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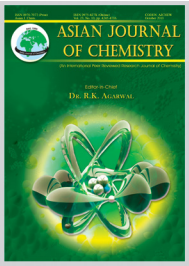

Characterization of Quercetin/Hydroxypropyl-β-Cyclodextrin Inclusion Complex

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The objective of the present study is to prepare and characterize quercetin/hydroxypropyl-β-cyclodextrin inclusion complex to improve its aqueous solubility. Quercetin/hydroxypropyl-β-cyclodextrin inclusion complex with 1:1 molar ratio was prepared by rotational electromagnetic stirring and rotating evaporation methods. Characterization of quercetin/hydroxypropyl-β-cyclodextrin inclusion complex was done by differential scanning calorimetry, fourier-transform infrared spectroscopy, X-ray powder diffractometry and scanning electron microscopy. All these approaches showed that quercetin/hydroxypropyl-β-cyclodextrin inclusion complex indicated the formation of new phase. The ability of water solubility of quercetin/hydroxypropyl-β-cyclodextrin inclusion complex was analyzed by high pressure liquid chromatography. Results showed that the ability of water solubility of quercetin in quercetin/hydroxypropyl-β-cyclodextrin inclusion complex was improved six times than quercetin monomer by the inclusion technique.

Key Words: Quercetin, Hydroxypropyl-β-cyclodextrin, Quercetin/hydroxypropyl-β-cyclodextrin inclusion complex, Phase behaviour.

INTRODUCTION

Quercetin is a typical antioxidative flavonoid ubiquitously distributed in various foodstuffs, herbs and dietary supplements[1]. It is likely to act as a bioactive compound by exerting reactive oxygen species (ROS)-scavenging activity and/or binding to specific proteins such as oxidative enzymes and transcriptional factors in signal transduction pathways[2]. However, the utility of quercetin in pharmaceutics is greatly limited because of its extremely low solubility in water.

Many efforts have been made to improve the solubility and dissolution rate of poorly water-soluble drugs. Among them, particularly in recent years, the successful utilization of complex technique with cyclodextrins to form inclusion complex substantially improves the solubility, chemical stability and bioavailability of poorly water-soluble substances[3,4].

Different techniques are adopted in drug delivery with thermosensitive gels, including dispersing the drug in the gel with a concentration higher than its solubility value and dispersing drug-loaded nanoparticles, liposomes and drug: cyclodextrin complexes[5-9]. Cyclodextrins are used in the pharmaceutical field to form inclusion complexes with drug molecules to enhance their aqueous stability or photostability, to mask unwanted characteristics or to reduce side effects[10,11]. Cyclodextrins are also reported to convey controlled-release properties to certain active ingredients[12].

The main objective of the present study was to evaluate the effect of hydroxypropyl-β-cyclodextrin on the aqueous solubility of quercetin and put forward a kind of rapid determination method for the content of quercetin in quercetin/hydroxypropyl-β-cyclodextrin inclusion complex by chromatographic analysis technology. For the wide application of quercetin in medicine provided a theoretical basis.

EXPERIMENTAL

Quercetin and HPLC-grade methanol was purchased from Sigma (Steinheim, Germany) and hydroxypropyl-β-cyclodextrin was purchased from Tianji Bodi Chemical Holding Co. Ltd., (Tianji, China). Analytical-reagent grade anhydrous alcohol, disodium phosphate and sodium dihydrogen phosphate and phosphoric acid were purchased from Shanghai Reagent Co. (Shanghai, China). An Ultra-pure Water System (SG Ultra Clear system, Wasseraufbereitung und Regenerierstation GmbH, Germany) was used to produce ultra pure water with specific conductivity down to 0.055 μS/cm for the assay analysis.

Differential scanning calorimetry (DSC, Q10, TA Instruments, New Castle, DE). Fourier transform infrared spectroscopy (FT-IR, TENSOR27, BRUKER, Germany). X-ray

diffractometry (XRD, BRUKER.axs, Germany). Scanning electron microscopy (SEM, Quan200, FEI, USA). High-performance liquid chromatography (HPLC, Surveyor, Thermo Finnigan, MA, USA).

Preparation of quercetin/hydroxypropyl-β-cyclodextrin inclusion complex: The inclusion complex of quercetin with hydroxypropyl-β-cyclodextrin in a 1:1 molar ratio was prepared according to the colyophilization technique[13]. A fixed amount of quercetin was weighed and transferred into a volumetric flask and made up to 50 mL with anhydrous ethyl alcohol. Hydroxypropyl-β-cyclodextrin was also dissolved in ultra-pure water. Then, quercetin solution was added drop by drop onto hydroxypropyl-β-cyclodextrin solution. Inclusion complex with 1:1 molar ratios was prepared by rotational electromagnetic stirring appropriate amounts of quercetin and hydroxypropyl-β-cyclodextrin mixed solution at 25 ± 1 ºC, for 24 h. After equilibrium and removal of the anhydrous ethyl alcohol with a rotating evaporation at 75 ºC. The evaporation solution was transfered to freeze-dry machine to dry 24 h when its quality basic didn't change. Yellow inclusion complex was synthesized and stored at -20 ºC until further used.

Quercetin/hydroxypropyl-β-cyclodextrin physical mixtures were prepared by mixing the exactly weighed amounts of quercetin and hydroxypropyl-β-cyclodextrin in a 1:1 molar ratio.

Characterization of quercetin/hydroxypropyl-β-cyclodextrin inclusion complex: Differential scanning calorimetry (DSC) analyses were performed on quercetin/hydroxypropyl-β-cyclodextrin complex, quercetin/hydroxypropyl-β-cyclodextrin physical mixture, hydroxypropyl-β-cyclodextrin and quercetin samples. Each sample weighing 2 mg was heated in hermetically sealed aluminum pans at a rate of 10 ºC/min up to 350 ºC in a dynamic nitrogen atmosphere.

Fourier transform infrared spectra of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin complex, quercetin/hydroxypropyl-β-cyclodextrin physical mixture and quercetin were recorded using a Bruker Tensor 27 FT-IR spectrometer using discs of each sample and previously prepared potassium bromide containing 0.01 g of sample in 0.1 g of potassium bromide between the wavelengths of 400 and 4000 cm^{-1}.

Powder X-ray diffractometry of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin complex, quercetin/hydroxypropyl-β-cyclodextrin physical mixture and quercetin were carried out using a BRUKER.AES scanner with filter Ni, CuK_α radiation over the interval 10-80º/2θ. The operation data were as follows: voltage 40 kV, current 20 mA, filter Ni and scanning speed 1º/min.

Scanning electron microscopy was performed to of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin complex, quercetin/hydroxypropyl-β-cyclodextrin physical mixture and quercetin. The samples were mounted on metal stubs with a doublesided adhesive band and then sputtered with a 100 Å thick layer of gold. They were examined with a Quanta 200 environmental scanning electron microscope at an acceleration voltage of 80 kV.

High pressure liquid chromatography (HPLC) analysis was performed to quercetin and quercetin/hydroxypropyl-β-cyclodextrin complex. These analyses were carried out employing an on-line coupling between UV and RP-HPLC. The chromatographic system consisted of an online Finnigan surveyor degasser, Finnigan surveyor pump plus equipped with a six-way ratary valve sampler with a 20 µL sample loop and a diode array UV detection (DAD) system (Thermo Finnigan, MA, USA). A EclipseXDB-C_{18} column (250 × 4.6 mm I.D.) was used for the analysis.

RESULTS AND DISCUSSION

Differential scanning calorimetry: Representative differential scanning calorimetry thermograms, measuring the rate of heat absorbed by quercetin/hydroxypropyl-β-cyclodextrin complex, physical mixture, hydroxypropyl-β-cyclodextrin, quercetin are shown in Fig. 1. Quercetin had two obviously endothermic peak at 140.08 ºC and 324.89 ºC. In the thermogram of hydroxypropyl-β-cyclodextrin, one endothermic peak was observed due to the loss of crystal water at 188.62 ºC. In the differential scanning calorimetry curve of physical mixture, two weakly endothermic peaks at 142.56 ºC and 278.85 ºC were found, which suggested the weak interaction between the two components without a formation of a new phase. As in the inclusion complex, an endothermic absorption peak was detected at 108.42 in the differential scanning calorimetry curve and the two peaks of quercetin at 140.08 ºC and 324.89 ºC disappeared at the same time. The phenomenon showed the presence of a new phase without quercetin crystal in the inclusion complex.

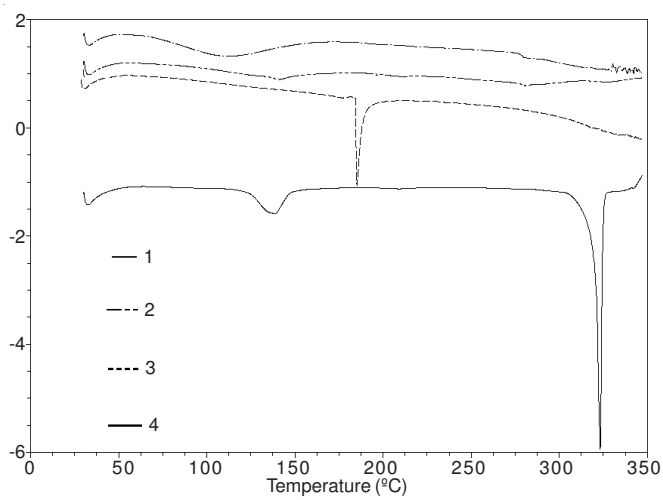

Fig. 1. Differential scanning calorimetry analysis of (1) quercetin/hydroxypropyl-β-cyclodextrin complex (2) quercetin/hydroxypropyl-β-cyclodextrin physical mixture (3) hydroxypropyl-β-cyclodextrin (4) quercetin

Fourier transform infrared spectroscopy: Fourier transform infrared spectroscopy of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin physical mixture, quercetin/hydroxypropyl-β-cyclodextrin complex, quercetin are shown in Fig. 2. The spectra of physical mixture in 3480, 2900, 1190 and 1050 cm^{-1} showed correspondence to superposition of its parent products with relatively weak absorption. From the spectrum of quercetin/hydroxypropyl-β-cyclodextrin complex, the characteristic absorption band at 1613, 1562, 930, 805, 541 cm^{-1} disappeared and the new characteristic absorption band at 1658, 852 and 415 cm^{-1} happened

at the same time. The spectra of inclusion complex demonstrated a relatively large difference compared with its parent products. The results further indicated the presence of a new phase.

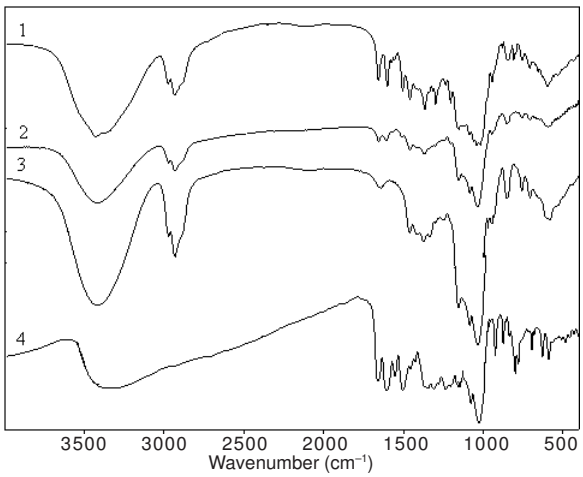

Fig. 2. FT-IR of (1) hydroxypropyl-β-cyclodextrin (2) quercetin/hydroxypropyl-β-cyclodextrin physical mixture (3) quercetin/hydroxypropyl-β-cyclodextrin complex (4) quercetin

X-ray diffractometry: The XRD patterns of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin physical mixture, quercetin/hydroxypropyl-β-cyclodextrin complex and quercetin are shown in Fig. 3. The X-ray diffraction spectrum of pure quercetin showed sharp and intense peaks at 10.70, 12.40, 17.14, 21.96, 23.80 and 27.30 with a diffraction angle of 2θ, suggesting that the quercetin was present as a crystalline material. Some of the quercetin crystallinity peaks were still present in the spectra of quercetin/hydroxypropyl-β-cyclodextrin physical mixture, especially at 12.40, 17.14, 21.96 and 27.30. In the meanwhile, some peaks were covered because some absorptive signals of hydroxypropyl-β-cyclodextrin itself were also detectable with the presence of quercetin. In contrast, there were no sharp peaks attributable to the crystalline form of quercetin in the inclusion complex, indicating that quercetin was no longer present as a crystalline, but was converted into an amorphous state.

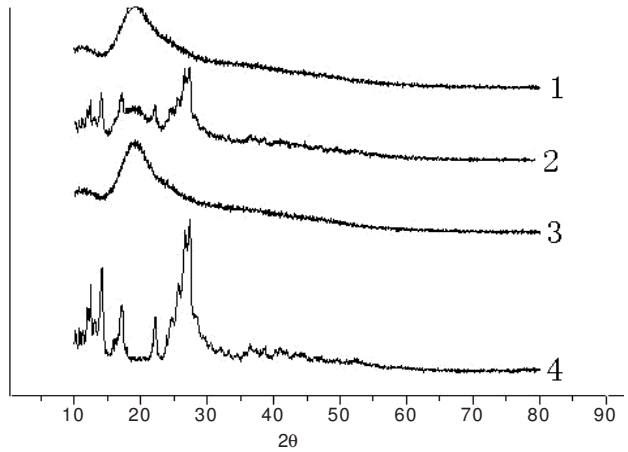

Fig. 3. XRD patterns of (1) hydroxypropyl-β-cyclodextrin (2) quercetin/hydroxypropyl-β-cyclodextrin physical mixture (3) quercetin/hydroxypropyl-β-cyclodextrin complex (4) quercetin

Scanning electron microscopy: SEM photomicrographs of hydroxypropyl-β-cyclodextrin, quercetin/hydroxypropyl-β-cyclodextrin physical mixture, quercetin/hydroxypropyl-β-cyclodextrin complex and quercetin are shown in Fig. 4. Fig. 4(1) represented amorphous state with many pores on its surface. Fig. 4(4) showed that quercetin was present as needle-like crystals assembling together. In quercetin/hydroxypropyl-β-cyclodextrin physical mixture, both needle-like and amorphous crystals were observed. In contrast, the quercetin/hydroxypropyl-β-cyclodextrin complex exhibited a totally different crystalline structure than the quercetin or the hydroxypropyl-β-cyclodextrin, with flake-type crystals suggesting the formation of a complex.

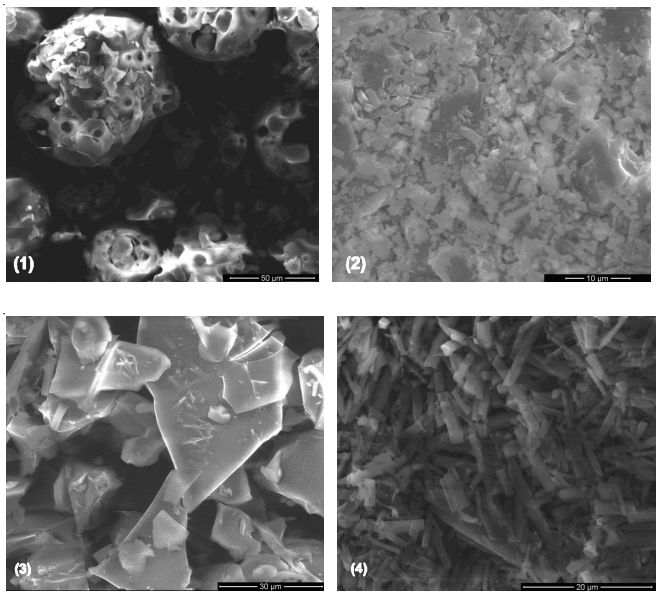

Fig. 4. SEM photomicrographs of (1) hydroxypropyl-β-cyclodextrin (2) quercetin/hydroxypropyl-β-cyclodextrin physical mixture (3) quercetin/hydroxypropyl-β-cyclodextrin complex (4) quercetin

HPLC analysis: A EclipseXDB-C_{18} column (250 mm × 4.6 mm, 5 μm) was used for the content determination of the quercetin aqueous solubility by HPLC. The column was maintained at 25 ºC throughout the analytic process and the eluate was monitored at 370 nm. The mobile phase was methanol-phosphate (60:40, v/v) at a flow rate of 1 mL/min. The injection volume was 20 μL. Fig. 5 showed the chromatogram of the standard solution of quercetin under the optimal chromatographic conditions.

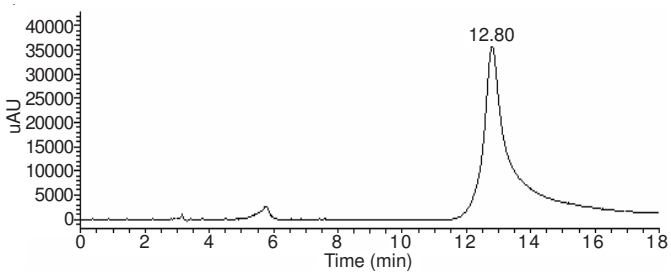

Fig. 5. Chromatogram of the standard solution of quercetin

To get the best response, the quercetin standard solution was scanned between 200 and 600 nm on the HPLC detector

in Fig. 6. The UV spectra showed that the characteristic absorbance of quercetin at 211, 253 and 372 nm. But there were lower sensitivities at 211 nm, 253 nm than at 372 nm, the suitable absorbance was approximately at 372 nm for quercetin. Therefore, the detection wavelength was set at 372 nm in this method.

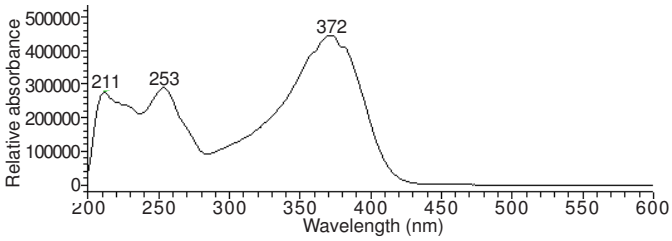

Fig. 6. UV absorbance spectra of quercetin at 200-600 nm

Solubility analysis of quercetin, quercetin/hydroxypropyl-β-cyclodextrin complex in water and methanol: Saturated quercetin and quercetin/hydroxypropyl-β-cyclodextrin complex aqueous solution and methanol solution were made and filtered through 0.45 μm microporous membrane, respectively. The content of soluble quercetin was analyzed for quercetin samples, quercetin/hydroxypropyl-β-cyclodextrin complex samples in water and methanol by HPLC. The results were listed in Table-1.

TABLE-1
SOLUBILITY ABILITY ANALYSISI OF QUERCETIN, QUERCETIN/HYDROXYPROPYL-β-CYCLODEXTRIN (HP-β-CD) COMPLEX IN WATER AND METHANOL

Sample	Quercetin	Quercetin/ HP-β-CD complex	Quercetin	Quercetin/ HP-β-CD complex
Solvent	Water	Water	Methanol	Methanol
Peak area (mAu min)	24194	138421	5424973	175401

It could be seen that the ability of water solubility of quercetin/hydroxypropyl-β-cyclodextrin complex was improved six times by the inclusion technique than quercetin monomer in Table-1, But the ability of solubility of quercetin/hydroxypropyl-β-cyclodextrin complex in methanol was less than quercetin monomer in methanol. The polarity of quercetin affected may lead to the change of hydrophilicity and hydrophobicity of quercetin because of the formation of quercetin/hydroxypropyl-β-cyclodextrin complex.

Conclusion

The preparation, physicochemical characterization of the quercetin/hydroxypropyl-β-cyclodextrin complex were reported. The results showed that a stable inclusion complex with a new phase was prepared. The hydrophilicity and hydrophobicity of quercetin were improved because of the formation of quercetin/hydroxypropyl-β-cyclodextrin complex. This fact could be explained by the effect of the surface hydroxyl groups of hydroxypropyl-β-cyclodextrin, which increased from hydrogen bond formation with surrounding water molecules. Thus, intermolecular hydrogen bonding can result in relatively high aqueous solubilities. Meanwhile, the formation of quercetin/hydroxypropyl-β-cyclodextrin complex also provided a solution for other water-soluble flavonoid medicine prepared.

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