Determination of *trans* resveratrol and different types of cyclodextrins by SAXS and WAXS at B1

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Resveratrol (Res) can be found in several plants e.g. grapes, peanuts or mulberries. Res has been investigated in various research works because of its numerous pharmacological effects. Because of the low water solubility Res has a low bioavailability in human body. It is well known that the solubility of a drug can be increased by a complexation with cyclodextrins (Cd). Therefore, in our project we have prepared different Cd-complexes as well as physical mixtures with different types of cyclodextrins. In order to understand the solid state behaviour various techniques were applied i.e. small and wide angle X-ray scattering (SWAXS), DSC, and ATR-FTIR method.

Experimental methods

3.1 Materials

Resveratrol (trans, 98% content, Polygonum cuspidatum) was purchased from Behr GmbH, Stuttgart, Germany. The cyclodextrins (alpha-, beta-, 2-hydroxypropyl-beta- "2HP-beta-Cd" and 2,6-di-o-methyl-beta-cyclodextrin "DM-beta-Cd") were purchased from ABCR GmbH & Co. KG Karlsruhe, Germany. Ethanol for analysis was purchased from Merck KGaA, Germany.

3.2 Preparation of complexes and physical mixtures

The complexes of Res and different types of cyclodextrins were prepared by mixing the powders (Res & Cd) in an intended molar ratio (1:1, 1:3 or 1:5). Both ingredients were mixed in a mortar and absolute ethanol was added drop wise until a paste-like consistency occurred. After 1 h reaction time in the dark, the mixture was dried for 3 days in a vacuum compartment dryer at 25 °C and 0 bar. Finally, the dried mixture was grinded and sieved (200 µm) to obtain fine-grain powder. The physical mixtures were prepared by simple 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 determined afterwards with the following techniques (3.3-3.5).

3.3 Differential scanning calorimetry (DSC)

The DSC thermograms of the different samples were recorded on a Perkin Elmer DSC 7 differential scanning calorimeter which was calibrated with indium. The thermal behaviour was studied by heating 2-4 mg sample in aluminum pans with holes (30 µl) under nitrogen gas at the temperature range of 50 – 350°C using heating rates of 20 °C/min. An empty aluminium pan served as a reference.

3.4 Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were obtained using a Perkin Elmer spectrophotometer equipped with a crystal diamond universal ATR sampling accessory (UATR). During the measurement the sample was in contact with the Universal diamond ATR top-plate. For each sample, the spectra represented an average of 4 scans recorded in the 4000-400 cm^{-1} range with a 4 cm^{-1} resolution.

3.5 Small and wide angle X-ray scattering (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 (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. The samples as powders were 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. Peak positions were determined using Origin-Program by fitting a Gaussian equation to the data.

Results and Discussion

The results from DSC showed that pure Res melt at ~ 254°C and pure Cd decomposed over 350°C. Modified Cds i.e. hydroxylpropyl or dimethyl did not show free Res in their mixtures. There was also no peak around 100°C, which suggested that Res replaced the water and the formation of a real inclusion complex took place. In contrast the natural Cds alpha and beta showed peaks around 260°C and 100°C in their mixtures with Res. This indicated that there was still free Res and water in the Cds cavity. The complexation was not completely performed. The IR-spectra also confirmed the formation of complexation by showing the shifting of bands or even disappearance of peaks. The SWAXS-scattering patterns showed the differences of the pure Res and the complexation. The structure was amorphous in a particular mixture i.e. Res with hydroxypropyl beta cyclodextrin.

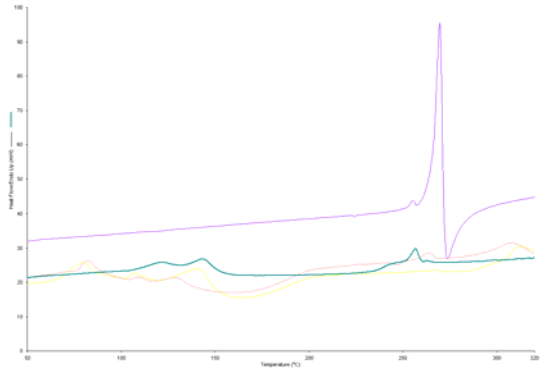

Figure 1: DSC thermograms of complexes and physical mixture of Res and alpha-cyclodextrin (1:1); violet = pure Res, green = complex, yellow = pure cyclodextrin, orange = physical mixture.

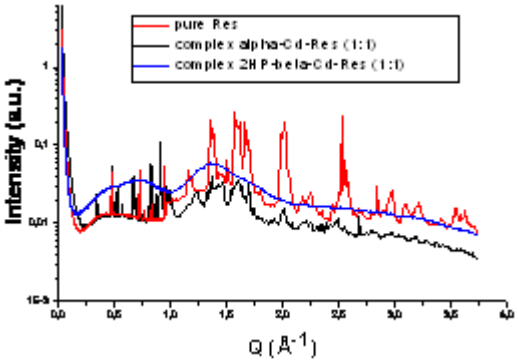

Figure 2: SWAXS pattern of complexes of Res and different cyclodextrins (1:1).

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References

[1] Kumpugdee-Vollrath M., et al. (2012). Increasing solubility of poorly water soluble drug resveratrol by surfactants and cyclodextrins, Advanced Materials Research, vol. 418-420, pp 2231-2234

[2] Bouwstra, J. A., et al (1992). The influence of alkyl-azones on the ordering of the lamellae in human stratum corneum, International Journal of Pharmaceutics, vol. 79, pp. 141-148