Solubilization of Hydroxypropyl-β-Cyclodextrin on Cholesterol in Aqueous Solution

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Abstract: Hydroxypropyl-β-Cyclodextrin (HP-β-CD), prepared *via* reaction of β-Cyclodextrin (β-CD) and propylene oxide (PO), is utilized to research solubilization of HP-β-CD on cholesterol in aqueous solution. HP-β-CD is characterized by Fourier Transform Infrared Spectrometry (FT-IR), and concentrations of cholesterol solution are measured by ultraviolet and visible (UV VIS) spectrophotometer. The research on optimal synthesis conditions of HP-β-CD indicates that sodium hydroxide amounts have the most effect on yields of product. The maximum solubilization multiples of HP-β-CD reaches 15, below which molecular rate of HP-β-CD and cholesterol in inclusion complex is 1:1.

Keywords: Hydroxypropyl-β-Cyclodextrin, Cholesterol, Solubilization, Optimal condition, Inclusion.

INTRODUCTION

Cholesterol, also known as cholesteryl, widely exists in animals especially in their kidney, spleen, skin, liver, and bile with higher content, as well as in brain and nerve tissue with the highest content. The solubility of cholesterol is similar to fat, freely soluble in ether and chloroform [1-3]. Cholesterol is mainly utilized to produce hormones [4-6] at present, while low solubility in aqueous solution limits its application to some extent [7].

It is reported in literature that water solubility of cholesterol could be greatly improve by inclusion interaction of cyclodextrin (CD) [8-9]. However complete hydrogen bond network of CD makes itself lower solubility that is just 1.85% at temperature of 25 ˚C, which results in low solubility of its inclusion complex as well. Hence, cyclodextrin modification becomes a hotspot in research.

In our study, HP-β-CD is firstly prepared according to literature method [10] and then it is utilized for inclusion of cholesterol. Reaction process is shown in Figure **1**. Ultimately, the solubilization of HP-β-CD on cholesterol in aqueous solution is studied through the discussion of solubilization curve.

1. EXPERIMENTAL

1.1. Materials and Apparatus

β-CD $(C_6H_{10}O_5)_7$ was purchased from Kelong chemical reagent factory (Chengdu, China) and its average molecular weight was 1134.98. β-CD was chemical pure, being recrystallized twice in water. PO was supplied by Chemical reagent Co., Ltd., Shanghai, China with chemical pure and its average molecular weight was 58.08. Cholesterol was obtained from Xinxing reagent institute (Shanghai, China) with analytical reagent grade. Other reagents were all chemical pure.

UV VIS spectrophotometer (UV2102C) was provided by Dragon Nick Instrument CO., Ltd., Shanghai, China. FT-IR spectrometer (170SX) was from USA Nicolet Company.

1.2. Synthesis of HP-β-CD

A certain amount of sodium hydroxide was dissolved in distilled water, and then obtained aqueous solution was added into three-necked flask. Next, 6 g β-CD was added in sodium hydroxide solution with stirring at a speed of 300 rmp. After β-CD completely dissolving, 2.5 ml PO was slowly dropped into three-necked flask by constant pressure titration funnel (6 drops per minute) at room temperature. Later the solution was heated to react and toluene was used to determine whether β-CD was consumed completely each hour. Reaction stopped until no transparent gel precipitation was generated in the tested reaction solution sample. Hydrochloric acid was added into solution for adjusting PH to 7 and then water was removed from reaction mixture by vacuum distillation. Tiny dimethylformamide (DMF) dissolved the residual mixture to further remove insoluble inorganic salt. The obtained solution was washed for several times with acetone and product was precipitated as white solid, which was dried in an oven at 70 ˚C. The solid was further grinded for powdered HP-β-CD.

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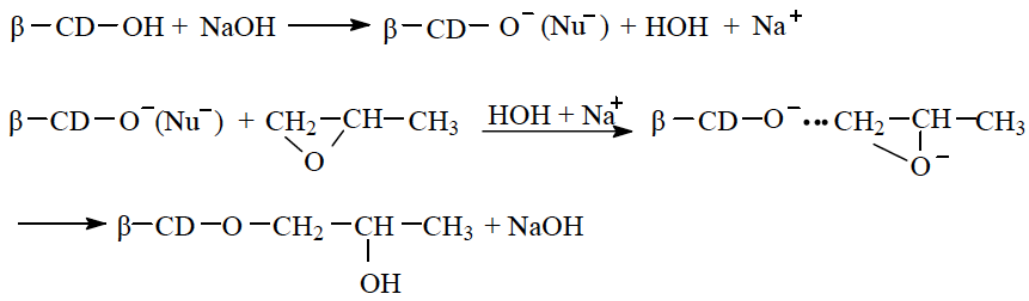

Figure 1: Reaction Process of β-CD and 1,2-propylene Oxide.

1.3. Calibration Curve

A group of cholesterol ethanol solutions with different concentration were added into cuvettes respectively for determining solution absorbance at 206 nm by UV VIS spectrometer, using blank ethanol solution as a reference. Ultimately, a calibration curve was drawn according to absorbance data.

1.4. Measurement of Solubilization Multiple

Using blank aqueous solution as a reference, a series of HP-β-CD aqueous solutions with different concentration were added into centrifuge tubes with plug respectively, and then excess cholesterol were put into each centrifuge tube. Later, the solutions were oscillated at constant temperature of 25 ˚C for 48 h. After equilibrium system was reached, upper supernatant without undissolved cholesterol was removed and diluted to 100ml by 80% ethanol aqueous solution. Finally, absorbance of saturated solutions under varied conditions were obtained *via* UV VIS spectrometer at 206 nm, thus calculating solubilization multiple according to it.

2. RESULTS AND DISCUSSION

2.1. Optimum Reactive Conditions of HP-β-CD

According to relevant literatures, optimization of HP-β-CD reactive conditions is mainly focused on solvent volume, sodium hydroxide amount and reactive temperature. Experiment was carried out in the light of two-level simplex tableau of three factors (U (2) 3). Reaction time was all fixed at 18 h in single factor experiments and results were listed in Table **1**.

From Table **1**, it was analyzed that effect of three factors on yields was in the order of sodium hydroxide amount, solvent volume and reactive temperature. Therefore, the key to improve the yield of HP-β-CD was to control concentration of sodium hydroxide solution, which is consistent with literatures [11].

2.2. Characteristic

HP-β-CD was crushed and pressed into a KBr tablet, which was analysis on FT-IR spectrometer in the range of 4000-400 $cm^{-1}$and spectroscopy was given in Figure **2**.

As shown in Figure **2**, the characteristic absorption peaks of -CH_2 and C=O were observed at 2931 and 1654 cm^{-1} respectively, and three absorption peaks between 1330 ~ 1417 cm^{-1} confirmed the existence of C - O group. Compared with β-CD, three weak peaks at 1387, 1460 and 2870 cm^{-1} characterized $-CH_3$, which indicates the synthesis of hydroxypropyl group. Furthermore, the main absorption peaks were summarized in Table **2** as follows, agreeing well with the literatures [12].

Table 1: Experimental Table for Reactive Conditions

Temperature [˚C]	Sodium hydroxide amounts [g]	Solvent volume [mL]	Yield [%]
57	1.0	10	60.9
67	1.0	10	64.9
62	1.2	10	65.4
62	1.1	15	69.3
70	1.2	13.4	67.7
62	1.34	15.6	67.9
67	1.23	19.3	69.8

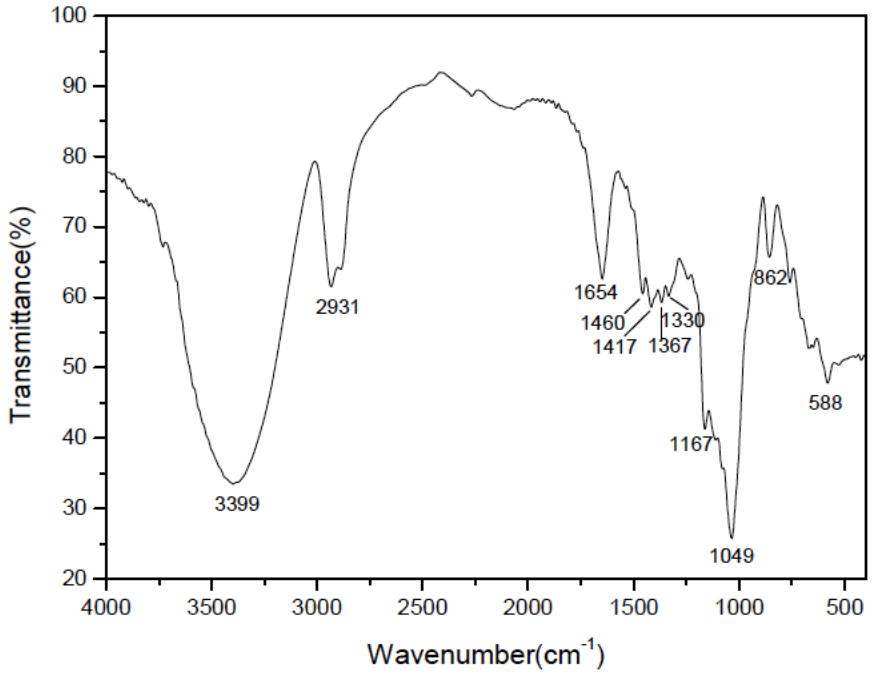

Figure 2: The infrared spectra of HP-β-CD.

Table 2: The Infrared Spectrum Characteristics of HP-β-CD

Frequency [cm-1]	Functional group	Assignment	Intensity
3399.503、3399859	—OH	stretching vibration	S
2928.574	—CH_2—	stretching vibration	m
2870	—CH_3	stretching vibration	m
1661.367	C=O	stretching vibration	S
1387.863	—CH_3	flexural vibration	m
1254.105	C—O—C	stretching vibration	w
1156.734	C—O—C	stretching vibration	S
1084.468	—CH—OH	stretching vibration	S
1033.468	CH_2—OH	stretching vibration	S
759.300		ring vibration	
708.820	—OH	ring vibration	
660.382		flexural vibration	

2.3. Calibration Curve

A working curve of absorbance (A) and concentration of cholesterol solution (C) was plotted in Figure **3**, and an equation was obtained *via* regression of experimental data as follows

$$C = 1.8681 \times -10^{-4} A + 9.2282 \times 10^{-6} (R = 0.9990) \quad (1)$$

2.4. Solubilization Multiple of Cholesterol

It is reported that solubilization of CD on guests was achieved by forming inclusion complex. According to kinetic equations of CD inclusion [13], the relationship between soubilization multiple (S_t/S_n) and initial concentration of HP-β-CD (C_{CD}) is summarized in Eq. 2 if the inclusion radio is 1:1

$$S_t/S_n = K_1 C_{CD} + b \quad (2)$$

where S_t is the total concentration of guests in equilibrium solution after adding HP-β-CD, representing the sum of guest-free and guest-in-inclusion concentration; S_n is the solubility of guest in aqueous solution; S_t/S_n is soubilization multiple of HP-

β-CD on cholesterol; K_1 is the slope of line, characterizing the stability of inclusion complex.

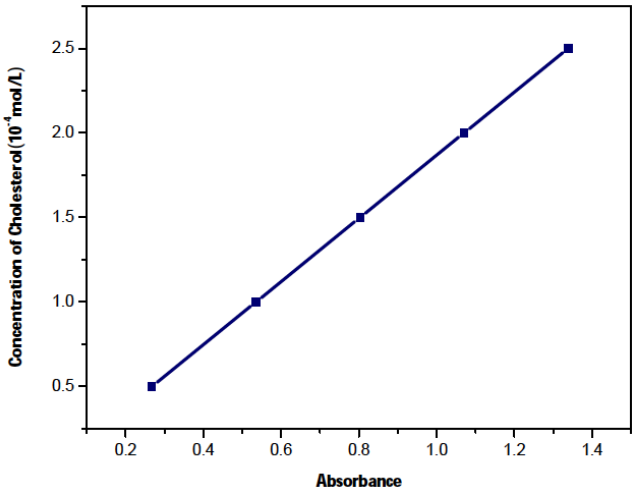

Figure 3: Calibration Curve of Cholesterol.

If the inclusion radio of HP-β-CD and guest is 2:1, the relationship of S_t/S_n and C_{CD} is summarized in Eq. 3

$$S_t/S_n = K_2C_{CD}^2 + b \quad (3)$$

where K_2 presents the stability of 2:1 inclusion complex.

The solubilization curve of HP-β-CD on cholesterol was plotted in Figure **4** [14-17]. As indicated in Figure **4**, maximum of S_t/S_n is 14.8, that is solubility of cholesterol in aqueous solution could reach 0.2g/L when concentration of HP-β-CD was 6×10^{-4} mol/L. The result shows a good solubilization property of HP-β-CD on cholesterol. In addition, before S_t/S_n reaches maximum, concentration of HP-β-CD solution is in good line relationship with S_t/S_n, which is consistent with Eq. 1, thus indicating that HP-β-CD and cholesterol formed an inclusion complex as radio of 1:1 in this condition. However, when the concentration of HP-β-CD is over 6×10^{-4} mol/L, S_t/S_n presents an obvious downtrend. It can be explained that when the inclusion complex aqueous solution reaches saturation, ion effect is enhanced as HP-β-CD continues to be added into the solution, which inhibits dissolving capacity of inclusion complex.

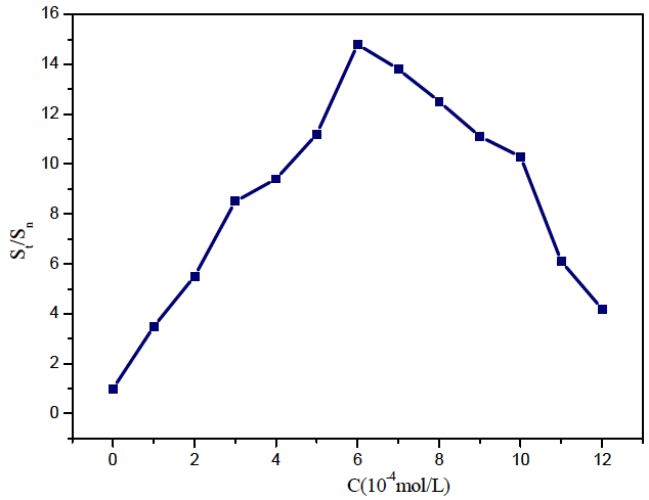

Figure 4: Solubilization Curve of HP-β-CD on Cholesterol.

3. CONCLUSION

A new type of water-soluble polymer HP-β-CD was synthesized and the optimum conditions were investigated by means of the single variable method, indicating that sodium hydroxide amount plays a important role in the modification of β-CD. Meanwhile, FT-IR confirmed the synthesis of HP-β-CD. Furthermore, solubilization multiple could reached 14.8 and the results reflect a good solubilization of HP-β-CD on cholesterol, while the solubility of cholesterol in polymer solution would decrease if the concentration of HP-β-CD is over a critical vaule.

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