Journal of Food and Drug Analysis, Vol. 18, No. 6, 2010, Pages 391-397 藥物食品分析 第十八卷 第六期

Physicochemical Characterizations of Osthole-hydroxypropyl-β-cyclodextrin Inclusion Complexes with High-Pressure Homogenization Method

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(Received: April 27, 2010; Accepted: October 28, 2010)

ABSTRACT

The purpose of this research was to evaluate the inclusion complex composed of osthole and hydroxypropyl-β-cyclodextrin (HP-β-CD) by using two preparative techniques, neutralization and high-pressure homogenization (HPH) method. The solid complexes were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), particle size determination, and dissolution test. The DSC diagrams indicated that the solid complex from the HPH method led to more complete complexation than that of physical mixture and neutralization method. The formation of inclusion complex between drug and cyclodextrin (CD) was verified by FTIR analysis. The smallest mean particle size of solid complex was acquired from the HPH method. The results proved that the rate of drug release for the inclusion complex obtained from the HPH method was much faster than those of neutralization, physical mixture, HP-β-CD and osthole alone.

Key words: osthole, hydroxypropyl-β-cyclodextrin, high-pressure homogenization method, neutralization, solubility

INTRODUCTION

The dried fruits of *Cnidium monnieri* (L.) Cusson, *Cnidium monnieri* Fructus, belonging to the Umbelliferae family were called "She chuang zi" in China and have been used in oriental countries for centuries as a philter or tonic[1]. Osthole [$C_{15}H_{16}O_3$, 7-Methoxy-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-2-one], a coumarin derivative, is an effective ingredient extracted from the fruit of *Cnidium monnieri* and possesses several curative properties[2]. Previous studies on osthole revealed various pharmacological activities, such as anti-allergic effects[3], anti-inflammatory effects[4], anti-proliferative effects[5], vasorelaxation[6], anti-tumor effects[7], improvement of aspects of spatial performance[8], nonspecific relaxant effects on the trachealis[9], respiratory stimulant effects[10,11], relaxation of corpus cavernosum tissue[12] and treatment of osteoporosis[13].

As far as physicochemical properties are concerned, applications of osthole are still confined on account of its poor water solubility. Chemical and physical modifications have been employed to enhance the solubility of poor water-soluble drugs or enlarge available surface areas of dissolution[14]. In recent decades, applications of CDs in the pharmaceutical field, analytical chemistry, agriculture and cosmetics were developed. Numerous advantages of CD complex for drug delivery systems were also verified, such as enhancement of solubility, improvement of bioavailability of hydrophobic drugs, domination of crystallization and polymorphic transition of drugs, controlled release of hydrophilic drugs, stabilization of peptide, oxygen-, and light-sensitive drugs, disguise of indisposed taste and smell, fixation of extremely volatile drugs and prevention of degradation of drugs[15,16]. The chemical structures of CD derivatives, cyclic oligosaccharides consist of (α-1, 4)-linked glucopyranose subunits, similar to a skirt-shape construction, produce a hydrophobic internal cavity and hydrophilic outer surface. CDs are able to interact with suitable drugs to generate the formation of inclusion complexes by various preparation methods[16-18].

The objective of this research is to study physicochemical characterizations of CD inclusion complexes prepared by physical mixture, neutralization and HPH method. Phase-solubility measurement, FTIR analysis, DSC study, particle size analysis and dissolution test were utilized for evaluation. The HPH method of preparing osthole-HP-β-CD inclusion complexes has not been

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published before. This method does not use toxic chemicals, so there is no concern of noxious chemicals residues. The aim of CD inclusion complexes was to increase the solubility and dissolution rate of osthole. The results indicated that the HPH method may be applied to improve the solubility and dissolution of osthole.

MATERIALS AND METHODS

I. *Chemicals*

Osthole was kindly supplied by Dr. Chien-Chih Yu from Sheng Chun Tang Pharmaceutical Industrial Co., Ltd. (Tainan, Taiwan, R.O.C.). The purity of osthole was above 99%. HP-*β*-CD was purchased from Roquette Ltd. (Lestrem, France). Honokiol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other materials were of analytical or high performance liquid chromatography (HPLC) grade.

II. *Phase-Solubility Measurement*

Phase-solubility measurement was performed based on the formula from Higuchi and Connors[19] to investigate the preferable complex ratio of osthole and HP-*β*-CD. A surplus of osthole was added to 10 mL of deionized water, which consisted of diverse concentrations of HP-*β*-CD (1 - 100 mM). The resulting mixtures were sufficiently equilibrated in a thermostatic shaking water bath for 72 h at 25°C. The supernatant was passed through a 0.45-μm membrane filter to eliminate undissolved solid after equilibration and then analyzed by high performance liquid chromatography (HPLC) system. Six determinations were accomplished for each sample. The result obtained was of 1 : 1 stoichiometry and the apparent stability constant (K_s) was estimated from the straight line portion of the phase-solubility diagram in accordance with the equation:

$$K_s = \text{slope} / [\text{intercept} (1 - \text{slope})] \quad (1)$$

where intercept refers to the saturation concentration of the drug appraised without cyclodextrin.

III. *HPLC Analysis of Osthole*

The analysis of osthole used a LiChrospher® C_{18} reversed phase column (5 μm, 250 mm × 4.6 mm i.d., Merck, Darmstadt, Germany) at room temperature. A Hitachi LaChrom Elite HPLC system equipped with a Hitachi L-2420 UV-Vis Detector (Hitachi, Japan) at 322 nm was used. The mobile phase, adjusted to pH 3.0 with orthophosphoric acid, consisted of 82% methanol and 18% deionized water at a flow rate of 1 mL/min. The injection volume was 20 μL and honokiol was employed as the internal standard. The inter-day precision was evaluated on fresh osthole standard solutions at 7:00 am, 9:00 am, 11:00 am, 1:00 pm, 3:00 pm and 5:00 pm for 6 successive days, while the intra-day precision was calculated using 6 replicate measurements of osthole within the same day. The mean, standard deviation (S.D.) and coefficient of variation (C.V. %) of precision as well as the relative differences of accuracy were determined to estimate the stability of HPLC assays.

IV. *Preparation of Solid Inclusion Complex*

The ideal molar ratio of osthole : HP-*β*-CD (1 : 1) for the formation of inclusion complexes were on the strength of consequences of phase-solubility measurement.

(I) *Neutralization Method*

Osthole and HP-*β*-CD were weighed accurately and added to 100 mL of 1 N sodium hydroxide solution. The mixture was stirred with a magnetic stirrer in a hood at 37 ± 2°C until a transparent solution was obtained. Exactly 100 mL of 1 N hydrochloric acid was added into the solution and agitated for 3 h. The precipitate was separated from the solution and washed three times with absolute ethanol. The precipitate was then evaporated to dryness at ambient temperature.

(II) *Physical Mixture*

Osthole and HP-*β*-CD were precisely weighed respectively, passed through a 425-μm sieve and then thoroughly mixed in a mortar.

(III) *HPH Method*

HP-*β*-CD was dissolved in deionized water. Then, osthole dissolved in absolute ethanol was added to the solution in the molar ratio mentioned above. The whole solution was immediately passed through a model EmulsiFlex-C3 high-pressure homogenizer (Avestin, Canada) 3 times under an operating pressure of 10,000 psi at room temperature. The eventual solution was filtered and evaporated to dryness until the solid complex was acquired.

V. *FTIR Analysis*

Samples were ground and blended with potassium bromide. A Perkin- Elmer Spectrum System 2000 FTIR spectrometer (Norwalk, USA) was used for the analysis in the range between 4000 cm^{-1} and 400 cm^{-1} at a resolution of 4 cm^{-1}. The FTIR spectra were obtained from dried samples and the data were averages of 10 scans.

VI. *DSC Study*

DSC thermogram studies were achieved using a Perkin Elmer model DSC-7 (Norwalk, USA).

Temperature calibrations were performed with indium and zinc before analyzing specimens. Four mg of osthole, HP-β-CD, physical mixture and 2 inclusion complexes were respectively weighed accurately and heated under a nitrogen gas flow at a scanning rate of 20°C/min over a temperature range of 40 - 200°C. Aluminum lids and pans were utilized for whole samples.

VII. *Particle Size Analysis*

The mean sizes of osthole, HP-β-CD, physical mixture and 2 inclusion complexes were measured by LS 230 laser diffraction particle size analyzer from Beckman Coulter (Miami, USA). Size distributions of all samples were evaluated by fitting the intensity of the scattering light (λ = 750 nm) and the scattering angle (θ = 0.017 - 34°). Ethylene glycol (η = 16.1 mPa·s at 25°C) was used as the background medium and its index of refraction was 1.41856. One hundred milligrams of each sample was adequately dispersed into 200 mL of ethylene glycol before analysis. Triplicate determinations were performed for each sample.

VIII. *Dissolution Test*

The USP 31 paddle method of dissolution studies were accomplished with the pharmaceutical dissolution testing model Hanson SR8 Plus (Chatsworth, USA). Nine hundred mL of deionized water was used as the dissolution medium. The stirring speed was 100 rpm and the temperature was maintained at 37 ± 0.5°C. All powder samples containing an equivalent amount of 10 mg of osthole were respectively placed in the dissolution medium. One milliliter of each sample was collected at 1, 5, 10, 15, 30, 45, 60 and 120 min. Thereafter, the HPLC method was applied to determine the sample concentrations. All dissolution tests were executed six times. Dissolution data were estimated in view of dissolution efficacy (DE), as an index of totality of processes and the dissolved percentage of drug (DP), as an indication of the dissolution rate. DE was defined as the area under the dissolution curve at time (t) by utilizing the trapezoidal rule and shown as a percentage of the area of the rectangle represented by whole dissolution in the same time[20].

RESULTS AND DISCUSSION

I. *Phase-Solubility Measurement*

The phase solubility diagram of osthole with HP-β-CD determined in water at 25°C was displayed in Figure 1. The profile of the diagram was similar to an Ap system[19] and the solubility of osthole measured at 25°C was 1.81×10^{-5} M. The solubility of osthole was elevated slowly following the increase of HP-β-CD concentration at the initial stage in Figure 1(a). The solubility of osthole increased rapidly when HP-β-CD concentration was further raised in later part of Figure 1(b). On the assumption that the complex formed was of a 1 : 1 stoichiometric ratio, the apparent stability constant (*K*s) of the complex calculated from the slope of the initial linear portion in Figure 1(a) of the phase solubility diagram was 6191.1 M^{-1}. Briefly, the 1 : 1 molar ratio of osthole and HP-β-CD was the preferred composition for forming the inclusion complex.

II. *Validation of HPLC Method*

The precision and accuracy of intra-day and inter-day osthole quantification were determined using HPLC. The calibration curve, ranging between 0.1 and 10 μg/mL, constructed from osthole exhibited exceptional linearity with $R^2 > 0.999$. The coefficient of variance of precision was from 0.096 to 4.347 and the relative error of accuracy was from 0.000 to 4.632. Therefore, the quantification of osthole was adequately acceptable.

III. *Demonstrations of Osthole Inclusion Complex*

(I) *DSC Studies*

DSC curves obtained from osthole, HP-β-CD, physical mixture, complexes from neutralization and HPH method were shown in Figure 2. Proof of interactions between osthole and HP-β-CD was acquired by applying thermal analytical study to verify the formation of solid complex. The DSC thermogram of pure osthole revealed a unique representative endothermic melting peak at the onset temperature of 88.0°C, and the polymorph of osthole was not detected. The thermal curve of HP-β-CD displayed a small elevatory curve around 96°C. Furthermore, the amorphism of HP-β-CD was presented. At 88.0°C, the thermal behavior of the physical mixture presented a

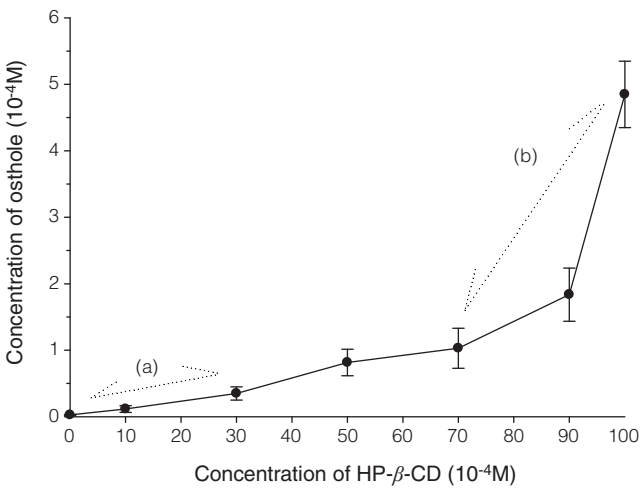

Figure 1. Phase solubility diagram of osthole-HP-β-CD in deionized water at 25°C (n = 6). (a) represent the initial stage of the diagram of solubility. (b) represent the last stage of the diagram of solubility.

conspicuous endothermic melting peak according to thermogram of osthole. The upward diagram of physical mixture was analogous to the curve of HP-*β*-CD and the apex of curve was transferred from 96°C to 111°C in virtue of interactions between osthole with HP-*β*-CD.

For the thermogram of solid complex prepared by neutralization, a slight melting peak at 88.0°C may indicate the existence of small residuals of osthole crystal in this preparation. No melting peak of inclusion complex from HPH method was present in whole time intervals, which is ascribed to the encapsulation of osthole molecules inside HP-*β*-CD cavities and the formation of amorphous solid complexes. This signified that sturdy interactions between osthole and HP-*β*-CD in the HPH method and recrystallization was obstructed.

(II) *FTIR Analysis*

FTIR spectra of osthole, HP-*β*-CD, physical mixture, complex from neutralization and HPH method are displayed in Figure 3 and Table 1 to substantiate evidence of solid inclusion complexes between drug and HP-*β*-CD.

In accordance with Figure 3, it was found that the FTIR spectrum of carbonyl stretching for pure osthole signified an angular concave peak at 1721 cm^{-1}. In comparison with the peak of pure osthole, a shift to higher frequency and a narrower shape for both inclusion complexes were shown. The curve of the complex from neutralization was moved from 1721 to 1722 cm^{-1}. Meanwhile, the peak of the complex from HPH method was shifted from 1721 to 1729 cm^{-1}. The consequence might be a result of interaction between the carbonyl group of osthole and the hydroxylpropyl group of HP-*β*-CD.

With regard to the band of alkenyl group, the characteristic of pure drug was maintained at 1606 cm^{-1}, while it shifted to 1607 cm^{-1} for the complex from neutralization and to 1614 cm^{-1} for the complex from HPH method. The curves of C=C stretching vibration for both inclusion complexes were also narrower with higher frequency. The causes of these observations might be due to the interaction between carbon molecules of drug and hydrogen molecules of HP-*β*-CD.

The spectrum of physical mixture could be regarded as the outcome of the blending of osthole and HP-*β*-CD, hence verifying lack of interactions between drug and CD, as implied by DSC thermogram. However, the FTIR spectra of inclusion complexes obtained by HPH method and neutralization revealed some dissimilarities with reference to those of physical mixture and accordingly exhibited some interactions between drug and CD. The shift of peaks shown in FTIR spectra relied on the preparation technique, indicating dissimilar level of interactions for different products. In brief, no significant feature of osthole was displayed in the spectra of both inclusion complexes obtained from neutralization and HPH method, revealing that the two inclusion complexes were excellently formed.

Table 1. Comparison of FTIR spectra of osthole, physical mixture and inclusion complexes from neutralization and HPH method

Osthole (cm^{-1})	Physical mixture (cm^{-1})	Inclusion complexes from neutralization (cm^{-1})	Inclusion complexes from HPH method (cm^{-1})	Remark
1606	1606	1607	1614	Change in C = C stretching vibration
1721	1721	1722	1729	Change in C = O symmetric vibration

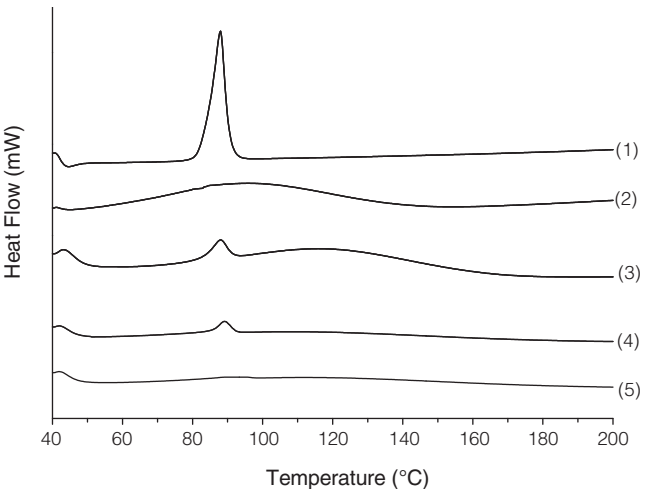

Figure 2. Thermograms of (1) osthole, (2) HP-*β*-CD, (3) physical mixture, (4) complex from neutralization method and (5) complex from HPH method.

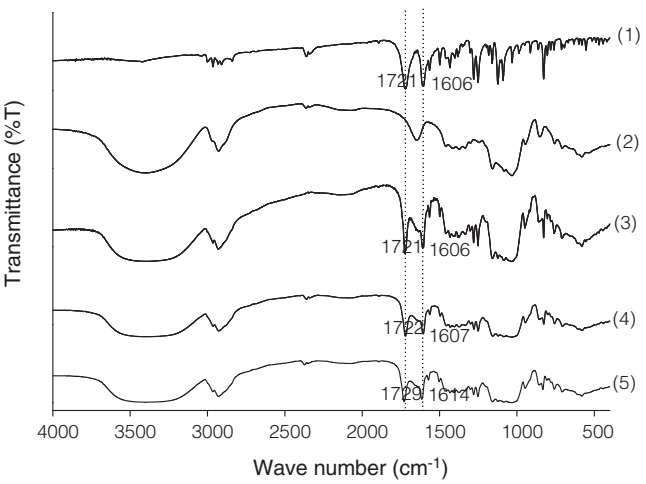

Figure 3. FTIR spectra of (1) osthole, (2) HP-*β*-CD, (3) physical mixture, (4) complex from neutralization and (5) complex from HPH method.

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Table 2. Comparison of DE and DP from osthole, physical mixture and inclusion complexes from neutralization and HPH method at 5, 30 and 60 min, respectively

	5 min		30 min		60 min	
	DE	DP	DE	DP	DE	DP
Osthole	0.0011	0.23	0.0050	0.66	0.0065	1.10
Physical mixture	0.0250	5.66	0.1266	20.65	0.1870	29.68
Inclusion complexes from neutralization	0.0604	12.2	0.2992	49.04	0.4308	62.28
Inclusion complexes from HPH method	0.1002	21.3	0.4833	69.02	0.6216	81.09

Table 3. Comparison of particle sizes from osthole, physical mixture and inclusion complexes from neutralization and HPH method

	Osthole	HP-β-CD	Physical mixture	Inclusion complexes from neutralization	Inclusion complexes from HPH method
Particle size Mean ± S.D. (μm)	241.9 ± 8.7	154.1 ± 3.3	197.0 ± 3.8	90.6 ± 4.6	60.2 ± 2.6

IV. *Particle Size Analysis*

The average and standard deviation of particle size for osthole, HP-β-CD, physical mixture, complex from neutralization and HPH method are shown in Table 3. The mean particle size of osthole and HP-β-CD was 241.9 ± 8.7 and 154.1 ± 3.3 μm, respectively. Nevertheless, the mean particle size was 60.2 ± 2.6 μm for the complex from HPH method and 90.6 ± 4.6 μm for the complex from neutralization. Owing to bonding collectively from hydrogen bonding, the particle size of HP-β-CD was larger than that of two solid complexes. Besides, the particle size of osthole was also larger than others due to aggregation during storage. The particle size of the physical mixture was between that of osthole and HP-β-CD. From Table 3, the particle size of the complex from HPH method was smaller than that of the complex from neutralization, implying that fractional complexation was imperfect from the neutralization technique.

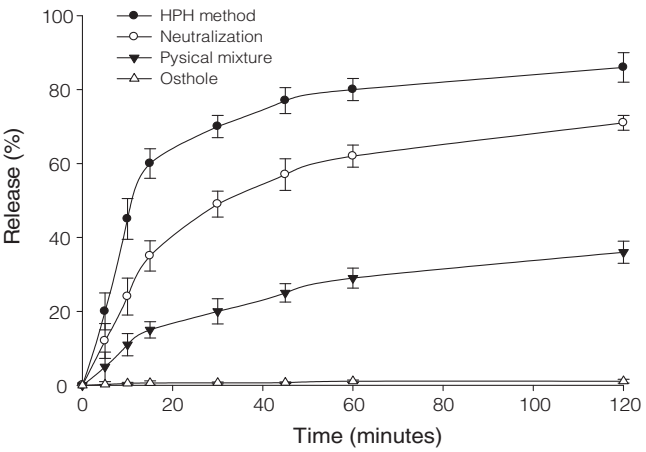

Figure 4. Dissolution curves of complex from HPH method (●), complex from neutralization (○), physical mixture (▼) and osthole (△).

V. *Dissolution Test*

Dissolution curves of complex from HPH method, complex from neutralization, physical mixture and osthole were exhibited in Figure 4. Dissolution data presented as DE and DP at 5, 30 and 60 min were shown in Table 2. According to the data of Table 2, the DP of solid complex from HPH method at 5 min was 21.3%, while the DP of solid complex from neutralization, physical mixture and osthole was 12.2, 5.66 and 0.23%, respectively. Even after an hour, the DP of the solid complex from HPH method (81.09%) was still apparently higher than those of the solid complexes from neutralization (62.28%), physical mixture (29.68%) and osthole (1.10%). The results demonstrated that solid complex from HPH method dissolved faster than the solid complexes from neutralization, physical mixture and osthole alone. Based on the data from DE, the DE of the solid complex from HPH method was much better than others. In terms of the DP and DE at 5, 30 and 60 min, all manifested that the solid complex from HPH method possessed higher dissolution rate than others, and the ranking of dissolution rates was complex from HPH method > complex from neutralization > physical mixture > osthole alone. The increment of dissolution rate for unsophisticated physical mixture may be ascribed to the decrement of interfacial tension between the hydrophobic drug and dissolution medium.

Furthermore, the improved dissolution performance of solid complexes could be attributed to the ameliorative wettability of the drug. Preparation methods utilized for solid complexes also apparently influenced the performance of dissolution efficacy. The technique of preparative method for the complex from HPH method was superior to that of the complex from neutralization.

CONCLUSIONS

In terms of phase-solubility measurements, 1 : 1 molar ratio of osthole-HP-β-CD inclusion complexes was designed to prepare for physicochemical characterizations. DSC studies and FTIR analyses were well applied to substantiate formations of both inclusion complexes. Solid complexes prepared by neutralization and HPH method brought about analogous FTIR spectrum, signifying that both complexes possessed similar characters of solid state. In addition, it could be reasonably inferred that molecular interactions of drug and CD might be due to formations of bonding according to the data from FTIR. From DSC thermograms, the solid complex obtained by HPH method completely lacked the melting peak of osthole. On the contrary, a small peak was present of the inclusion complex prepared by neutralization method. DSC curves showed that the technique of HPH preparation method resulted in more thorough complexation than that of neutralization.

Depending upon particle size analysis, the smallest particle size of solid complexes was also obtained from HPH method. Concerning dissolution tests, the dissolution rate of the inclusion complex from HPH method was more rapid than those of neutralization and physical mixture. In summary, the superiority of the HPH technique may be principally ascribed to the decrement of particle size of drug and the improvement of drug solubility. The study indicated that osthole-HP-β-CD complex prepared by using the HPH method may substantially improve the solubility and dissolution rate of osthole in contrast to those from physical mixture and neutralization.

Owing to the increase in solubility, the formulation of osthole-CD inclusion complexes may be applied in various dosage forms, such as ointment for local anti-allergic effects, cream for local anti-inflammatory effects, gel for relaxation of corpus cavernosum tissue and powder for relaxant effects on the trachealis. *In vitro* or *in vivo* studies, toxicology, pharmacokinetic and related investigations of innovative drug-CD inclusion complexes obtained from various techniques will be more extensively studied. The HPH method ought to be experimented with more drugs and different kinds of CDs to better study the developmental possibilities of this method.

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