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Research Article

Formulation of Berberine Hydrochloride and Hydroxypropyl-β-Cyclodextrin Inclusion Complex with Enhanced Dissolution and Reduced Bitterness

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Abstract

Purpose: To prepare and characterize inclusion complex of berberine hydrochloride with hydroxypropyl-β-cyclodextrin in order to achieve enhanced solubility and reduced bitterness of the former.
Methods: The inclusion complex of berberine hydrochloride with hydroxypropyl-β-cyclodextrin (1:1) was prepared by freeze-drying method and compared with the the physical mixtures of the compounds. Inclusion complexation was studied by phase solubility diagram, differential thermal analysis, spectrophotometric characterization and dissolution rate. The solubility of the formulations was evaluated by saturation solubility studies while their bitterness was tested in human volunteers.
Results: Differential thermal analysis and spectrophotometric characterization indicate that berberine hydrochloride formed inclusion complex with hydroxypropyl-β-cyclodextrin. The phase solubility diagram of berberine hydrochloride with hydroxypropyl-β-cyclodextrin was of A_L-type, with a stability constant was 694.5 L/mol at 25 ^oC. The solubility of berberine hydrochloride was increased by 5.27 times for the complex at a concentration of 0.01 mol/L. The dissolution of berberine hydrochloride after 20 min from the inclusion complex, physical mixture and pure berberine hydrochloride was 89.6, 69.8 and 58.8 %, respectively. The bitterness of the inclusion complex was considerably lower than that of the drug alone or the physical mixture with hydroxypropyl-β-cyclodextrin.
Conclusion: The inclusion complex demonstrated improved dissolution properties and lowered the bitterness of berberine hydrochloride.

Keywords: Berberine hydrochloride, Hydroxypropyl-β-cyclodextrin, Inclusion complex, Solubility, Bitterness

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INTRODUCTION

Berberine hydrochloride (BH), a natural product isolated from the coptidis rhizome and amoorcorn tree bark, is a novel anti-infective drug, and can now be chemically synthesized. It has become an important drug in the treatment of intestinal infections and bacillary dysentery. BH has potentials to be used in the treatment of high blood pressure, coronary artery disease and hyperlipemia[1]. Due to its poor solubility and bitterness, current dosage form suffers from disadvantage of low bioavailability, high dose and bitter taste. It is, therefore, to enhance its poor solubility and lower its bitterness. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a hydroxypropylated derivative of β-cyclo-dextrins (β-CD) whose solubility in water is more than 50% (w/v) at 25 ^{o}C. It can form inclusion complexes and play an important role in improving the therapeutic efficacy of drugs with poor solubility and/or stability problems [2]. HP-β-CD is also capable of reducing the undesirable properties of drug molecules such as bitterness through the formation of inclusion complexes [3].

Our previous attempts to prepare suitable inclusion complexes using β- CD were not satisfactory, for example, the solubility of BH/β-CD inclusion complexes was only 2.6 ± 0.3 mg/ml [4]. Hence, the objective of this study was to assess the suitability of HP-β-CD for the preparation of BH inclusion complex.

EXPERIMENTAL

Materials

BH (purity: 99.52 %) was refined by the Department of Medical Chemistry, School of Pharmacy, Xinxiang Medical University, Xinxiang, China. HP-β-CD, and other reagents and solvents of analytical grade were purchased from Zhengzhou Bafang Chemicals Ltd, Zhengzhou, China. Double distilled water was used throughout the study.

Preparation of BH/HP-β-CD inclusion complex

The inclusion complex of BH with HP-β-CD was prepared by freeze-drying method [5]. BH and HP-β-CD (molar ratio 1:1) were accurately weighed. BH was dispersed in aqueous solution of HP-β-CD (20 %w/v), and stirred magnetically at 60 ^{o}C until completely dissolved. The solution was cooled to room temperature and then lyophilized with a vacuum freeze dryer (FD-1C-50, China).

Preparation of BH/HP-β-CD physical mixture

A physical mixture of BH and HP-β-CD (molar ratio 1:1) was prepared by thoroughly mixing the two components with a stirrer (VH-5, China). Both the inclusion complex and physical mixture were stored in a refrigerator at 5 ^{o}C pending further characterization.

Phase solubility diagram

Phase solubility studies were carried out in water according to the method described by Higuchi and Connors [6]. Excess amount of BH was added to 10 ml of its aqueous solution containing HP-β-CD (0 - 0.01 mol/L). The suspension was mechanically shaken at 25 ± 0.5 ^{o}C for 72 h (SHA-C, China). After attainment of equilibrium, the mixture was centrifuged at 4000 rpm for 5 min, filtered through a 0.45 µm cellulose acetate membrane filters and suitably diluted. The absorbance of BH was recorded spectrophotometrically (TU-1810PC, China) at a λ_{max} of 263 nm. Stability constant (*Ks*) was calculated from the slope of the phase solubility diagram using Eq 1.

$$Ks = \frac{Slope}{S_0(1 - Slope)} \quad \text{.....(1)}$$

where S_0 is the solubility of drug in water.

Saturation solubility studies

Saturation solubility studies were carried out in water at room temperature [7]. Pure BH (200 mg), a quantity of BH/ HP-β-CD inclusion complex and the physical mixture (molar ratio 1:1) equivalent to 200 mg of BH were taken in separate sealed vials with 10.0 ml water and stirred vigorously in a shaker water bath at 25 ± 0.5 ^{o}C for 72 h. The samples were then centrifuged and filtered through 0.45 µm cellulose acetate membrane filter. After suitable dilution, the absorbance was recorded at 263 nm.

Spectrophotometric characterization

Approximately 4.13 g HP-β-CD was placed in a 200 ml volumetric flask and diluted with distilled water to volume. Varying quantities (0, 5.0, 10.0, 15.0, 20.0, 24.0 ml solution were withdrawn and transferred into separate 25 ml volumetric flasks and made up to volume to give concentrations of 0, 3.0, 6.0, 9.0, 12.0, 14.4 mmol/L, respectively. An appropriate amount of BH solution was added to each of the HP-β-CD solutions to yield a BH concentration of 7 µg/ml. The absorption spectra of the mixtures were obtained by scanning spectrophotometrically from 400 to 200 nm using HP-β-CD solution as blank. The spectra were compared with one another in terms of peak position and wavelength shifts [8].

Dissolution rate studies

In vitro dissolution studies of BH, BH/HP-β-CD inclusion complexes and the physical mixtures (molar ratio 1:1) were carried out in a dissolution apparatus (ZRC-6FT, China) using method 1 described in United States Pharmacopoeia at 37 ^{o}C, with the paddle rotating at 100 rpm. In the test, 100 mg of BH, or its equivalent for the physical mixture or inclusion complexes, was added to 900 ml distilled water. Five milliliters of the solution was withdrawn at 2.0, 5.0, 10.0, 15.0, 20.0, 30.0 and 45.0 min. The solution was immediately filtered (0.45 µm pore filter), suitably diluted, and absorbance recorded spectrophotometrically at 263 nm. Equivalent volume of fresh dissolution medium, pre-warmed to 37 ^{o}C, was used to replenish the medium after each sampling. The cumulative percentage of BH dissolved was calculated from the regression equation generated from the standard data [9]. The experiment was conducted in triplicate.

Differential thermal analysis (DTA)

DTA thermograms of BH raw material, HP-β-CD, BH/HP-β-CD inclusion complexes and their physical mixtures (molar ratio 1:1) were obtained with a DTA instrument (CRY-32P, China). Each sample (5 mg, accurately weighed) was heated in an aluminum pan at a rate of 10 ^{o}C/min from 40 to 340 ^{o}C under air flow. The thermograms were compared with one another regarding in terms of peak position, peak shift, and presence/absence of peaks at particular temperatures [10].

Bitterness test

The study involved 20 healthy Chinese adult male volunteers, aged 21- 25 years (mean ± SD, 22.7 ± 0.6 years), weighted 55- 75 kg (61.3 ± 5.0 kg), with a height of 163-179 cm (171.8 ± 5.7 cm), giving a mean Body Mass Index (BMI) of 20.58 ± 1.37 kg/m^2. The study was carried out in accordance with the regulations for biomedical research involving human ethics review [11] under the jurisdiction of the Ministry of Health of the People's Republic of China and approved by the Research Ethics Committee of Xinxiang Medical University (approval no. 2010- 046).
The volunteers were provided written informed consent prior to study participation and they all completed the study. They were instructed to abstain from using any medications for at least 2 weeks prior to and during the study, and from smoking and taking alcohol, caffeine or related drugs for at least 48 hours before administering the study drug and throughout the study.

The volunteers were divided into two groups. A solution (200 ml) of the drug (1.0 g/L) was administered orally to the volunteers. Group A received the solution of the physical mixture (molar ratio 1:1) in the first round of the test and BH/HP-β-CD inclusion complex solution in the second round while Group B received BH/HP-β-CD inclusion complex solution in the first round and a solution of the physical mixture (molar ratio 1:1) in the second round. The two treatment rounds were separated by a 7-day washout period. On the study day, assessment of the gustatory sensation of the subjects on the drug was facilitated by qualified nurses [12]. The classification of the results was as follows: 0 (not bitter); 1 (bitter, i.e., bitterness is obvious); 2 (very bitter (bitterness is high, i.e., bitterness is so high that it was hard to swallow, and/or even cause vomiting). Volunteers were given up to 10 s to identify the taste.

Statistical analysis

Data analysis was carried out using SPSS 13.0 software (SPSS, Chicago, IL, USA). Statistical differences between taste (bitterness) values of two groups was assessed by Chi-square test (χ^2) (2×3). $P < 0.05$ was considered statistically significant.

RESULTS

Phase solubility diagram

The phase solubility behavior of BH in HP-β-CD solution is shown in Figure 1. The diagram shows that the aqueous solubility of BH increased in a linear manner as a function of HP-β-CD concentration, which resulted in A_L-type phase solubility diagram based on the Higuchi and Connors model [6] .The stability constant (*Ks*) was 694.5 L/mol and BH solubility for the inclusion complex increased 5.27 times at HP-β-CD concentration of 0.01 mol/L.

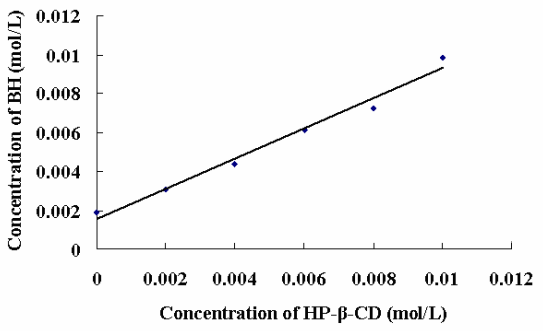

Figure 1: Phase solubility diagram of BH/HP-β-CD system in water

Saturation solubility

The saturation solubility of BH, BH/ HP-β-CD inclusion complexes and their physical mixtures at room temperature were 0.81, 10.35 and 1.92 mg/ml, respectively.

UV-visible spectra

The UV-visible spectra of BH in HP-β-CD solution is shown in Figure 2. The absorption wavelengths of BH at 228, 263 and 341 nm shifted to 229, 264 and 342 nm, respectively, when the concentration of HP-β-CD was varied (Figure 2).

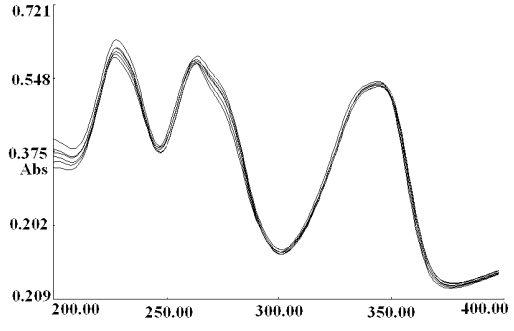

Figure 2: UV-visible absorption spectra of BH dissolved in HP-β-CD solution of varying concentrations (0, 3.0, 6.0, 9.0, 12.0, 14.4 mmol/L). ***Note:*** The lower concentration the higher the absorption

Dissolution rate

The dissolution profiles of BH, its inclusion complex and physical mixtures are shown in

Figure 3. It is evident that both the inclusion complex and the physical mixture exhibited faster dissolution rate than BH alone.

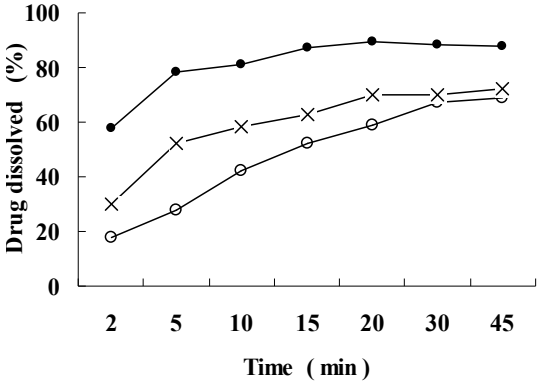

Figure 3: Dissolution profile of samples of BH (○), physical mixture of BH/HP-β-CD (×) and BH/HP-β-CD inclusion complex (●)

Thermal characteristics

Thermal analysis provided evidence that an inclusion complex was formed (Fig 4).

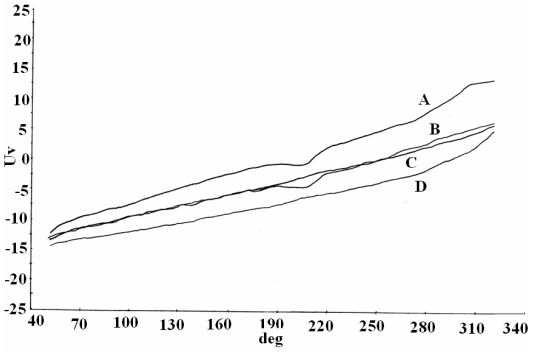

Figure 4: DTA thermograms of BH (A), physical mixture (B), HP-β-CD (C) and inclusion complex (D)

Inclusion of drug molecules in the HP-β-CD cavities or crystal lattice generally resulted either in the shift of their melting, boiling, or sublimation points to a different temperature. The DTA thermogram of BH exhibited a sharp endothermal peak at 204 ^{o}C (A), indicating its melting point. HP-β-CD (C) did not show any obvious absorption peak, but rather an oblique line. In the thermogram of the inclusion complex (D), the endothermal peak of BH was not observable, indicating physical interaction of BH with HP-β-CD, resulting in an almost complete loss of crystal form of the binary system. In contrast, the physical mixture (B) showed a clearly visible endothermal peak for BH.

Bitterness

Bitterness data are presented in Table 1. The volunteers subjected to physical mixture who feel very bitter, bitter, not bitter taste were 9, 9 and 2, respectively; The volunteers subjected to BH/ HP-β-CD inclusion complexes who reported the taste as very bitter, bitter, and not bitter were 3, 5 and 12., respectively

Table 1: Subjective bitterness of BH/HP-β CD preparations

Treatment (BH/HP-β-CD)	No. of volunteers		
	0 (Not bitter)	*1 (Bitter)*	*2 (Very bitter)*
Physical mixture	2	9	9
Inclusion complex	12	5	3

χ^2= 11.2857, $\chi^2_{1\text{-}0.01\ (2)}$ = 9.21, $\chi^2 > \chi^2_{1\text{-}0.01}$, $p < 0.01$ compared with the control group; physical mixture of BH and HP-β-CD = control group

DISCUSSION

BH/HP-β-CD inclusion complex with improved solubility and reduced bitterness was formed using freeze-drying method. This method is suitable for the preparation of inclusion complexes as it is easy to carry out on a small scale and at industrial level.

To the best of our knowledge, inclusion complexes in 1:1 molar ratio of drug : HP-β-CD have ever been prepared. DTA results indicate complete formation of inclusion complex in the above molar ratio, and this is

consistent with the phase solubility data obtained.

Although both the inclusion complex and physical mixture exhibited increased dissolution rate than BH alone, that of the complex was much faster, being 5-fold. The key reason for the solubility of BH/HP-β-CD inclusion complexes being higher than their physical mixtures might probably be the mutual action of BH and HP-β-CD. The increase in the dissolution rate of BH in the physical mixture may be attributed to improved wettability of the drug by HP-β-CD [13] while that of the drug in the inclusion complex may be due to several factors, including formation of soluble inclusion complex, solubility increase, greater wettability, and particle size reduction [14]. The higher drug dissolution from BH/HP-β-CD inclusion complex than from the equivalent BH/β-CD, as previously reported [4] underlines the importance of appropriate choice of carriers. The superiority of HP-β-CD is the result of its greater water solubility, higher wetting, and complexation to BH.

The main conditions for the formation of BH/HP-β-CD inclusion complex are the three-dimensional structure and polarity of BH and HP-β-CD. The stability of the inclusion complex formed by the two molecules depends on the strength of the van der Waals force, dispersion force, hydrogen bonding, charge transfer, etc. It is often the result of a single force or the synergy of several forces. The size and shape of the BH should be adaptive with HP-β-CD, otherwise it would have been difficult for a stable inclusion complex to be formed.

The volunteers who perceived BH/HP-β-CD is very bitter was significantly higher for the physical mixture than for the inclusion complex. Thus, formation of inclusion complex caused a significant reduction in the undesirable taste properties of BH which should render it more palatable to patients.

CONCLUSION

BH/HP-β-CD inclusion complex obtained by freeze-drying method is a promising approach for achieving vastly higher drug solubility and bitter taste reduction. These, in turn, would result in improved drug bioavailability decreased gastrointestinal-associated side-effects and potentially higher patient compliance.

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