of the complex. The released percentage of MOL from γ-CyD-F into water (at 37 °C and a stirring speed of 60 rpm) after 3 h was found to be about 45% and slowly reached to the maximum at 8.5 h, whereas the pure drug and the physical mixture show 100% release of MOL within 2 h. The combination of properties such as the amphiphilicity and hydrophobicity conferred by fluoroalkyl groups and environmentally benign methods could make the γ-CyD-F as a novel carrier material in the injectable and chemical residue-free sustained release formulations of clinically important drugs.

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