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ENHANCING WATER-SOLUBILITY OF POORLY SOLUBLE DRUG, *ASIATIC ACID* WITH HYDROXYPROPYL-Β-CYCLODEXTRIN

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The purpose of this research was to improve the solubility of Asiatic acid, a poorly water soluble drug, by complexation with hydroxypropyl-β-cyclodextrin (HPβCD). The effect of Asiatic acid: HPβCD feed ratio by molar on the aqueous solubility was investigated, the aqueous solubility of Asiatic acid reached 2100μg/ml when the molar ratio of Asiatic acid to HPβCD was 1:2. The aqueous solubility of Asiatic acid was increased by 21-fold in Asiatic acid/ HPβCD solid complex. The Asiatic acid/ HPβCD solid complex system was characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM). The FTIR and XRD spectra of Asiatic acid/ HPβCD solid complexes showed that Asiatic acid could form inclusion complex with HPβCD in solid state. The SEM, DSC and XRD spectra of Asiatic acid/ HPβCD solid complexes indicated Asiatic acid existed in amorphous state, this could be explained the fact that the aqueous solubility of Asiatic acid was increased.

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Keywords: Water solubility, B-cyclodextrin, Asiatic acid

1. Introduction

Asiatic acid is one of the component of the titrated extract of Centella asiatica (TECA) [1-2]. Asiatic acid (figure 1) is known to be clinically effective on systemic scleroderma, abnormal scar formation, and keloids [3-5]. However, one major problem associated with administration of Asiatic acid is its low solubility in aqueous and oil medium which may hinder dissolution causing decreased bioavailability of the drug.

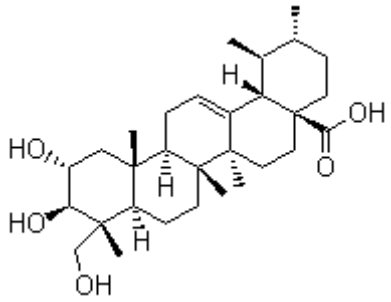

Figure 1. Structure of Asiatic acid.

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One of the main interests associated with cyclodextrins refers to the enhancement of solubility and /or dissolution rate of lipophilic drugs in aqueous media, very often resulting in improved bioavailability [6-7].Among the commercially available cyclodextrins, hydroxypropyl-β-cyclodextrin (HP-β-CD) (figure 2) has been used in improving the aqueous solubility of a variety of compounds [8]. It is a cyclic oligosaccharide containing seven D-(+)-glucopyranose units, with an average of one hydroxypropyl group per unit. The circular arrangement of the glucose units produces a torus-shaped molecule and CH_2 groups and ether linkages of the molecule face the hollow interior of the configuration results in a nonpolar cavity and a polar exterior. When a compound with appropriate geometry and HP-β-CD are in the same solution, the non polar aromatic portions of the compound tend to enter the nonpolar interior of the HP-β-CD molecule. This complexation isolates the aromatic and heterocycle ring portion of the molecule from the water, thereby increasing its aqueous solubility.

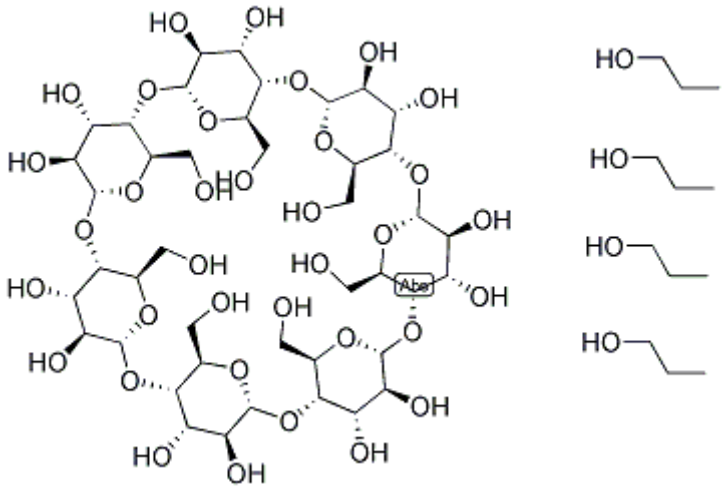

Fig. 2.Structure of hydroxypropyl-β-cyclodextrin.

The solubility of Asiatic acid in distilled water is only 0.1mg/ml [9]. Solubility of Asiatic acid has been dramatically improved by using ionionic and anionic surfactant systems [10]. However, no study to date has used hydroxypropyl-β-cyclodextrin by complexation to improve the solubility of Asiatic acid in the distilled water. In this study, it is pursued to increase the solubility of Asiatic acid in the distilled water by complexation with hydroxypropyl-β-cyclodextrin. XRD, FTIR, DSC and SEM were used to characterize the properties of Asiatic acid/ HPβCD solid complex systems.

2. Materials and methods

2.1 Materials

Reference Asiatic acid (purity 95%) and pharmaceutical grade asiatic acid were purchased from Guangxi Changzhou Natural Pharmaceutical Co., Ltd (Guangxi,China). hydroxypropyl-β-cyclodextrin was purchased from Shandong Xinda Fine Chemical Co.,Ltd (Shandong, China). Water was purified using a Milli-Q system (Millipore, BedfC co., Ltd (Tianjin, China). Analytical grade ethanol was purchased from Concord Technology Co., Ltd (Tianjin, China).

2.2 Methods

Preparation of Asiatic acid - HP-β-CD complex was prepared according to solvent evaporation technique [11]. Asiatic acid - HP-β-CD complex was prepared in the following way. Briefly, the physical mixtures (PM) of Asiatic acid and HP-β-CD was dissolved ethanol, the resulting solution was stirred fully to let them solute entirely. The Asiatic acid: HP-β-CD feed ratio by molar was varied from1:10 to 2:1. The solution was evaporated at controlled temperatures of 40-45℃, the content was then dried overnight in a vacuum dessicator and then passed through no. 80 sieve. Physical mixtures (PM) were prepared by mixing in geometric proportion followed by passing through no. 80 sieve with minimum abrasion. The aqueous solubility of Asiatic acid was determined by HPLC equipped with a UV detector at 205nm.Acetonitrile-water with gradient elution was used as mobile phase. The injection volume was 10μl and the flow rate was 0.6ml/min. The Extend-C_{18} column (4.6×250 mm, 5 μm particle size, Agilent, USA) was used.

2.3 Characterization of Samples

2.3.1 Solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors [12]. Excess of pure drug and inclusion complex were added to 20 ml of distilled water taken in stoppered conical flasks and shaken for 24 hrs in rotary flask shaker at room temperature. After shaking to achieve equilibrium, appropriate aliquots were withdrawn and filtered through whatman filter paper no.41. The filtrate so obtained was analysed by HPLC at 205nm.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared spectra of the samples were obtained in the range of 400 to 4000cm^{-1} using a Jasco-FTIR spectrophotometer (Jasco, Essex, UK) by the KBr disc method.

X-Ray Diffraction Studies

X-ray diffraction (XRD) patterns were recorded on a Philips X-ray diffractometer (PW 1710, Philips Analytical, Almelo, The Netherlands) with a copper target, voltage 40KV, current 30 mA, and a scanning rate of 1°/min.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry studies were performed on samples weighing 5mg in flat-bottomed aluminum pans using a Shimadzu DT-40 thermal analyzer. The samples were heated from 25 to 350℃ at a heating rate of 10℃/min.

Scanning Electron Microscopy (SEM)

Samples were mounted on brass stubs using double-sided tape and vacuum-coated with a thin layer of gold.

3. Results and discussion

3.1 Solubility studies

The systems of Asiatic acid with HPβCD showed enhancement in the solubility as compared to pure drug alone (table 1). The enhancement in aqueous solubility of Asiatic acid could be explained in terms of wetting property and hydrophillicity of HPβCD with simultaneous reduction in the crystallinity of the drug caused by the kneading process and inclusion into the hydrophobic cavity of the HPβCD [13]. As shown in table 1, the aqueous solubility of Asiatic acid increased with the increase of the HPβCD in the systems of Asiatic acid with HPβCD. However, when the molar ratio of Asiatic acid to HPβCD is 1:2, the aqueous solubility of Asiatic acid did not increase again. This phenomenon may be due to the fact that the saturated nonpolar cavity has been obtained when the molar ratio of Asiatic acid to HPβCD is 1:2. When the molar ratio of Asiatic acid to HPβCD exceeded 1:2, there was no enough nonpolar cavity to complex Asiatic acid. However, when the molar ratio of Asiatic acid to HPβCD was under 1:2, there was no enough Asiatic acid to complex with HPβCD.

Table1. Solubility study of Asiatic acid with HPβCD in water.

System	Solubility in water at 25℃ μg/ml* (Mean±S.D.)	S.E.M
Asiatic acid	120±11.01	6.34
2:1 KN	870±29.87	17.25
1:1KN	1100±40.67	23.48
1:2KN	2100±50.63	29.23
1:3KN	2077±51.02	29.46
1:5KN	2109±49.99	28.86
1:8KN	2104±50.07	28.91
1:10KN	2083±52.31	30.20

*Indicates mean of three readings; S.D.: standard deviation; S.E.M: Standard error of mean; KN: Kneaded product (complex); The ratio represents the molar ratio of Asiatic acid to HPβCD which is shown in the table 1.

Fourier Transform Infrared Spectroscopy

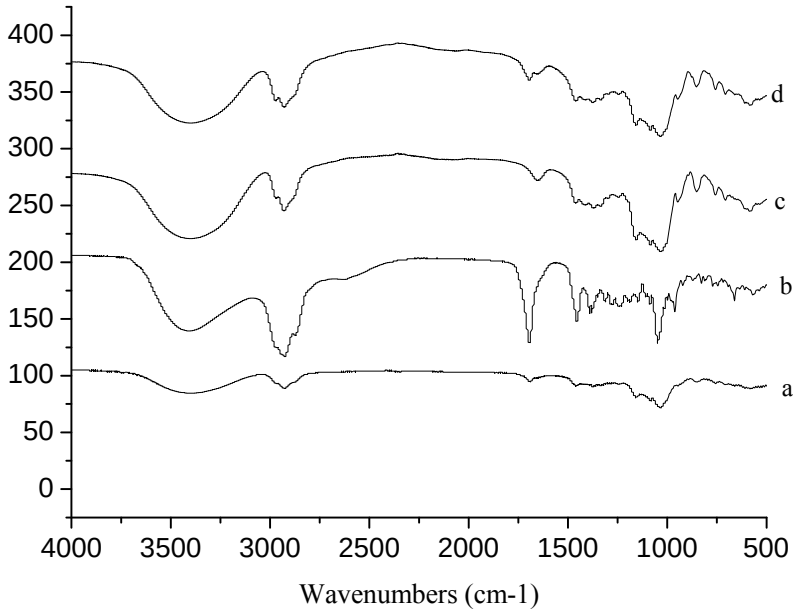

Fig.3 FTIR spectrum of Asiatic acid- HPβCD systems (a) physical mixture; (b) Asiatic acid; (c) HPβCD; (d) inclusion compex.

Fig.3 illustrates the FTIR spectra of Asiatic acid, HPβCD, physical mixture and inclusion complex. IR spectrum of Asiatic acid (b) was characterized by principal absorption peaks at 2926.14 cm-1 (C-H aliphatic asymmetric), 2869.57cm-1 (C-H aliphatic symmetric), 1694.12cm-1(C=O), 3404.61cm-1(O-H), 1049.28cm-1 (C-O).The IR spectrum of HPβCD (c) showed prominent peaks at 3397.22cm-1(O-H), 2930.03cm-1(C-H), 1652.17cm-1(H-O-H bending). In IR spectra of PM (a), all of the peaks appeared with decreased intensity. The peak at 2869.57cm-1 was not visible whereas the peak at 2926.14 cm-1, 1694.12cm-1, 3404.61cm-1 and 1049.28cm-1 was shifted to 2921.74cm-1, 1691.30cm-1, 3393.45cm-1 and 1021.74cm-1 respectively. This result suggested that strong physical interaction of Asiatic acid with HPβCD. In

IR spectra of inclusion complex (d) the peak at 2869.57cm-1 was completely disappeared. The peak at 2926.14 cm-1, 1694.12cm-1, 3404.61cm-1 and 1049.28cm-1 was shifted to 2928.21cm-1, 1695.65cm-1, 3396.79cm-1 and 1034.31cm-1 respectively with decrease in peak intensity. These changes occurred in IR spectrum of samples indicated that the non polar portions of Asiatic acid has been entrapped in the hydrophobic cavity of host molecule and formation of inclusion complex in solid state. Slight change was found in the water region for HPβCD represented by the 1652.17cm-1. It had shifted to 1643.48cm-1, with diminished intensity after complexation with Asiatic acid. This suggested that Asiatic acid could form inclusion complex with HPβCD in solid state.

X-Ray Diffraction

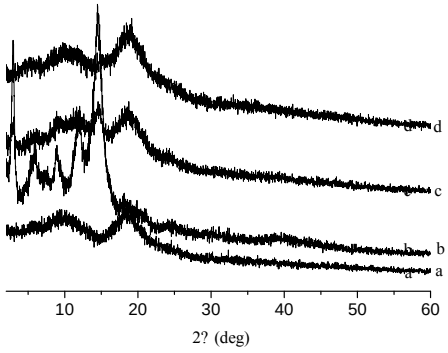

Fig.4 XRD spectrum of Asiatic acid- HPβCD systems (a) Asiatic acid; (b) HPβCD; (c) physical mixture; (d) inclusion complex.

The XRD pattern of Asiatic acid showed (Fig.4) intense and sharp peaks, indicating its crystalline nature. Asiatic acid (a) showed sharp peaks at 2.952°，5.920°, 8.901°,11.724°and 14.531°. The diffraction pattern of physical mixture (c) showed peaks of Asiatic acid with great decrease in the peak intensity of Asiatic acid indicating reduction in crystalllinity. In inclusion complex system (d) the crystallinity of Asiatic acid also was reduced to a greater extent as compared to physical mixture. Further, the peak at 2.952°of Asiatic acid in inclusion complex system was completely disappeared indicating formation of inclusion complex.

Differential Scanning Calorimetry

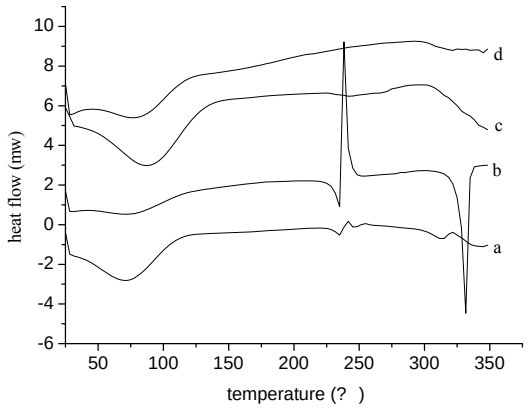

Fig.5 DSC spectrum of Asiatic acid- HPβCD systems (a) physical mixture; (b) Asiatic acid; (c) HPβCD; (d) inclusion complex.

The crystalline Asiatic acid displayed a single strong endothermic peak at 241.37℃ and two exothermic peaks at 236.73 and 334.32℃ respectively. HPβCD showed an large broad exotehmic band from 150 to 310℃, which was typical of HPβCD amorphous nature [14]. The DSC curve of the complex was similar to that of HPβCD. The characteristic peaks of the Asiatic acid at 236.73, 241.37 and 334.32℃ disappeared in the complex sample. This phenomenon suggested that the Asiatic acid existed in the complex in amorphous state.

Scanning Electron Microscopy

The photomicrographs of the samples obtained by scanning electron microscopy (SEM) are shown in the Fig. 6. The HPβCD powders (c) presented a spherical shape, whereas Asiatic acid (b) presented needle-like crystals. The physical mixture (a) also presented spherical shape. The Asiatic acid- HPβCD solid complex presented amorphous particles (d). This result was in accordance with the studies conducted by Calabro et al [15], Pralhad and Rajendrakumar [16] and Sri ea al [17]，which employed DSC and X-ray powder diffractometry to demonstrate that Asiatic acid/ HPβCD solid complexes existed in amorphous state.

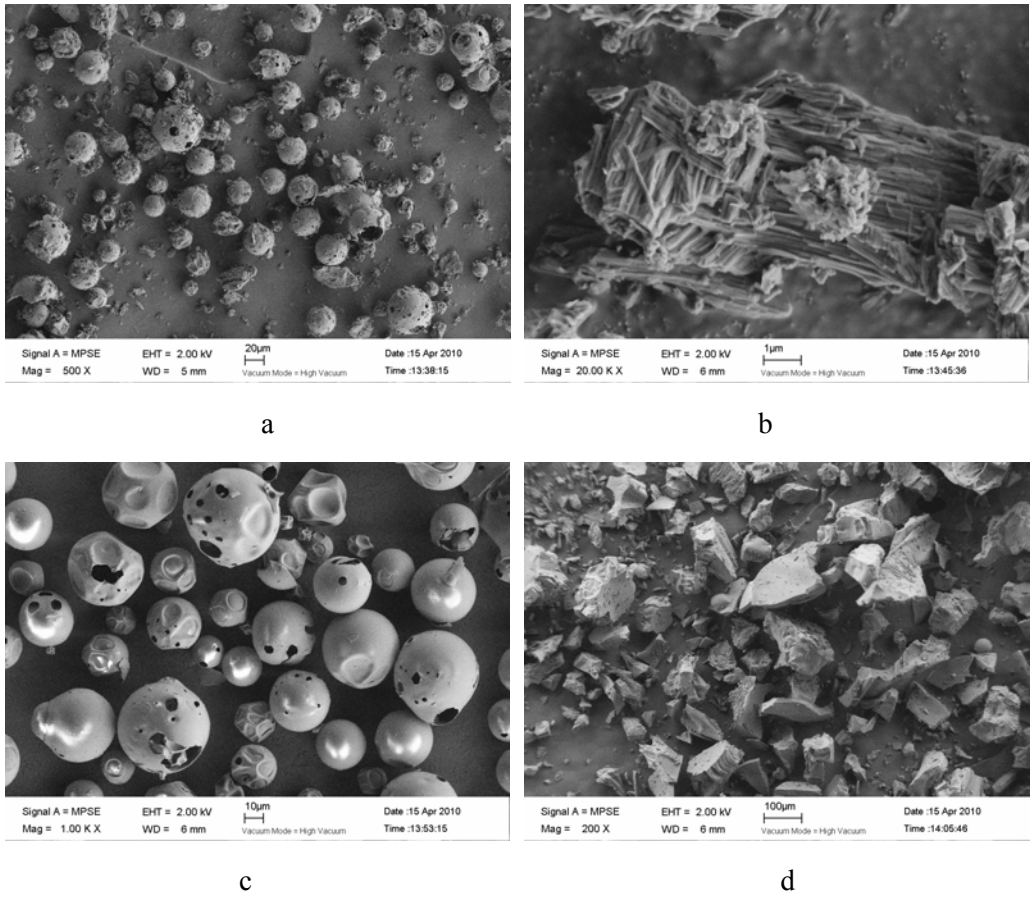

Fig.6 SEM spectrum of Asiatic acid- HPβCD systems (a) physical mixture; (b) Asiatic acid; (c) HPβCD; (d) inclusion complex.

4. Conclusions

The present study showed that aqueous solubility of Asiatic acid was successfully increased by complexation with complex with HPβCD. The aqueous solubility of Asiatic acid reached 2100 μg/ml when the molar ratio of Asiatic acid to HPβCD was 1:2. The aqueous

solubility of Asiatic acid was increased by 21-fold in Asiatic acid/ HPβCD solid complex. The FTIR and XRD spectra of Asiatic acid/ HPβCD solid complexes showed that Asiatic acid could form inclusion complex with HPβCD in solid state. The SEM, DSC and XRD spectra of Asiatic acid/ HPβCD solid complexes indicated that Asiatic acid existed in amorphous state; this can be explained by the fact that the aqueous solubility of Asiatic acid is high.

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