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Ternary system of dihydroartemisinin with hydroxypropyl-β-cyclodextrin and lecithin: Simultaneous enhancement of drug solubility and stability in aqueous solutions

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ABSTRACT

The purpose of this study was to simultaneously improve the solubility and stability of dihydroartemisinin (DHA) in aqueous solutions by a ternary cyclodextrin system comprised of DHA, hydroxypropyl-β-cyclodextrin (HP-β-CD) and a third auxiliary substance. Solubility and phase solubility studies were carried out to evaluate the solubilizing efficiency of HP-β-CD in association with various auxiliary substances. Then, the solid binary (DHA–HP-β-CD or DHA–lecithin) and ternary systems were prepared and characterized by Fourier transform infrared (FT-IR), differential scanning calorimetry (DSC) and power X-ray diffraction (PXRD). The effect of the ternary system on the solubility, dissolution and stability of DHA in aqueous solutions was also investigated. As a result, the soybean lecithin was found to be the most promising third component in terms of solubility enhancement. For the solid characterization, the disappearance of the drug crystallinity indicated the formation of new solid phases, implicating the formation of the ternary system. The dissolution rate of the solid ternary system was much faster than that of the drug alone and binary systems. Importantly, compared with binary systems, the ternary system showed a significant improvement in the stability of DHA in Hank's balanced salt solutions (pH 7.4). The solubility and stability of DHA in aqueous solutions were simultaneously enhanced by the ternary system, which might be attributed to the possible formation of a ternary complex. For the ternary interactions, results of molecular docking studies further indicated that the lecithin covered the top of the wide rim of HP-β-CD and surrounded around the peroxide bridging of DHA, providing the possibility for the ternary complex formation. In summary, the ternary system prepared in our study, with simultaneous enhancement of DHA solubility and stability in aqueous solutions, might have an important pharmaceutical potential in the development of a better oral formulation of DHA.

1. Introduction

The combination of dihydroartemisinin (DHA)-piperaquine is recommended to cure plasmodium infections by World Health Organization (WHO) in 2010. However, an increased treatment failure with DHA has been reported in the western Cambodia from 8.1% to 27.6% in 2010 [1]. The major reason is the Plasmodium resistance to antimalarial medicines, which makes the transmission of malaria gain momentum in turn and brings new challenges to the malaria prevention. The long term use of drugs with relative low solubility, stability and bioavailability is possibly a negatively important factor to malaria drug resistance. The use of effective drugs with relative high solubility, stability and bioavailability will be of great benefit to reduce the incidence of malaria drug resistance.

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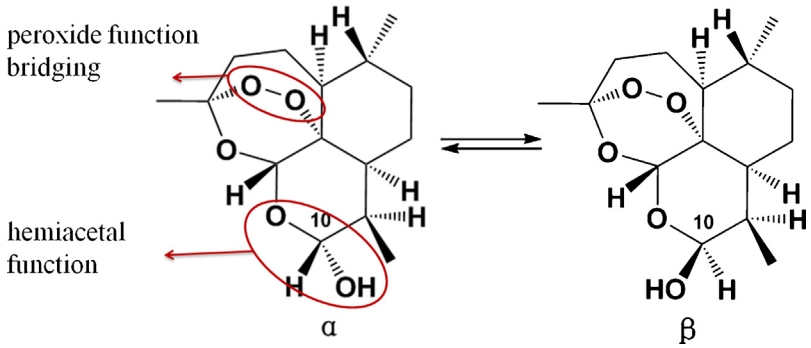

Fig. 1. Structure of dihydroartemisinin.

Among artemisinin derivatives, DHA provides improved antimalarial potency in vitro and in vivo compared to other artemisinin analogues. However, the aqueous solubility of DHA is very low due to the glucopyranose rings in its chemical structure [2,3]. It contains peroxide bridging over the seven-membered ring, which is known to be rather unstable. The hemiacetal functional group, which is more susceptible to acidic situations and moisture, is another contributor to its low stability [4,5]. DHA is also physically unstable due to the conversion of the lactone carbonyl group at C-10 (Fig. 1).

It is reported that artemisinin products in the market have hardly met the criteria of WHO, especially in Africa. The active pharmaceutical ingredient content is below the criterion of 90% at the end of shelf life [2]. In addition, the huge demand of artemisinins has created a global shortage of artemisinins. Therefore, it is urgently needed to develop DHA products with high solubility, stability and bioavailability. Several studies have been carried out to enhance the solubility and stability of DHA by preparing solid dispersions [6], nanosuspensions [7], micronization with supercritical solutions [8], and cyclodextrin complexes [9,10]. Among them, cyclodextrin inclusion is one of the most efficient ways to improve the solubility and stability of DHA. It has been reported that the aqueous solubility of DHA increased 77 folds after complexation with HP-β-CD. The hydrolysis rate constant of DHA in solid complexes of HP-β-CD (275.1 mM) decreased 29 folds compared with DHA alone at 50 °C after 3 months [5], and the degradation rate constant of DHA in aqueous solutions decreased approximately 10 folds in the presence of HP-β-CD (57 mM) at 60 °C [11].

However, due to the relatively high molecular weight of HP-β-CD, a large amount of cyclodextrins used in the formulation limits its application into a convenient and cost-effective dosage form. Moreover, high dose of cyclodextrins may lead to potential toxicity and other related side effects, which also impedes its application [12]. It is of importance to improve the complexation of DHA and cyclodextrins. Recently, it is reported that ternary system with drug, cyclodextrin and the third auxiliary substance can reduce the dose of cyclodextrins and increase the complexation efficiency (Table 1) [13–28].

To our literature research, there is no report available about the ternary system of DHA with HP-β-CD and auxiliary substances up to now. In this study, the simultaneous enhancement of solubility and stability of DHA through ternary system was investigated. The auxiliary substance added in the ternary system was screened through solubility studies. Then, the binary and the ternary systems for DHA constituted with HP-β-CD and the optimal auxiliary substance were prepared by the solvent evaporation method. Finally, the properties of DHA in the binary system, the ternary system and their physical mixtures were characterized by FT-IR, DSC and PXRD techniques in solid state, whilst their solubility, stability and dissolution behaviours in aqueous state were evaluated. Finally, combined with the solid state characterization, the possible molecular formation mechanism of the ternary complex was investigated by the molecular docking study.

2. Experimental methods

2.1. Materials

Dihydroartemisinin (DHA, 99% purity) was obtained from Chongqing Huali Wulingshan Medicine Co., Ltd. (Chongqing, China). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution = 4.5) was obtained from China Shijiazhuang Pharmaceutical Group Co., Ltd. (Shijiazhuang, Hebei, China). Soybean lecithin (phosphatidyl choline, 94% purity) was supplied by Lipoid GmbH (Ludwigshafen, Germany). Polyethylene glycol 4000 (PEG4000), polyethylene glycol 6000 (PEG6000), polyvinyl pyrrolidone K30 (PVP) and lactose were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Polyethylenglycol 660-12-hydroxystearate (Solutol HS15) was procured from BASF (Germany). Lauroyl polyoxylglycerides (Gelucire 44/14) was purchased from Gattefosse (France). Hydroxypropyl methylcellulose (HPMC K4M) was obtained from Dow Chemical Company (USA). Acetonitrile and methanol were high performance liquid chromatography grade from Merck Co., Ltd. (Germany). Hank's balanced salts were purchased from Sigma-Aldrich Co. LLC. (USA). Water was prepared by ultra pure water system (Milli-Q). Other reagents were of analytical grade.

2.2. Methods

2.2.1. HPLC analysis

Quantitative analysis of DHA was performed using HPLC method listed in Chinese Pharmacopoeia (2010 edition). The HPLC instruments consisted of a binary pump, G1311C; autosampler, G1329B; and a diode-array detector G4212B (Agilent, Palo Alto, USA). The separation was achieved on a Diamonsil C18 analytical column (150 × 4.6 mm ID, 5 μm, Dikma, Beijing, China) equipped with an Easyguard ii C18 guard column (Dikma, Beijing, China) maintained at 20 °C. The mobile phase consisted of acetonitrile: ultra pure water (65:35, v/v) and the flow rate was set to 1 mL min^{-1}. The detection wavelength of the detector was set to 210 nm.

2.2.2. Stability studies

The stability tests of pure DHA in water or Hank's balanced salt solutions were carried out at 37 °C indicating the dissolution test temperature. The Hank's balanced salt solution was prepared with 8.00 g L^{-1} NaCl, 0.40 g L^{-1} KCl, 0.35 g L^{-1} $NaHCO_3$, 0.06 g L^{-1} KH_2PO_4, 0.05 g L^{-1} Na_2HPO_4, 0.19 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.09 g L^{-1} $MgSO_4$, and 1.00 g L^{-1} glucose and the pH values were adjusted to 6.8, 7.0, 7.2 and 7.4 with $NaHCO_3$ (3.8 mM, pH11.2) solution.

Table 1
Lists and characteristics of ternary systems of pharmaceutical interest containing cyclodextrin and an auxiliary substance.

Drug	Cyclodextrins	Auxiliary substances	Ternary system composition	Solubility increasing folds[a]	Dissolution time[b] (min)
Melcoxicam (MC)	*β*-CD	HPMC	MC + 6% (w/v) *β*-CD + 0.12% (w/w) HPMC	1000	70
DRF-4367	HP-*β*-CD	Meglumine	DRF-4367: HP-*β*-CD: Meglumine 1:2:1 (m/m/m)	900	30
Flubiprofen	HP-*β*-CD	Triethanolamine (TEA)	Flubiprofen + 0.08 M HP-*β*-CD + 0.013 M TEA	540	25
Clarithromycin (CLM)	*β*-CD	Citric acid (CA)	CLM-*β*-CD + 0.5% (w/v) CA	104	/
Piroxicam (PX)	DM-*β*-CD	HPMC	PX: DM-*β*-CD (1:1 m/m) + 0.1% (w/v) HPMC	20	15
Naproxen (NAP)	HP-*β*-CD	Arginine (ARG)	NAP: HP-*β*-CD: ARG 1:1:1 (m/m/m)	13	15
Famotidine	SBE-*β*-CD	PVP	Famotidine: SBE-*β*-CD (1:1 m/m) + 1% (w/v) PVP	7	5
Lansoprazole (LSP)	HP-*β*-CD	PVP	LSP + 9% (w/v) HP-*β*-CD + 6% (w/v) PVP	8	/
Gemfibrozil (GFZ)	*β*-CD	Triethanolamine (TEA)	GFZ: *β*-CD (1:1.7 m/m) + 6.25% (w/v) TEA	36	100
Dehydroepiandrosterone (DHEA)	*α*-CD	Glycine	DHEA: *α*-CD: Glycine 1:2:3 (m/m/m)	/	60
Vinpocetine (Vin)	*β*-CD	Tartaric acid (Tar)	Vin: *β*-CD: Tar 1:2:2 (m/m/m)	910	5
Irbesartan (IRB)	*β*-CD	PVP	IRB: *β*-CD (1:1 m/m) + 1% (w/v) PVP	5	5
Ketoprofen	Me-*β*-CD	Phospholipid	Ketoprofen: Me-*β*-CD: Phospholipid 20:4:76 (w/w/w)	/	/
Dexamethasone	*γ*-CD	HDMBr	Dexamethasone-*γ*-CD + 0.25% (w/v) HDMBr	58	/
Daidzein	HP-*β*-CD	PVP	Daidzein-HP-*β*-CD + 1% (w/v) PVP	13	/
Famotidine	*β*-CD	HPMC	Famotidine: *β*-CD (1:1 m/m) + 0.75% (w/v) HPMC	6	15

[a] The folds of drug solubility improved by adding auxiliary substances in phase solubility studies.
[b] The time for the percentages of drug dissolution >90%.

For the stability tests in water and in Hank's balanced salt solutions (pH6.8, 7.0, 7.2, 7.4), DHA solution with the concentration of 100 μg mL^{-1} was firstly put into 10 mL test tubes with plugs. Then, aliquots of 0.5 mL were taken out at intervals of 1 h at 37 °C in 6 h. The concentration of DHA was measured by HPLC. Each test was performed in triplicate.

Since the degradation of DHA followed first-order kinetics in the presence of HP-*β*-CD and lecithin, the apparent degradation rate constants (k) used to evaluate the stability of DHA were calculated from the slope of the degradation diagrams according to the following Eq. (1) [29].

$$\ln[C] = \ln[C_0] - kt \quad (C \neq C_0) \tag{1}$$

where $[C_0]$ was the initial concentration of DHA, $[C]$ was the concentration of DHA at time t, and k was the slope of the fitted linear regression for the first order reaction.

2.2.3. Solubility and phase solubility studies

Solubility studies were carried out to screen the auxiliary substances. An excess amount of DHA was added to 0.5% (3.6 mM, w/v) HP-*β*-CD solutions containing concentrations of auxiliary substances (0–0.2%, w/v). The mixtures were shaken in a thermostatic oscillator (THZ-D, Suzhou Pei Ying Experimental Equipment Co., Ltd., Jiangsu, China) at 25 °C for 72 h to achieve the equilibrium. Appropriate aliquots of the mixture were then withdrawn and filtered with a polyvinylidene fluoride syringe-filter (pore size 0.22 μm), and the concentrations of DHA in the filtrates were analyzed by HPLC.

The phase solubility studies were carried out according to the Higuchi-Connors procedure [30]. An excess amount of DHA was added to 5 mL aqueous solutions containing various concentrations of HP-*β*-CD (0–18 mM) and lecithin (0%, 0.5% and 1.0%, w/v). The mixtures were shaken in the thermostatic oscillator at 25 °C for 72 h to achieve the equilibrium. Appropriate aliquots of the mixture were then withdrawn and filtered with a polyvinylidene fluoride syringe-filter (pore size 0.22 μm), and the total concentrations of DHA in the filtrates were analyzed by HPLC.

Phase solubility diagrams were obtained by plotting the molar concentration of DHA versus the molar concentration of HP-*β*-CD. For the 1:1 drug/HP-*β*-CD complex, the complexation efficiency and the apparent stability constant could be estimated from the slope of the phase solubility diagram (Eqs. (2) and (3)) [31].

$$CE = \frac{slope}{1 - slope} \tag{2}$$

$$K_{1:1} = \frac{slope}{[S_0(1 - slope)]} \tag{3}$$

where CE was the complexation efficiency, *slope* was the slope of the phase solubility diagram, $K_{1:1}$ was the apparent stability constant and S_0 was the equilibrium solubility of DHA in water in the absence of HP-*β*-CD and the lecithin.

2.2.4. Preparations of the binary system and the ternary system

The binary system and the ternary system were prepared by the solvent evaporation method as follows. For the preparation of DHA–HP-*β*-CD binary system, DHA (molecular weight = 284 g moL^{-1}) and HP-*β*-CD (molecular weight = 1396 g moL^{-1}) were dissolved in anhydrous methanol with a mass ratio of 1:5 (w/w) and stirred at 800 rpm for 1 h to achieve equilibrium. The solution was evaporated using rotary evaporator (IKA, Germany) under reduced pressure to remove the organic solvent at 30 °C. The DHA–HP-*β*-CD binary residues were dried in vacuum at 30 °C over-night. The preparation of DHA–lecithin binary system (1:5, w/w) was the same to DHA–HP-*β*-CD binary system.

For the ternary system preparation, after the equilibrium reached, a weighted amount of lecithin equal to the amount of HP-β-CD was added in the mixture and agitated until the suspension was transparent. The mass ratio of DHA: HP-β-CD: lecithin was 1:5:5 (w/w/w). The solution was evaporated under reduced pressure to remove the organic solvent at 30 °C. The ternary residues were dried in vacuum at 30 °C over-night.

Appropriate amounts of the dried residues obtained from above preparations of the binary system and the ternary system were added in water and the suspensions were filtered through a 0.22 μm polyvinylidene fluoride membrane to remove the insoluble DHA. The filtrates were lyophilized to obtain the finished products of the binary system and the ternary system. The drug loading (DL) percentages of the binary system and the ternary system were determined and calculated according to Eq. (4).

$$\mathrm{DL} = \left(\frac{W_{\mathrm{DHA}}}{W_0}\right) \times 100\% \quad (4)$$

where W_{DHA} is the weight of DHA in the lyophilized powders of the binary system and the ternary system and W_0 is the weight of the lyophilized powders of the binary system and the ternary system. Both the lyophilized binary system and the lyophilized ternary system were further used to perform the solid properties characterizations. The physical mixtures in equal proportions to the compositions of the lyophilized binary system and the lyophilized ternary system were prepared as reference for the characterization.

2.2.5. Characterization of the binary system and the ternary system in solid state

The properties of DHA, HP-β-CD, lecithin, DHA–HP-β-CD physical mixture, DHA–HP-β-CD binary system, DHA–lecithin physical mixture, DHA–lecithin binary system, ternary physical mixture and DHA–HP-β-CD–lecithin ternary system in solid state were characterized by FT-IR, DSC and PXRD.

2.2.5.1. Fourier transform infrared spectroscopy. The FT-IR spectroscopy was acquired with a Nicolette 6700 FT-IR apparatus. The samples were mixed with KBr and a tablet was prepared and the FT-IR spectroscopy was detected at 400–4000 cm^{-1}. The instrument was operated under dry air purge, and the scans were collected at the scanning speed of 2 mm s^{-1} with the resolution of 4 cm^{-1}. The number of scans was adjusted automatically as a function of sample concentration in the disc.

2.2.5.2. Differential scanning calorimetry. The DSC thermograms were recorded using a differential scanning calorimeter (DSC 822, Mettler Toledo, Switzerland). About 2.0 mg of each sample was heated in a pierced aluminium pan from 50 to 250 °C at a heating rate of 10 °C min^{-1}. Thermal data analysis of the DSC thermograms was conducted using the Mettler Toledo STAR system software.

2.2.5.3. Powder X-ray diffraction. Diffraction patterns were detected with a Bruker D8 Advance diffractometer at ambient temperature. The samples were irradiated with monochromatized Cu Kα radiation and analyzed at a 2 theta angle range 3–40°. The pattern was collected with a tube voltage of 40 kV and a tube current of 40 mA in step scan mode (8° min^{-1}).

2.2.6. Evaluation of the ternary system in aqueous state

2.2.6.1. In vitro dissolution studies. Dissolution studies were performed in triplicate according to the paddle method in Chinese Pharmacopoeia (2010 edition) at 37 °C. Samples containing 10 mg of DHA or its equivalent as a binary system or a ternary system were added to 250 mL of water and stirred at 100 rpm. At time points of 5, 10, 15, 30, 60, 90, 120, 180 and 360 min, 2 mL of samples were withdrawn and replaced with an equal volume of fresh medium. The samples were filtered through 0.22 μm polyvinylidene fluoride membrane and analyzed by HPLC method at 210 nm.

2.2.6.2. Stability analysis. For stability studies, the solutions of the binary system or the ternary system containing 100 μg mL^{-1} of DHA were prepared with Hank's balanced salt solution (pH 7.4). The triplicate samples were incubated at 37 °C in water bath for 4 h. The stabilities were tested by the same method defined in Section 2.2.2.

3. Results and discussion

3.1. Method validation for the quantification of DHA

Two isomers of DHA, namely, α- and β-epimers, were separated by HPLC determination. Because the two isomers of DHA were apt to transform into each other, the reference materials of the two epimers were not available. Thus, the peak area of DHA was estimated by the sum of α-epimer and β-epimer.

3.1.1. Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing auxiliary substances and DHA with that obtained from DHA. In the presence of HP-β-CD and auxiliary substances, it was found that there was no significant change in peak shape and retention time of DHA.

3.1.2. Linearity

The limit of detection (LOD) and the limit of quantitation (LOQ) of DHA were 0.5 μg mL^{-1} (S/N > 3) and 1.0 μg mL^{-1} (S/N > 10). The calibration curve was linear over the concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 μg mL^{-1} (R^2 = 0.9999).

3.1.3. Recovery

The recovery of the method was evaluated at the DHA concentrations of 100, 250 and 500 μg mL^{-1} in the presence of HP-β-CD (0.5%, w/v) and the other auxiliary substances (0.2%, w/v). The results showed that the recoveries of samples at three concentrations of DHA for all of the auxiliary substances were 98.2–101.9% (8 groups, for each group n = 9). The results indicated that there were no significant influence of ternary compounds used (HP-β-CD, polymers and lecithin) on the quantification of DHA and the method was accurate and precise.

3.2. Solubility and phase solubility studies

DHA was slightly soluble in water (134.8 μg mL^{-1}, 25 °C). In the presence of 0.5% (w/v) HP-β-CD, the solubility of DHA increased to 302.7 μg mL^{-1}. Except soybean lecithin showed certain improvement of the solubility of DHA in the presence of HP-β-CD, whilst the addition of the other selected third auxiliary substances did not obviously improve the solubility of DHA compared with the HP-β-CD solution (0.5%, w/v) (Table 2).

As shown in Table 2, the solutions containing 0.5% (w/v) HP-β-CD and soybean lecithin showed some enhancement of the solubility of DHA. The system containing 0.2% (w/v) lecithin and 0.5% (w/v) HP-β-CD exhibited the highest solubility of DHA (413.4 μg mL^{-1}). However, for solutions with other auxiliary substances, the solubility of DHA increased slightly, even if increasing the concentrations of the auxiliary substances. For solution containing HPMC, the solubility of DHA decreased about 15% compared to the DHA–HP-β-CD binary system. Furthermore, the auxiliary substances like PEG4000, PEG6000, PVP K30, lactose and Solutol HS15 had slight effects on the solubility improvement of DHA in the DHA–HP-β-CD binary system. The favourable effect of lecithin

Table 2
Solubility of DHA[a] ($\mu g\,mL^{-1}$) in the presence of 0.5% HP-β-CD and the third auxiliary substances (mean ± SD).

The third auxiliary substance	Concentration of auxiliary substance (w/v)		
	0.05%	0.10%	0.20%
Soybean lecithin	319.0 ± 5.8	348.4 ± 9.8	413.4 ± 16.0
PEG4000	304.7 ± 1.7	301.3 ± 3.2	305.2 ± 8.7
PEG6000	317.7 ± 4.3	323.4 ± 9.1	321.2 ± 6.1
PVP K30	314.1 ± 5.2	323.0 ± 4.3	308.0 ± 10.2
Lactose	300.9 ± 1.0	307.8 ± 2.9	313.4 ± 5.0
Solutol HS15	303.5 ± 4.1	305.5 ± 4.3	303.5 ± 0.4
Gelucire 44/14	291.5 ± 5.6	265.1 ± 3.6	258.9 ± 7.3
HPMC	259.5 ± 8.5	266.0 ± 11.3	248.7 ± 9.9

[a] The solubility of DHA in water was 134.8 ± 0.3 $\mu g\,mL^{-1}$, and the solubility of DHA in the presence of 0.5% (w/v) HP-β-CD was 302.7 ± 5.1 $\mu g\,mL^{-1}$.

on the solubility improvement of DHA might be ascribed to its amphiphilic property.

The phase solubility study was also performed at 25 °C in aqueous solutions with different concentrations of HP-β-CD in the presence of lecithin (0%, 0.5% and 1.0% (w/v)). The phase solubility diagram displayed an A_L type, showing a linear increase of drug solubility. The slopes in all cases were less than unity, indicating the formation of 1:1 binary and ternary system. It was reported that the hydrophilic polymers could improve the complexation efficiency (CE) and the stability constants ($K_{1:1}$) of cyclodextrin inclusions for drugs [31]. In our study, slope values for DHA–HP-β-CD binary system and DHA–HP-β-CD–lecithin ternary systems were 0.161, 0.160 (with the lecithin concentration of 0.5%, w/v) and 0.148 (with the lecithin concentration of 1.0%, w/v). The corresponding CE values were 0.192, 0.190 and 0.174. The CE value might indicate the similar solubilizing efficiency of DHA–HP-β-CD binary system and DHA–HP-β-CD–lecithin ternary system. The S_0 (Eq. (3)) in the presence of 0.5% (w/v) lecithin and 1.0% (w/v) lecithin were 252.3 ± 2.6 $\mu g\,mL^{-1}$ and 538.9 ± 22.5 $\mu g\,mL^{-1}$. According to Eq. (3), the corresponding $K_{1:1}$ values were 415, 214 and 91 M^{-1}. The $K_{1:1}$ value decreased from 415 M^{-1} for the DHA–HP-β-CD binary system to 91 M^{-1} for the DHA–HP-β-CD–lecithin ternary system (1.0%, w/v, lecithin), with an improvement of S_0 in the present of lecithin. A sharp reduction of the $K_{1:1}$ value suggested that the addition of lecithin might decrease the interaction between HP-β-CD and DHA. The reduced affinity for the HP-β-CD cavity might due to the interaction between DHA and lecithin.

3.3. Characterization of the binary systems and the ternary system

For the preparation of binary and ternary systems, lecithin can hardly be dissolved in water. Low molecular weight alcohols as methanol and ethanol were added as the solvent. Methanol was chosen in consideration of its high drug loading. The drug loading of DHA–HP-β-CD binary system prepared with methanol and ethanol were 5.35% and 4.51%, respectively. This may be attributed to the stronger polarity of methanol and the tendency of DHA to be encapsulated by the hydrophobic cavity of HP-β-CD in the strong polarity of solvents [31]. The residual methanol in the final products was determined by gas chromatography (Agilent6890, Palo Alto, USA). The residual methanol in the final products was 2.15 ppm, which was much less than the limitation specified as 3000 ppm in the European Pharmacopoeia (7th edition), indicating that the ternary system was safe for the case of methanol impurity.

The drug loading of DHA–HP-β-CD binary system, DHA–lecithin binary system and the ternary system were 5.35% (DHA: HP-β-CD, 1:17, w/w), 5.28% (DHA: lecithin, 1:18, w/w) and 5.02% (DHA: HP-β-CD–lecithin, 1:19, w/w) respectively.

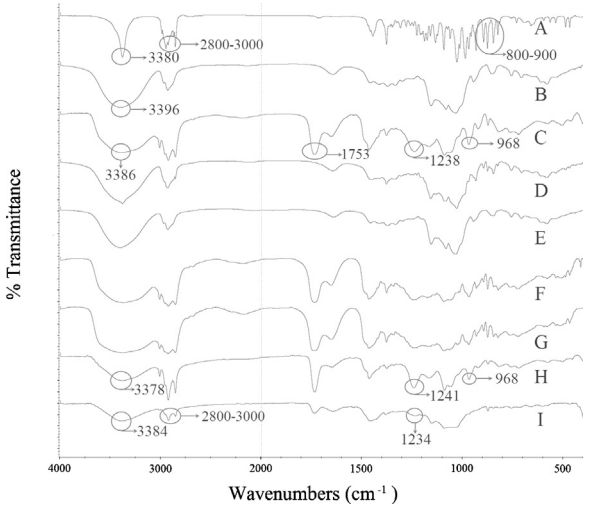

Fig. 2. FTIR spectra of pure DHA (A), HP-β-CD (B), lecithin (C), DHA–HP-β-CD physical mixture (D), DHA–HP-β-CD binary system (E), DHA–lecithin physical mixture (F), DHA–lecithin binary system (G), DHA–HP-β-CD–lecithin physical mixture (H) and DHA–HP-β-CD–lecithin ternary system (I).

3.3.1. Fourier transform infrared spectroscopy

FT-IR spectra of pure DHA, HP-β-CD, lecithin, and the two binary systems, the ternary system were shown in Fig. 2. For the FT-IR spectrum of pure DHA (Fig. 2A), sharp peaks at 3380 cm^{-1}, 2800–3000 cm^{-1}, 800–900 cm^{-1} could be considered as the main characteristic peaks for the stretching vibrations of —OH groups, C—H bond and C—O bond (in the seven-membered ring) in DHA [32,33]. Compared with DHA (Fig. 2A), the changes to these three sharp characteristic peaks were observed in DHA–HP-β-CD binary system (Fig. 2E), DHA–lecithin binary system (Fig. 2G) and the DHA–HP-β-CD–lecithin ternary system (Fig. 2I). It indicated that the host-guest interactions may exist in these systems.

For the ternary system (Fig. 2I) and its corresponding physical mixture (Fig. 2H), the former showed band shifting at 3384 cm^{-1} while the latter exhibited peaks at 3378 cm^{-1}. Furthermore, the ternary system showed smaller peaks of the C—H stretching in the range 2800–3000 cm^{-1} than that for DHA and the binary systems. Additionally, the intensity of the C=O peak for lecithin at 1753 cm^{-1} (Fig. 2C) greatly decreased in the ternary system (Fig. 2I) [34]. In the fingerprint region, the peaks of lecithin (Fig. 2C) at 1238 and 968 cm^{-1} could be assigned to the absorbance of P=O and $^{+}N(CH_3)_3$ stretching vibrations respectively [35]. These peaks were also found in the ternary physical mixtures. While for the ternary system, the P=O peak shifted from 1238 to 1234 cm^{-1} and the peak at 968 cm^{-1} disappeared. It might indicate that host–guest interactions existed and new solid phases formed in the ternary system compared with its corresponding physical mixture.

3.3.2. Differential scanning calorimetry

The DSC thermogram of DHA showed typical characteristics of a crystalline substance, exhibiting two exothermic peaks at 155.03 and 170.08 °C which might be assigned as the melt of decomposition of peroxide, while HP-β-CD and lecithin did not show any endothermic or exothermic peaks (Fig. 3).

The physical mixtures of the binary systems and the ternary system also showed exothermic peaks (DHA–HP-β-CD binary physical mixture at 167.55 °C; DHA–lecithin binary physical mixture at 153.12 °C; ternary physical mixture at 157.25 °C). However, the exothermic peaks of DHA obviously disappeared in the binary system and the ternary system, which might be ascribed to the new amorphous solid formed or the molecular inclusion formed in the binary system and the ternary system.

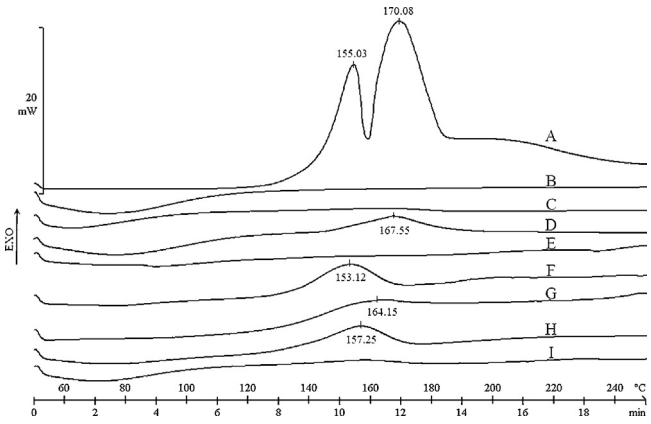

Fig. 3. DSC thermograms of pure DHA (A), HP-β-CD (B), lecithin (C), DHA–HP-β-CD physical mixture (D), DHA–HP-β-CD binary system (E), DHA–lecithin physical mixture (F), DHA–lecithin binary system (G), DHA–HP-β-CD–lecithin physical mixture (H) and DHA–HP-β-CD–lecithin ternary system (I).

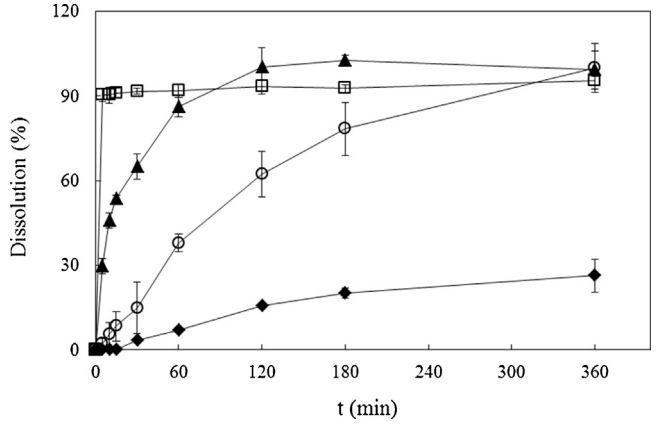

Fig. 5. Dissolution curves of pure DHA (♦), DHA–HP-β-CD binary system (□), DHA–lecithin binary system (○) and DHA–HP-β-CD–lecithin ternary system (▲) at 37 °C. Error bars are s.d. (n = 3).

3.3.3. Powder X-ray diffraction

The PXRD patterns of DHA, lecithin, DHA–HP-β-CD–lecithin physical mixture and the ternary system showed obvious difference. The peaks in the diffraction pattern of DHA powder were sharp and high with the main diffraction angles (2θ) at 7.7°, 9.3° and 11.2°, suggesting the significant crystallinity of DHA. The lecithin showed a flat pattern as typical diffraction pattern of amorphous substance. For the ternary physical mixture, lower intensities of the crystalline diffraction peaks of DHA could be observed at 7.9°, 9.5° and 11.3°, in which the typical peaks from DHA were presented and no new peaks were observed, suggesting the absence of interaction among drug, HP-β-CD and lecithin in their physical mixture. For the ternary system, there was neither typical DHA diffraction peak at 5–15° nor any new peaks, indicating the complete disappearance of DHA crystallinity and new solid phase formed in the ternary system [32]. The result was consistent with the results obtained from FT-IR and DSC studies (Fig. 4).

3.4. Property of the binary system and the ternary system in aqueous state

3.4.1. In vitro dissolution

Compared to the DHA powders, the dissolution profiles of the binary system and the ternary system showed great improvements of the cumulative release amounts and the release onsets were in the order of DHA–HP-β-CD binary system > DHA–HP-β-CD–lecithin ternary system > DHA–lecithin binary system > DHA. The dissolution rates of DHA in the HP-β-CD based binary and ternary system were obviously faster than that in the DHA–lecithin binary system and DHA. The dissolution percentages of DHA in the HP-β-CD based binary system and ternary system were larger than 90% at 120 min. In addition, the dissolution percentages of DHA in its pure form and in the DHA–lecithin binary system were only 15.5 ± 0.9% and 62.2 ± 8.1% at 120 min (Fig. 5).

The good dissolution performance for the DHA–HP-β-CD binary system and the ternary system might be attributed to the concurrence of several factors, e.g., the reduction of the DHA crystallinity in these two systems, which were confirmed by DSC and PXRD studies, and possibly increased particle wettability in the presence of hydrophilic HP-β-CD and lecithin.

3.4.2. Stability studies

3.4.2.1. Stability of DHA in aqueous solution. The concentration of DHA in water was nearly unchanged within 6 h and 96% left at 37 °C. Although DHA was stable in water in our experiment condition, it was unstable in Hank's balanced salt solutions (pH7.4). The degradation of DHA in Hank's balanced salt solutions (pH7.4) followed first order reaction described by Eq. (1) (R^2 > 0.98). The degradation rate constant values were calculated by linear regression of ln[C] and t. The changes of DHA concentration percentage as a function of time were shown in Fig. 6.

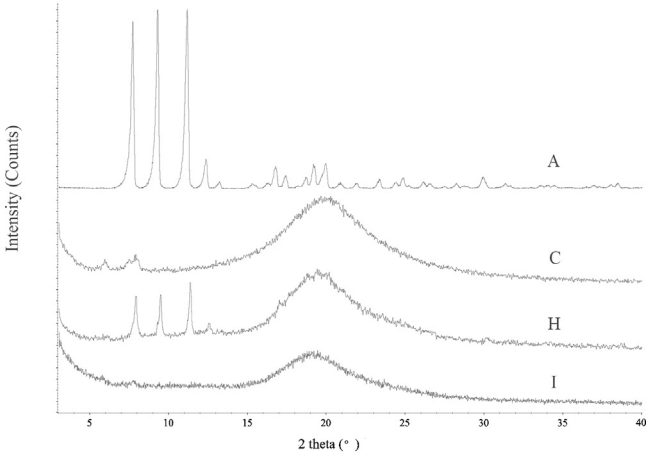

Fig. 4. X-ray powder diffraction patterns of pure DHA (A), lecithin (C), DHA–HP-β-CD–lecithin physical mixture (H) and DHA–HP-β-CD–lecithin ternary system (I).

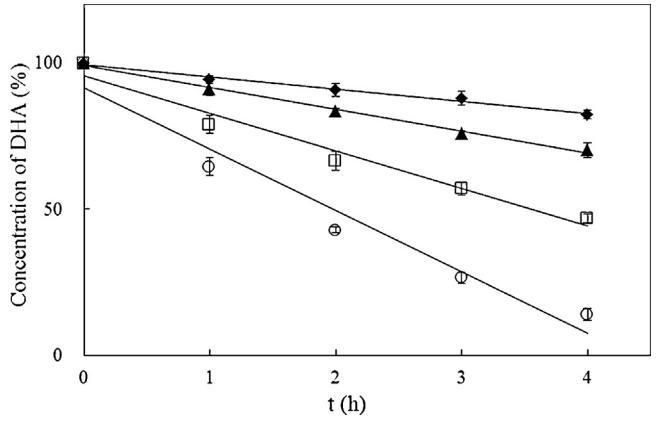

Fig. 6. The changes of DHA concentration percentage as a function of time in Hank's balanced salt solution (pH6.8 (♦), pH7.0 (▲), pH7.2 (□), pH7.4 (○)) at 37 °C. Error bars are s.d. (n = 3).

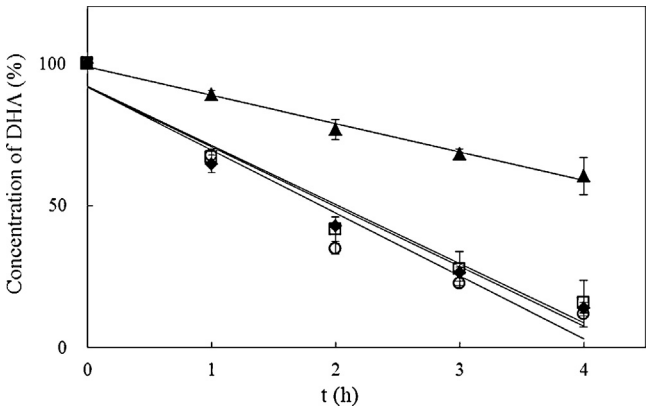

Fig. 7. The changes of DHA concentration percentage as a function of time in Hank's balanced salt solution (pH7.4) at 37 °C for DHA (♦), DHA–HP-β-CD binary system (□), DHA–lecithin binary system (○) and DHA–HP-β-CD–lecithin ternary system (▲). Error bars are s.d. ($n = 3$).

The degradation rate constants of DHA at 37 °C in pH6.8, 7.0, 7.2 and 7.4 Hank's balanced salt solutions were 0.04 ± 0.006, 0.09 ± 0.008, 0.18 ± 0.001 and $0.48 \pm 0.035\ h^{-1}$ respectively. With the pH increasing, values of k tended to rise. At pH 7.4, there were only 14% percentages of DHA left in the Hank's balanced salt solution at 4 h.

3.4.2.2. Stability of binary and ternary system in aqueous solutions. As shown in Section 3.4.2.1. Stability of DHA in aqueous solution", the stability of DHA in pH7.4 Hank's balanced salt solution was very poor and only 14% of DHA was left at 4 h. Therefore, the pH7.4 Hank's balanced salt solution was chosen as the solvent for the stability analysis of DHA in the binary system and the ternary system. The changes of DHA concentration percentage as a function of time and the degradation rate constant values were shown in Figs. 7 and 8.

As shown in Fig. 8, the solution of the ternary system was the most stable sample, with the lowest k value of $0.13\ h^{-1}$. The rank order of the k values was DHA–lecithin binary system > DHA > DHA–HP-β-CD binary system > ternary system. Apparently, the stability of DHA in Hank's balanced salt solution with pH 7.4 was significantly improved by the ternary system, with the k value decreased from 0.48 to $0.13\ h^{-1}$. While the k value of the DHA–HP-β-CD binary system decreased from 0.48 to $0.38\ h^{-1}$.

Zhang et al. [11] had investigated the effect of HP-β-CD on the stability of DHA in water at 60 °C. The value of k for DHA decreased by 75% (from $0.001842\ min^{-1}$ to $0.000461\ min^{-1}$) with the presence of HP-β-CD (57 mM). Compared with their study, the concentration of HP-β-CD in our ternary system was about 0.7 mM and the k value decreased by 73%. Thus, the addition of lecithin had markedly enhanced the stability of DHA and reduced the dose of HP-β-CD significantly.

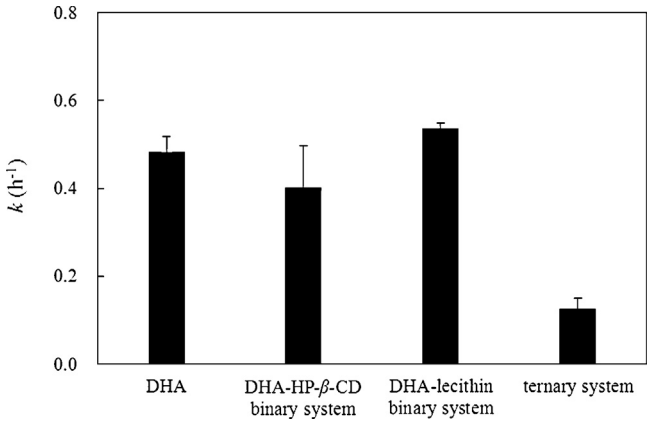

Fig. 8. The apparent degradation rate constants of DHA in Hank's balanced salt solution (pH7.4) at 37 °C for binary and ternary system. Error bars are s.d. ($n = 3$).

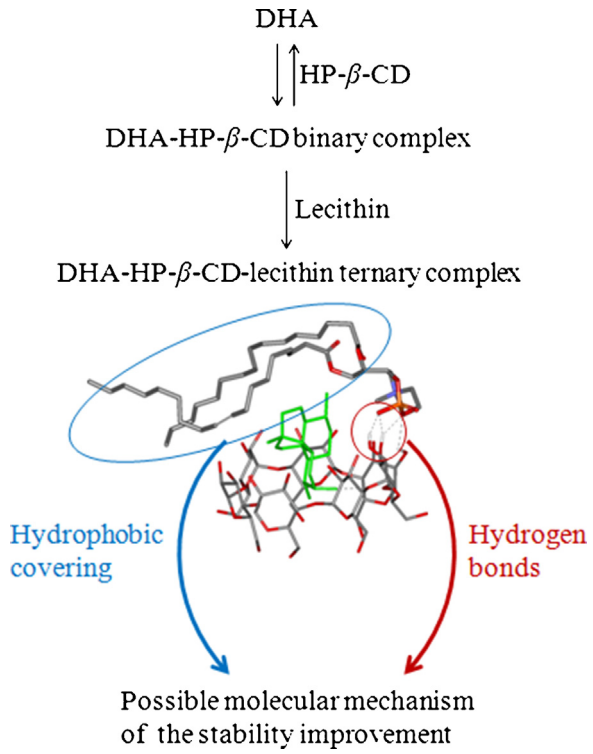

Fig. 9. The possible molecular mechanism of interactions among DHA, HP-β-CD and lecithin.

In summary, with the consideration of simultaneous enhancement of DHA solubility and stability in aqueous solutions, DHA–HP-β-CD–lecithin ternary system had significant advantages over DHA–HP-β-CD and DHA–lecithin binary systems. Such an effect, which was greater than the simple sum of the effects due to the individual components, might be ascribed to the possible formation of a ternary complex. According to the amphiphilic surfactant properties of lecithin, it might be assumed that the lecithin possibly interacted with DHA via the hydrophobic interactions and with HP-β-CD via hydrogen bonding. Simultaneously, DHA was encapsulated by the hydrophobic cavity of HP-β-CD.

The possibility of the above assumption was further investigated by molecular docking studies (Fig. 9). Based on the calculation of molecular charges with Gaussian software (version 9), the molecular docking was carried out with AutoDock Vina [36]. The simulated annealing and optimization of the obtained complex were performed with the force field of CHARMm by Discovery Studio (Accelrys Inc.). As shown in Fig. 9, DHA was included into the hydrophobic cavity of HP-β-CD via hydrogen bonding, formed by the —OH group of DHA and the —O— of HP-β-CD. There was also a hydrogen bond between the —OH group outside the cavity of HP-β-CD and the $-PO_2^-$ of the lecithin. The hydrophobic tails of lecithin covered the top of the wide rim of HP-β-CD, and surrounded around the peroxide bridging of DHA.

Therefore, interactions among DHA, HP-β-CD and lecithin existed in the ternary system: (1) hydrophobic interactions between DHA and HP-β-CD; (2) hydrophobic interactions between DHA and lecithin; (3) hydrogen bonding of lecithin with HP-β-CD or its complex. The molecular evidence for the ternary complexation

assumption might also implicate the mechanism for the simultaneous improvement of DHA solubility and stability in aqueous solutions by the ternary system.

4. Conclusions

In this study, the solubility and stability of DHA in aqueous solutions were enhanced by ternary system of DHA with HP-β-CD and soybean lecithin. The solid state characterizations illustrated the formation of a new solid phase, indicating the formation of the ternary system. In aqueous state, the solubility, dissolution and the stability of DHA in ternary system were better than that in the pure form and binary systems, which might be ascribed to the ternary complexation. The ternary complexation assumption was further implicated with the aid of molecular docking studies. In summary, the ternary system prepared in our study, with simultaneous enhancement of DHA solubility and stability in aqueous solutions, might have an important pharmaceutical potential in the development of a better oral formulation of DHA.

Acknowledgments

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