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ORIGINAL ARTICLE

The effects of water-soluble polymers on hydroxypropyl-β-cyclodextrin solubilization of oleanolic acid and ursolic acid

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Abstract The objective of this study was to investigate the influence of water-soluble polymers on the complex formation of hydroxypropyl-β-cyclodextrin (HP-β-CD) with oleanolic acid (OA) and ursolic acid (UA), two insoluble isomeric triterpenic acids. The interactions of OA and UA with HP-β-CD in water-soluble polymer solutions were studied by ultraviolet (UV) spectrophotometry. Phase-solubility studies were carried out to evaluate the solubilizing power of HP-β-CD, in association with water-soluble polymers, towards OA and UA. UV spectra indicated that the absorption intensity decreased upon the addition of HP-β-CD both in the absence and presence of water-soluble polymers. Stability constants were also calculated from the UV spectra. When water-soluble polymers were added, both the solubilization effect and the complexation efficiency (CE) of HP-β-CD with the isomers decreased. The experimental results suggested that the addition of water-soluble polymers inhibit the solubilization effect of HP-β-CD with OA and UA.

Keywords Hydroxypropyl-β-cyclodextrin · Solubilization · Water-soluble polymers · Oleanolic acid · Ursolic acid

Introduction

Oleanolic acid (OA) and ursolic acid (UA) are pentacyclic triterpenoid isomers, see (Fig. 1). The difference between the two isomers is only the location of one methyl group on ring E, but they show a great difference in physicochemical properties. Both OA and UA have numerous pharmacological effects, such as hepatoprotection and chemotherapy, but their clinical use is limited by their poor aqueous solubility [1].

It is considered that the solubility of a drug in aqueous media determines many aspects of its efficacy for delivery and absorption [2]. In recent years, the usefulness of a number of drugs and drug candidates is hampered by their insolubility in water, so the solubilization of water insoluble compound is becoming more and more important in pharmaceutical preparations. Solubilization can be achieved by: pH adjustment, particle size reduction, salt formation, solid dispersion, complexation, cosolvency, micellization, or a combination effect of any of the above [3].

Cyclodextrins (CDs) are capable of forming an inclusion complexes with many drugs. This can result in improved chemical stability, an increase in the apparent aqueous solubility, and higher bioavailability without changing their pharmacokinetic properties [4–8]. CDs have mainly been used as complexing agents in the pharmaceutical industry to increase aqueous solubility of poorly water-soluble drugs [9]. However, the efficiency of complexation is often not very high, and therefore, relatively large amounts of CDs must be used to obtain the desired effect [10]. On the other hand, for a series of reasons (e.g. including relatively high cost, possible toxicity, problems of bulk formulation, etc.), pharmaceutical dosage forms should contain as small amounts of CDs as possible. It is therefore important to develop methods which can be applied in order to enhance the efficiency of drug:CD complexation.

Typical pharmaceutical preparations are complex mixtures of drugs and excipients which may include antimicrobial agents, surfactants, polymers, etc. [11, 12]. Polymers are used widely in pharmaceutical systems as

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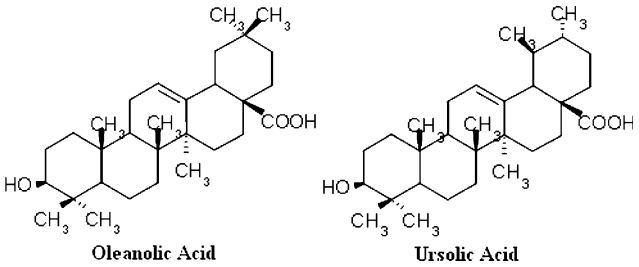

Fig. 1 Chemical structures of OA and UA

adjuvants, suspending and emulsifying agents, flocculating agents, adhesives, packaging and coating materials. Polymers are known to interact with CDs [13], enhancing drug availability in aqueous solutions [14]. The favourable effect of hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) on the poorly soluble drugs, leading to an increase in the drug solubility in conventional aqueous medium and CD solutions [10, 15–18] has been demonstrated previously. It is also reported that water-soluble polymers can increase the complexation efficacy of CDs [18]. As this technique method has the ability to further increase drug solubility, the potential of using small quantities of HPMC and PVP with HP-β-CD complexed with OA and UA will be investigated in this study.

Experimental

Materials

Oleanolic acid (OA, QDGSXC-060218, 98.07%) and ursolic acid (UA, XGSBS-060330, 98.07%) were obtained from Shanxi Huisheng Medicament Technology Corporation Limited. Hydroxypropyl-β-cyclodextrin (Kleptose® HP) with an average molecular weight of 1480.695 was kindly provided by Roquette Cyclodextrin Technologies Development Incorporation. Polyvinylpyrrolidone (PVP k30) and hydroxypropyl methylcellulose (HPMC E5) were purchased from Shanghai Colorcon Coating Technology Corporation Limited. HPLC grade methanol was obtained from Shanghai Ludu Reagent Company. Acetic acid (AR grade) was purchased from Shanghai Lingfeng Chemical Reagent Corporation Limited. All water used was de-ionized water.

A Shimadzu UV-2401 visible spectrophotometer was used to record absorption spectra. High-performance liquid chromatography (HPLC) system consisted of a pump (Shimadzu LC-10AT), a detector (SPD-10A) and a reversed-phase column (Dikma Kromasil, 100 A C_{18} 5 μm 250 × 4.6 mm). The weight measurements were performed with an AY-220 electronic analytic weighting scale (Shimadzu, Japan). Constant Temperature Shaker (Hua Li Da HZ-9310K Refrigerator Shakers, Tai Cang Shi Ke Jiao Qi Cai Chang, China) was used in this experiment.

Chromatographic conditions

The concentrations of OA and UA were determined by HPLC method. Dikma Kromasil was used as the stationary phase. The column temperature was controlled at 30 °C, the flow rate was 1.0 mL/min, and the sample injection volume was 20 μL. According to the UV absorption spectra, both the maximum absorption wavelengths of the isomers were around 200 nm. Simultaneously, considering the interference of mobile phase constituents and potential impurities took place when the wavelength was too short, the UV detector was set at 210 nm. Various mobile phase compositions (with a variety of methanol and water components) were tested with OA and UA in our preliminary experiments. The mobile phase composed of methanol–water–acetic acid (95:5:0.2, v/v/v) was a satisfactory one. Stock solutions of OA and UA (1 mg/mL) were prepared in methanol. Working solutions of OA and UA were obtained by diluting the stock solution with the same solvent.

Linearity

At the wavelength of 210 nm the linearity was tested over the concentration range from 1 to 40 μg/mL for both isomers. Measurements at all concentration levels were carried out in triplicate and all the values of peak areas were subjected to linear regression.

Precision

In order to evaluate the precision of the HPLC method, repeatability and reproducibility of measurements were studied at the wavelength of 210 nm. The repeatability of the peak areas of the two isomers was determined as relative standard deviations (RSD) for six consecutive injections of the standard substance with the concentration of 20 μg/mL. The reproducibility of the chromatographic data (peak areas) of the two isomers was measured in 3 days by three different operators.

Recovery test

A series of solutions containing OA and HP-β-CD (molar ratio 1:1) with and without water-soluble polymers were analyzed by HPLC. UA was treated similarly. The concentrations of OA and UA were 5, 20, 35 μg/mL, respectively.

Stability of sample solutions

Stability of the sample solutions kept under various storage conditions was tested by the optimized HPLC method during the period of 3 weeks. Three equal solutions of OA and UA were prepared by dilution of the stock solution with methanol to the final concentration 20 μg/mL. The first sample solution was stored at low temperature (in the refrigerator), the second one at room temperature and in darkness, and the last solution was kept also at room temperature but on daily light.

UV spectrophotometric measurement

In aqueous solutions, the complexation of OA and UA with HP-β-CD in the absence and presence of water-soluble polymers were demonstrated by UV spectrophotometry at 25 ± 0.1 °C. In case of absorption spectra scan, the slit width was set at 1.0 nm and the wavelength was set between 195 and 300 nm. Samples were contained in 1.0 cm path length quartz cuvettes. Because of poor solubility of the isomers in distilled water, the UV spectrophotometric analysis of OA and UA (0.05 mM) were performed in ethanol/water (50%, v/v) to ensure that both the chemical compounds will not precipitate from the solutions. Stability constants of the complexes (the isomers with HP-β-CD in the absence and presence of water-soluble polymers) were calculated from the concentration dependences of absorbance on the basis of well-known Benesi-Hildebrand equation [19]:

$$A = A_0 + \frac{(\Delta\varepsilon K[\mathrm{D}][\mathrm{L}])}{(1 + K[\mathrm{L}])} \quad (1)$$

where A and A_0 are the absorbance of drug in the presence and absence of CD, respectively; $\Delta\varepsilon = \varepsilon_{DL} - \varepsilon_D$ is the difference in the molar absorptivities between free (ε_D) and complex drug (ε_{DL}); [D] and [L] are the initial concentrations of the drug and CD, respectively; K is stability constant of the complex.

For the calculation of the binding constants, the changes of absorption of chemical compounds were measured at 202 nm as a function of HP-β-CD concentrations. The concentrations of OA and UA were fixed at 0.05 mM and the concentrations of HP-β-CD were changed from 0.01 to 0.05 mM. The absorbance of PVP is stronger than HPMC in 50% v/v ethanol/water. In order to reduce the influence of water-soluble polymers on the UV absorption, the final concentrations of HPMC and PVP were set at 0.02% (w/v) and 0.001% (w/v), respectively.

Phase-solubility studies

Phase-solubility studies were carried out according to the method of Higuchi and Connors [20]. A series of combination media containing increasing amounts of HP-β-CD (0–50 mM), with or without a fixed concentration of water-soluble polymers (0.10%, 0.25%, and 0.50% w/v) were obtained by adding HP-β-CD and HPMC or PVP into distilled water. The phase-solubility samples were prepared by adding excess OA/UA into each 10 mL combination media in the test tube. The samples were filtered with a 0.45 μm membrane after keeping shaking at 25 °C for 48 h in Constant Temperature Shaker. The filtrates were diluted for proper times and then injected into the HPLC system.

Theoretical background

The most common type of CD complexes is the 1:1 drug/CD complex where one drug molecule (D) forms a complex with one CD molecule:

$$[\mathrm{D}] + [\mathrm{CD}] \Leftrightarrow [\mathrm{D/CD}] \quad (2)$$

The value of the stability constant ($K_{1:1}$) is used to compare the affinity of drugs for different CDs or CD derivatives. The total solubility of drug (S_t) in aqueous CD solutions will then be:

$$S_t = S_0 + \frac{K_{1:1}S_0}{1 + K_{1:1}S_0}[\mathrm{CD_t}] \quad (3)$$

where S_0 is the intrinsic solubility of the drug, and [CD_t] is the total concentration of CD in the aqueous medium. A plot of S_t vs. [CD_t] will give a straight line with a slope ($K_{1:1}S_0/(1 + K_{1:1}S_0)$) less than unity and an intercept (S_{int}) equal to S_0. Then $K_{1:1}$ is calculated from the slope and S_0 [20]:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (4)$$

According to Eq. 3 the intrinsic solubility should be equal to the intercept but there are numerous exemptions from this (Fig. 2).

For poorly soluble drugs (aqueous solubility <0.1 mM) the intrinsic solubility (S_0) is in general much larger than the intercept of the phase-solubility diagram (S_{int}) resulting in non-linearity of otherwise linear (A_L-type) phase-solubility diagram. This can lead to erroneous $K_{1:1}$ value. A more accurate method for determination of the solubilizing efficiency of CD is to determine their complexation efficiency (CE), i.e. the concentration ratio between CD in a complex and free CD.

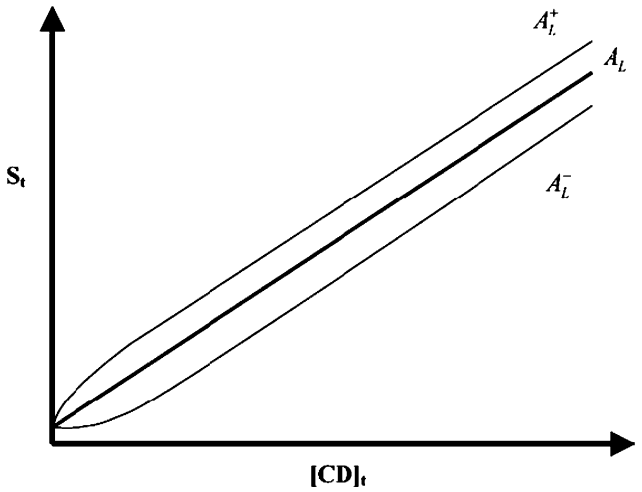

Fig. 2 Linear phase-solubility diagrams. A normal diagram (A_L), a diagram with a positive deviation at low CD concentrations ($A_L{}^+$), and a diagram with a negative deviation at low CD concentrations (A_L^-)

$$CE = \frac{[D/CD]}{[CD]} = K_{1:1} * S_0 = \frac{\text{slope}}{(1 - \text{slope})} \quad (5)$$

CE is calculated from the slope of the phase-solubility diagrams according to Eq. 5, but it is independent of both S_0 and S_{int}. CE is more reliable when the influences of different pharmaceutical excipients on the solubilization are being investigated [2].

Results and discussion

The HPLC assay method of OA and UA

All the OA and UA solutions were proved to be stable over a three week period, irrespective storage conditions. The repeatability of the peak areas of the isomers was obtained: RSD of the areas 0.56% for OA; and RSD of the areas 0.86% for UA. The reproducibility of the peak areas of the isomers was obtained: RSD of the areas 1.86% for OA; and RSD of the areas 1.98% for UA. These results suggest that the method is suitable for quantitative analysis of OA and UA.

Linear relationships between the peak areas and the concentrations of the two isomers were observed in the concentration range tested. The following linear regression equations were obtained: $y = 8797.3x - 1300.7$ ($R^2 = 0.9999$, OA) and $y = 7596.3x + 1724.1$ ($R^2 = 0.9999$, UA), where x is the concentration (μg/mL) and y is the peak area, R^2 is assessment coefficient.

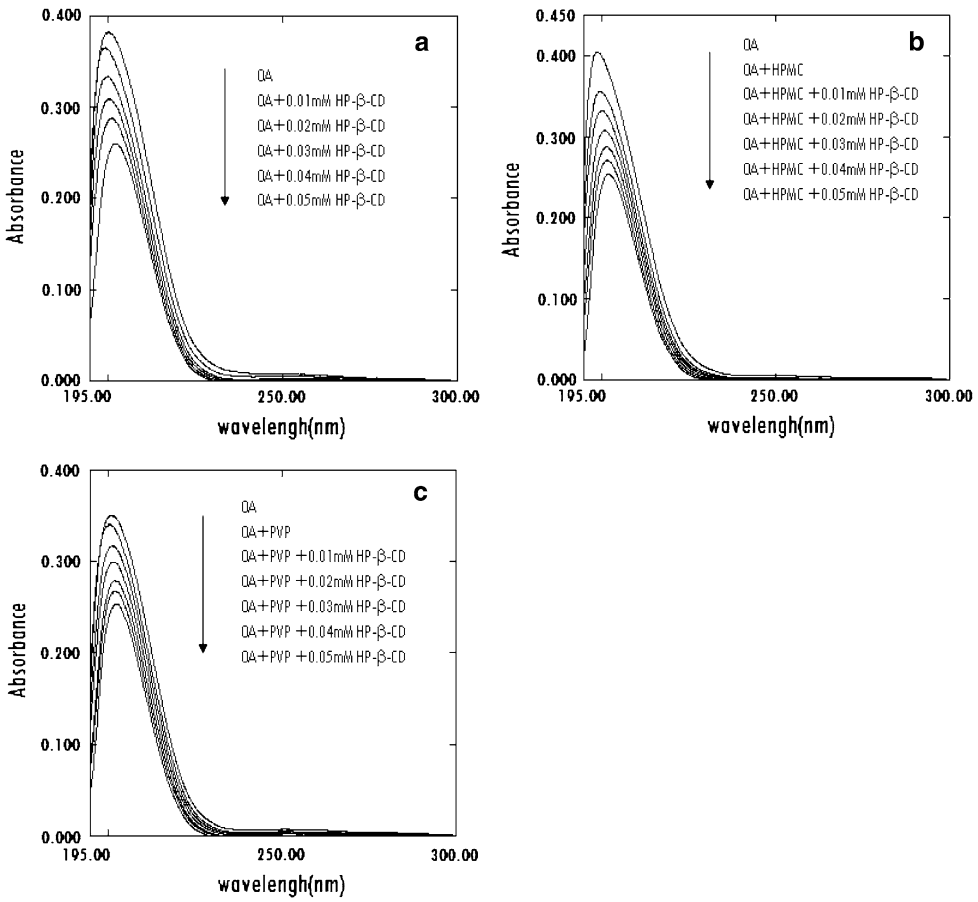

Fig. 3 Absorption spectra of OA (0.05 mM) containing various concentration of HP-β-CD in the absence and presence of HPMC (0.02%) or PVP (0.001%)

Somewhat higher RSD values at 210 nm are due to higher possibility of interference of impurities present in the separation system at this lower wavelength. Average recovery of OA was 100.4% and RSD was 0.60% ($n = 9$); average recovery of UA was 99.5% and RSD was 0.98% ($n = 9$). The concentration of OA and UA from 1 to 40 μg/mL could be calculated by the established HPLC assay method. And the precision of this method was good.

A highly selective and sensitive HPLC method was developed for the determination of OA and UA.

UV spectrophotometric analysis

The UV absorption spectrum of each chemical compounds studied is altered in the presence of water-soluble polymers. It can be seen from Fig. 3a that OA exhibited an absorption peak at 202 nm in 50% v/v ethanol/water solution. Upon the addition of HP-β-CD, the absorption of OA at 202 nm decreased gradually, which suggested that the formation of HP-β-CD/OA inclusion complexes, but the maximum absorbance wavelength was unchangeable.

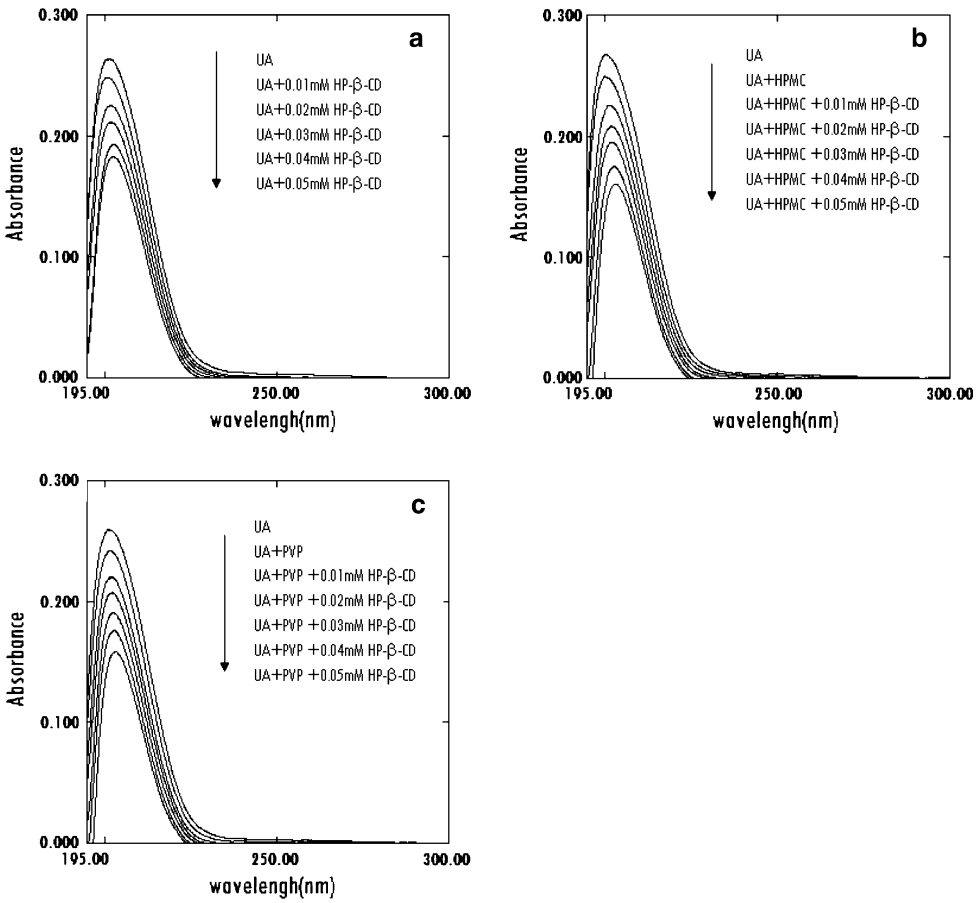

Fig. 4 Absorption spectra of UA (0.05 mM) containing various concentration of HP-β-CD in the absence and presence of HPMC (0.02%) or PVP (0.001%)

Table 1 The stability constant calculated from UV absorption spectra

Group	Equation	R^2	Stability constant (M^{-1})
OA	$y = -0.0004x - 0.8938$	0.9987	2,235
OA with HPMC	$y = -0.0005x - 1.3525$	1.0000	2,705
OA with PVP	$y = -0.0005x - 1.7834$	0.9989	3,569
UA	$y = -0.0006x - 0.78$	0.9956	1,300
UA with HPMC	$y = -0.0004x - 3.2104$	0.9951	8,026
UA with PVP	$y = -0.0004x - 4.8236$	0.9958	12,059

As shown in Fig. 3b and c, when water-soluble polymers (HPMC and PVP) were added alone or simultaneously with HP-β-CD, the absorption of OA decreased, and the maximum wavelength did not change.

Figure 4 shows UV absorption spectra of UA in HP-β-CD solution in the absence and presence of water-soluble polymers. It can be seen that UA shared similar changes with OA in the UV absorption spectra. Following the progressive addition of HP-β-CD and water-soluble polymers into the UA solution, characteristic phenomena of the complexes formation were noticed: reduction of the absorbance and a significant narrowing.

Absorbance values of the isomers decreased when HP-β-CD was added, both in the absence and presence of water-soluble polymers, which could be attributed to the complex formation via inclusion of OA and UA into to HP-β-CD hydrophobic cavities. The presence of water-soluble polymers (without HP-β-CD) also decreased the absorption of OA and UA indicated that there was interaction between OA/UA and water-soluble polymers.

Stability constants of the complexes calculated from the Benesi-Hildebrand equation were presented in Table 1. The applications of absorbance changes to Benesi-Hildebrand equation showed linear plots, the linear correlation coefficients were all >0.997, indicating 1:1 stoichiometry. Stability constants revealed that the stabilities of the complexes followed the order: PVP + HP-β-CD > HPMC + HP-β-CD > HP-β-CD. The larger K values for the former two complexes might be because the formation of ternary complex among CD, water-soluble polymer and OA/UA with higher stability constants than that of the binary systems.

Phase-solubility studies

Due to poor solubility of OA and UA, the calculation of the stability constant and thermodynamic parameters of the complexes were problematic in the phase-solubility studies. Because the isomers are insoluble and also show sharp A_L^--type phase-solubility diagrams in aqueous solutions, CE was applied to evaluate the solubilizing efficiency of HP-β-CD. Considering the detection limit of the HPLC system, the concentration of HP-β-CD in phase-solubility studies of UA was set from 20 to 50 mM.

The phase-solubility diagrams obtained with both drugs (OA and UA) with and without water-soluble polymers (PVP and HPMC) are shown in Figs. 5 and 6. They displayed A_L-type [20] phase solubility diagrams for both OA and UA binary and ternary systems, showing that OA and UA solubility increase linearly as a function of HP-β-CD concentration at a certain concentration of water-soluble polymers. The addition of water-soluble polymers into the HP-β-CD solution did not change the type of phase-solubility diagrams obtained for binary systems. It also can be

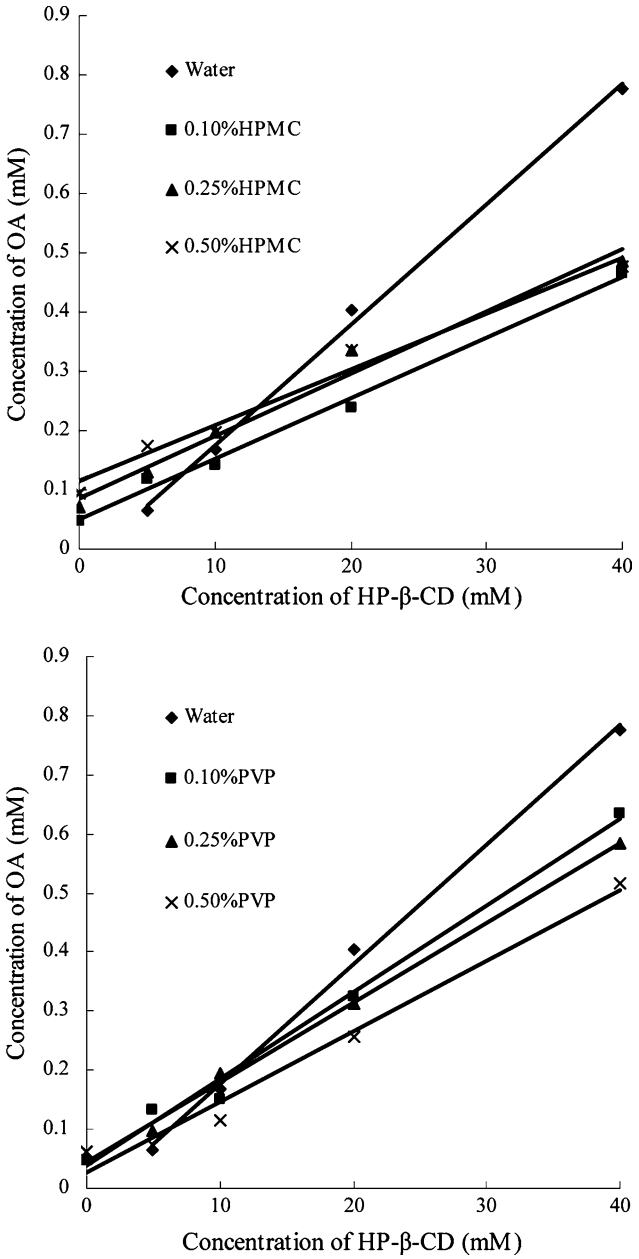

Fig. 5 Phase-solubility diagrams for OA in the presence of HP-β-CD without water-soluble polymers and with HPMC or PVP

seen from the phase-solubility diagrams that both HPMC and PVP can increase the aqueous solubility of OA without the presence of HP-β-CD. Because of the detection limit of the HPLC system, the solubilization effect of water-soluble polymers on UA was not been observed. However, the simultaneously existing of HP-β-CD and water-soluble polymers decreased the complex efficacy of HP-β-CD, which was shown in Table 2. It should be noted that the CE values of OA and UA with PVP is higher than HPMC in the presence of HP-β-CD. The results also implicated that CE values of OA in the presence of HP-β-CD without and with HPMC or PVP all higher than the CE values of UA.

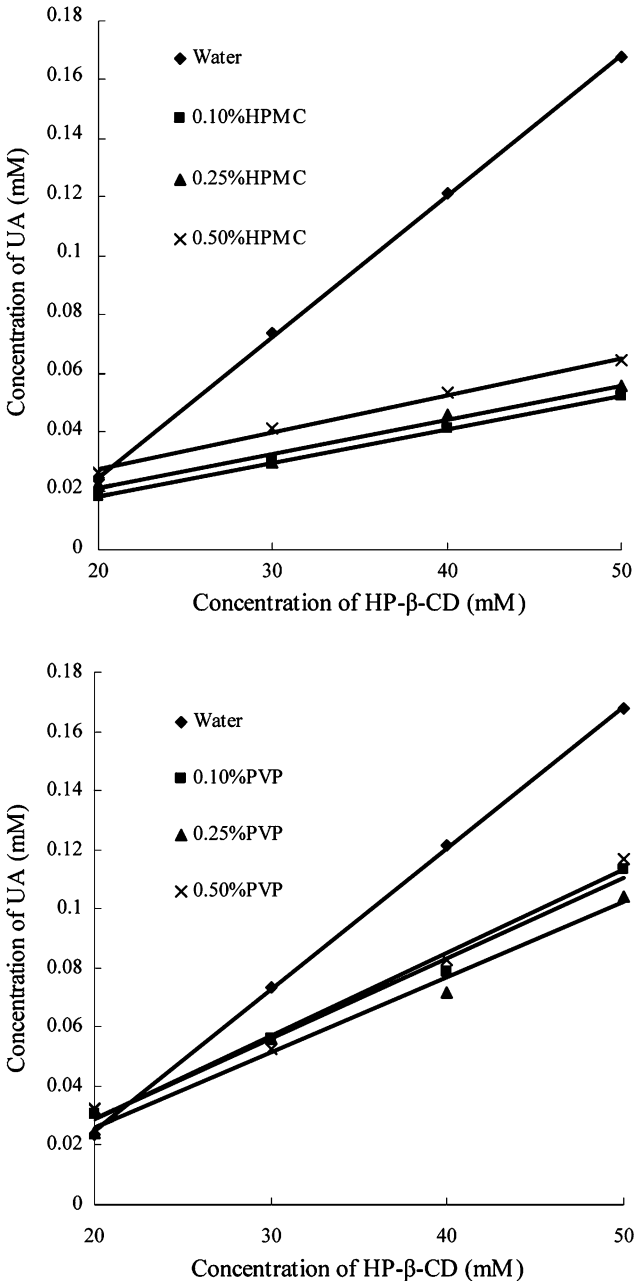

Fig. 6 Phase-solubility diagrams for UA in the presence of HP-β-CD without water-soluble polymers and with HPMC or PVP

Consequently, inhibitory effects on OA and UA solubility were observed in the presence of HP-β-CD and water-soluble polymers since the solubility values achieved in the presence of both HP-β-CD and polymers were lower than the sum of the contribution solubility values obtained with the HP-β-CD and polymer solutions. Similar effects have been reported for the influence of other additives such as PEG [21] and alcohols [22, 23] binding to β-CD. This is usually interpreted as reflecting upon the reduction in the concentration of free CD when the additive is present which, in turn, inhibits binding of the drug to the CD cavity [23, 24].

Table 2 The CE values of OA and UA with HP-β-CD in the presence of HPMC or PVP

Concentration of polymers (w/v)	CE	
	OA	UA
Water	0.020825	0.004823
0.10% PVP	0.014919	0.002707
0.25% PVP	0.013685	0.002607
0.50% PVP	0.012043	0.002808
0.10% HPMC	0.010305	0.001101
0.25% HPMC	0.010611	0.001201
0.50% HPMC	0.009591	0.001302

The stability constants calculated from the UV absorbance spectrum show that the presence of water-soluble polymers produces an increase in the affinity of the HP-β-CD to OA and UA. For this reason, two possible causes for the decrease in appearance solubility must be taken into account: (1) exclusion of the drug from the CD cavity can occur via formation of more favorable drug-polymer interactions rather than occupation of the cavity by hydrophilic polymer parts; and/or (2) ternary complex formation between CD, polymers and OA/UA with a lower solubility than that of binary system [21].

In our case, taking into account that chemical compounds interact with polymers and the presence of polymers produces an increase in the stability constants, the possible reason that water-soluble polymers result in a lower appearance solubility is that the solubility of the ternary system in distilled water is poor. In this case, when the phase-solubility samples were filtered, a great part of the drugs were discarded. As the concentration of the water-soluble polymers increased, the slightly increase of appearance solubility of OA and UA may be because of the solubilization effect of polymers itself.

Although the difference between the two chemical compounds is only the location of one methyl group, quite different physicochemical properties (initial solubility, wettability, fluidity, etc.) are shown. Different solubility in aqueous solutions were also shown in the phase-solubility studies. All the differences are caused by the different locations of one methyl group which indicates that the complex process is affected greatly by the structure of the ring E.

Conclusions

The result of UV spectrophotometric analysis indicated that stability constants of HP-β-CD can be significantly

enhanced by including a small amount of water-soluble polymer in ethanol/water (50%, v/v) complexation medium. The polymers increase the stability constant of drug/CD complexes might through the formation of ternary drug/CD/polymer complexes. A significant decrease in both the solubilization effect and the CE values of HP-β-CD in phase-solubility studies was an unexpected finding. It is probably because the solubility of the ternary system in distilled water is poor. The different complexing properties found for the isomers suggest the complex process may be affected greatly by the structure of the ring E of the two isomers. The inhibitory effects of water-soluble polymers with HP-β-CD will be further investigated in subsequent studies.

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