Preparation and property evaluation of vitamin A cyclodextrin inclusion complex

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Abstract. Hydroxypropyl-β-cyclodextrin (HP-β-CD) is widely used in drug encapsulation and cosolvent of insoluble drugs, modified HP-β-CD derivatives may to make pH sensitive by introducing functional groups. In this study, HP-β-CD derivative was prepared by oxidation cross-linking method with urea as the crosslinker agent. HP-β-CD derivatives and vitamin A (VA) inclusion complex were characterized using FT-IR, DSC, XRD and SEM. The solubilization effect, encapsulation efficiency (EE,%), loading content (LC,%) and in vitro release behavior of cyclodextrin derivatives on VA also investigated at different pH. The experimental results show that urea linked HP-β-CD can significantly increase the solubility of VA up to 32 times then pure water, the EE of the VA inclusion complex reach 66.88±4.56%, and the LC was 15.38±1.12%, The 24h in vitro cumulative release reaches 95.91%(pH=4.5) and 66.68%(pH=7.0) when the ratio of urea to HP-β-CD was 3:1. The HP-β-CD derivatives can increase the solubility of VA and the drug release has pH sensitivity.

Keywords: Vitamin A, β-CD derivatives, Inclusion complex, Solubilization, pH sensitivity.

1 Introduction

CD is an oligosaccharide composed of glucopyranose, which is connected to a hydrophilic outer surface and a hydrophobic central cavity through α-1,4-glucoside bonds. The most common natural cyclodextrins are α-CD, β-CD and γ-CD, which contain 6, 7 and 8 glucopyranose units respectively[1,2]. Recently, CD containing 3 and 4 glucose units has been synthesized successfully[3]. CD can encapsulate compounds with appropriate size and molecular polarity in the lipophilic cavity through van der Waals force, hydrophobic interaction and hydrogen bonding, so as to enhance the solubility and stability of a variety of insoluble drugs in aqueous solution [4-6]. Malihe et al. [4] used β-CD grafted magnetic graphene oxide nanocomposites(β-CD-MG) as nano carrier to encapsulate methotrexate, which had good drug release behavior and was non-toxic to K562 cells, and could be used as carrier of anticancer drugs. Shuang et al. [7] synthesized the inclusion complex of β-CD and Difenoconazole by ultrasonic method, which increased the water solubility of Difenoconazole, and the killing effect of the inclusion complex on *Gibberella sp*. was

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stronger than that of the bulk drug. Anuj et al. [8] prepared thymol and HP-β-CD and added it to the hydrogel system for external use, showing high dissolution rate (98.73%) and high stability of thymol. For some fat soluble vitamins, such as vitamin E and vitamin D, their water solubility and bioavailability can be greatly improved after they are made into inclusion complexes [9,10].

In this study, urea is used as crosslinking agent to prepare β-Cyclodextrin derivatives, and then the solubility and release behavior of the inclusion complex of VA will be studied.

2 Materials and methods

2.1 Experimental Materials

Dialysis bag (cut-off of 500D, purchased from United Carbon Co., Ltd.); HP- β- CD (purchased from Tianjin Bodi Chemical Co., Ltd.); The other reagents used were analytically pure grade.

2.2 Preparation of HP-β-CD derivatives.

Oxidation: The aldehyde group containing cyclodextrin derivatives were prepared by $NaIO_4$ oxidation method. Prepare four 100mL flasks contained 6g (4 mmol) HP-β-CD, 60 ml of 80% ethanol is added respectively, and then sodium periodate (4 mmol, 8 mmol, 12 mmol, 16 mmol) is added respectively, and leave them for 2 h in dark.

Crosslinking: Add 0.24g (4 mmol), 0.48g (8 mmol), 0.72g (12 mmol) and 0.96g (16 mmol) urea to the above reaction solution respectively, add 2 drops of glacial acetic acid, react at 60 ℃ for 4 h, filter, wash with 80% ethanol and dry to obtain urea linked HP-β-CD (Note as 1:1 Urea-HP-β-CD, 2:1 Urea-HP-β-CD, 3:1 Urea-HP-β- CD, 4:1 Urea-HP-β-CD).

2.3 Preparation of Urea-HP-β-CD inclusion complex.

Dissolve cyclodextrin and its derivatives in acetone to make saturated solution, the same amount of VA was dissolved in a small amount of acetone and drop into above saturated solution, followed stirring at room temperature for 24h. The mixture was allowed to stand in refrigerator (4 ℃) for 24h. And then filter it to get white powder solid. Next, the sample was washed with acetone to remove the uncoated VA, dried in vacuum at 50 ℃ obtained VA loaded HP-β- CD.(Note as VA@ HP-β-CD, VA@ 1:1 Urea-HP-β-CD, VA@ 2:1 Urea-HP-β-CD, VA@ 3:1 Urea-HP-β-CD, VA@ 4:1 Urea-HP-β-CD).

Physical Mixture (PM) is a homogeneous mixture of VA and cyclodextrin at 1:1(w/w) rate.

2.4 Characterization of cyclodextrin and its derivatives

2.4.1 Differential scanning calorimetry (DSC)

Using Malvern differential scanning calorimeter (DSC 240F1, Germany) to analyze the thermal behaviors of VA, HP-β-CD, Urea- HP-β-CD, VA@ Urea- HP-β-CD and PM under nitrogen protection. Around 2 mg of the sample are placed in aluminum pans and heat from 20℃ to 200℃ at a rate of 10℃/min.

2.4.2 X-ray diffraction analysis(XRD)

The nickel filtered Cu-Kα (Kα = 1.5460 Å) is used to radiate samples at rate of 10°/min between 3° and 60°, with the conditionof 40 kV and 150 mA, and the scanning step is 0.02°(D-MAW 2500/PC, Japan).

2.4.3 Determination by Fourier transform infrared spectroscopy (FT-IR).

Infrared spectra are obtained by KBr tableting method using a FTIR spectrometer (Shimadzu FTIR-8400, Japan).

2.4.4 Scanning electron microscopy (SEM)

The morphological characteristics of Urea-HP-β-CD,VA, PM and VA@ Urea- HP-β-CD are observed using scanning electron microscopy (SEM, VEGA3 TESCAN) after treating the dried sample with conductive adhesive.

2.5 Evaluation of solubilization and drug release performance

2.5.1 Saturate solubility studies

The solubility of VA in different carriers is determined by saturated solubility method with HP-β-CD and Urea-HP-β-CD as carrier. Add excess VA to the appropriate amount of deionized water and then the same mass of HP-β-CD or Urea-HP-β-CD, stir for 24 hours at room temperature slowly, centrifuge at 4000 rpm for 15 minutes, measure the absorbance of the supernatant at 392 nm, which filtered through 0.45 um filter, and calculate the concentration of VA by standard curve. Use Solubilization multiple to describe the solubilization effect of VA in cyclodextrin.

$$\text{Solubilization multiples} = \frac{\text{Solubility of experimental group}}{\text{Solubility of blank group}} \quad (1)$$

2.5.2 Phase solubility studies

The phase solubility studies of HP-β-CD and Urea-HP-β-CD are performed according to the method of Higuchi et al.[11] with slight modification. Excessed VA is add to a series of solutions containing increasing concentrations of HP-β-CD and different proportions of Urea-HP-β-CD, the concentration ranges of the HP-β-CD and Urea-HP-β-CD are 0-5 mg/mL. The suspension is stirred slowly for 24 hours at room temperature. After equilibrium, the solution is centrifuged at 3000 rpm for 10 min to remove undissolved VA, and the supernatants are measured at 392 nm. With the concentration of HP-β-CD and Urea-HP-β-CD as abscissa and the concentration of VA as ordinate, draw the phase solubility curve. The binding strength (K_f) and recombination efficiency (CE) are calculated from phase solubility curve.

K_f is the binding strength of the complex of cyclodextrin and VA. K_f value is calculated as equation (2).

$$K_f = \frac{\text{slope}}{S_0(1-\text{slope})} \quad (2)$$

CE is the solubilization efficiency of cyclodextrin for VA. The calculation formula is as equation (3).

$$\mathrm{CE} = \frac{\mathrm{slope}}{1-\mathrm{slope}} = \mathrm{K_f} \times S_0 \quad (3)$$

Where S_0 is the water solubility of VA in the absence of cyclodextrin; slope is the slope of the phase solubility curve.

2.5.3 Determination of entrapment efficiency and drug loading of inclusion complex

To determine the total content of VA, all kinds of complexes are weighted accurately and dissolved in 20mL DMSO with ultrasonicating for 30 min, then centrifuged at 12000 rpm for 15 min. To determine the free content, 10 mg of powder is dispersed with 10 mL of distilled water and vortexed for 30 s then centrifuged at 3000 rpm for 15 min. The dilution of supernatant is measured at 392 nm. The amount of VA in the inclusion complex is calculated by the difference between the total amount of the drug and recovered one. The encapsulation efficiency(EE, %) and loading content(LC, %) are respectively calculated using the equations (4) and equation (5).

$$\mathrm{EE\%} = \frac{\text{total amount of VA} - \text{free VA}}{\text{total amount of VA}} \times 100\% \quad (4)$$

$$\mathrm{LC\%} = \frac{\text{amount of encapsulated VA}}{\text{total amount of VA}} \times 100\% \quad (5)$$

2.5.4 Release behavior under different pH conditions

The release behavior of VA from cyclodextrin and its derivatives is studied using dialysis bag (500D). At room temperature, the cumulative release at different time points (0.5-24 h) is monitored in pH 4.5 and pH 7.0 phosphate buffer solution; 5mL of medium are taken out and replaced by the fresh buffer of equal volume. Then the collected samples are filtered and measure the cumulative release rate of VA at 392 nm by UV.

3 Results and discussion

3.1 Differential scanning calorimetry (DSC)

As shown in Figure 1, Urea-HP-β-CD, HP-β-CD, VA, PM and VA@ Urea-HP-β-CD were characterized. The DSC curve (b) for HP-β-CD shows a wide endothermic peak at 60-120℃ due to the evaporation of water from its inner cavity, while the Urea-HP-β-CD shows an endothermic peak at 141.8℃ which corresponds to the melting process, indicating that HP-β-CD is successfully crosslinked under the action of urea. The VA shows a wide endothermic peak at 65-105℃. The DSC curve obtained for the PM shows the sum of the characteristic absorption peaks of VA and Urea-HP-β-CD, and there is no cross-linking at the molecular level. In Figure.1(c), VA inclusion complex VA@ Urea-HP-β-CD appears melting peak at 135.8 ℃, which temperature is below then 141.8℃, result indicates probable interaction between VA and Urea-HP-β-CD.

3.2 X-ray diffraction analysis (XRD)

The XRD graph of HP-β-CD, Urea-HP-β-CD, VA, PM and VA@ Urea-HP-β-CD are shown in Figure 2. HP-β-CD and VA Non-display crystalline structure. However, Urea-HP-β-CD, PM and VA@ Urea-HP-β-CD have a certain crystallinity. PM is almost the superposition of the diffraction peaks of VA and cyclodextrin. After mixing, VA shows a strong characteristic peak at 2θ=18.64°. In VA@ Urea-HP-β-CD, the characteristic peak is sharply weakened, which indicates that VA is contained in cyclodextrin and forms a new phase.

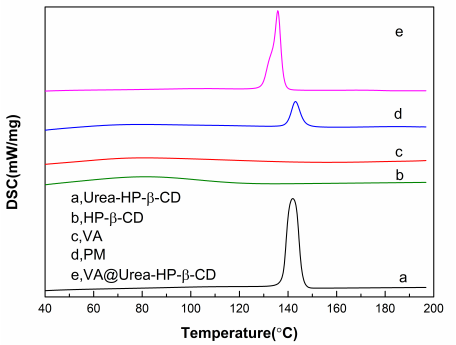

Fig. 1. DSC Thermograms of (a) Urea-HP-β-CD (b) HP-β-CD (c)VA (d) PM (e) VA@ Urea-HP-β-CD.

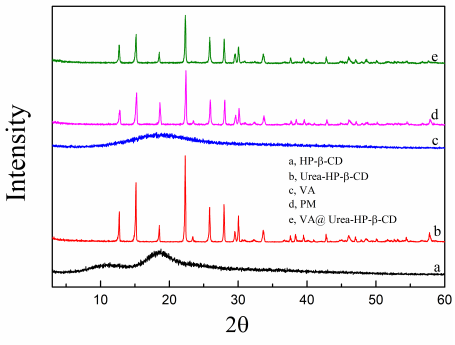

Fig. 2. The XRD graph of (a) HP-β-CD (b) 3:1 Urea-HP-β-CD (c) VA (d) PM (e) VA@ Urea-HP-β-CD.

3.3 Fourier transform infrared spectroscopy

In the infrared spectrum of Figure 3, compared with HP-β-CD (a), there are new characteristic peaks at 1632 cm^{-1} and 1677 cm^{-1} in Urea-HP-β-CD (b), indicating that the carbon of two adjacent hydroxyl groups of cyclodextrin oxidized by periodate is aldehyde group. The spectrum of vitamin shows peak at 3423 cm^{-1} from the carbonyl group (C=O), and peak at 1734 cm^{-1} belongs to the stretching of the O-C=O bond. The infrared spectrum (d) of vitamin and Urea-HP-β-CD is the superposition of ester group of vitamin A (1745 cm^{-1}) and peak of Urea-HP-β-CD (1678, 1632 cm^{-1}), although slight changes in their intensity could be observed, which proves that there is weak interaction between VA and inclusion complex. In contrast, the characteristic peak (1734 cm^{-1}) of VA in the infrared spectrum (e) of the inclusion complex does not exist, which indicates that the carbonyl group of VA is located in the cavity of the cross-linked cyclodextrin molecule.

3.4 Morphological characteristics

Figure 4 (a-e) shows the SEM image of Urea-HP-β-CD, VA inclusion complex, physical mixture and VA. HP-β-CD (a) shows a kind of amorphous spherical particles with cavity structure. The difference of Urea-HP-β-CD (b) is long rod structure, which is different from that of HP-β-CD (a) spherical structure. The reason for this difference may be that urea is added to cross link HP-β-CD into chain. However, the physical mixture PM (d) presents spherical and rod-shaped mixed morphology, which indicates that there is no interaction between the two substances in the physical mixture; In the complex VA@ Urea-HP-β-CD

(E), it is shown as uniform long strip structure particles with irregular size. The change of particle morphology may be due to the interaction between VA and cyclodextrin, which indicates that VA is incorporated into cyclodextrin. The results showed that the drug is uniformly contained in the cyclodextrin derivatives after the inclusion complex is formed.

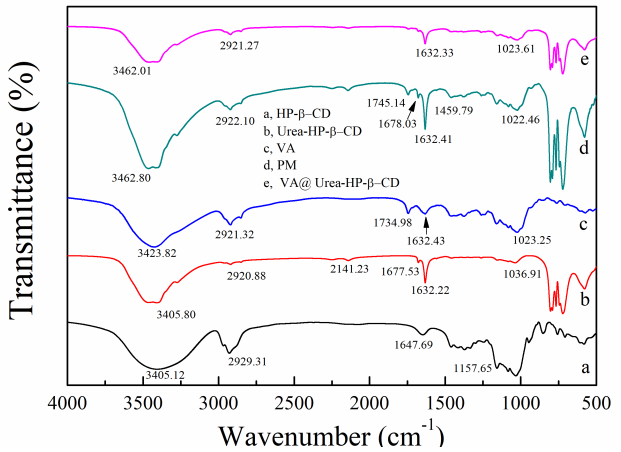

Fig. 3. Infrared spectroscopy of (a) HP-β-CD (b) Urea-HP-β-CD (c) VA (d) PM (e) VA@ Urea-HP-β-CD.

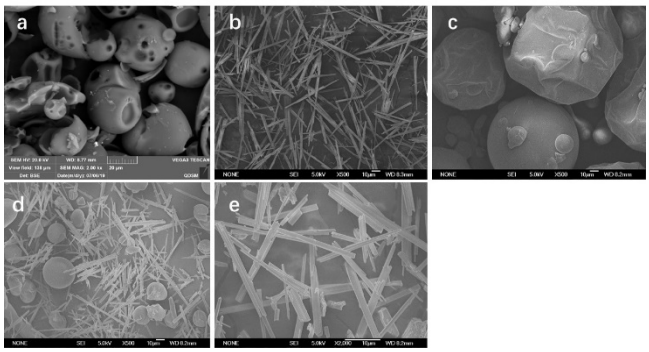

Fig. 4. The SEM images of (a)HP-β-CD (b) Urea-HP-β-CD (c) VA (d) PM (e) VA@ Urea-HP-β-CD.

3.5 Solubility determination of VA

With dimethyl sulfoxide as blank control, the UV absorption curve of VA has a good linear relationship in the concentration range of 0.4~2.4 mg/mL, (A=0.2153C-0.0132, R^2 =0.998). Figure 5 shows the solubility of VA in different proportions of Urea-HP-β-CD. It can be seen when the ratio of urea: HP-β-CD is 3:1, the solubility of VA increased by Urea-HP-β-CD is higher than that of HP-β-CD (about 20%), which is 32 times of that of pure water. This may be due to the fact that VA is not only encapsulated in the cavity of cyclodextrin, but also load into the cross-linked porous structure.

3.6 Determination of phase solubility

As shown in Figure 6 the solubility curves can be classified as the A-L type, which conforms to the description of Higuchi and Connors[11]. The solubility of VA increases linearly with the increase of the concentration of HP-β-CD and different proportions of

Urea-HP-β-CD, confirming the stoichiometric ratio of the inclusion complex is 1:1. K_f mainly indicates the bonding strength between Urea-HP-β-CD and VA, CE indicates the solubilization effect. From Table 1, it can be seen that the 3:1 Urea-HP-β-CD, K_f value is 1.115 L/g , CE value is 0.086, which has the best solubilizing effect on VA. This may be due to the size and shape of the porous structure of cyclodextrin derivatives formed by 3:1 Urea-HP-β-CD cross-linking, which is more suitable for binding with VA.

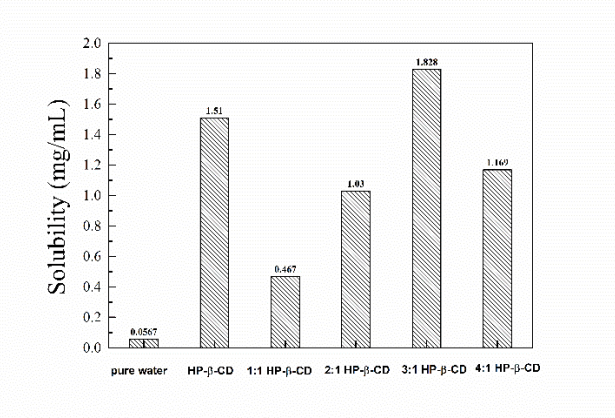

Fig.5. Effect of Urea-HP-β-CD in Different Proportions on VA Solubility.

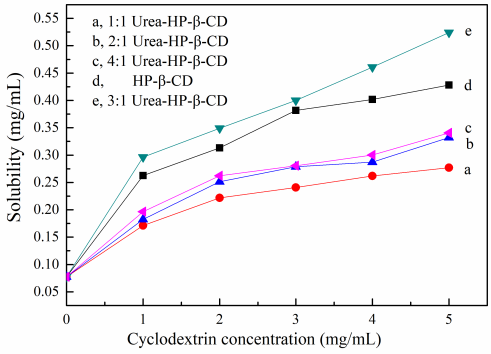

Fig.6. Phase solubility curves of VA at different concentration.

Table 1. Phase Solubility and encapsulation and drug loading efficiency ($\bar{X}$±SD, n=3).

Medium	Kf (L/g)	CE	LE, %	EE, %
HP-β-CD	0.884	0.068	9±0.75	55.96±5.11
1:1 Urea- HP-β-CD	0.495	0.038	7.44±0.42	45.64±4.12
2:1 Urea- HP-β-CD	0.626	0.048	7.04±0.66	50.51±3.77
3:1 Urea- HP-β-CD	1.115	0.086	15.38±1.12	68.88±4.56
4:1 Urea- HP-β-CD	0.639	0.049	8.43±0.86	55.61±4.78

3.7 Determination of entrapment efficiency and drug loading of inclusion complex

The EE and LC of VA inclusion complexes loaded with five cyclodextrins and its derivatives are shown in Table 1. The EE and LC of 3:1 Urea-HP-β-CD are 68.88%±4.56% and 15.38%±1.12% respectively, which higher than other carriers. The reason may be that the porous structure formed by 3:1 Urea-HP-β-CD is more suitable for the inclusion of VA than other four kinds of cyclodextrins.

3.8 Study on release behavior under different pH conditions

The release curves of cyclodextrin inclusion complex at pH 4.5 and pH 7.0 are shown in Figure 7 and Figure 8. In the cumulative release within 24 hours, the cumulative release rate of VA at two pH conditions is very low, which is related to it's low solubility in water. Both HP-β-CD and 3:1 Urea-HP-β-CD show sudden release in the initial 2 h, but the cumulative release of HP-β-CD is significantly lower than that of urea cross-linked cyclodextrin in the same time period, which is related to the different encapsulation efficiency of the two cyclodextrin inclusion complexes. The cumulative release of 3:1 Urea-HP-β-CD is the highest in 24h (pH 4.5 to 95.91%, pH 7.0 to 66.68%), which may be related to the strongest interaction of 3:1 Urea-HP-β-CD and VA, and its EE is the highest. The cumulative release of VA inclusion complex prepared by urea cross-linked cyclodextrin derivatives increases significantly within 24 h when the pH is acidic, which is due to the instability of C=N- bond in acidic solution, which will lead to different degrees of fracture, so that more VA are released from cyclodextrin molecules.

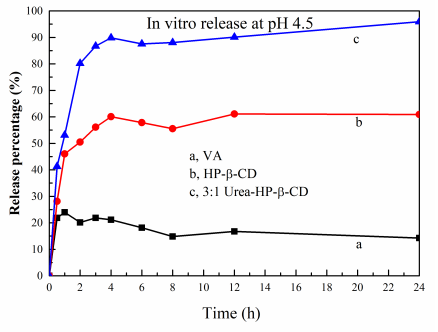

Fig. 7. VA Release profiles (a) VA (b) HP-β-CD (c) 3:1 Urea-HP-β-CD at pH 4.5.

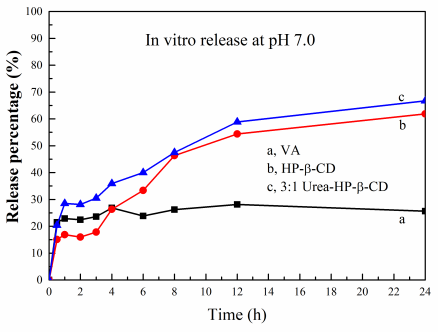

Fig. 8. VA Release profiles (a) VA (b) HP-β-CD (c) 3:1 Urea-HP-β-CD at pH 7.0.

4 Conclusion

Using urea as crosslinking agent, HP-β-CD is oxidized by four different crosslinking ratios (1:1, 2:1, 3:1, 4:1) and sodium periodate to prepare cross-linked cyclodextrin derivatives of VA. The cyclodextrin derivatives are characterized by DSC, IR, XRD and SEM, evaluating the encapsulation efficiency, loading content, solubility and release in vitro under different pH conditions. The water solubility of VA is significantly increased by the obtained cyclodextrin derivatives. Compared with pure water, 3:1 Urea-HP-β-CD can increase the solubility of VA by 32 times, and increase by 20% compared with HP-β-CD. In vitro release evaluation at different pH values shows that the cumulative drug release within 24

hours is 95.91% in the medium of pH 4.5, and 66.68% in the medium of pH 7.0. This means that the cyclodextrin derivatives we prepared are pH sensitive.

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