

RESEARCH PAPER

Solid state characterization of trans resveratrol complexes with different cyclodextrins

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Abstract

Trans resveratrol (Res) has been investigated numerously because of having interesting pharmacological effects but Res has low bioavailability. As the solubility, stability and bioavailability of a drug can be increased by a complexation with cyclodextrin (CD), it was herein to prepare Res-CD complexes and their physical mixtures different types of CD. In order to understand the solid state behavior, various characterization techniques were applied. The results from differential scanning calorimetry showed double peaks at 254°C and 269°C, demonstrating the melting point of pure Res. Most of the pure CDs decomposed over 300°C. Beta-CD and 2-hydroxypropyl-beta-CD had a significant peak around 100°C which demonstrated the evaporation of water. Alpha-CD had many peaks between 80-140°C and also 269°C which differed from dimethyl-beta-CD. Res-alpha-CD complex showed peaks at 130°C and 269°C, whereas Res-beta-CD only showed peak at 269°C. The modified CDs did not show the significant peak of Res or water. This indicated that Res replaced water in the cavity and a real inclusion complex was formed. The infrared spectra also confirmed the formation of complex. The small and wide angle X-ray scattering patterns showed the difference of Res, CD and different complexes.

Introduction

Trans resveratrol (trans-3,5,4'-trihydroxystilbene; Res) has a relative molecular weight of 228.25 g/mol and can be found in several plants, e.g., grapes, peanuts, mulberries. Res was first isolated from leaves of the white lily (*Lilium candidum*) and was already well known in the traditional Japanese medicine, as an active ingredient in the Japanese knotgrass (*Polygonum cuspidatum*), for a long time. Res has received more attention nowadays in the prevention or therapy of many diseases including cancers, cardiovascular diseases. It has also shown that Res can modulate lipid metabolism and inhibit platelet activation and aggregation (Baur and Sinclair, 2006). Because of high hydrophobicity of Res, it has low water solubility (0.03 g/L) and consequently has a low bioavailability in human body. Many researchers have tried to improve the solubility and/or bioavailability of Res by different techniques, e.g., nanoemulsification, micellization, inclusion complex formation with cyclodextrin (CD), etc (Bertacche *et al*, 2006; Amri *et al*, 2012; Das and Ng, 2012; Hung *et al*, 2008; Lu *et al*, 2009; Lucas-Abellan *et al*, 2007; Lucas-Abellan *et al*, 2008; Lucas-Abellan *et al*, 2011; Kumpugdee-Vollrath *et al*, 2012; Santos *et al*, 2011).

In recent years, applications of CD as pharmaceutical additive have been growing rapidly. CD is cyclic oligosaccharides derived from starch with six, seven or eight glucose residues linked by $\alpha(1\rightarrow4)$ glycosidic bonds and denominated alpha-, beta-, and gamma-CD, respectively. It can be represented as a truncated cone structure with a hydrophobic cavity while the rims of the surrounding walls are hydrophilic (Brewster and Loftsson, 2007). The hydrophobicity forms inclusion complexes with a wide range of guest molecules. Inclusion of

a drug molecule will alter physicochemical properties and reduce the undesirable side effects. It was demonstrated that complexation can considerably increase the solubility, stability and bioavailability of poorly water-soluble drugs (Lu *et al*, 2009; Lucas-Abellan *et al*, 2007). Currently, inclusion of Res with CD has been reported in very few publications. Most of them focus on the use of beta-CD or the modified beta-CD. The formation of inclusion complexes, in the solution state, has been investigated by different methods, such as nuclear magnetic resonance, enzymatic and fluorimetric assays (Lu *et al*, 2009; Lucas-Abellan *et al*, 2008). However, the solid state experiments of inclusion of Res with CD have not well reported. Although Bertacche *et al* (2006) have already reported the solid state characteristics of Res-CD inclusion complexes that were prepared by suspension method, while other techniques to prepare such complexes have not yet reported.

In this study, the Res-CD complexes were prepared by wetting method which requires less solvent and may give new information in relation to solid state properties of Res-CD complexes. Moreover, the study also used small and wide angle X-ray scattering (SWAXS) technique which might give the evidence of the solid state structure of the substances (Gramdorf *et al*, 2008; Wonglerntirant *et al*, 2012). Other techniques, i.e., differential scanning calorimetry (DSC) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) were also used to confirm the formation of the inclusion complexes.

Materials and Methods

Materials: Resveratrol (trans, 98% content, *Polygonum cuspidatum*) was purchased from Behr GmbH, Stuttgart Germany. Different types of CD (alpha-, beta-, gamma-, 2-hydroxy propyl-beta-, dimethyl-beta-CD) were purchased from ABCR GmbH & Co. KG (Karlsruhe, Germany). Ethanol (analytical grade) was purchased from Merck KGaA (Germany).

Preparation of complexes and physical mixtures: The complexes of Res and different types of CD were prepared by mixing the powders (Res and CD) in an intended molar ratio (1:1, 1:3 and 1:5). Both ingredients were mixed in a mortar and absolute ethanol was added dropwise until a consistent paste-like mixture occurred. After 1-h reaction time in the dark, the mixture was dried for 3 days in a vacuum compartment dryer (Model P0096, Thermo Electron Corporation, Massachusetts, USA) at 25°C and 0 bar. Finally, the dried mixture was ground and sieved to obtain fine powders (200 µm). The physical mixtures were prepared by simply mixing Res with the relevant CD in a molar ratio of 1:1 in a mortar without ethanol. Consequently, the final product was received as powder. The complexes and physical mixtures were kept for further characterization.

Solid state characterization by DSC: The DSC thermograms of the different samples were observed by a Perkin Elmer DSC 7 (Perkin Elmer, Massachusetts, USA), which was calibrated with indium. The thermal behavior was studied by heating 2-4 mg of sample in aluminum pans (maximum volume of 30 µl) with holes under nitrogen gas at the temperature range of 50 – 350°C using heating rates of 20°C·min⁻¹. An empty aluminium pan was used as a reference.

Solid state characterization by ATR-FTIR spectroscopy: ATR-FTIR spectra were obtained using a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Massachusetts, USA) equipped with a crystal diamond universal ATR sampling accessory (UATR). Before each measurement, the ATR crystal was carefully cleaned with ethanol. During the measurement, the sample was in contact with the universal diamond ATR top-plate. For each sample, the spectrum represented an average of 4 scans was recorded in the range of 4000-400 cm⁻¹ with a 4 cm⁻¹ resolution.

Solid state characterization by SWAXS: The experiments were performed at the small and wide angle X-ray scattering (SAXS and WAXS) instrument, beamline B1, installed at the DORIS III synchrotron source at HASYLAB/DESY in Hamburg, Germany. The SAXS scattering patterns (0-1 Å) were acquired using a large area pixel detector (Model PILATUS 1M, Dectris, Switzerland) with pixel size of 172 µm × 172 µm. The WAXS (1-4 Å) was measured simultaneously using a Mythen strip detector (Dectris, Switzerland). The distance from sample to detector was 0.885 m and the X-ray energy was 14 keV. Each sample as powder was put in between the special tape (Scotch Magic®, 3M, France) and then fixed onto a metallic holder, which was then placed into a vacuum chamber and measured for SWAXS. The raw scattering data were background corrected, integrated and calibrated using a MATLAB-based analysis suite, which was available at the beamline.

Results and Discussion

The melting points of pure Res are shown as double peaks at 254°C and 269°C, while most of pure CD decomposed over 300°C (Figure 1).

Solid state characterization of resveratrol complexes

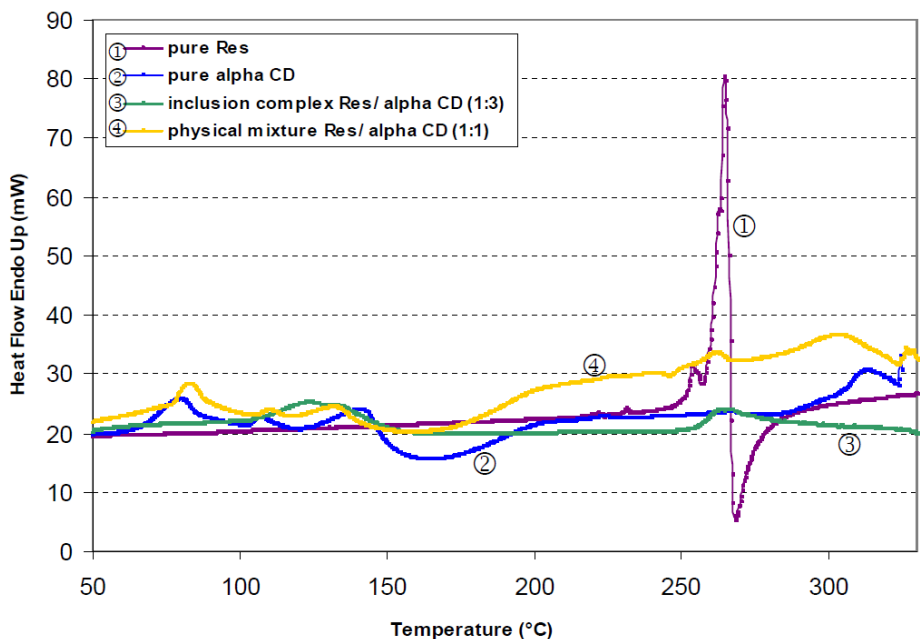


Figure 1 DSC thermograms of Res, alpha-CD, their complex and physical mixture

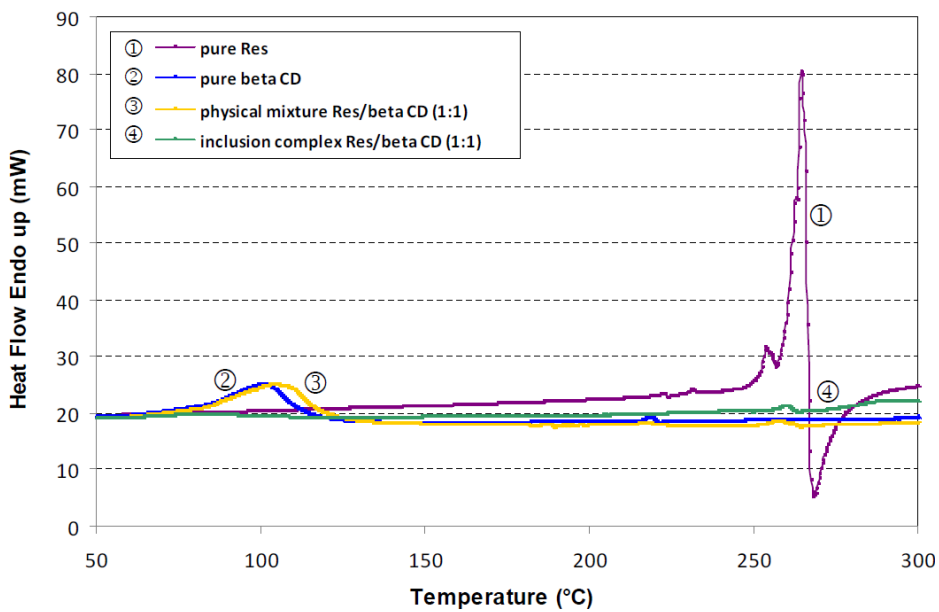


Figure 2 DSC thermograms of Res, beta-CD, their complex and physical mixture

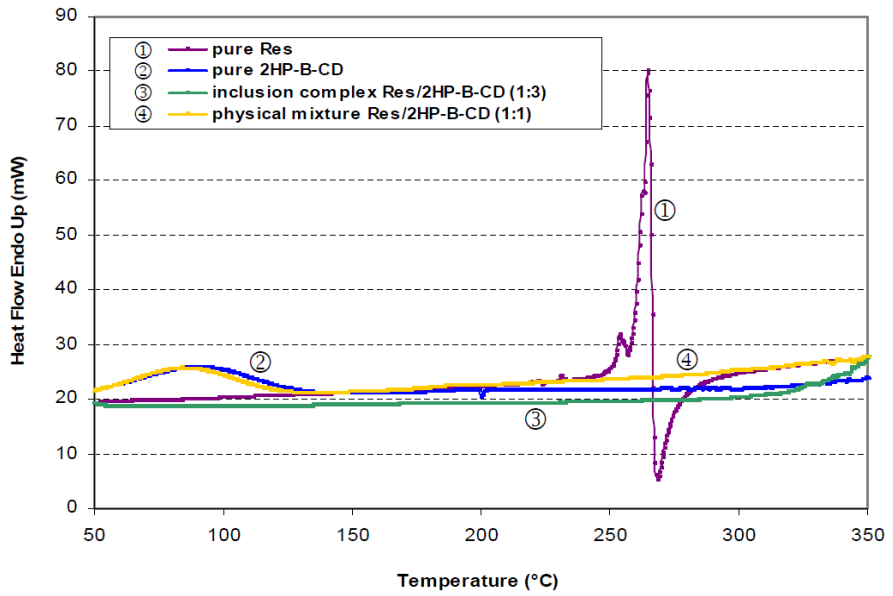


Figure 3 DSC thermograms of Res, 2HP-B-CD, their complex and physical mixture

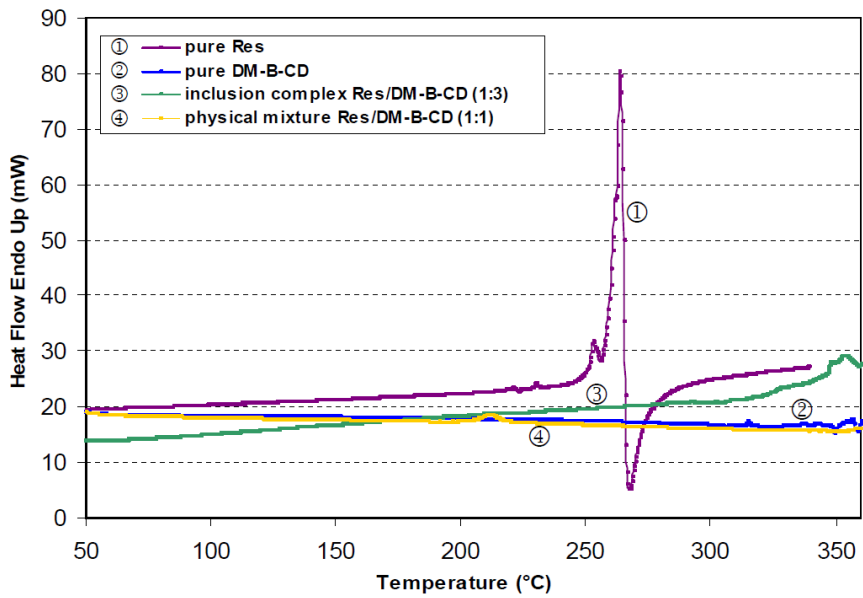


Figure 4 DSC thermograms of Res, DM-B-CD, their complex and physical mixture

Solid state characterization of resveratrol complexes

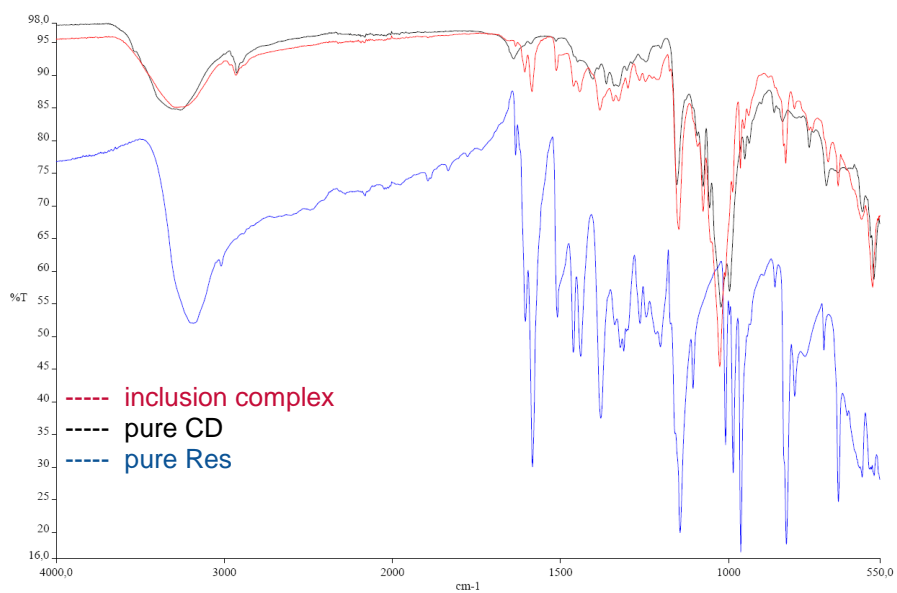


Figure 5 IR spectra of Res, alpha-CD, their complex (1:1)

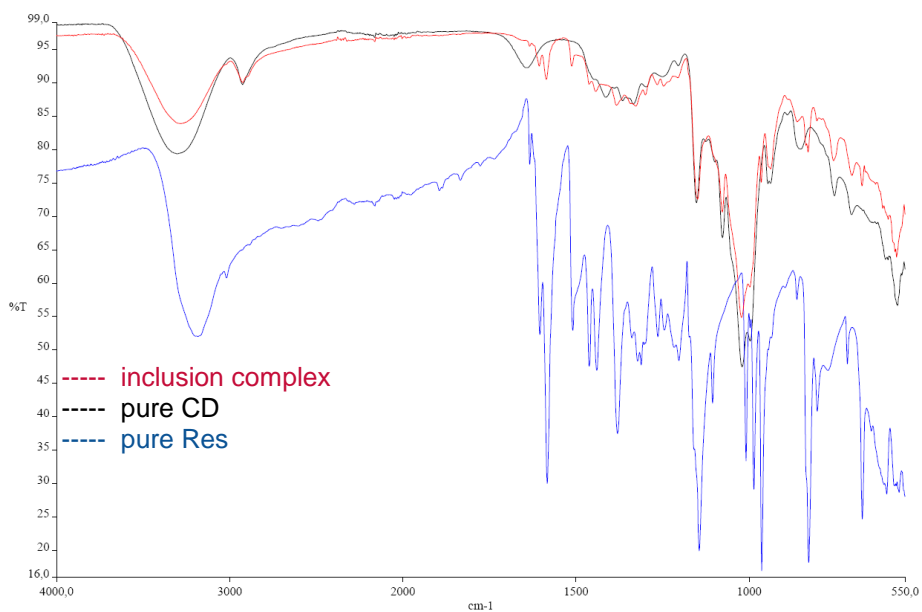


Figure 6 IR spectra of Res, beta-CD, their complex (1:1)

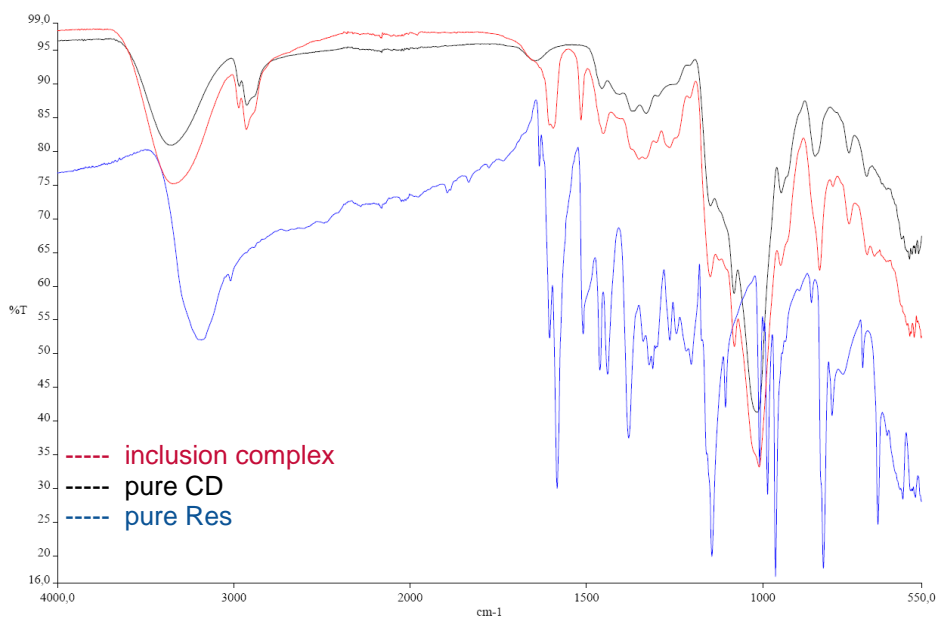


Figure 7 IR spectra of Res, 2HP-B-CD, their complex (1:1)

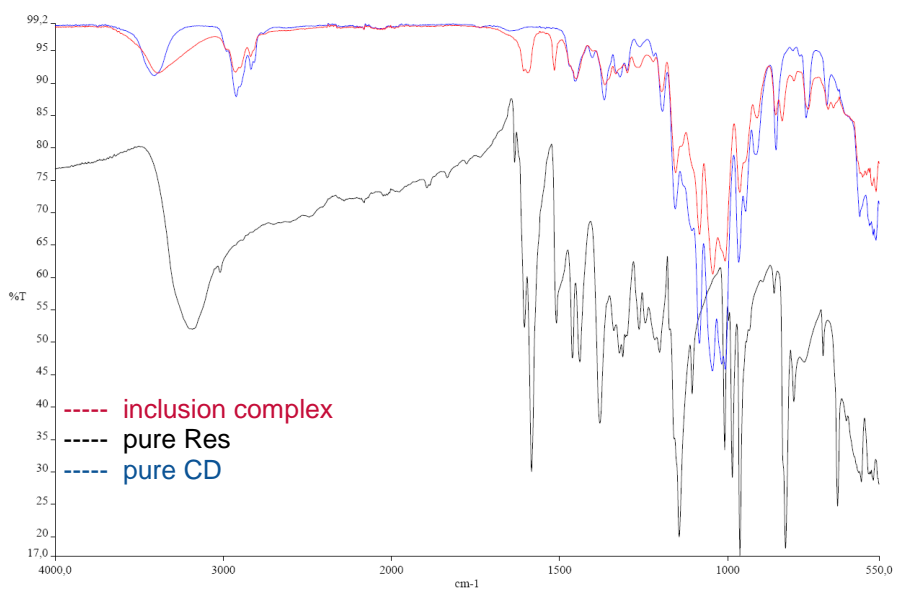


Figure 8 IR spectra of Res, DM-B-CD, their complex (1:1)

Solid state characterization of resveratrol complexes

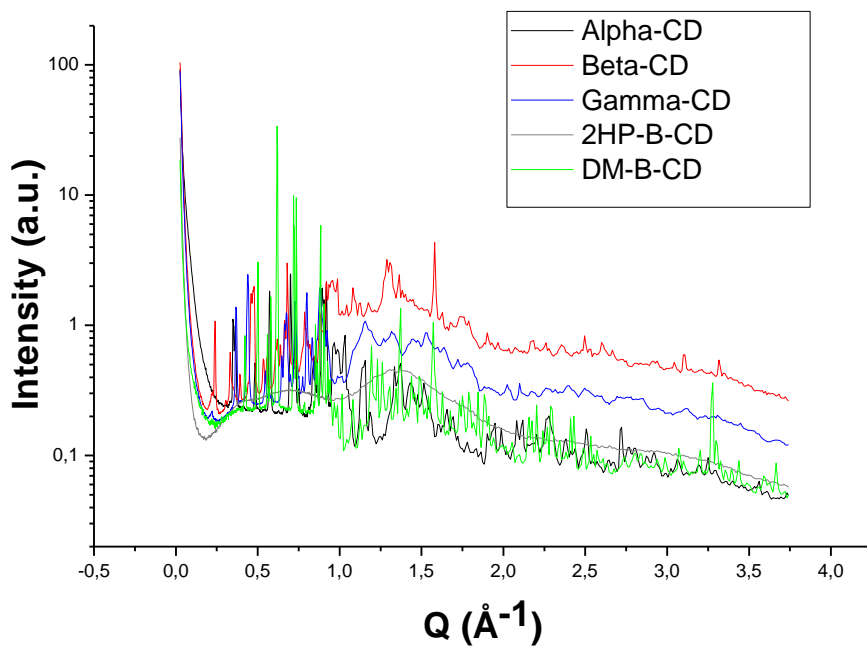


Figure 9 SWAXS patterns of different CDs

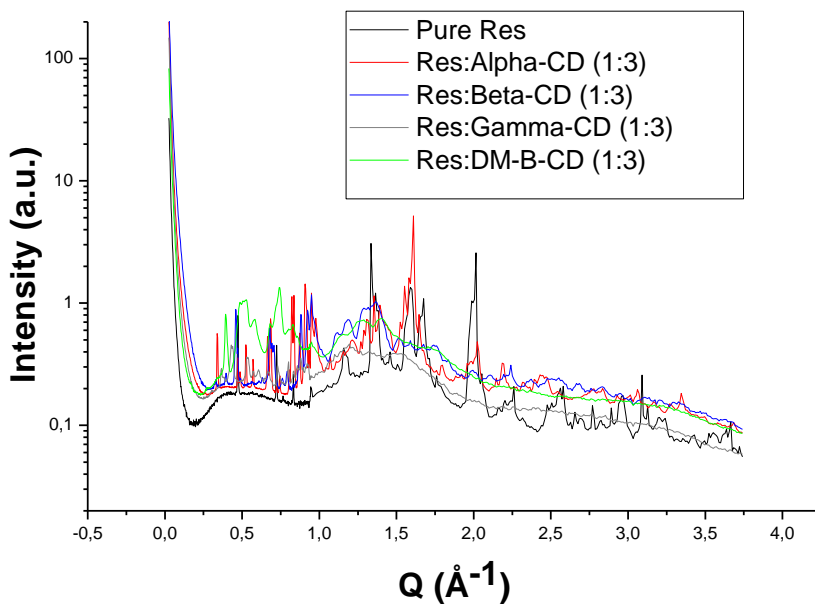


Figure 10 SWAXS patterns of Res, and Res-CD complexes using different CDs

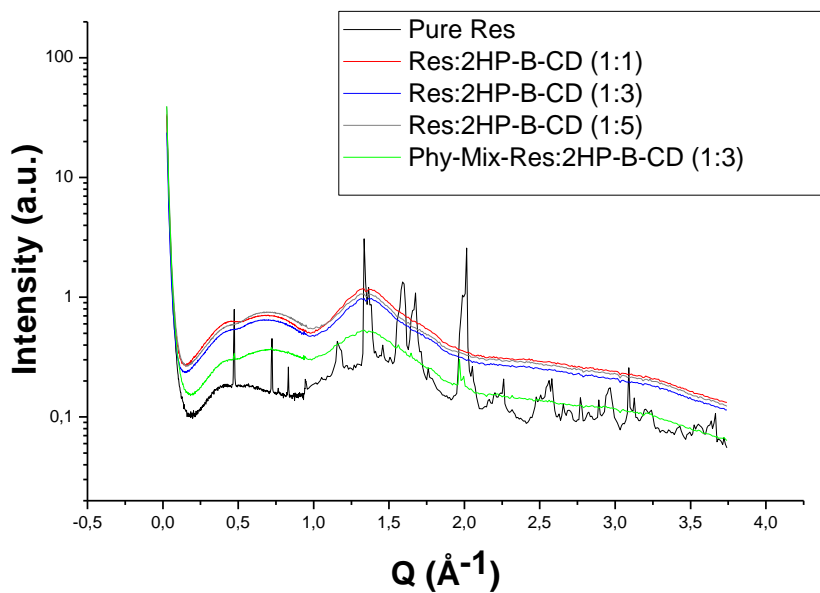


Figure 11 SWAXS patterns of Res, Res-2HP-B-CD complexes at different molar ratios, and physical mixture of Res and 2HP-B-CD

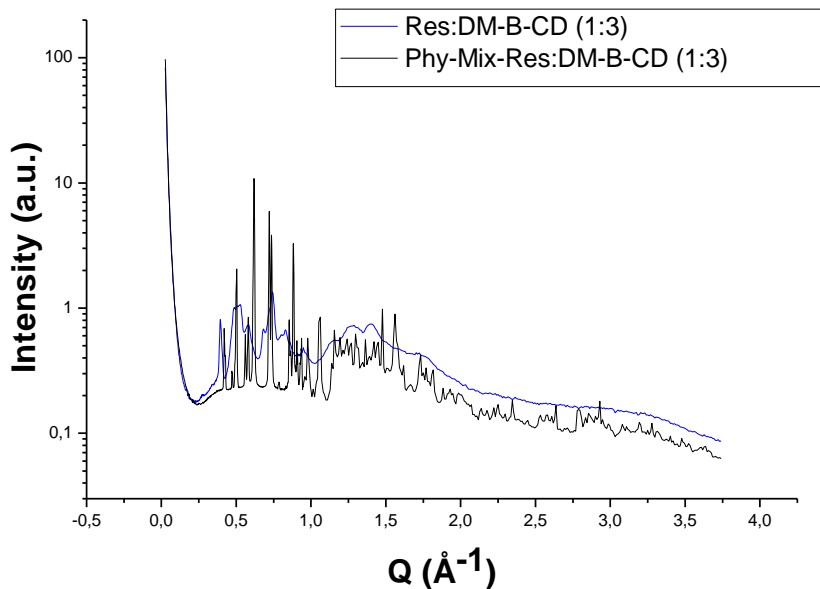


Figure 12 SWAXS patterns of Res-DM-B-CD-complex, and physical mixture of Res and DM-B-CD

Beta-CD (Figure 2) and 2-hydroxypropyl-beta-CD (2HP-B-CD) (Figure 3) had a significant peak at around 100°C, which demonstrated the evaporation of water. This indicated that water was included in the cavities of CD. Alpha-CD (Figure 1) had many peaks between 80-140°C and also 269°C which differed from dimethyl-beta-CD (DM-B-CD) (Figure 4). DM-B-CD did not show any peak. If the complexation was compared, it can be noticed that Res-alpha-CD complex shows peaks at 130°C and 269°C (Figure 1), whereas Res-beta-CD (Figure 2) only showed peak at 269°C. This means that water in beta-CD was removed but the complexation with Res was not completely achieved. The incompleteness of complexation was also found with Res-alpha-CD. The modified CDs, i.e., 2HP-B-CD (Figure 3) and DM-B-CD (Figure 4), do not show the significant peaks of Res at 254°C or 269°C or of water at 100°C. This indicates that Res replaced the water in the cavity and a real inclusion complex was formed. If the physical mixtures of native CDs, i.e., alpha-CD and beta-CD were compared (Figures 1 and 2), the significant peaks of water (~100°C) and of Res (269°C) can be detected in both native CDs. It is surprising that the physical mixture of Res and modified CDs, i.e., 2HP-B-CD and DM-B-CD, did not show the significant peaks of Res at 269°C. Only one small peak was detected, in RES-DM-B-CD, at about 210°C. The small peak of water at about 100°C can be detected in RES-DM-B-CD similar to that of the complex. It is likely that the physical mixing process can already incorporate Res into the cavity of 2HP-B-CD. This result should be confirmed with other techniques. Moreover, the results obtained in this work are different from the published paper (Bertacche *et al*, 2006), resulting from the different inclusion

complex preparation techniques. Thus, it is important to use the appropriate technique to achieve the expected output.

The ATR-FTIR method makes it possible to monitor the slightest changes in the state of the sample, since there is no sample preparation, unlike in the case of the KBr pellet method, when grinding and pressing mostly unifies the samples with weak interactions. The fingerprints of Res and CDs were clearly demonstrated (e.g., Figure 5). Res showed three typical strong absorption bands at 1605 cm⁻¹, 1583 cm⁻¹ and 1380 cm⁻¹ corresponding to C-C aromatic double bond stretching, C-C olefinic stretching and C-C stretching, respectively. The band at 964 cm⁻¹ demonstrated the trans-form of Res (Bertacche *et al*, 2006). Different CDs (native or modified) also have significant absorption bands in the regions of 1600-700 cm⁻¹ (Figures 5-8). However, they did not have high intensities as Res.

The FTIR spectra of the complexes between Res and CD mostly show the significant bands of CD (e.g., Figure 7). The significant bands of Res (964 cm⁻¹ and 3184 cm⁻¹) and 2HP-B-CD (1645 cm⁻¹) disappeared but the new bands at 1595 and 1516 cm⁻¹ were observed. This may be due to the formation of new chemical bond between Res and CD inside the cavity. Some bands (e.g., 1585 cm⁻¹ and 1606 cm⁻¹) of Res can be seen in the spectra of complex with native CDs (Figures 5 and 6). The data are consistent with that reported in the literature with only small difference (Bertacche *et al*, 2006). The FTIR spectra pointed out that the formation of complex occurred in all CD types but not all CDs can give the complete inclusion complex. There is no significant difference between 1:1 and

1:3 molar ratio; only the intensities of bands were different but not the shifting or formation of new bands.

All pure substances, except 2HP-B-CD, showed many significant peaks in the region of SAXS (0-1 Å) and WAXS (1-4 Å) (Figures 9-10). This result suggested that the well-ordered crystalline structure was formed.

Only 2HP-B-CD was amorphous, because of broad peaks. The complexes between Res and 2HP-B-CD at different ratios (Figure 11) existed as an amorphous state. It means that the inclusion complex completely formed in a wide range of molar ratio (i.e., 1:1, 1:3, 1:5). It is possible that 2HP-B-CD exhibits stronger inclusive ability than native CDs, implying that the cavity of modified CD provides a better protective microenvironment. The substitution by hydroxypropyl may lead to the enlargement of the bigger opening of native CDs and the break of strong intramolecular hydrogen bond network, leading to the strong inclusive ability of 2HP-B-CD (Lu *et al*, 2009). The physical mixture of Res and 2HP-B-CD at molar ratio of 1:3 showed some significant peaks of Res (0.4 and 2 Å), indicating that the complex was not completely formed. Res-DM-B-CD complex show different SWAXS patterns (Figure 12), which can be observed by disappearance of typical peaks of CD (1.3-3.5 Å) or Res (0.4 and 1.3-3.5 Å). This indicated that the complexation may be formed but the structure was still crystalline.

Conclusion

The complexation of Res and different types of CD can be confirmed and analyzed by using different physical techniques. Modified CDs (e.g., 2HP-B-CD and DM-B-CD) can completely form complexes with Res. Other native CDs, i.e., alpha-CD and beta-

CD, seem to build only partially inclusion complexes, in which Res is not completely incorporated. All applied techniques complement each other very well. The DSC data can show whether there was any free Res by indicating the melting peak around 254 and 269°C. The IR data provided information about the functional groups involved in the complexation. The SWAXS data indicated the difference between amorphous and crystalline structure of the pure substances and the inclusion complexes. According to the present observation, 2HP-B-CD seems to be the best host for the Res. A complete inclusion can be observed without any free Res and no crystalline structure can be detected in a complex with 2HP-B-CD. This solid state structure should be benefit to improve solubility and stability of Res. Consequently, the bioavailability of Res in human body can be increased.

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