

Enhanced solubility of fullerene (C₆₀) in water by inclusion complexation with cyclomaltonnaose (δ -CD) using a cogrinding method

Takayuki Furuishi · Yasunao Ohmachi ·
Toshiro Fukami · Hiromasa Nagase · Toyofumi Suzuki ·
Tomohiro Endo · Haruhisa Ueda · Kazuo Tomono

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Abstract The solubilizing effects of cyclomaltonnaose (δ -CD), a cyclic oligosaccharide composed of nine α -1,4-linked D-glucose units, on C₆₀ were investigated by using a ball-milling method based on a solid–solid mechanochemical reaction. The complex between C₆₀ and δ -CD was characterized by UV–VIS spectrometry, ¹³C-NMR, and fast atom bombardment mass spectrometry (FAB-MS). Coloration of the C₆₀/ δ -CD system was yellowish-brown in aqueous solution, and the UV–VIS spectrum was in agreement with that of C₆₀ in hexane solution. The ¹³C-NMR spectrum of C₆₀/ δ -CD system in aqueous solution revealed the presence of free δ -CD and dissolved C₆₀ giving one sharp peak at 144.4 ppm, which was close to that reported for C₆₀ in organic solvents. The FAB-MS spectrum of the C₆₀/ δ -CD system showed a negative ion peak corresponding to the molecular weight of a complex between two δ -CDs and one C₆₀. Moreover, the phase solubility diagram of C₆₀ with δ -CD at 10 °C was classified as the Bs-type, resulting in a stoichiometric ratio of 1:2 (C₆₀: δ -CD), in agreement with the stoichiometry obtained from FAB-MS. These findings suggest that the solubilization of C₆₀ in water was due to complex formation of C₆₀ with δ -CD and that the stoichiometric ratio of the complex was 1:2 (C₆₀: δ -CD).

Keywords Large-ring cyclodextrin · Fullerene · Solubilization · ¹³C-NMR · Fast atom bombardment mass spectrometry (FAB-MS)

Introduction

Conventional cyclodextrins (CDs) are cyclic oligosaccharides consisting of 6 (α -CD), 7 (β -CD), and 8 (γ -CD) D-glucose units linked by α -1,4 bonds. The structure of CD molecules resembles truncated cones with the secondary hydroxyl groups located at the wider edge of the ring and the primary groups on the narrower edge. Hydrogen atoms are directed to the inner part of the ring, resulting in a hydrophobic cavity along with a hydrophilic character outside the ring. Hence, CDs are soluble in water and at the same time may accommodate a broad range of hydrophobic species inside the cavity with formation of inclusion complexes. This property accounts for the great interest in CDs and it was shown that complex formation can be improved by chemical modifications of native CDs [1]. On the other hand, there have been few papers concerning large-ring CDs (LR-CDs) composed of more than nine α -1,4-linked D-glucose units, except for a paper by French et al. in 1965 which provided the first definitive evidence for the existence of such molecules. LR-CDs with 9, 10, 11, 12, and 13 D-glucose units were named δ -, ϵ -, ζ -, η -, and θ -CD, respectively [2]. However, 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 and purification methods, physicochemical properties, and complex-forming ability of LR-CDs with 9–50 D-glucose units [3–6]. We have already determined the crystal structures for ϵ - and ι -CD [7, 8]. Most importantly, we have established an

T. Furuishi · T. Fukami · T. Suzuki · K. Tomono
Research Unit of Pharmaceutics, College of Pharmacy, Nihon
University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555,
Japan

Y. Ohmachi · H. Nagase · T. Endo · H. Ueda (✉)
Department of Physical Chemistry, Hoshi University,
2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
e-mail: ueda@hoshi.ac.jp

isolation and purification method to obtain relatively large amounts of δ -CD [9, 10] and have characterized its physicochemical properties and complex-forming abilities with a number of guest compounds [11–13].

The fullerene family, especially C_{60} , has very appealing photo-, electro-, and physico-chemical properties, which can be exploited in many different biological fields [14]. However, the predominant hydrophobic character of fullerenes causes low solubility in polar media, such as water. Several groups reported successful techniques to overcome this problem that relied on embedding C_{60} in α -, β -, and γ -CD, respectively [15–17]. In addition, a C_{60}/γ -CD complex exhibits significant inhibitory effects on the oxidative discoloration of β -carotene in scavenging harmful peroxy radicals and reactive oxygen species [18]. Therefore, water-soluble fullerene can be expected as a new potential antioxidant tool in pharmaceutical and cosmetic fields.

Previously, we have reported that C_{70} , a member of the fullerene family, could be solubilized in water by complexation with δ -CD [19, 20]. It was considered that C_{70} was better fitted to δ -CD than other conventional CDs since δ -CD has a larger cavity.

In this paper, the preparation method of the inclusion complex of C_{60} with δ -CD using a ball-milling method based on a solid–solid mechanochemical reaction is reported. The inclusion complex was characterized by UV–VIS spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and fast atom bombardment mass spectrometry (FAB-MS) in both the solution and solid state. Moreover, phase solubility studies were performed, which confirmed that complexation with δ -CD, including pretreatment by ball-milling, actually enhanced the C_{60} solubility and that the stoichiometric ratio between C_{60} and δ -CD in aqueous solution was 1:2 ($C_{60}:\delta$ -CD).

Materials and methods

Materials

δ -CD in excess of 98% purity was prepared by the method described previously [9]. γ -CD was obtained from Wacker Chemicals East Asia Ltd. (Tokyo, Japan). δ -CD and γ -CD were used after recrystallization from water. C_{60} was purchased from Term USA (Berkeley, CA, USA), and purity of C_{60} was greater than 99%. All other chemicals and solvents were commercial grade and used without further purification. Milli-Q water was used in all experiments.

Instruments

A lightproof ball mill (model V-1M; Irie Shokai Co., Ltd., Tokyo, Japan) was used. A pot's volume was 50 mL and

inside diameter was 35 mm. The enclosed glass balls had about 7 mm diameter. 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 60 kV and 200 mA at room temperature. UV–VIS spectrometry was measured using a Ubest-30 Double Beam spectrophotometer (JASCO Co., Ltd., Tokyo, Japan). FAB-MS was performed in negative ion mode by a JEOL SX-102A mass spectrometer (JEOL, Tokyo, Japan) with a Magic Bullet matrix and acceleration voltage of -6 kV. ^1H - and ^{13}C -NMR spectra at 500 MHz were taken on a JNM-LA500 spectrometer (JEOL, Tokyo, Japan) at 50 °C.

Determination of the time for a ground mixture of C_{60} with δ -CD

A mixture of 16.9 mg of C_{60} (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.

Preparation of the complexes of C_{60} with δ -CD in aqueous solution and analysis by spectrophotometry

Initially, a mixture of 16.9 mg of C_{60} (2.4×10^{-5} mol) and 35.0 mg of δ -CD (2.4×10^{-5} mol) was ball-milled for 24 h 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 $12,000 \times g$) for 1 h. The supernatant was examined by UV–VIS spectroscopy at a wavelength of 338.5 nm. The C_{60}/γ -CD system was studied in the same way as that of C_{60}/δ -CD system.

Characterization of the C_{60}/δ -CD system by NMR spectrometry

A mixture of 16.9 mg of C_{60} (2.4×10^{-5} mol) and 175.0 mg of δ -CD (1.2×10^{-4} mol) was ball-milled, incubated and centrifuged as described in the previous section. Then, water in the supernatant was removed by freeze-drying. The solid sample of C_{60}/δ -CD system was dissolved in 99.95% deuterium oxide (D_2O). Chemical shifts were reported in δ -units (ppm) downfield from the signal of external tetramethylsilane.

Determination of the stoichiometry of the C_{60}/δ -CD system by FAB-MS

FAB-MS analysis of the C_{60}/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.

Phase solubility diagram of C₆₀ with δ -CD

The phase solubility study was carried out according to Higuchi and Connors [21]. Excess amounts (10 mg) of C₆₀ were added to 0.01–0.05 M δ -CD aqueous solutions. The solutions were dissolved under sonication and incubated at 10 and 25 °C. After equilibrium was attained (approximately 4 days), the solution was filtered through a membrane (0.45 μ m, KURABO INDUSTRIES LTD., Osaka, Japan). After adding toluene (2 mL) to the filtrate (2 mL), the suspension was centrifuged at 20 °C, 3,000 rpm for 10 min. This procedure was repeated 10 times. Through the extraction process, C₆₀ was completely transferred into the organic phase. The extract was evaporated to dryness under reduced pressure and re-dissolved in 10 mL toluene. Each 10 μ L toluene solution was analyzed by HPLC. HPLC analysis was performed on a LC-6A liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) using an Inertsil ODS-2 (150 \times 4.6 mm i.d.) column (GL-Sciences Inc., Tokyo, Japan) with a SPD-6A UV detector at 260 nm (Shimadzu). Hexane/2-propanol (50:50 v/v) was used as the mobile phase (flow rate = 1 mL/min).

Results and discussion

Determination of the time taken to obtain a ground mixture of C₆₀ with δ -CD

A cogrinding technique 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. Reduced particle size is a consequence of 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. [22] reported the preparation method to dissolve C₆₀ in water by grinding between C₆₀ and γ -CD. Hence, the theory and method of cogrinding were applied for the solubilization of C₆₀ with δ -CD.

XRD patterns of δ -CD, C₆₀ and a physical mixture of δ -CD and C₆₀ (molar ratio 1:1) are presented in Fig. 1a. The pattern of the physical mixture could be interpreted as an approximate superposition of δ -CD and C₆₀. It was revealed that no interaction occurred between δ -CD and C₆₀ by physical mixing alone. The peak intensity of each line decreased with increasing grinding time and a broad pattern was observed after 6, 12, 24, and 48 h of grinding, as shown in Fig. 1b. The changes in XRD patterns upon grinding indicated a decrease in proportion of crystalline

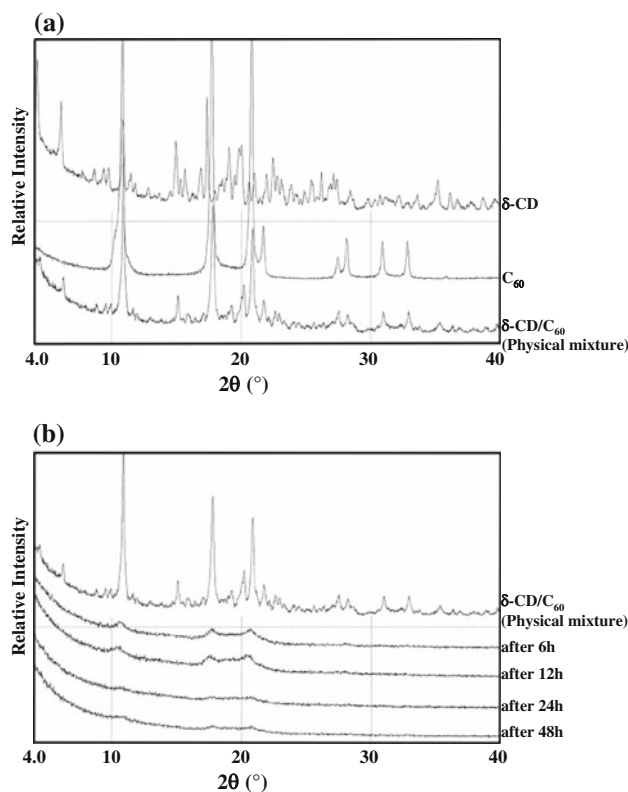


Fig. 1 XRD patterns of δ -CD, C₆₀ and the C₆₀/ δ -CD system (physical mixture) (a) and effect of ball-milling time on C₆₀/ δ -CD system on the XRD patterns (b)

forms of δ -CD and C₆₀, and no new diffraction peak appeared. This suggests that the crystalline structure of C₆₀ and δ -CD were disrupted and that both C₆₀ and δ -CD molecules were substantially converted into amorphous states, but did not form a crystalline complex. Moreover, the diffraction patterns did not alter after 24 h of grinding; suggesting adequate duration for obtaining a ground mixture of δ -CD and C₆₀ under our experimental conditions.

UV–VIS spectrum of the C₆₀/ δ -CD system

The UV–VIS spectrum of the C₆₀/ δ -CD system is shown in Fig. 2a. The spectrum of the C₆₀/ δ -CD system was in agreement with that of C₆₀ hexane solution [23, 24] and was similar to that of the C₆₀/ γ -CD complex in aqueous solution [17]. A maximum UV–VIS absorption (λ_{max}) for this system was observed at 262 nm and the spectrum of the C₆₀/ δ -CD system in water was slightly red-shifted compared to those of the C₆₀ hexane solution [23, 24] and the C₆₀/ γ -CD complex in aqueous solution [25]. This phenomenon could be explained by the intermolecular interaction between the cavity of δ -CD and the π -systems of C₆₀. Furthermore, the C₆₀/ δ -CD system solution appeared yellowish-brown; similar to the coloration of C₆₀

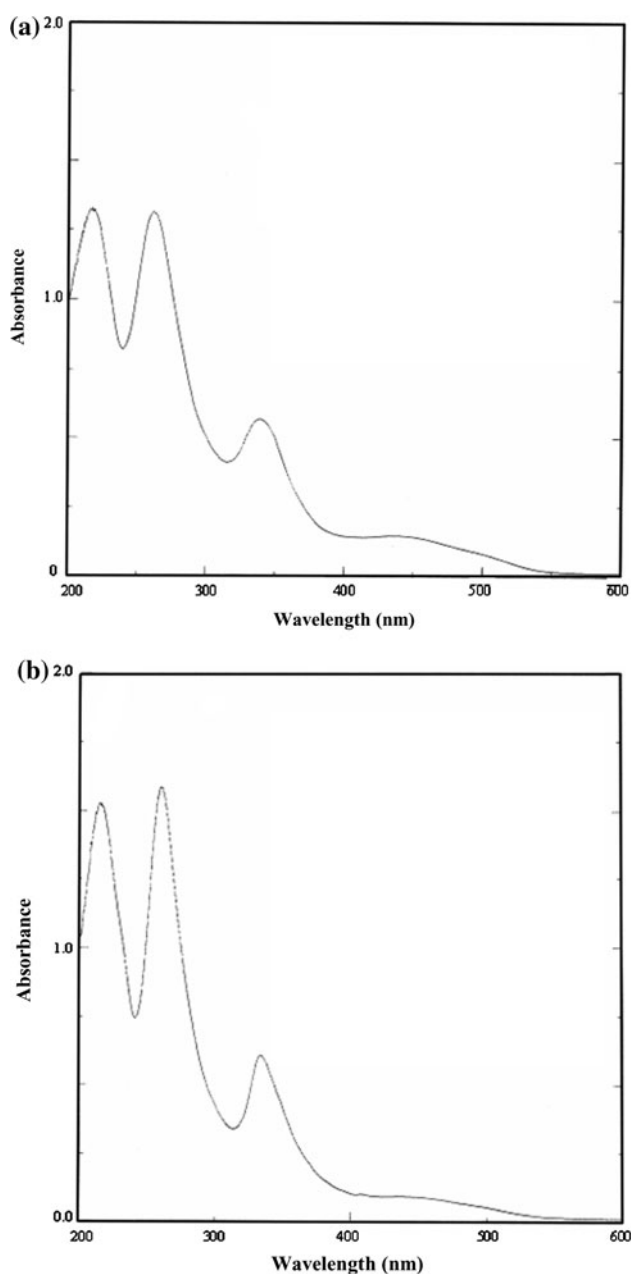


Fig. 2 UV–VIS spectra of C_{60}/δ -CD complex (diluted 20 times) (a) and C_{60}/γ -CD complex (diluted 10 times) (b) in aqueous solution

in 1,2-dimethoxy-benzene solution [26]. These results suggested that it was possible to dissolve C_{60} in water to allow formation of a complex between C_{60} and δ -CD.

Figure 2b shows the UV–VIS spectrum of the C_{60}/γ -CD system under our experimental conditions and is consistent with spectra of the C_{60}/δ -CD system and C_{60} in hexane solution. This result demonstrates that interactions between C_{60} and the γ -CD in water could be reproduced under our experimental conditions. However, the spectral intensity of the C_{60}/γ -CD system was lower than that of the C_{60}/δ -CD system and shows that the solubility of complexed C_{60} with

γ -CD in water was lower than with that of δ -CD. The concentration of C_{60} in the C_{60}/δ -CD system in water was found to be about 1.57×10^{-4} M using the UV absorption method; which was higher than the value of C_{60}/γ -CD complex [17]. δ -CD would incorporate C_{60} more efficiently than γ -CD due to its larger cavity.

Characterization of C_{60}/δ -CD system by NMR spectrometry

In order to clarify the interaction between δ -CD and C_{60} in aqueous solution, ^1H -NMR and ^{13}C -NMR spectra of the C_{60}/δ -CD system were measured in D_2O at 50 °C. When the sample was prepared at a molar ratio of 1:1 ($C_{60}:\delta$ -CD), the ^1H - and ^{13}C -NMR spectra of the C_{60}/δ -CD system were almost the same as that of δ -CD alone. It was considered that this sample did not give adequate solubility of C_{60} in D_2O to be measured by NMR spectrometry. Hence, the sample was prepared at a molar ratio of 1:5 ($C_{60}:\delta$ -CD).

From the ^1H -NMR spectroscopy, the chemical shifts of the C_{60}/δ -CD system in D_2O were almost the same as that of δ -CD alone (data not shown). It is well known that protons at the H-5 and H-3 positions in D-glucose units are upfield shifted by the insertion of a guest compound in the inner hydrophobic space of the CD ring [27]. If the C_{60} molecule inserts into the inner space of the δ -CD molecule by inclusion complex formation, the protons H-5 and H-3 would be most influenced by the insertion. However, we did not observe this phenomenon. Similar observation of the ^1H -NMR on C_{60}/γ -CD system was reported by several groups [28–30].

The ^{13}C -NMR spectrum of C_{60}/δ -CD system is shown in Fig. 3. A signal at 144.4 ppm, which was close to that reported for C_{60} in organic solvents [31], and the other six signals derived from δ -CD were observed. The signals of all carbons moved an average of 0.2 ppm toward the lower field region than δ -CD alone (data not shown). Kanazawa et al. [29] reported similar observations in the C_{60}/γ -CD system. They considered that the ^{13}C -NMR changes indicated that a part of the C_{60} molecule intrudes into the inner space of the γ -CD and that rotation of the C_{60} molecule within the complex occurs rapidly on the NMR time scale. It seemed appropriate to consider our results in a similar way, that is, the result indicated the formation of a water-soluble supramolecular complex between δ -CD and C_{60} .

Determination of the stoichiometry of the C_{60}/δ -CD system by FAB-MS

From UV–VIS and ^{13}C -NMR measurements, it was suggested that an interaction between C_{60} and δ -CD had been revealed, however this was not verified by the stoichiometry of the C_{60}/δ -CD system.

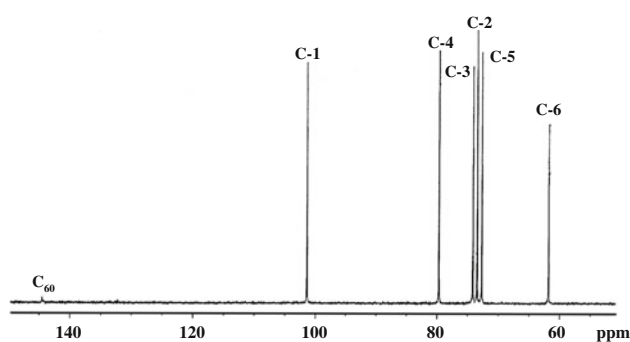


Fig. 3 ^{13}C -NMR spectrum of $\text{C}_{60}/\delta\text{-CD}$ complex in D_2O at $50\text{ }^\circ\text{C}$

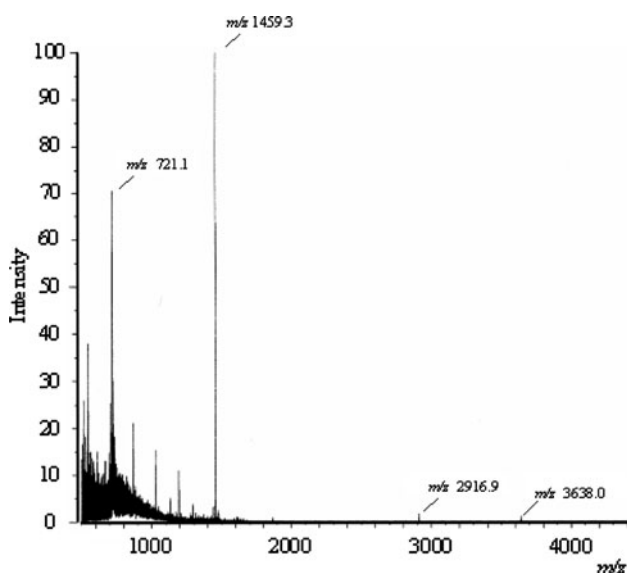


Fig. 4 FAB-MS spectrum of $\text{C}_{60}/\delta\text{-CD}$ complex

As a soft ionization technique, fast atom bombardment (FAB), electrospray ionization (ESI), and matrix assisted laser desorption/ionization (MALDI) mass spectrometry have been important tools in research of non-covalent complexes in recent years due to many advantages over other techniques [32]. High sensitivity, specificity, and speed are the proverbial virtues. Furthermore, FAB, ESI, and MALDI-MS can provide stoichiometric information for the complex directly and detect multiple components simultaneously.

Andersson et al. [33] reported that a complex consisting of two γ -CDs and one molecule of C_{60} was identified by negative fast atom bombardment/liquid secondary ion (FAB/LSI) mass spectroscopy. Here the use of FAB-MS for determination of the stoichiometric ratios of $\text{C}_{60}/\delta\text{-CD}$ system is described.

The FAB-MS spectrum of the $\text{C}_{60}/\delta\text{-CD}$ system is shown in Fig. 4. The peaks at m/z 721.1 and m/z 1459.3

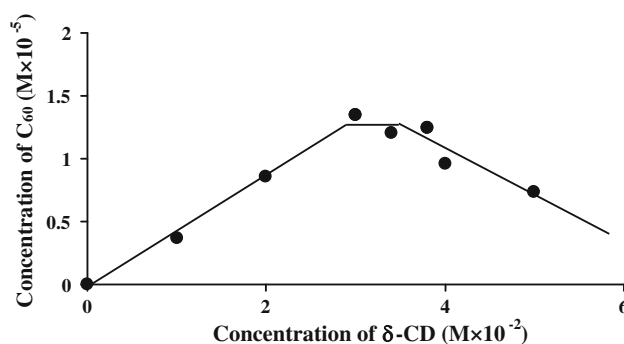


Fig. 5 Phase solubility diagram of $\text{C}_{60}/\delta\text{-CD}$ system in water at $10\text{ }^\circ\text{C}$

correspond to the negative ions for C_{60} and $\delta\text{-CD}$, respectively. In addition, the peak at m/z 2916.9 corresponds to the dimer $\delta\text{-CD}$. Furthermore, a peak at m/z 3638.0 was observed and corresponds to a complex of two $\delta\text{-CD}$ and C_{60} . This result suggested a stoichiometric ratio of the $\text{C}_{60}/\delta\text{-CD}$ complex was 1:2 ($\text{C}_{60}:\delta\text{-CD}$).

Phase solubility diagram of C_{60} with $\delta\text{-CD}$

From the FAB-MS spectrum, the formation of the $\text{C}_{60}/\delta\text{-CD}$ complex with a stoichiometry of 1:2 ($\text{C}_{60}:\delta\text{-CD}$) in solid state was identified. However, the stoichiometric ratio of the $\text{C}_{60}/\delta\text{-CD}$ complex in aqueous solution had not been observed yet. In order to understand the mechanism of inclusion complex formation of C_{60} with $\delta\text{-CD}$, the behavior of inclusion complex formations for C_{60} with $\delta\text{-CD}$ in aqueous solution was studied using the phase solubility method. Although attempts were made to develop a phase solubility diagram of the $\text{C}_{60}/\delta\text{-CD}$ system, it could not clearly be classified as A or B type at $25\text{ }^\circ\text{C}$. It was suggested that the driving force of inclusion complex formation would decrease with increasing temperature due to the decrease in interactions, such as van der Waals and hydrophobic forces [34]. Thus, the phase solubility diagram of $\text{C}_{60}/\delta\text{-CD}$ system was carried out at $10\text{ }^\circ\text{C}$ (Fig. 5). The system showed typical Bs-type solubility curve, where the initial rising portions are followed by plateau regions and then the total C_{60} concentration decreases with the precipitation of complexes. From the diagram obtained, the stoichiometry of the complex was found to be 1:2 ($\text{C}_{60}:\delta\text{-CD}$), in agreement with the stoichiometry in solid state as suggested from the FAB-MS study. Furthermore, the solubility of C_{60} was about $1.3 \times 10^{-5}\text{ M}$ in this system in a preparation method without ball-milling. This value was lower than that with the ball-mill process ($1.57 \times 10^{-4}\text{ M}$). Therefore, the cogrinding with CD using ball-mill method could be powerful tool for solubilization of water-insoluble fullerenes into water.

Conclusion

A successful dissolution of C₆₀ in a water inclusion complex with δ -CD, an LR-CD composed of nine α -1,4-linked D-glucose units, has been achieved using a ground mixture preparation. A duration of 24 h was found to be adequate to obtain ground mixtures between δ -CD and C₆₀. From the result of ¹³C-NMR spectrometry, an interaction between δ -CD and C₆₀ in aqueous solution was revealed. Furthermore, FAB-MS data and a phase solubility diagram showed the formation of 1:2 complexes of the C₆₀/ δ -CD system in both the solid state and aqueous solution. The solubility of C₆₀ in water was about 1.57×10^{-4} M using UV-VIS spectrometry, which was improved in comparison with that derived from the preparation method without ball-milling. These results showed that δ -CD was more suitable host molecule for C₆₀ than conventional CDs and may show a potential of LR-CDs for dissolving fullerenes in water.

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