

Research Unit of Pharmaceutics¹, College of Pharmacy, Nihon University, Chiba; Department of Physical Chemistry², Hoshi University, Tokyo, Japan

Improvement of solubility of C₇₀ by complexation with cyclomaltonaose (δ-cyclodextrin)

T. FURUISHI¹, T. FUKAMI¹, H. NAGASE², T. SUZUKI¹, T. ENDO², H. UEDA², K. TOMONO¹

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Professor Haruhisa Ueda, Department of Physical Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
ueda@hoshi.ac.jp

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We investigated the solubilizing effects of cyclomaltonaose (δ-CD), a cyclic oligosaccharide composed of nine α-1,4-linked D-glucose units, on C₇₀ by using the ball-milling method based on a solid-solid mechanochemical reaction. The complex between C₇₀ and δ-CD was characterized by UV-VIS spectrometry and fast atom bombardment mass spectrometry (FAB-MS). Coloration of the C₇₀/δ-CD system was red-brown in aqueous solution, and the UV-VIS spectrum was in agreement with that of C₇₀ in hexane solution. The FAB-MS spectrum of the C₇₀/δ-CD system showed a negative ion peak corresponding to the molecular weight of a complex between two δ-CD and one C₇₀. These findings suggest that the solubilization of C₇₀ in water was due to complex formation of C₇₀ with δ-CD, and the stoichiometric ratio of this complex was 1 : 2 (C₇₀ : δ-CD).

1. Introduction

Cyclodextrin (CD) is a common name for cyclic oligosaccharides composed of a number of α-1,4-linked D-glucose units, of which 6, 7 and 8 are well known as α-, β- and γ-CD, respectively. Owing to their average cavity diameter of 5–8 Å, they are able to form an inclusion complex with a variety of guest molecules. They and their derivatives have been well studied and are used in many fields (Dodziuk 2006). In contrast, there have been few papers concerning large-ringed CDs (LR-CDs) composed of more than nine α-1,4-linked D-glucose units. However, a paper by French et al. presented the first definitive evidence of such molecules, and those with 9, 10, 11, 12 and 13 D-glucose units were named δ-, ε-, ζ-, η- and θ-CD, respectively (French et al. 1965). Yet, due to low yields and difficulties in their purification, they have not been studied in detail. We have focused on LR-CDs for several years and have previously reported on the isolation, purification methods, physicochemical properties, and abilities of LR-CDs with 9–45 D-glucose units to form inclusion complexes (Miyazawa et al. 1995; Endo et al. 1995; Ueda et al. 1996; Endo et al. 1997a; Endo et al. 1997b; Endo et al. 1998; Onodera et al. 2002; Shimizu et al. 2003; Nakadate et al. 2004; Taira et al. 2006; Katsura et al. 2006). In addition, we have determined the crystal structures for cyclomaltodecaose and cyclomaltotetradecaose (Ueda et al. 1996; Harata et al. 1998). Above all, we have established an isolation and purification method to obtain relatively large amounts of δ-CD (Miyazawa et al. 1995; Wakamiya et al. 1997), and characterized its physicochemical properties and complex-forming abilities with a number of drugs (Miyazawa et al. 1995; Ueda et al. 1999).

Fullerenes (C₆₀, C₇₀) were successfully prepared in measurable quantities by Krätschmer et al. (1990), which led to extensive studies in organic and inorganic chemistry and material sciences (Kroto et al. 1991). Following an exciting report on the inhibition of HIV-1 proteases by C₆₀ derivatives (Friedman et al. 1993), many research groups have begun to investigate the biological uses of fullerenes over the past few years (Jensen et al. 1996). However, the predominant hydrophobic character of fullerenes causes low solubility in polar media, especially water.

Beginning with the work of Andersson et al. (1992), several groups reported a successful technique to overcome this problem that relied on embedding C₆₀ in γ-CD (Zhang et al. 1994; Kuroda et al. 1995). The reports suggest that γ-CD, which has a cavity size similar to the diameter of C₆₀, is the most suitable host molecule for solubilization. However, there has been little information on the dissolving of C₇₀, a member of the fullerene family, in water using CDs (Andersson et al. 1994). Our preliminary experiment revealed that C₇₀ could be solubilized in water by complexation with δ-CD (Furuishi et al. 1998). It was considered that C₇₀ showed better fit to δ-CD than γ-CD since δ-CD has a larger cavity. However, details of the interaction mechanism and complex structure between C₇₀ and δ-CD were unknown.

In this paper, we described the solubilizing effects of δ-CD on C₇₀ in aqueous solution via complex formations on the basis of data from the UV-VIS spectrum. Furthermore, we determined the stoichiometry of this complex by fast atom bombardment mass spectrometry (FAB-MS).

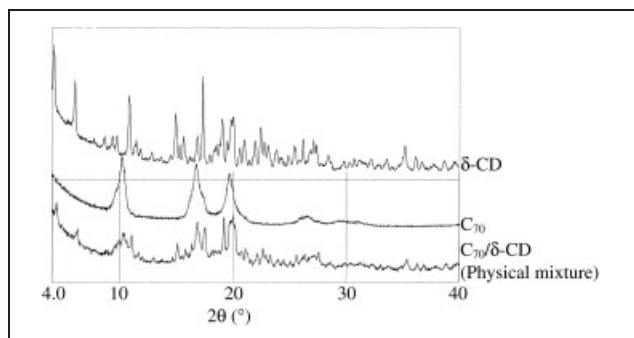


Fig. 1: XRD patterns of δ -CD, C_{70} and the C_{70}/δ -CD system (physical mixture)

2. Investigations, results and discussion

2.1. Determination of the time taken to obtain a ground mixture of C_{70} with δ -CD

The method of grinding a mixture is widely used for reducing the particle size of a solid to enable dissolution of water-insoluble compounds in water, since dissolution rate depends strongly on particle size. Reducing the size is a consequence of a strong grinding force on a solid, and causes an increase in activation energy on the surface and distortion of the crystal lattice. Braun et al. reported a preparation to dissolve C_{60} in water by grinding between C_{60} and γ -CD (Braun et al. 1994). Hence, the theory and methods of obtaining ground mixtures were applied for the solubilization of C_{70} by use of δ -CD.

Powder X-ray diffraction (XRD) patterns of δ -CD, C_{70} and a physical mixture of δ -CD and C_{70} (molar ratio 1:1) are presented in Fig. 1. The pattern of the physical mixture could be interpreted as an approximate superposition of the δ -CD and C_{70} . As with the C_{70}/δ -CD system, no interaction was found between δ -CD and C_{70} by physical mixing alone. The intensity of each peak decreased with increasing grinding time, and a broad pattern was observed after 6, 12, 24 and 48 h (Fig. 2). The changes in XRD patterns upon grinding indicated a decrease in proportion of crystalline forms of δ -CD and C_{70} , and no new diffraction peak (implying the formation of a crystalline complex) appeared. This suggests that the crystalline structure of C_{70} and δ -CD were disrupted, and that both C_{70} and δ -CD molecules were substantially converted into amorphous states. Moreover, the diffraction patterns did not alter after 6 h of grinding; this was found to be an adequate duration for obtaining a ground mixture of δ -CD and C_{70} under our experimental conditions.

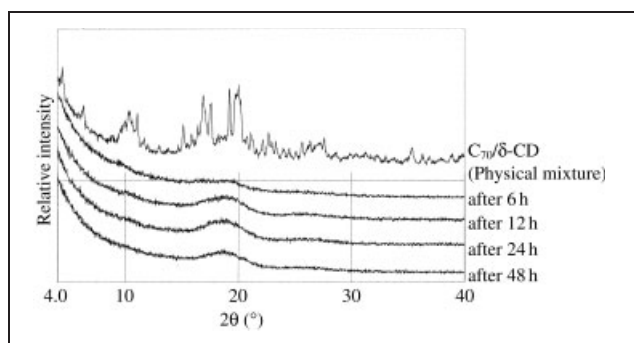


Fig. 2: Effect of ball-milling time on XRD pattern of the C_{70}/δ -CD system

2.2. UV-VIS spectrum of the C_{70}/δ -CD system

C_{70} has strong absorption in the ultraviolet region when dissolved in aqueous and organic media, thus can be easily detected by UV-VIS spectroscopy. Figure 3 shows the UV-VIS spectra of the C_{70}/δ -CD and C_{70}/γ -CD systems. The spectrum of the C_{70}/δ -CD system was in agreement with that of C_{70} in hexane solution (Ajie et al. 1990; Hare et al. 1991). The λ_{\max} of this system was observed at 237.5 nm, and the spectrum of the C_{70}/δ -CD system in water was slightly red-shifted compared with that of C_{70} in hexane solution. It can therefore be presumed that an intermolecular interaction between the cavity of δ -CD and the π -system of C_{70} is involved. The C_{70}/δ -CD system solution appeared red-brown, similar to the color of C_{70} in toluene solution, however the C_{70}/γ -CD system was colorless. From these results, it was suggested that the solubilization of C_{70} into water was due to formation of a complex between C_{70} and δ -CD. The concentration of C_{70} in water was about 8.36×10^{-5} M. This concentration was almost the same as that of the C_{70}/γ -CD system reported by Andersson et al. (1994). The solubilization of C_{70} with γ -CD was achieved by use of surplus γ -CD. On the contrary, the spectral intensity of the C_{70}/γ -CD system in

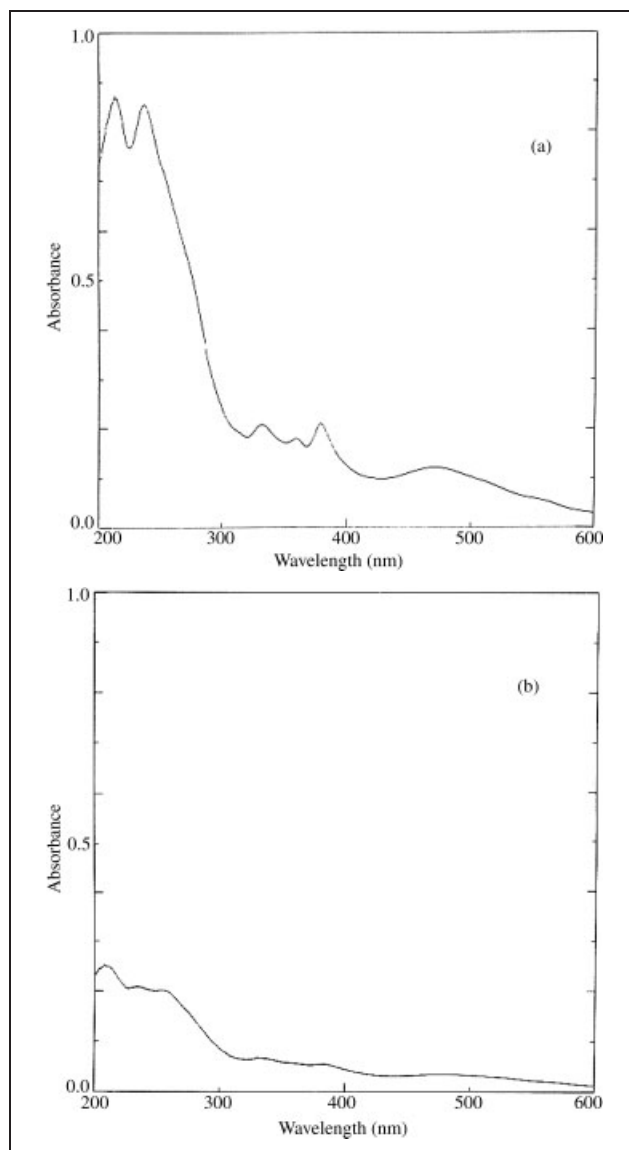


Fig. 3: UV-VIS spectra of (a) C_{70}/δ -CD complex (diluted 12.5 times) and (b) C_{70}/γ -CD complex (not diluted) in aqueous solution

Fig. 3(b) was lower than that of the C_{70}/δ -CD system. This difference was due to the better fit of C_{70} to the inner hydrophobic space of δ -CD than to that of γ -CD, because δ -CD had a larger cavity.

In order to clarify the interaction between δ -CD and C_{70} in aqueous solution, we investigated it in detail by ^1H NMR and ^{13}C NMR spectroscopy at 50 °C. The ^1H and ^{13}C NMR spectra of the C_{70}/δ -CD system (molar ratio of 1 : 1) were almost the same as that of δ -CD alone. It was considered that this sample could not give adequate solubility of C_{70} in D_2O to be measured by NMR spectrometry. Hence, the sample was prepared as a molar ratio of 1 : 5 ($C_{70} : \delta$ -CD) in DMSO-d_6 , since the solubility of the complex was too low to give high-quality NMR spectra in D_2O . The essential feature of the interaction of C_{70} with δ -CD in DMSO may be similar to that which occurs in water, because the relative magnitude of dielectric constants of the solvents ($\epsilon = 46.8$ and 80, respectively) against the hydrophobic guest molecule is not significantly different between DMSO and water (Jyothirmayi et al. 1991; Matsui et al. 1994; Gafni et al. 1997). The chemical shifts of all protons in a D-glucose unit in the presence of C_{70} moved about 0.025 ppm toward the higher field region than those in the absence of C_{70} , although a large signal change was not observed for each proton (data not shown). It is well known that protons at the H-5 and H-3 positions in D-glucose units are in the inner hydrophobic space of the CD ring. If the C_{70} molecule inserts into the inner space of the δ -CD molecule by inclusion complex formation, the protons H-5 and H-3 will be most influenced by the insertion. This result suggests that a complex between δ -CD and C_{70} could be formed, however the interaction is very weak.

Moreover, we found the chemical shifts of all carbons in the D-glucose unit of δ -CD in the presence of C_{70} moved on average 0.02 ppm toward the lower field region than those in the absence of C_{70} (data not shown). However, we did not observe the five carbon signals of C_{70} in the DMSO-d_6 solution. In organic solution, ^{13}C NMR spectrum for C_{70} consisted of five signals at 150.1, 147.5, 146.8, 144.7 and 130.3 ppm (Taylor et al. 1990). From the data described above, we suggest the possibility of an interaction between C_{70} with δ -CD in aqueous solution, but are unable to obtain strong evidence.

2.3. Determination of the stoichiometry of C_{70}/δ -CD systems by FAB-MS

From UV-VIS and NMR measurements, it was suggested that an interaction between δ -CD and C_{70} had been revealed, however this was not verified by the stoichiometry of the C_{70}/δ -CD system.

The MS spectrometric detection of host-guest complexes, where two or more molecules are held together by weak non-covalent interactions, has become possible with the development of softer ionization techniques, such as fast atom bombardment (FAB) and electrospray. Andersson et al. reported that a complex consisting of two γ -CD and one molecule of C_{60} was identified by negative fast atom bombardment/liquid secondary ion (FAB/LSI) MS spectroscopy (Andersson et al. 1995). Here we describe the use of FAB-MS for determination of the stoichiometric ratios of C_{70}/δ -CD system.

The spectrum of the C_{70}/δ -CD system is shown in Fig. 4. The peaks at m/z 841.1 correspond to the negative ion for C_{70} , and the peak at m/z 3756.9 corresponds to the complex of two δ -CD and one C_{70} . This result suggests a

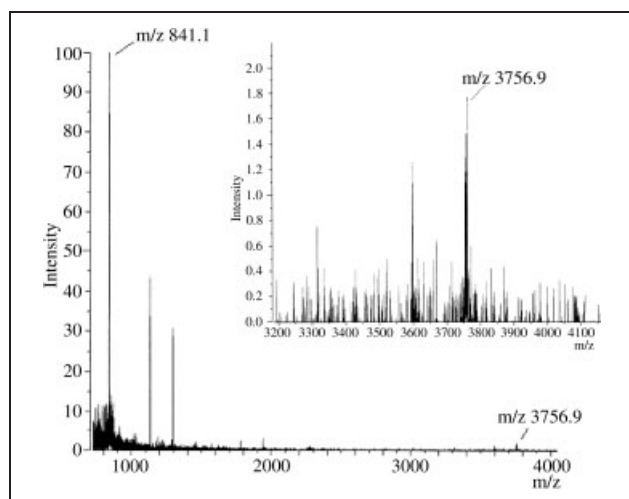


Fig. 4: FAB-MS spectrum of C_{70}/δ -CD complex

stoichiometric ratio of the C_{70}/δ -CD complex of 1 : 2 ($C_{70} : \delta$ -CD).

In conclusion, we have succeeded in dissolving C_{70} in a water complexation with δ -CD, an LR-CD composed of nine α -1,4-linked D-glucose units, using a preparation of ground mixture. A duration of 6 h was found to be adequate to obtain ground mixtures between δ -CD and C_{70} . The solubility of C_{70} in water was approximately 8.36×10^{-5} M using UV-VIS spectrometry. FAB-MS showed the formation of a 1 : 2 complex of the C_{70}/δ -CD system.

Finally, we hope the δ -CD is a candidate for a highly selective phase in HPLC separation of C_{60} , C_{70} and other fullerenes, especially chiral molecules (Cabrera et al. 1993). We continue to isolate other LR-CDs composed of more than ten α -1,4-linked D-glucose units, and the interactions between these and higher fullerenes will be published elsewhere in the near future.

3. Experimental

3.1. Materials

δ -CD in excess of 98% purity was prepared by the method described previously (Miyazawa et al. 1995). γ -CD was obtained from Wacker Chemicals East Asia Ltd. (Tokyo, Japan). δ -CD and γ -CD were used after recrystallization from water. C_{70} was purchased from Term USA (Berkeley, CA, USA), and purity of C_{70} was greater than 99%. All other chemicals and solvents were commercial grade and used without purification. Milli-Q water was used in all experiments.

3.2. Instruments

A lightproof ball mill (model V-1M; Irie Shokai Co., Ltd., Tokyo, Japan) was used; its volume was 50 mL and height 78 mm. The enclosed glass balls had a diameter of 7 mm. Powder X-ray diffraction (XRD) was recorded using an RINT 1400 X-ray diffractometer (Rigaku Co., LTD., Tokyo, Japan). X-Ray measurement was carried out at 1°/min from 4° to 40° of the diffraction angle at room temperature at 60 kV and 200 mA. UV-VIS spectrometry was measured using a Ubest-30 Double Beam spectrophotometer (JASCO Co., Ltd., Tokyo, Japan). FAB-MS was performed in a negative mode by a JEOL SX-102A mass spectrometer (JEOL, Tokyo, Japan) with a Magic Bullet matrix and acceleration voltage of -6 kV.

3.3. Determination of the time taken to obtain a ground mixture of C_{70} with δ -CD

A mixture of 20.2 mg of C_{70} (2.4×10^{-5} mol) and 35.0 mg of δ -CD (2.4×10^{-5} mol) was ball-milled at room temperature. The mixture was measured by XRD after ball-milling for 6, 12, 24, and 48 h.

3.4. Preparation of the complex of C_{70} with δ -CD in aqueous solution and analysis by spectrophotometer

Initially, a mixture of 20.2 mg of C_{70} (2.4×10^{-5} mol) and 35.0 mg of δ -CD (2.4×10^{-5} mol) was ball-milled at 230 rpm at room temperature.

After adding 25 mL of water, the solution was incubated at 25 °C for about 3 days. The suspension was centrifuged at 25 °C and 10,000 rpm (maximum gravity approximately 12,000 G) for 1 h. The supernatant was examined by UV-VIS spectroscopy at a wavelength of 237.5 nm. The C₇₀/γ-CD system was studied in the same manner.

3.5. Determination of the stoichiometry of the C₇₀/δ-CD system by FAB-MS

FAB-MS analysis of the C₇₀/δ-CD system was performed after freeze drying to remove water from the aqueous samples, in a similar manner as the sample preparation for UV-VIS spectrometry.

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