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Studies on Water-soluble α-, β- or γ-Cyclodextrin Prepolymer Inclusion Complexes with C_{60}

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Abstract. Treatment of water-soluble α-,β- and γ-cyclodextrin-epichlorohydrin prepolymer (CDP) with C_{60} by kneading leads to the formation of six distinct water-soluble inclusion complexes: α-CDP/C_{60} (1 : 1), β-CDP/C_{60} (1 : 1), γ-CDP/C_{60} (1 : 1), α-CDP(2 : 1), β-CDP(2 : 1) and γ-CDP(2 : 1). Their formation and structures have been examined by X-ray powder diffraction (XRD), differential scanning calorimeter (DSC) and UV-vis spectrosocopy. The effect of side chains of the CDPs has also been studied.

Key words: C_{60}, cyclodextrin, prepolymer, water-soluble inclusion.

1. Introduction

From the time that C_{60} could be prepared to a high degree of purity, the physical and chemical properties of C_{60} have attracted growing interest [1, 2]. However, the solubility of C_{60}, poor in most organic solvents, and negligible in water, has been one of the greatest impediments in studying its reactions and biological functions.

Cyclodextrins (CDs) and their derivatives seem to be very promising agents in making C_{60} soluble in water. According to the crystal structure of C_{60}: the nearest-neighbor distance is 10.02 Å and the calculated diameter of the carbon cage is 7.1 Å [3, 4]. Cyclodextrins are composed of α-1,4 linked D-glucopyranose units, these ring-shaped molecules enclose cavities of approximately 6, 8 and 10 Å diameter for α-, β- and γ-CD respectively. Due to these hydrophobic cavities, CDs and their derivatives are capable of forming inclusion complexes with many organic molecules [5]. Among α-, β- and γ-CD, only γ-CD can include C_{60} and two distinct water-soluble inclusion complexes [γ-CD/C_{60} (1 : 1) and γ-CD/C_{60}(2 : 1)] have been obtained [6, 7]. The chemical modification of CDs can result in enhanced solubility and inclusion properties, dimethyl-β-cyclodextrin (DM-β-CD) has been found to be very effective in binding the C_{60} molecule [8].

The cyclodextrin-epichlorohydrin polymer is one kind of cyclodextrin polymer (CDP) produced from the reaction of CD with epichlorohydrin. The special properties of CD are largely retained in this polymer, and the CDPs of low degree of polymerization are soluble [9]. In this work, we chose the water-soluble α-,

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β- or γ-cyclodextrin-epichlorohydrin prepolymers (α-, β- or γ-CDP) [10] as the inclusion hosts, and by kneading, obtained six distinctive solid C_{60} complexes. The formation and structures of these CDP/C_{60} products were examined by UV-vis, DSC and XRD. The solubility of these complexes in water and the effect of side chains of CDPs were also studied. Some of these findings have already been presented as a prelimiary communication [11].

2. Experimental

2.1. MATERIALS

α-CD is a product of Aldrich Chemical Company and γ-CD is a product of Chinon Pharmaceutical and Chemical Works Ltd., Budapest. β-CD is a product of Shaanxi Zhidan Biochemical Plant and its purity is 99% or higher, it was recrystallized twice from water before use. The fullerene C_{60} is a product of the Department of Chemistry, Peking University, and the purity is above 99%. AR-grade epichlorohydrin, hydrochloric acid, sodium hydroxide and *n*-hexane were obtained from Shanghai Chemical and Pharmaceutical Co., Ltd. Distilled water was used throughout the study.

2.2. SYNTHESIS OF CYCLODEXTRIN EPICHLOROHYDRIN PREPOLYMERS

The prepolymers were prepared from α-, β- or γ-CD with epichlorohydrin respectively according to a published method [12]. 4.0 mmol CD was dissolved in 20 mL 0.05 mol·dm^{-3} NaOH solution. 60.0 mmol epichlorohydrin was added slowly dropwise at 60 °C under stirring. After 30 minutes the reaction was stopped by the addition of 2 mol·dm^{-3} HCl until the solution reached pH 7.0. The mixture was desalted with Sephadex G-10 gel. The crude product was separated with Ultrogel ACA 54 and 34 gel (eluent: water containing 0.02% w/v NaN_3; monitor: Sepa-200 high Sensitive Polarimeter (Horiba)), and the fraction of the molecular weights between 3,500 and 4,500 was collected, then the prepolymer was obtained by freeze drying as a white powder. The CD content of these CDPs was measured after acidic hydrolysis by the determination of the liberated reducing sugars [13]. The average molecular weights of three kinds of CDPs were not more than 4,000, as determined by gel chromatography of Ultrogel ACA 34 and 54 on polyacrylamide gels. Table I gives the the CD content of the CDPs. The CD content of the CDPs and the molecular weight range of the CDPs, indicate that every CDP obtained is mainly a mixture of monomers (CD glyceryl ethers [5]) and dimers [10], with more dimers than monomers.

2.3. PREPARATION OF THE INCLUSION COMPLEXES

α-CDP/C_{60} (1 : 1): 8.7 mg (1.2×10^{-5} mol) C_{60} and 29.4 mg α-CDP (in the α-CDP there are 1.2×10^{-5} mol α-CD units, this number can be calculated as below: the

Table I. The CD contents of CDPs

Sample	The content of CD (%)
α-CDP	40
β-CDP	46
γ-CDP	53

wt. of α-CD in 29.4 mg α-CDP is 29.4 mg $\times$ 40% = 11.8 mg, here 40% is the α-CD content (see Table I). So the molar amounts of α-CD units is 11.8 mg/972 = 1.2×10^{-2} mmol, i.e. 1.2×10^{-5} mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 37.5 mg. C_{60} content: 22.3%.

α-CDP/C_{60}(2 : 1): 4.5 mg (6.3×10^{-6} mol) C_{60} and 30.8 mg α-CDP (containing α-CD 1.3×10^{-5} mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 34.8 mg. C_{60} content: 12.3%.

β-CDP/C_{60} (1 : 1): 9.4 mg (1.3×10^{-5}mol) C_{60} and 32.0 mg β-CDP (containing β-CD 1.3×10^{-5}mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 40.9 mg. C_{60} content: 22.4%.

β-CDP/C_{60}(2 : 1): 4.7 mg (6.5×10^{-6} mol) C_{60} and 32.2 mg β-CDP (containing β-CD 1.3×10^{-5} mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 36.4 mg. C_{60} content: 12.6%.

γ-CDP/C_{60} (1 : 1): 8.0 mg (1.1×10^{-5}mol) C_{60} and 26.9 mg γ-CDP (containing γ-CD 1.1×10^{-5} mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 34.3 mg. C_{60} content: 22.6%.

γ-CDP/C_{60}(2 : 1): 4.2 mg (5.8×10^{-6}mol) C_{60} and 28.4 mg γ-CDP (containing γ-CD 1.2×10^{-5} mol) were homogenized and kneaded for 1 hr with dropwise addition of *n*-hexane and water (about 2 mL *n*-hexane and 1 mL water). The product was vacuum-dried at 117 °C for 3 hours. Yield: 32.1 mg. C_{60} content: 12.8%.

2.4. PHYSICAL MEASUREMENTS

UV-VIS spectra were recorded on a HP-8452A UV spectrometer.

X-ray powder patterns were obtained with a Rigaku-D/max-RB diffractometer with a Ni monochromator utilizing CuKα radiation with 40 kV and 100 mA at a scan rate of 4°/min.

Table II. The UV-VIS data of CDP/C_{60} inclusion complexes

Sample	λ_{max} (nm)		
C_{60} cyclohexane solution	216	258	330
α-CDP/C_{60} (1 : 1) aq. sol.	220	268	350
α-CDP/C_{60} (2 : 1) aq. sol.	222	272	354
β-CDP/C_{60} (1 : 1) aq. sol.	220	266	344
β-CDP/C_{60} (2 : 1) aq. sol.	220	268	346
γ-CDP/C_{60} (1 : 1) aq. sol.	222	270	354
γ-CDP/C_{60} (2 : 1) aq. sol.	222	274	358

DSC measurements were performed on a Dupont 1090 DSC-TGA System at a heating rate of 5°/min under a nitrogen atmosphere.

3. Results and Discussion

3.1. UV-VIS SPECTRA

The UV-vis spectra of the CDP/C_{60} samples shown in Figures 1–3, indicate the formation of the CDP/C_{60} complexes. The maximum absorption wavelengths are listed in Table II. In every CDP/C_{60} system, the respective positions of the absorption peaks of the 1 : 1 and 2 : 1 CDP/C_{60} complexes are different, although the difference is rather small, it may be proof of the formation of two distinct inclusion complexes corresponding to the two different structures: 1 : 1 and 2 : 1. The UV-vis peaks of the 1 : 1 complex in aqueous solution are blue-shifted as compared to those of the 2 : 1 complex in aqueous solution and slightly red-shifted as compared to those of a C_{60} cyclohexane solution. In the preparation of every CDP/C_{60} complex, the C_{60} and CDP were kneaded at a molar ratio of 1 : 1 or 2 : 1 (cyclodextrin units in CDP: C_{60}), and all of these complexes can be completely dissolved in water without any C_{60} precipitate (these CDP/C_{60} aqueous solutions are transparent and yellow).

When C_{60} and CDP were kneaded at a molar ratio of 3 : 1, 4 : 1, or 10 : 1 (cyclodextrin units in CDP: C_{60}), the samples obtained are also soluble in water, and their spectra are the same as those of their respective CDP/C_{60} (2 : 1) aqueous solutions.

When using toluene as an extraction agent, C_{60} can be extracted from the CDP/C_{60} aqueous solutions,the toluene extracts are red-purplish. The UV-vis spectra of the β-CDP/C_{60} (1 : 1) aqueous solution, on extraction with toluene, are shown in Figure 4. As the C_{60} concentration in the water phase decreases (as shown by absorption (about 344 nm)), the absorptive peaks red-shift, indicating decomposition of β-CDP/C_{60} (1 : 1) and the formation of β-CDP/C_{60} (2 : 1).

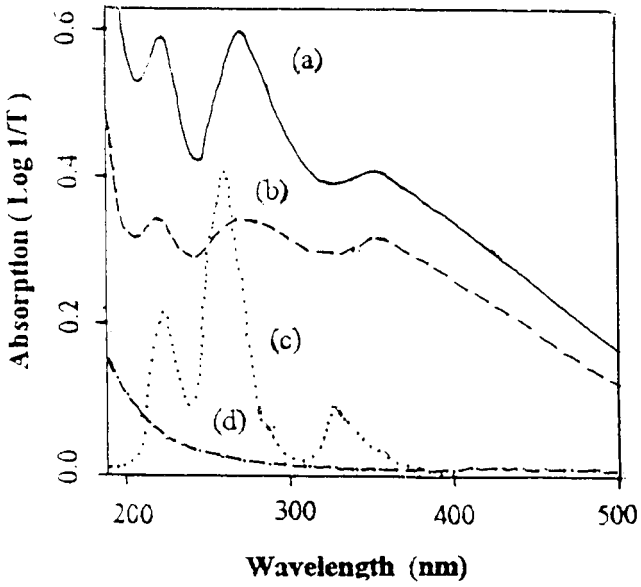

Figure 1. UV-vis spectra of (a) α-CDP/C_{60} (1 : 1) aq. solution (1.3×10^{-5} mol·dm^{-3}), (b) α-CDP/C_{60} (2 : 1) aq. solution (8.6×10^{-6} mol·dm^{-3}), (c) C_{60} cyclohexane, (d) α-CDP aq. solution.

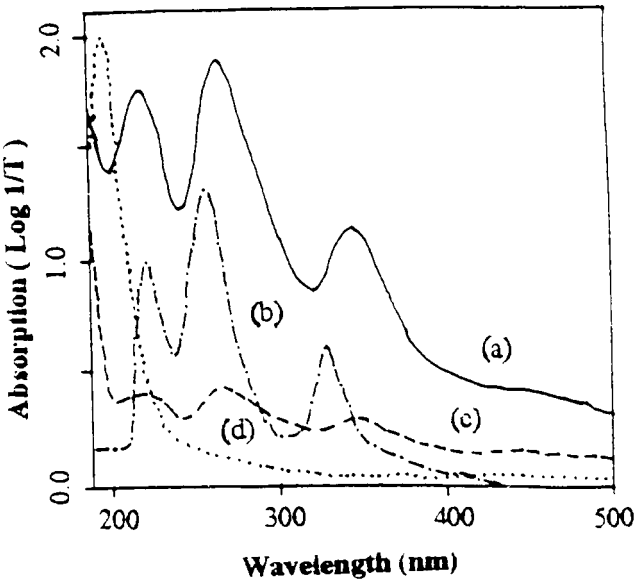

Figure 2. UV-vis spectra of (a) β-CDP/C_{60} (1 : 1) aq. solution (3.8×10^{-5} mol·dm^{-3}), (b) C_{60} cyclohexane, (c) β-CDP/C_{60} (2 : 1) aq. solution (8.9×10^{-6} mol·dm^{-3}), (d) β-CDP aq. solution.

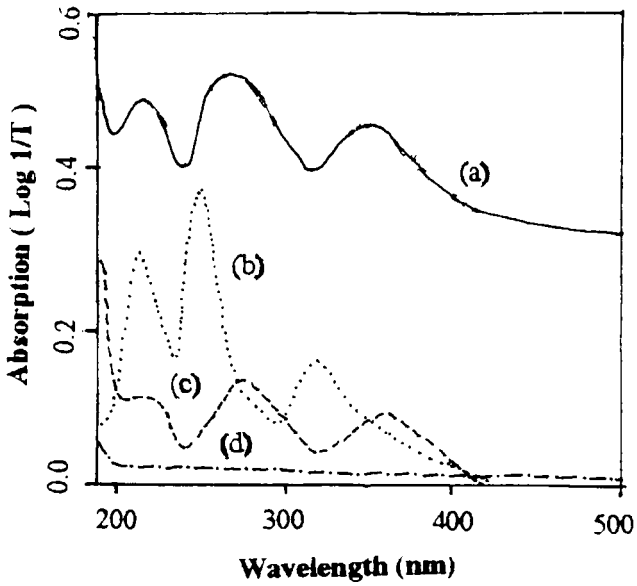

Figure 3. UV-vis spectra of (a) γ-CDP/C_{60} (1 : 1) aq. solution (1.2×10^{-5} mol·dm^{-3}), (b) C_{60} in cyclohexane, (c) γ-CDP/C_{60} (2 : 1) aq. solution (2.4×10^{-6} mol·dm^{-3}), (d) γ-CDP aq. solution.

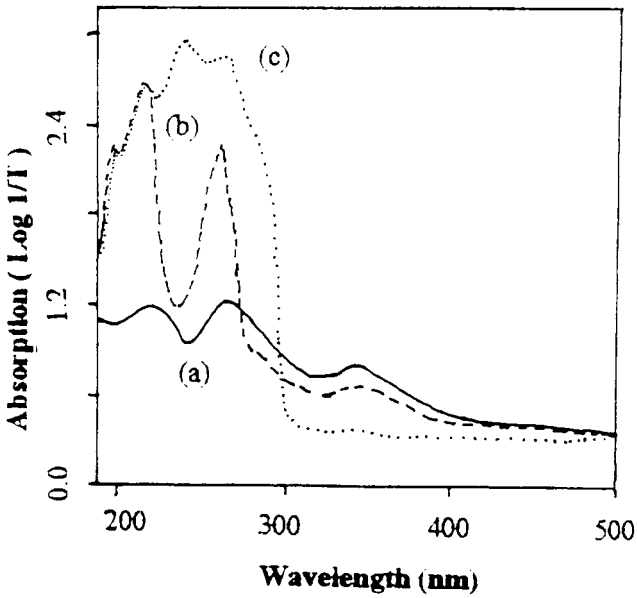

Figure 4. The UV-vis spectra of (a) β-CDP/C_{60} (1 : 1) aq. solution (4.2×10^{-5} mol·dm^{-3}), (b) after extraction by the same volume of toluene twice, (c) the toluene extract.

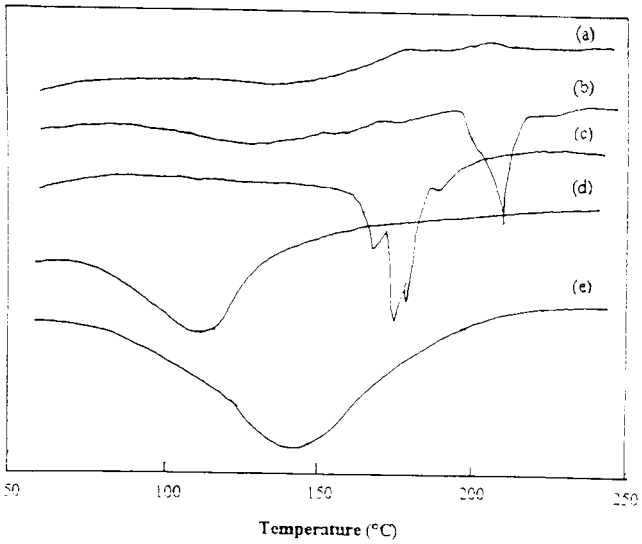

Figure 5. The DSC curves. (a) C_{60}, (b) β-CDP, (c) physical mixture of β-CDP and C_{60} (1 : 1), (d) β-CDP/C_{60} (1 : 1), (e) β-CDP/C_{60} (2 : 1).

3.2. DSC CURVES OF THE CDP/C_{60} SYSTEMS

The DSC curves of CDP/C_{60} are presented in Figure 5. The curves of the materials (C_{60} and β-CDP) and their physical mixture compared with those obtained by kneading imply that there is an interaction between C_{60} and β-CDP. Furthermore, the curve of β-CDP/C_{60} (1 : 1) is different from that of β-CDP/C_{60} (2 : 1), which is circumstantial evidence, indicating different structures.

3.3. X-RAY DIFFRACTION

The X-ray powder patterns for the individual components, the β-CDP/C_{60} (1 : 1) complex, the β-CDP/C_{60} (2 : 1) complex, and a physical mixture (molar ratio 1 : 1) are presented in Figure 6. These indicate that the two solid products are new inclusion-forming substances. The difference in the X-ray diffraction patterns between the two products provide proof for the formation of two kinds of β-CDP/C_{60} complexes: β-CDP/C_{60} (1 : 1) and β-CDP /C_{60} (2 : 1).

3.4. SOLUBILITY

All of the CDP/C_{60} complexes obtained can be completely dissolved in water. Their solubilities were measured at 25 °C by spectrometeric analysis in aqueous solution with determination at the UV-VIS peak near 268 nm. The results are listed in Table III. In every CDP/C_{60} system, there is a difference between the 1 : 1 and 2 : 1 complex, the solubility of the 2 : 1 complex is larger than that of the 1 : 1 complex. Among the three CDPs, β-CDP is the best one to increase C_{60} water solubility.

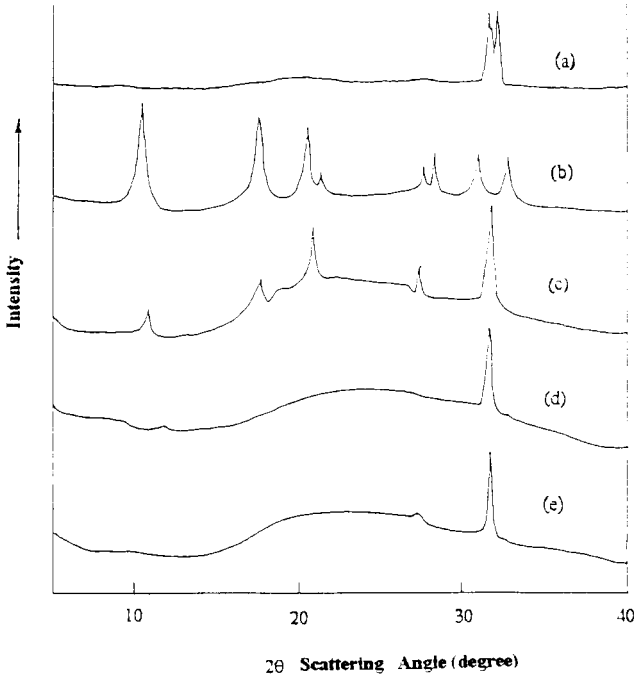

Figure 6. X-ray diffraction patterns. (a) β-CDP, (b) C_{60}, (c) physical mixture of β-CDP and C_{60} (1 : 1), (d) β-CDP/C_{60} (1 : 1), (e) β-CDP/C_{60} (2 : 1).

Table III. The solubility of the samples in water

Sample	Solubility of the complex (mg/100 mL)	Highest concentration of C_{60} in aq. solution (mol·dm^{-3})
α-CD/C60 (1 : 1)	16.1	5×10^{-5}
α-CD/C60 (2 : 1)	46.8	8×10^{-5}
β-CD/C60 (1 : 1)	64.3	2×10^{-4}
β-CD/C60 (2 : 1)	171.3	3×10^{-4}
γ-CD/C60 (1 : 1)	15.9	5×10^{-5}
γ-CD/C60 (2 : 1)	45.0	8×10^{-5}

3.5. THE EFFECT OF SIDE CHAINS OF CDP ON THE FORMATION OF CDP/C_{60} COMPLEXES

The formation of γ-CDP/C_{60} complexes can easily be explained by the cavity diameter of γ-CD. It is however known that α-CD and β-CD are too small to accommodate a large hydrophobic molecule like C_{60}, thus why could the α-CDP and β-CDP include C_{60}? It might be the side chains of the CDPs which enlarge the cavities of CDs in α-CDP and β-CDP. As mentioned in Section 2.2, the dimers

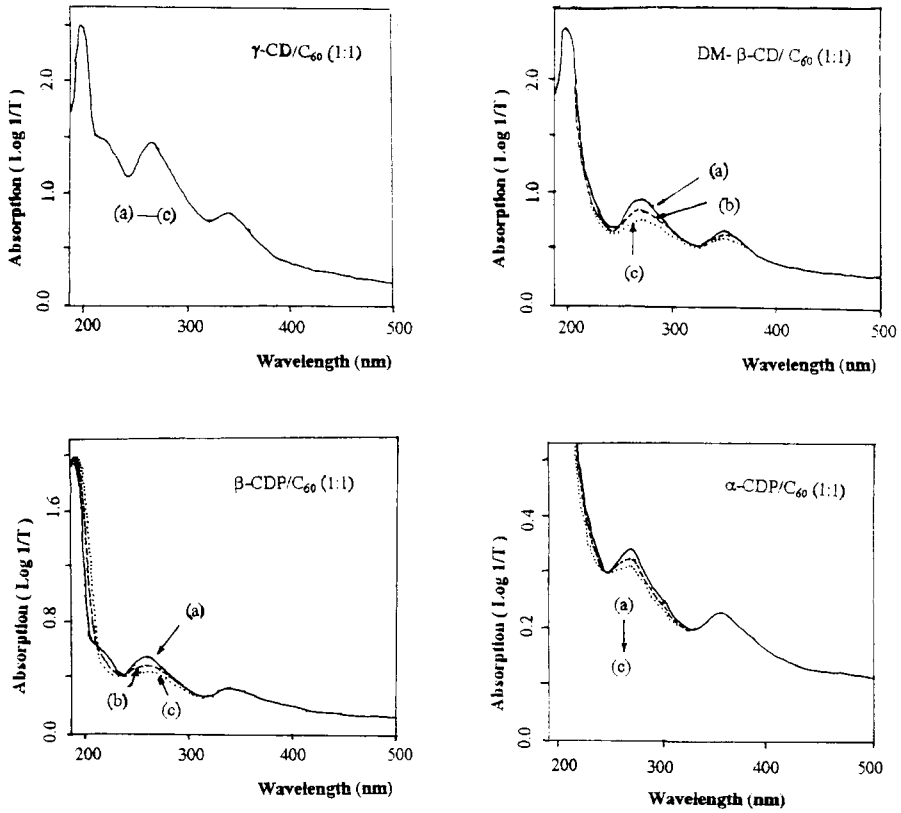

Figure 7. The UV-vis spectra of γ-CD/C_{60} (1 : 1), DM-β-CD/C_{60} (1 : 1), β-CDP/C_{60} (1 : 1) and α-CDP/C_{60} (1 : 1) aq. solution when treated by the same volume of 0.25 mol·dm^{-3} HCl at 50°C for (a) 0 minute; (b) 10 minutes; (c) 20 minutes.

should be the main components in these CDPs. The main structures of the CDPs might be formulated [10]:

$$Xn(CD)—O—CH_2—CHOH—CH_2—O—(CD)Xm$$

where X represents the side chain: —O—CH_2—CHOH—CH_2—OH or its condensed polymer chain; m and n, the number of side chains; and CD, the ring of cyclodextrin. In DM-β-CD, the outward pointing methyl groups enlarge the cavity of β-CD according to CPK models [8]. In our CDP molecules, there should be many similar outward pointing 'arms', and these 'arms' would enlarge the cavities of CDs and enable them to accommodate C_{60}. To verify this assumption, we performed the experiment below.

Figure 7 gives the spectra of aqueous solutions of γ-CD/C60 (1 : 1), DM-β-CD/C60 (1 : 1), α-CDP/C60 (1 : 1) and β-CDP/C60 (1 : 1) complexes when treated by the same volume of 0.25 mol·dm^{-3} HCl at 50 °C. The UV-VIS absorption of γ-CD/C_{60} (1 : 1) remains unchanged before and after the treatment, indicating that

the CD ring has not been destroyed. But under the same conditions, the UV-vis absorption of DM-β-CD/C60 (1 : 1) is apparently reduced, implying the hydrolysis of the outward pointing methoxy-groups and the decomposition of the complex.

The hydrolysis reactions of DM-β-CD and β-CD under the same condition have also been followed by TLC (Sil G/UV_{254}, benzene : methanol = 7 : 3, monitored by iodine, the Rf of DM-β-CD is 0.60 and the Rf of β-CD is 0.05). When a β-CD aqueous solution is treated by the same volume of 0.25 mol·dm^{-3} HCl at 50 °C for 20 min, β-CD remained as one spot (Rf 0.05). However, under the same conditions DM-β-CD showed a few spots, among them one spot of Rf 0.60 and one spot of Rf 0.05. These suggest the hydrolysis of the outward pointing methoxy-groups of DM-β-CD, and also imply that an enhanced hydrolysis of the glycosidic bonds does not happen.

Similar to DM-β-CD/C_{60}, the absorptions of the α-CDP/C_{60} and β-CDP/C_{60} complexes are also reduced after being treated by the HCl solution. From these observations, we can assume that the side chains of the CDP would be hydrolysed and the complex decomposed. It is reasonable that the side chains of CDP would play an important role in the formation of the CDP/C_{60} inclusion complexes.

4. Conclusion

Six different cyclodextrin prepolymer C_{60} complexes have been formed under proper experimental conditions. All of these complexes can be dissolved in water easily. The present method might be a potentially useful one to increase the water solubility of C_{60}.

Acknowledgements

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References

1. H.W. Kroto, A.W. Allaf, and S.P. Balm: *Chem. Rev.* **91**, 1213 (1991).
2. A.D. Darwish, H.W. Kroto, R. Taylor, and D.R.M. Walton: *J. Chem. Soc. Chem. Commun.* 15 (1994).
3. N. Sivaraman, R. Dhamodaran, I. Kaliappan, T.G. Srinivasan, P.R. Vasudeva Rao, and C.K. Mathews: *J. Org. Chem.* **57**, 6077 (1992).
4. W. Kratschmer, L.D. Lamb, K. Fortiropoulos, and D.R. Huffman: *Nature* **347**, 354 (1990).
5. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Netherlands (1988).

6. T. Anderson, K. Nilsson, M. Sundahl, G. Wenstman, and O. Wennerstrom: *J. Chem. Soc. Chem. Commun.* **8**, 604 (1992).
7. D.D. Zhang, Q. Liang, J.W. Chen, M.K. Li, and S.H. Wu: *Supramol. Chem.* **3**, 235 (1994).
8. D.D. Zhang, J.W. Chen, Y. Yang, R.F. Cai, X.L. Shen: *J. Incl. Phenom.* **16**, 245 (1993).
9. D. Koradecki and W. Kutner: *J. Incl. Phenom.* **10**, 79 (1991).
10. W. Kutner, W. Storck, and K. Doblhofer: *J. Incl. Phenom.* **13**, 257 (1992).
11. Y. Liu, L.F. Wang, and D.D. Zhang: *Chinese Science Bulletin* **40**, 1760 (1995).
12. E. Fenyvesi, M. Szilasi, B. Zsadon, J. Szejtli, and F. Tudos: in J. Szejtli (ed.), *Proceedings of the 1st International Symposium on Cyclodextrins*, D. Reidel, Dordrecht, 345 (1982).
13. N. Wiedenhof, J.N.J. Lammers, and C.L. van Panthaleon van Eck: *Staerke* **21**, 119 (1969).