Preparation of pH-Sensitive β-Cyclodextrin Derivatives and Evaluation of Their Drug-Loading Properties

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Abstract. Hydroxypropyl β-cyclodextrin (HP-β-CD) is widely used in the encapsulation of drugs and the increase of solubility of poorly soluble drugs. The modified hydroxypropyl cyclodextrin can make cyclodextrin have pH and temperature sensitive and other characteristics. The purpose of this study was to oxidize and crosslink HP-β-CD to prepare a derivative of the network structure and evaluate its drug-loading properties. First, the β-cyclodextrin with aldehyde group was obtained by periodate oxidation, and then crosslinked with different ratios of ethylenediamine (EN) to obtain a network structure of hydroxypropyl β-cyclodextrin derivative (EN-HP-β-CD). The β-cyclodextrin derivatives were characterized by FT-IR, DSC, XRD and SEM. Vitamin E (VE) was used as a model drug to study the solubilization effect, phase solubility and in vitro release behaviour under different pH conditions. The results showed that EN-HP-β-CD could significantly improve the solubility of VE, and 1:1 EN-HP-β-CD could increase the solubility by 25 times. In vitro release experiments under different pH conditions within 24 h showed that the cumulative release at pH 4.5 was 78.25%, which was significantly higher than the pH 7 release effect (43.94%). The result indicated that EN-HP-β-CD had solubility increasing effect and pH-sensitive properties.

Keywords. Cyclodextrin derivative, VE, solubility, pH sensitive.

1. Introduction
Cyclodextrin (CD) is a cyclic molecule which is consisted of glucopyranose units linked by alpha-1,4-glycosidic linkage including a hydrophilic outer surface and a hydrophobic central cavity. The most common natural cyclodextrins contain 6, 7 and 8 glucopyranose units, named α-CD, β-CD and γ-CD, respectively [1]. CD can enhance the stability and water solubility of a lot of insoluble drugs by entrapping the drug molecule into the hydrophobic cavity. CD can encapsulate compounds of appropriate size and molecular polarity in lipophilic cavities, especially β-CD can be a guest molecule to make inclusion complexes with a lot of organic compounds [2, 3]. HP-β-CD is a common β-CD derivative. The structure of HP-β-CD is shown in figure 1a. Compared with natural β-cyclodextrin, HP-β-CD has 20 times more water solubility, better complexation performance and lower toxicity. It is more suitable for pharmaceutical applications [4, 5]. The HP-β-CD inclusion complex can enhance the stability of the active ingredient during processing, storage and use [6].
VE is a lipid-soluble vitamin with many biological functions. Brigelius Flohe et al. [7] studied its role in neurological function, smooth muscle growth, platelet aggregation and immune response. Tocopherol acetate (Figure 1b) is a schematic diagram of the structure of VE acetate, usually used in food and diet.

VE is greatly limited using in food cosmetics and pharmaceuticals because VE has low water solubility and instability to alkali or oxygen. Therefore, it is necessary to study the methods of increasing the solubility and stability of VE. Kuttiyawonga et al. [8] synthesized a macrocyclic cyclodextrin from starch by maltogenic starch and formed a complex of VE with cyclodextrin to improve the solubility of VE. Some researchers believe that the biological effects of VE may be limited by the lack of solubility and stability of the VE in solution [9]. Therefore, the addition of cyclodextrin to VE enhances its solubility and stability without affecting its antioxidant activity is an effective way to improve its biological activity [10]. Karim Benhemia et al. [9] found that the combination of VE and cyclodextrin had a greater protective effect on male semen during freezing and thawing than VE alone. VE is also encapsulated in sodium starch octenyl succinate to make a VE nanoparticle beverage [11]. VE nanoparticle products not only improve stability in beverages, but also do not significantly change beverages appearance. In addition, researchers have made VE into microemulsions or nanoemulsions [12, 13] or liposomes [14] to enhance the stability and water solubility of VE.

The purpose of this study was to oxidize HP-β-CD using sodium periodate (NaIO₄), and the resulting aldehyde group of β-CD was crosslinked with ethylenediamine to form C=N bond. C=N bond is easily broken under acidic conditions, thus the hydroxypropyl β-cyclodextrin derivative after ethylenediamine cross-linking can be used as a pH-sensitive excipient material. The β-cyclodextrin derivatives and the cyclodextrin complex loaded with VE were subsequently characterized, and its solubilizing effect on VE and the degree of release under different pH conditions were evaluated.

2. Experimental

2.1. Experimental Materials and Methods

2.1.1. Experimental Materials. Dialysis bags (EPK/MWCO: 500D, MD44) were purchased from Viskase Company (USA). 2-Hydroxypropyl β-cyclodextrin (HP-β-CD) was purchased from Shandong Binzhou Zhiyuan Biotechnology Co., Ltd. Tocopheryl acetate (Vitamin E, VE) was purchased from Shanghai Maclean Biochemical Technology Co., Ltd. Ethylenediamine and sodium periodate were purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals are of analytical grade.

2.1.2. Preparation of Hydroxypropyl β-Cyclodextrin Derivatives. Oxidation: A cyclodextrin derivative containing an aldehyde group was prepared by the NaIO₄ oxidation method [15]. Weigh 6g (4 mmol)
of HP-β-CD into 3 flasks, and add 60 mL of 80% ethanol, add amount of sodium periodate 4 mmol, 8 mmol and 12 mmol, respectively and avoid the light reaction for 2 h.

Cross-linking: 0.24, 0.48, 0.72g of ethylenediamine were added to the above reaction solutions, and 2 drops of glacial acetic acid were added dropwisely, and reacted at 60 °C for 4 h, suction filtration. Wash with 80% ethanol and dry to obtain 1:1 EN-HP-β-CD, 1:2 EN-HP-β-CD and 1:3 EN-HP-β-CD.

2.1.3. Drawing of the Tocopherol Acetate Standard Curve. The tocopherol acetate acetone solution was first scanned at a full wavelength of the ultraviolet spectrophotometer to determine a maximum absorption of 306 nm. The tocopherol acetate acetone solutions with different concentration gradients were prepared, and measured absorbance at 306nm. The concentration was plotted on the abscissa and the absorbance was plotted on the ordinate to obtain the tocopherol acetate standard curve.

2.1.4. Determination of Solubilization Effect of EN-HP-β-CD. Saturated solubility: The solubility of VE in pure water, aqueous solutions containing cyclodextrin, aqueous solutions containing cyclodextrin complexes (1:1 EN-HP-β-CD, 1:2 EN-HP-β-CD, and 1:3 EN-HP-β-CD) was evaluated by the saturation solubility method. The method is as follows [16]: adding excess VE to a beaker containing 10 mL of deionized water, and adding a known equimolar amount of cyclodextrin or cyclodextrin inclusion complex, stirring at 100 rpm for 24 h, centrifugating at 5000 rpm for 15 minutes. The supernatant was filtered through a 0.45 µm microporous membrane. The content of VE in the solution was determined by measuring the absorbance at 306 nm (n=3).

Phase solubility: The phase solubility study was implemented as Higuchi & Connors described [17]. Excess VE (0.2 g) was added to a 20 mL aqueous solution containing different concentrations of cyclodextrin and its derivatives. Then stirred them for 24 h. The concentrations of the cyclodextrin and its derivatives were 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, respectively. After equilibration, using 0.45µm membranes to filter the samples and analyzing them by spectrophotometry at 306 nm (n=3). Phase solubility curve was plotted by plotting the concentration of VE versus the concentration of cyclodextrin. Kf is the binding strength of cyclodextrin and VE complex. The Kf value of the composite is calculated as follows:

\[ K_f = \frac{\text{slope}}{S_0 (1 - \text{slope})} \]  

(1)

S0 is the solubility of VE in water without cyclodextrin. Slope is the slope of the phase solubility curve.

The solubilization efficiency of cyclodextrin to VE was evaluated by complex efficiency (CE). Its calculation formula is as follows:

\[ CE = \frac{\text{slope}}{1 - \text{slope}} = K_f \times S_0 \]  

(2)

2.1.5. Preparation of EN-HP-β-CD Inclusion Complex. The VE-loaded inclusion complex was prepared by a solvent evaporation method. The VE was dissolved in acetone, then the cyclodextrin and cyclodextrin derivatives were added to the VE solution and stirred. The solvent was evaporated, washed with acetone to remove the unencapsulated VE, and dried. The physical mixture (PM) was ground to obtain homogeneous mixture of VE and cyclodextrin complex.

2.2. Characterization of Cyclodextrin and Its Derivatives

2.2.1. Drug Entrapment and Drug Loading Efficiency. All kinds of cyclodextrin complexes were weighed quantitatively, added 20mL acetone, then ultrasonicated for 30min to release loaded VE. The supernatant was filtered through a 0.45 µm microporous membrane after centrifugation at 5000rpm. The UV absorbance was measured at 306 nm.

The formulas for drug entrapment and drug loading efficiency are as follows:
\[
\% \text{ Drug entrapment efficiency} = \frac{\text{Drug encapsulated}}{\text{Drug total}} \quad (3)
\]
\[
\% \text{ Drug loading efficiency} = \frac{\text{Drug encapsulated}}{\text{Inclusion complex total}} \quad (4)
\]

2.2.2. Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR was recorded at a resolution of 4 cm\(^{-1}\) using a spectrometer (Shimadzu FTIR-8400, Japan) at a wavenumber range of 4000-500 cm\(^{-1}\).

2.2.3. Differential Scanning Calorimetry (DSC). The thermal behaviour of HP-\(\beta\)-CD, EN-HP-\(\beta\)-CD, VE, PM and inclusion complexes were evaluated by DSC (DSC 240F1, Germany) under nitrogen purge conditions.

2.2.4. X-ray Diffraction (XRD). Samples whose particle size were ground less than 40mm were weighed, compacted and measured (D-MAW 2500/PC, Japan). The samples were operated at a voltage of 40 kV and a current of 150 mA using nickel-filtered Cu-K\(\alpha\) (K\(\alpha\)= 1.5460 Å) radiation between 5-35° at a rate of 10° min\(^{-1}\), with a corresponding scanned step of 0.02°.

2.2.5. Scanning Electron Microscope (SEM). Morphological characterization of HP-\(\beta\)-CD, EN-HP-\(\beta\)-CD, inclusion complex was performed by scanning electron microscopy (SEM, VEGA3 TESCAN).

2.2.6. In Vitro Release Behaviour. The release behaviour of the VE from cyclodextrin and its derivatives was studied using a dialysis bag (500D). Sampling 5mL from aqueous solutions of pH 4.5 and pH 7.0 at different time points (0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18, 24h) within 24 hours at room temperature, add 5mL buffer solution after each sampling. The cumulative release amounts of VE, HP-\(\beta\)-CD, HP-\(\beta\)-CD-CHO, 1:1-EN-HP-\(\beta\)-CD, 1:2-EN-HP-\(\beta\)-CD, 1:3 EN-HP-\(\beta\)-CD were determined by UV at 306 nm.

3. Results and Discussion

3.1. Solubility Effects of VE
The standard curve of VE was showed in figure 2.

The dissolution of VE in pure water, aqueous solution containing cyclodextrin, aqueous solution containing cyclodextrin complex (1:1 EN-HP-\(\beta\)-CD, 1:2 EN-HP-\(\beta\)-CD, and 1:3 EN-HP-\(\beta\)-CD) were showed in table 1.

Table 1 showed that the solubility of VE in cyclodextrin and its derivatives was increased compared with the pure aqueous solution, and the dissolution effect of the EN-HP-\(\beta\)-CD was better than HP-\(\beta\)-CD, which might be VE was not only encapsulated in the cavity of the cyclodextrin but also loaded into the crosslinked porous structure. The ratio of cyclodextrin to crosslinker also affected the solubility of VE. Among them, the 1:1 EN-HP-\(\beta\)-CD solubilization effect was the most obvious, up to 25 times.

The effect of HP-\(\beta\)-CD, 1:1 EN-HP-\(\beta\)-CD, 1:2 EN-HP-\(\beta\)-CD, 1:3 EN-HP-\(\beta\)-CD on the solubility of VE was shown in figure 3. The solubility of VE in pure water was 0.0063 mg/mL. The results showed that the solubility of VE increased linearly with the increase of cyclodextrin concentration, and each cyclodextrin derivative showed Higuchi and Connors [17]. The defined A-L type solubility curve indicates that the stoichiometric ratio of cyclodextrin to VE inclusion complex was 1:1.

When the stoichiometric ratio of the inclusion complex was 1:1, the apparent stability constant (K\(d\)) and the recombination efficiency (CE) of the inclusion complex could be calculated according to the equations (1) and (2), as shown in table 2. The greater the value of K\(d\) and CE, the better the solubilization effect of cyclodextrin on VE. Therefore, it could be seen from table 2 that 1:1 EN-HP-\(\beta\)-CD had the best solubilizing effect on VE.
Figure 2. Standard curve of VE.

Figure 3. Phase solubility curve of cyclodextrin and its derivatives.

| Medium                  | Solubility (mg/mL) | Solubility increase times |
|-------------------------|--------------------|---------------------------|
| pure water              | 0.0037             | 1                         |
| HP-β-CD                 | 0.0344             | 9                         |
| 1:1 EN- HP-β-CD         | 0.0929             | 25                        |
| 1:2 EN- HP-β-CD         | 0.0678             | 18                        |
| 1:3 EN- HP-β-CD         | 0.0753             | 20                        |

Table 1. Solubility of VE in different medium.

| Medium                  | $K_f$ (L/g) | CE        |
|-------------------------|------------|-----------|
| HP-β-CD                 | 1.43       | 0.009     |
| 1:1 EN- HP-β-CD         | 3.66       | 0.023     |
| 1:2 EN- HP-β-CD         | 2.71       | 0.017     |
| 1:3 EN- HP-β-CD         | 2.86       | 0.018     |

Table 2. Phase solubility of HP-β-CD and EN- HP-β-CD.

3.2. Drug Entrapment and Drug Loading Efficiency
The encapsulation efficiency and VE loading of various cyclodextrin derivatives VE inclusion complexes were calculated according to equations (3) and (4), as shown in table 3. Drug loading and encapsulation efficiency of 1:1 EN- HP-β-CD were the highest, 15.45% and 51.50%, respectively.

| Medium                  | Drug loading efficiency (%) | Drug entrapment efficiency (%) |
|-------------------------|----------------------------|-------------------------------|
| HP-β-CD                 | 3.84                       | 17.72                         |
| 1:1 EN- HP-β-CD         | 15.45                      | 51.50                         |
| 1:2 EN- HP-β-CD         | 12.80                      | 45.82                         |
| 1:3 EN- HP-β-CD         | 14.83                      | 49.81                         |

Table 3. Drug loading and encapsulation efficiency of VE inclusion complexes.

3.3. Fourier Transform Infrared Spectroscopy (FT-IR)
Various cyclodextrin derivatives were characterized with FT-IR by potassium bromide (KBr) tableting method. The infrared spectrum of the HP-β-CD and its derivatives was shown in figure 4.

In the infrared spectrum of HP-β-CD-CHO, a new characteristic peak of 1631 cm$^{-1}$ appeared, demonstrating that NaIO$_4$ has successfully oxidized the -OH moiety in HP-β-CD to an aldehyde group. In figure 4c, there was a distinct methylene stretching vibration of 2837 cm$^{-1}$ and a
characteristic peak of C=N double bond of 1677 cm\(^{-1}\), which proved that ethylenediamine had successfully crosslinked with cyclodextrin molecules.

The infrared spectra and characteristic peaks of the VE and VE inclusion complex were shown in figure 5.

It can be seen from figure 5 that the characteristic peaks of VE 1759 cm\(^{-1}\) (C=O) and 1208 cm\(^{-1}\) (C-O) partially disappeared in the infrared spectrum of the inclusion compound (d), demonstrating the C=O of VE. The group and C-O were encapsulated in the cavity of the crosslinked cyclodextrin molecule. The infrared spectrum of the physical mixture was only a superposition of VE and 1:1 EN-HP-β-CD.

3.4. Differential Scanning Calorimetry (DSC)
DSC was used to evaluate the physical state of guest molecules in CD complex systems [18]. The melting points, boiling or sublimation points of guest molecules might change or disappear when they formed inclusion complex with CD [19, 20].

The DSC curve of the sample was shown in figure 6. The DSC curve of HP-β-CD showed a broad endothermic peak from 60 °C to 120 °C, which was related to the evaporation of water. The DSC curve of EN-HP-β-CD (figure 6b) showed a sharp fusion of the 140 °C endothermic peak corresponding to the melting process. In the DSC curve of the HP-β-CD inclusion complex (figure 6e), the melting peak of EN-HP-β-CD appeared at 120 °C due to the formation of an amorphous product or inclusion complex. The endothermic peak similarly to the resultant complex also occurred in the case of physical mixing because the complex was also formed during the physical mixing by physical grinding.

3.5. X-ray Diffraction (XRD)
The X-ray diffraction patterns of HP-β-CD, EN-HP-β-CD, PM and inclusion complex were shown in figure 7. HP-β-CD was non-crystalline. Both EN-HP-β-CD, PM and inclusion complex had a certain degree of crystallinity. In the complex, since VE was contained in the cyclodextrin and formed a new crystal form, the structural characteristics of the new crystal form appeared in addition to the peaks appearing in the physical mixture compared to the physical mixture. The intensity of the diffraction peak was weakened or moved or disappeared.
3.6. Scanning Electron Microscope (SEM)

The surface morphology of HP-β-CD, 1:1 EN-HP-β-CD, inclusion complex was investigated by scanning electron microscopy (SEM). SEM images of HP-β-CD, 1:1 EN-HP-β-CD and inclusion complex were given in figures 8a-8c. HP-β-CD (a) exhibited its typical structure and was an amorphous spherical particle having a cavity structure. EN-HP-β-CD (b) exhibited a long rod-like structure completely different from the spherical structure of HP-β-CD, probably due to the addition of ethylenediamine to crosslink the HP-β-CD into a chain. In the electron micrograph of inclusion complex (c), the morphology was different with HP-β-CD and EN-HP-β-CD and showed uniform, organized long strips of structured particles having irregular sizes. The large change in particle morphology might be due to the interaction between VE and HP-β-CD. This indicated that after the complex was formed, the formation and crystalline form of the inclusions were retained. Thus, SEM images qualitatively demonstrated new entities that formed inclusion complexes from VE and EN-HP-β-CD.

3.7. In Vitro Release Behavior

In order to investigate the in vitro release behaviour of cyclodextrin complex of VE at different pH conditions, two buffer solutions of pH=4.5 and pH=7.0 were selected for experiments. The cumulative release profile of VE was shown in figures 9 and 10.
From the cumulative release within 24 h, the cumulative release rate of VE without any medium at the two pH levels were very low, which was related to the low solubility of VE in water. HP-β-CD, HP-β-CD-CHO, 1:1 EN-HP-β-CD, 1:2 EN-HP-β-CD, 1:3 EN-HP-β-CD had a burst release within the first 5 h, but during the same time period, the cumulative release of HP-β-CD and HP-β-CD-CHO were significantly lower than that of cross-linked cyclodextrin, which was related to the low encapsulation efficiency of the two kinds of cyclodextrin inclusion complexes. During clathrate release, VE was released slowly for 24 hours. This indicated that the drug was encased in the cyclodextrin cavity instead of just attached to the surface. 1:1 EN-HP-β-CD had the highest cumulative release at 24 h (pH 4.5 up to 78%, pH 7.0 up to 43%), probably because 1:1 EN-HP-β-CD might have the strongest effect on VE, and its entrapment efficiency was the highest. Moreover, when the pH was acidic, the cumulative release of the VE inclusion complex prepared by the ethylenediamine-crosslinked cyclodextrin derivative increased significantly within 24 h, because C=N was unstable in an acidic solution, might occur different degrees of fracture, so that the drug was more released from the cyclodextrin molecule.

4. Conclusion
Cross-linking cyclodextrin derivatives for VE delivery was prepared by using ethylenediamine as a cross-linking agent through three different cross-linking ratios (1:1, 1:2, 1:3) and oxidizing HP-β-CD by sodium periodate. Cyclodextrin derivatives were characterized by DSC, FT-IR, XRD and SEM. The encapsulation efficiency and drug loading, drug solubility, and drug release in vitro under different pH conditions were evaluated. The resulting cyclodextrin derivative significantly increases the water solubility of the drug. The degree of solubility increase is related to the ratio of cyclodextrin to crosslinker and the amount of cyclodextrin derivative added. The solubility of the drug indicates that 1:1 EN-HP-β-CD increases the solubility of VE by up to 25 times. Evaluation of drug release in vitro at different pH showed that the cumulative release rate of 1:1 EN-HP-β-CD was reachable at pH 4.5 (78%) above pH 7.0 (43%) within 24 h, meaning that we prepared the cyclodextrin derivative is pH sensitive. In conclusion, it was found that EN-HP-β-CD was a potential carrier, which could improve the solubility of VE and had pH-sensitive properties.

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