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# Nano-complex formation of cyclodextrin and azobenzene using supercritical carbon dioxide

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**We demonstrated the possibility, at temperatures lower than the melting point of an aqueous-insoluble dye, of synthesizing an inclusion complex of the dye and β-cyclodextrin using supercritical carbon dioxide.**

Cyclodextrins (CDs) as cyclic oligosaccharides are classified as α-, β- and γ-CD according to the number of (1,4) linked α-D-glucopyranose units; six, seven, and eight, respectively. CDs can include various organic guest molecules in their cavities to form inclusion complexes, and consequently, be widely employed to create supramolecules.[1] Additionally, because of their excellent biocompatibility, they have been studied extensively as pharmaceutical molecule capsules to improve the solubility, stability and bioavailability of drugs[2] or as a host for food aromatic substances to functionally maintain aroma.[3] Dye molecules have low stability against light, heating or oxidization.[4] This study indicated that a dye molecule, azobenzene (AB) can be included into β-CD, effectively improving the stability of the dye molecule. Moreover, because the exterior of CD is hydrophilic,[1] it is possible to improve the aqueous dispersibility of hydrophobic dye molecules after including them into CD. Thus, applications to paints and food dyes can also be expected.

In order to synthesize inclusion complexes, many studies have been carried out in solutions.[1–5] Solute species are limited to this method because CDs are dissolved only in a solvent with high polarity such as water. Conversely in aqueous solution some non-polar dyes are insoluble. Supercritical carbon dioxide (supercritical $CO_2$) is a fluid with low polarity. It exhibits both gas-like and liquid-like characteristics: its low viscosity and high diffusivity cause rapid mass transfer across a boundary, even through a solid matrix, and it has a density close to those of liquid solvents. After supercritical $CO_2$-treatment, a solid sample can be obtained easily by removing the carbon dioxide, thus making it unnecessary to consider the problem of a residual solvent. This report describes how dye molecules can be complexed with β-CD by the use of the supercritical $CO_2$ technique, even at temperatures below their melting point.

The inclusion experiments using supercritical $CO_2$ were performed in an apparatus (Nihon Bunko, Japan) consisting of a high-pressure pump, a backpressure regulator, a heater, and two high-pressure cells (50 cm$^3$) connected by a stainless steel pipe with inside diameter of 0.5 mm. One gram of β-CD powder was placed into one high pressure cell and 0.16 g AB (both obtained from Wako Pure Chemical Industries Ltd., Japan) into the other. $CO_2$ gas was pumped into the AB cell, and then into the β-CD cell until the pressure and temperature reached 30 MPa and 50 °C, respectively, and then maintained statically at this condition. The treatment temperature was deliberately selected as 50 °C, because this is lower than the melting point of AB of 68.5 °C.[6] After 24 h, the temperature was lowered to below the critical point of $CO_2$, 31.1 °C, and subsequently the $CO_2$ gas was slowly removed. The powders were agitated during this supercritical $CO_2$ treatment. The contents of C, H, and N in the obtained sample were analyzed as 40.1, 6.27, and 0.44 wt%, using a SUMIGRAPH NCH-21 nitrogen/carbon/hydrogen determination unit. Thus, the molar ratio of CD to AB was calculated as 5. The presence of AB molecules in the obtained sample implies that those AB molecules were dissolved in the supercritical $CO_2$, and delivered into the cell where β-CD was set.

The obtained powder was subjected to DSC analysis with a Perkin-Elmer Pyris 7 differential scanning calorimeter. Samples were prepared in P/N SSC000E031 AL15 DSC capsules. They were heated from 20 °C to 150 °C at 10 °C min$^{-1}$. As shown in Fig. 1, there was no thermal effect on the curve of β-CD as-provided and that which was supercritical $CO_2$-treated (a and b, respectively). The endothermic peak at 67 °C for AB (c in Fig. 1) is due to the melting of AB. This kind of melting peak was also observed in the DSC curves (d in Fig. 1) for the sample physically mixed using a mortar at the same β-CD/AB ratio as that of the supercritical $CO_2$-treated β-CD/AB sample. However, the supercritical $CO_2$-treated sample (e) did not show the endothermic peak corresponding to the melting of AB. This behavior suggests that β-CD was complexed with AB by inclusion at the molecular level. A strong exothermic peak (with enthalpy $\Delta H$ about $-19$ J g$^{-1}$) appears beginning at 83 °C. The difference in the DSC curve of the supercritical $CO_2$-treated sample from the other samples suggests the formation of an inclusion complex. It is interesting that when studying the interaction of the starch–glycerol complex system, Smits *et al.* observed an exothermal peak.[7] This behavior was explained as due to the development of a strong starch–polyol interaction. We assume that the exothermic peak in the present case can be explained as the rearrangement. The β-CD complexed with AB by inclusion proceeds to a more stable state by rearrangement of the inclusion structure.

The X-ray diffraction data were collected using a Rigaku RINT 2100 X-ray diffractometer. As shown in Fig. 2, all the peaks of β-CD, indexed to a monoclinic symmetry (JCPDS 17-1024) with $a$ = 15.3, $b$ = 10.2, $c$ = 21.0 and $\beta$ = 68.1°, indicated a highly crystallized structure. Peaks in the XRD pattern of the β-CD treated at the same supercritical $CO_2$ conditions as that of the complex indicated lower crystallinity (Fig. 2b). However, the pattern of the

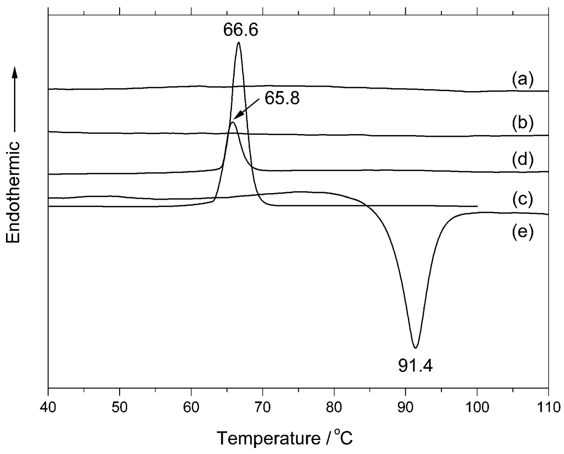

**Fig. 1** DSC curves for (a) provided β-CD, (b) supercritical $CO_2$-treated β-CD, (c) azobenzene, (d) physical mixture of β-CD and azobenzene, and (e) β-CD/azobenzene complex.

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complex has three main diffraction halos indicating the total suppression of the crystalline structure of β-CD. This is in accordance with the observations for the β-CD and fullerene inclusion complex.[8] The peaks of AB ($2\theta$ = 12.6, 19.0, and 25.3°) could not be observed, while in the profile for the mixture they are clearly distinguishable from β-CD.

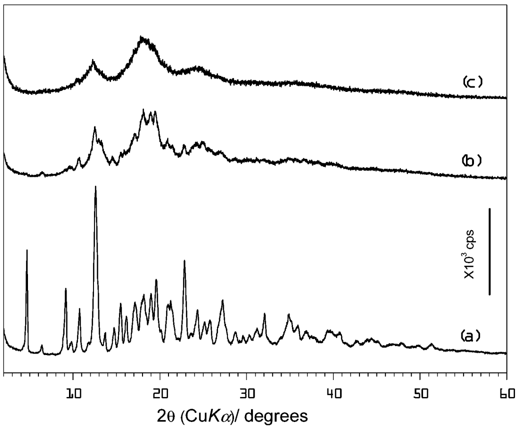

**Fig. 2** X-Ray diffraction patterns for (a) provided and (b) supercritical $CO_2$-treated β-CD and (c) β-CD/azobenzene complex.

The above results show that with supercritical $CO_2$-treatment below the melting point of azobenzene, aqueous-insoluble azobenzene molecules can be dissolved in the supercritical $CO_2$, and included into solid-state β-CD forming an inclusion complex.

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