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A donepezil/cyclodextrin complexation orodispersible film: Effect of cyclodextrin on taste-masking based on dynamic process and *in vivo* drug absorption



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ABSTRACT

The aim of this study was to develop a palatable donepezil (DP) orodispersible film (ODF) to facilitate the swallowing process and investigate the effect of cyclodextrin on taste-masking based on dynamic process and *in vivo* drug absorption. Complexation of DP with hydroxypropyl- β -cyclodextrin (HP- β -CD) was applied to mask the bitter taste then the prepared complexes were incorporated into ODF using solvent casting method. The taste-masking efficiency was evaluated by e-tongue; meanwhile the pharmacokinetic behavior of DP/HP- β -CD ODF was investigated by *in vivo* study. Results showed the optimized film was more palatable than donepezil hydrochloride (DH) film and was bioequivalent with DH. The molecular mechanism was revealed by phase solubility study, Fourier-transform infrared spectrometer (FT-IR), Differential scanning calorimeter (DSC), X-ray diffraction (XRD) and molecular modeling. Taste-masking was attributed to the formation of DP/HP- β -CD which was due to moderate interaction between DP and HP- β -CD. The stability of DP/HP- β -CD was decreased because of the acid environment in stomach, which facilitated the absorption of DP. These results extended our understanding about the application of cyclodextrin complexation and provided guidance for the design of ODF especially for drugs with disgusting taste.

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1. Introduction

Recently, orodispersible film (ODF) gets growing attention because of its unique superiorities for the target groups including children and the geriatric population who have difficulty in swallowing [1–2]. ODF allows drug to disperse rapidly in oral cavity [3], thus provides many advantages such as rapid disintegration, improved dosing accuracy and stability compared to ordinary oral dosage forms [4–5]. Donepezil (DP) is a commonly used drug for the treatment of Alzheimer's disease. Commercial oral products of DP (tablets or capsules) may result in swallowing difficulties, which causes distress to the major group of Alzheimer's patients, the elders. Therefore, in order to improve patient compliance, it is meaningful to develop donepezil oral orodispersible film as an alternative to commercial DP products. Moreover, the recommended dosages of donepezil are 5 mg and 10 mg per day [6], which is appropriate to develop DP ODF [7].

DP has unacceptable taste such as bitterness, leading to poor compliance during the development process of DP ODF due to its rapid dissolution in oral cavity. Adding flavors into the formulation is the simplest and most commonly used taste-masking method [8]. However, the addition of flavor is not effective enough to mask the taste of drugs like DP with extremely disgusting taste [9]. Cyclodextrin and its derivatives are widely used for preparing inclusion complexes due to its non-toxicity, high biodegradability and various other advantages [10]. It was reported that when drugs formed stable inclusion complexes with cyclodextrin, the disgusting taste of drugs could be reduced, even fully masked [11]. Therefore, cyclodextrin complexation was a useful method for taste-masking in the development of ODF. Additionally, HP- β -CD showed high solubility (60% or more, w/w) in water [12], which was suitable to be applied to develop ODF.

When drug/cyclodextrin inclusion complexes are used to mask taste in a formulation, the effect of cyclodextrin becomes complicated due to multiple factors influencing drug absorption. Except for the drug itself, one of the factors is the physicochemical properties of cyclodextrins. If cyclodextrin has a low solubility, it possibly results in a slow dissolution of drug *in vivo*. Another factor, which is considered as the most important one, is the *in vivo* dynamic process of drug/cyclodextrin inclusion complexes. It is significantly influenced by drug/cyclodextrin interaction and the environment *in vivo*. The existence of moderate interaction will achieve a successful taste-masking, but if the interaction force between drug and cyclodextrin is too strong, it will lead to the decrease of free drug concentration in the intestine, causing low absorption [13–14]. Additionally, drug molecules travel through various physiological environment from mouth to gastrointestinal tract. Changing of the location may affect the form of drug or the stability of complexes, which affects drug absorption.

In a previous research focusing on the effect of cyclodextrin concentration on the oral bioavailability, variations on pharmacokinetic parameters and profiles were observed after drug/cyclodextrin solution with different cyclodextrin concentration was dosed in rats [15]. Another research found the significant influence of HP- β -CD and sucrose on the absorp-

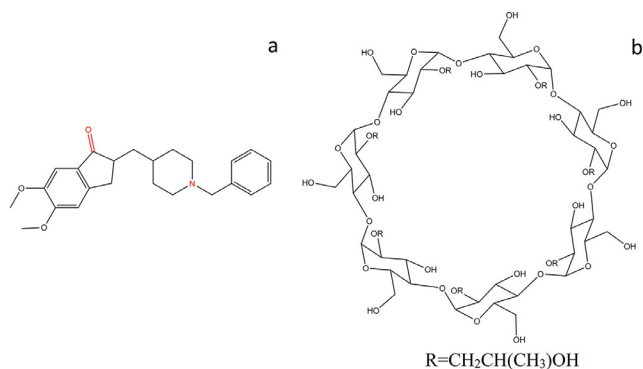


Fig. 1 – The structures of DP (a) and HP- β -CD (b).

tion of midazolam in rabbits [16]. These studies showed the important role of cyclodextrin in the enhancement of solubility or taste-masking and suggested the complicated effect of cyclodextrin on drug absorption. Nevertheless, the effect of drug/cyclodextrin dynamic process *in vivo* was rarely mentioned or studied. Therefore, the knowledge of how cyclodextrin affected drug absorption while taste-masking and how the dynamic process carried out was still limited.

In this study, DP/HP- β -CD inclusion complexes were prepared to mask the disgusting taste of DP, and subsequently the complexes were incorporated into ODF by solvent casting method. The structures of DP and HP- β -CD were shown in Fig. 1. The taste-masking efficiency was evaluated by e-tongue, and the pharmacokinetic behavior of the film was investigated by *in vivo* study. More importantly, the effect of cyclodextrin was explored via series of technologies based on dynamic process and *in vivo* drug absorption.

2. Materials and methods

2.1. Chemicals and animals

Donepezil hydrochloride (DH) was obtained from Jinan Dexinbia Biological Products Co., Ltd. (Jinan, China). HP- β -CD was purchased from Xian Deli Biological Chemical Co., Ltd. (Xi'an, China). Hypromellose (HPMC, Methocel E5 premium LV) was a gift from Colorcon (Shanghai, China). Polyethylene glycol 400 and glycerol were provided by Bodi Chemical Co., Ltd. (Tianjin, China). All other chemicals or solvent were of the highest reagent grade available.

Wistar rats (male, 200 ± 20 g) were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The experiments were performed in accordance with the Institutional Animal Care and Use Committee with the approval of No. SYPU-IACUC-C2016-1121-201.

2.2. Quantitative analysis of DP

DP in various samples was determined using a HPLC system (Hitachi High-Technologies Corporation, Tokyo, Japan) consisting of a L-2130 pump, a L-2420 ultraviolet absorbance detector and a L-2200 automatic injector. An ODS-C₁₈ column (200 mm \times 4.6 mm, 5 μ m, Dikma Technologies Inc., USA) was

used with the temperature set at 40 °C. For the *in vitro* samples, the mobile phase was 0.1% glacial acetic acid and 0.2% triethylamine in water–methanol (40:60, v/v). For the *in vivo* study, the mobile phase was 60% methanol and 40% glacial acetic acid solution (0.5%) adjusted to pH 6.5 with triethylamine. The quantitative analysis was performed under the condition of the flow rate at 1.0 ml/min and the wavelength at 271 nm. The injection volume was 20 µl. All of the analytical methods have been validated.

2.3. Development of DP/HP-β-CD ODF

2.3.1. Preparation of DP/HP-β-CD inclusion complexes

DP free base was prepared using liquid-liquid extraction method [17]. After that, 6.90 g of HP-β-CD and 1 g of DP were completely dissolved in alcohol and the solution was stirred for 12 h at room temperature. After the evaporation of alcohol, the product was collected for further use.

2.3.2. Formulation design of DP/HP-β-CD ODF

One-factor optimization method was used to obtain the final optimized formulation. Film forming materials including hydroxypropyl methyl cellulose (HPMC), pregelatinized starch (PS), polyethylene oxide (PEO) and low-substituted hydroxypropyl cellulose (L-HPC) were optimized according to the standards of film-forming capacity and appearance of films. Plasticizers such as polyethylene glycol (PEG), glycerin and propylene glycol, were optimized with the standards of dissolution within 3 min and folding endurance value, which was determined by repeatedly folding the film at the same point until broken [18]. Moreover, the drug content was controlled by adjusting the drug loading.

2.3.3. Preparation of DP/HP-β-CD ODF

Solvent casting method was used to prepare DP/HP-β-CD ODF. Certain amount of polymer, plasticizer and DP/HP-β-CD inclusion complexes were dissolved in distilled water and stirred for 1 h at room temperature to obtain the casting solution. After degassing, the casting solution was casted onto a plastic plate using a laboratory coating unit (SLT200; Kaikai Company, Ltd., Shanghai, China) with casting height of 1 mm, and then the formed film was dried at 54 °C for 1 h to obtain the DP/HP-β-CD ODF. Then the film was cut into 2 × 3 cm².

As control samples, DH ODF, the film containing DH and sucralose, blank film (pure polymer) were prepared using the same method.

2.4. Critical quality attributes of DP/HP-β-CD ODF

Some critical quality attributes were controlled in the development of ODF, including thickness, weight, drug content and mechanical properties using micrometer (Mitutoyo, Japan) (*n* = 6), electronic analytical balance CP225D (Sartorius Instruments system, Ltd., Beijing, China) (*n* = 20), HPLC (*n* = 20) and auto stripping tester (Labthink instruments Co., Ltd., China) (*n* = 6) [19], respectively.

2.5. *In vitro* dissolution test

In vitro dissolution test was performed to investigate the dissolution behavior of film in different conditions. According to

the United States Pharmacopeia (USP), the paddle method was applied, and distilled water as well as 0.1 mol/l HCl were used as dissolution medium. DP/HP-β-CD ODF (2 × 3 cm², *n* = 6) was placed at the bottom of the dissolution cup (250 ml), then distilled water of 100 ml at 37 ± 1 °C was poured into the cup and stirred at a rotation speed of 50 rpm. At preselected time intervals of 1, 3, 5, 10, 15, 20, 30 and 60 min, dissolution medium of 5 ml was withdrawn and replaced by equal volume of fresh distilled water immediately. After centrifuging at 16 000 rpm, the withdrawn samples were analyzed by the HPLC. For the dissolution medium of 0.1 mol/l HCl, all the operations were the same as that of distilled water.

2.6. E-tongue assessment

E-tongue assessment was conducted to evaluate the taste of samples using a Taste Sensing System SA402B (Intelligent Sensor Technology, Ltd., Japan). Four kinds of medicinal sensors, BT0 (for specific bitterness of hydrochloride drugs), AN0 (for alkaline bitterness), CO0 (for acidic bitterness) and AE1 (for astringency) were used separately. Before the assessment, the samples were dissolved, and the concentration of DP was at 0.05 mg/ml. Potassium chloride solution of 10 mM was used as the reference and rinse solution.

According to relative sensor outputs, the difference between the test sample and reference solution in potential ($V_s - V_r$) and CPA values which was defined as the difference ($V_r' - V_r$) between the potentials of the reference solution before and after sample assessment (change of membrane potential caused by adsorption of samples) were obtained [20–21]. Principal component analysis (PCA) was used to analyze these data [22–23]. Meanwhile, Euclidean distance from samples to DH was calculated according to the following Eq. 1:

$$d(p, q) = \sqrt{\sum_{i=1}^n (p_i - q_i)^2} \quad (1)$$

Where *p* and *q* were the $V_s - V_r$ value of samples and DH, respectively, *i* represented different sensors.

2.7. *In vivo* pharmacokinetic study

2.7.1. Administration and sampling

Eighteen Wistar rats were divided into three groups randomly, No.1, No.2 and No.3 (6 rats for each group), which were fastened and only allowed free access to water for 12 h prior to the experiment.

DH aqueous solution was administered to the rats of group No.1 intravenously (3 mg/kg). The rats of the group No.2 was given DH aqueous solution via intragastrical administration route (10 mg/kg), and for the group No.3, DP/HP-β-CD ODFs strips were set on the tongue of the rat (10 mg/kg). After administration, blood samples of 0.2 ml were taken from orbit at 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 600 and 720 min for group No. 1 and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h for group No.2 and No.3.

2.7.2. Plasma sample and pharmacokinetic analysis

Plasma was separated immediately by centrifuging at 16,000 rpm and stored at –60 °C before analysis. The Win-

Nonlin (Version 5.2.1; Pharsight Co., Mountain View, USA) was used to calculate pharmacokinetic parameters based on the non-compartmental model.

2.8. Statistical analysis

Results were expressed as the mean \pm standard deviation. Statistical analysis of data was carried out based on two-way analysis of variance (ANOVA) and 90% confidence interval analysis. The level of significance was taken as $p < 0.05$. For parameters of animal study, AUC and C_{\max} were logarithmic, and then simply used to evaluate the bioequivalence of these two preparations.

2.9. Exploration of drug/cyclodextrin interaction

2.9.1. Phase solubility study

In order to investigate the effect of HP- β -CD on the solubility of DP and explore the stability of inclusion complexes in different conditions, phase solubility study was conducted according to the method established by Higuchi and Connors [24]. Aqueous solution and 0.1 mol/l HCl solution of HP- β -CD were used as medium, respectively. Excess amounts of DP were added into 10 ml of aqueous HP- β -CD solution with different concentrations (from 0 to 100 mM). The obtained suspensions were shaken at 37 °C for 48 h. The samples were collected and filtered through a Millipore filtration of 0.22 μ m to remove the undissolved DP. The amount of DP in filtrate was then determined by HPLC and all experiments were carried out in triplicate. The solubility diagram was plotted with the molar concentration of DP as a function of the molar concentration of HP- β -CD. The value of binding constant (K) was calculated through the Eq. 2.

$$K = \left(\frac{\text{slope}}{S_0} \right) \times (1 - \text{slope}) \quad (2)$$

Where S_0 was the solubility of DP in water. For the medium of 0.1 mol/l HCl solution of HP- β -CD, all the operations were the same as aqueous HP- β -CD solution.

2.9.2. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR study was conducted to explore the interaction between DP and HP- β -CD. The FT-IR spectra of DP, HP- β -CD, physical mixtures and DP/HP- β -CD inclusion complexes were obtained in the region of 400–4000 cm^{-1} by Bruker Vertex 70 spectrometer (Billerica, USA). All the powders were measured by the KBr disk technique.

2.9.3. Thermal analysis (DSC)

Thermal analysis was conducted using a DSC-1 system (Mettler Toledo International Inc., Switzerland). The samples of DP, HP- β -CD, physical mixtures and DP/HP- β -CD inclusion complexes were accurately weighed (about 5 mg) and placed in an aluminum pan with the cover sealed. The heat procedure was at a scanning rate of 10 °C/min from 25 to 150 °C under a nitrogen purge.

2.9.4. X-ray diffractometry (XRD)

XRD study was conducted to investigate the crystal form of substance. XRD patterns of DP, HP- β -CD, physical mixtures and DP/HP- β -CD inclusion complexes were obtained at room temperature using a DX2700 X-ray diffractometer (Aolong Radiative Instrument Group Co. Ltd, Dandong, China) under a voltage of 40 kV and a current of 40 mA. All the samples were analyzed in an angle range of 5–50° (2θ) with a scanning step width of 2° per minute.

2.9.5. Molecular modeling

Molecular modeling was conducted to investigate interaction between HP- β -CD and DP. AutoDock 4.2 software was used to calculate the molecular model. The initial structures of DP and HP- β -CD were obtained using ChemBioDraw Ultra 14.0, and then these structures were optimized using Discovery Studio 4.0 software. Additionally, the Lamarckian Genetic Algorithm (LGA) in AutoDock 4.0 was used to perform the docking study in a vacuum environment. Furthermore, in order to probe the DP/HP- β -CD dynamic process *in vivo*, the DP/HCl interaction was explored using same operations as DP/HP- β -CD.

3. Results and discussion

3.1. Formulation design of DP/HP- β -CD ODF

Results of the optimization process were listed in Tables 1 and 2. HPMC had proper film forming capacity and the film prepared by HPMC had a good appearance; meanwhile, the film containing glycerin of 40% (accounting for the proportion of polymer, 16.7% of the formulation) was excellent in both mechanical properties and dissolution behavior. Therefore, HPMC was selected as the optimized polymer, and glycerin of 40% was chosen as the optimized plasticizer. Additionally, DP content was controlled as 5 mg/2 \times 3 cm² by adjusting DP/HP- β -CD inclusion complexes loading as 41.4% of the formulation. The optimized formulation of DP/HP- β -CD ODF contained DP/HP- β -CD inclusion complexes (41.4%, DP accounted for 4.7% of the formulation), HPMC (41.9%), and glycerol (16.7%).

3.2. Critical quality attributes of DP/HP- β -CD ODF

The critical quality attributes of the optimized film were listed in Table 3. The DP content and the weight of film were 4.96 \pm 0.21 mg, 129.02 \pm 6.29 mg (RSD < 5%) ($n = 20$), respectively, which would be used for further studies.

3.3. In vitro dissolution test

An orodispersible film should dissolve quickly to ensure the rapid dissolution of drug. In this study, two different dissolution media were selected to investigate the effect of HCl on dissolution behavior. Meanwhile, 0.1 mol/l HCl solution was used to simulate the gastric environment. The dissolution behaviors of DP/HP- β -CD ODF were shown in Fig. 2. DP dissolved rapidly from the film in both distilled water and 0.1 mol/l HCl solution. The dissolution rate in HCl solution was significantly

Table 1 – The result of polymer optimization (n = 3).

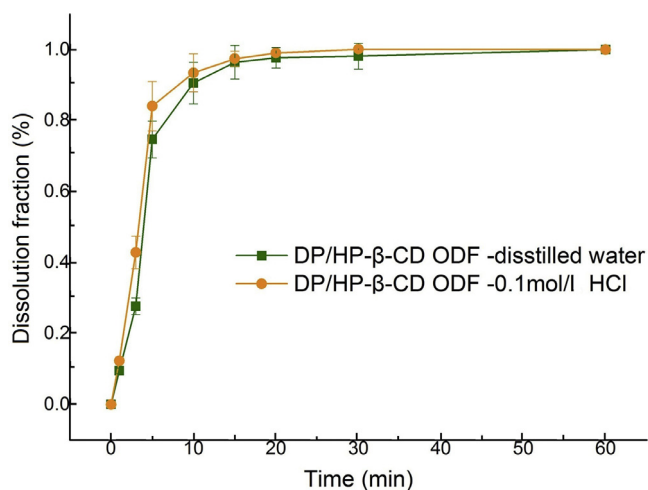
Formulation no.	Polymers	Viscosity of coating solution (mPa-s)	Coating process	Film-forming capacity	Appearance of film
1	PS	Pasty	Difficult	Bad	—
2	L-HPC	13	Difficult	Bad	White opacity
3	HPMC	762	Easy	Good	Colorless transparent
4	PEO	258	Medium	Medium	White translucent

Table 2 – The result of plasticizer optimization (n = 3).

Formulation no.	Plasticizer (w/w: plasticizer/polymer)	Polymer	DP content (mg/2 × 3 cm ²)	Folding endurance value	Dissolution within 3 min
1	20%PG	HPMC	5	1	—
2	30%PG	HPMC	5	3	41.5%
3	40%PG	HPMC	5	>20	33.4%
4	20%GI	HPMC	5	1	—
5	30%GI	HPMC	5	8	38.3%
6	40%GI	HPMC	5	>20	50.8%
7	20%PEG 400	HPMC	5	1	—
8	30%PEG 400	HPMC	5	1	—
9	40%PEG 400	HPMC	5	>20	30.4%

Table 3 – Critical quality attributes of the optimized DP/HP-β-CD ODF.

Thickness (μm)	Weight (mg)	DP content (mg)	Tensile strength (N·mm ²)	Elongation (%)
100.80 ± 1.68	129.02 ± 6.29	4.96 ± 0.21	45.72 ± 1.98	10.31 ± 1.02

**Fig. 2 – The dissolution profiles of DP/HP-β-CD ODF in distilled water and 0.1 mol/l HCl solution.**

faster than that in distilled water at 3 min, which was attributed to the effect of HCl on DP/HP-β-CD complexes. Complete dissolution was achieved in 15 min irrespective of the pH value of dissolution medium, which suggested that DP/HP-β-CD ODF had a favorable dissolution profile [19].

3.4. E-tongue assessment

Taste-masking was the key point to the design of DP ODF. Electronic tongue was sensor array based robotic system [21],

which was often applied to assess the efficiency of taste-masking effects [25]. Based on the potentiometry technique, Taste Sensing System SA402B equipping different sensors was used to evaluate taste-masking efficiency, and each lipid membrane sensor was associated with a specific taste [25]. Liew et al. [26] screened some flavors and DH ODF containing 7 mg of sucralose was reported to be superior in terms of taste, aftertaste, mouthfeel and acceptance. In order to investigate the taste-masking effect of HP-β-CD, the film containing DH and sucralose was prepared according to the previous study.

The responses and CPA values of samples including DH, DP/HP-β-CD inclusion complexes, the physical mixtures, DP/HP-β-CD ODF, the film containing DH and sucralose, DH ODF and the blank film were obtained. The PCA map displaying 4 sensors outputs was shown in Fig. 3. According to the scale of every sensor in the extracted principal component, the PC1 (69.88%) tended to express the bitterness (AN0: 0.976, C00: 0.959), while the PC2 (29.13%) mainly showed the astringency of the substances (AE1: 0.997). It was found that samples with similar taste gathered together and formed a cluster. The samples including DH, the physical mixtures, DH ODF and the film containing DH and sucralose gathered into a cluster, which suggested that the taste was unpleasant, or taste-masking effect was weak. DP/HP-β-CD inclusion complexes and DP/HP-β-CD ODF clustered together, which indicated that cyclodextrin complexation was significantly effective for taste-masking and the DP/HP-β-CD inclusion complexes were stable in the film. Another separate cluster formed by the blank film showed slightly astringent taste, which was ascribed to the taste of the film-forming material (HPMC) itself. In other samples with drug, this astringent taste

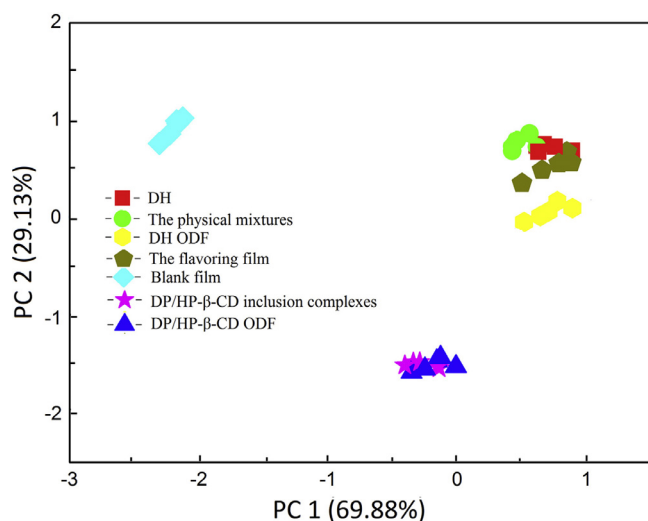


Fig. 3 – PCA map displaying 4 sensors outputs in a two-dimensional graph.

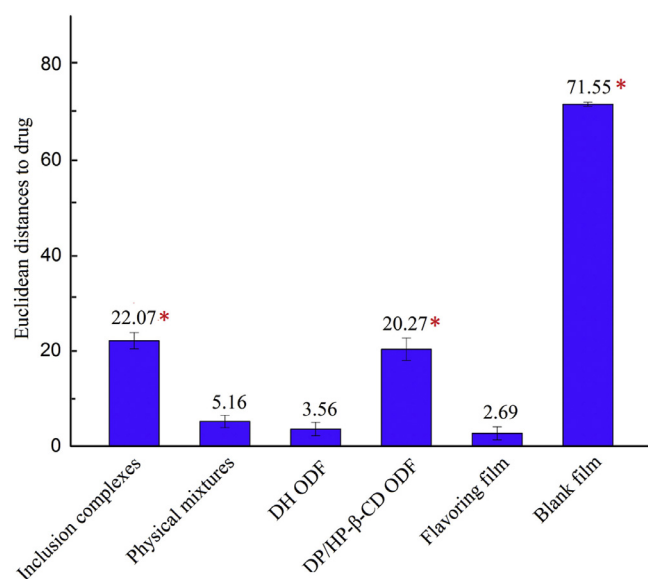


Fig. 4 – Euclidean distances from samples to the drug substance (DH) (b). * indicated a significant difference in statistics.

was possibly diluted by the addition of drug or flavor, so they exhibited a different degree of astringency.

Euclidean distances from samples to DH were shown in Fig. 4. Usually, larger distance indicated better taste-masking [21]. Euclidean distance of inclusion complexes and DP/HP- β -CD ODF were significantly larger than that of other samples. Therefore, it was further proved that cyclodextrin complexation was more effective than common flavors for taste-masking in designing orodispersible film of DP.

3.5. In vivo pharmacokinetic study

The plasma concentration-time profiles were shown in Fig. 5, and pharmacokinetic parameters were listed in Table 4.

Table 4 – Pharmacokinetic parameters of DH after injection of DH (3 mg/kg), following oral gavage of DH (10 mg/kg) and oral cavity administration of DP/HP- β -CD ODF (10 mg/kg) ($n = 6$, mean \pm SD).

Parameter	i.v.	ODF	Oral gavage
C_{max} (ng/ml)	738 \pm 264	385 \pm 110	439 \pm 108
T_{max} (h)	0.08 \pm 0.00	0.83 \pm 0.61	0.58 \pm 0.20
AUC (h·ng/ml)	959 \pm 185	1836 \pm 498	1669 \pm 249
MRT (h)	3.39 \pm 0.35	6.74 \pm 0.89	7.38 \pm 1.39

Usually, orodispersible film exhibited a fast disintegration in oral cavity, then it was swallowed and absorbed in gastrointestinal tract [1]. In this study, it was found that DP was detectable immediately after the oral administration of DP/HP- β -CD ODF (5 min), which demonstrated that DP was dissolved from DP/HP- β -CD ODF and entered into blood circulation rapidly. The absolute bioavailability and relative bioavailability of DP/HP- β -CD ODF were 57.46% and 110.05%, respectively. In addition, AUC and C_{max} of DP/HP- β -CD ODF and DH solution showed no significant difference based on ANOVA and 90% confidence interval analysis. The result indicated that the DP/HP- β -CD ODF was bioequivalent with DH.

3.6. Exploration of drug/cyclodextrin interaction

The effect of HP- β -CD on DP was explored in terms of phase solubility study, FT-IR, DSC, XRD and molecular modeling. It could be concluded that the aqueous solubility of DP increased as the concentration of HP- β -CD increased by the results of phase solubility study. For the medium of aqueous HP- β -CD solution, the regression equation was $Y = 0.2039X + 0.00007$, $r^2 = 0.9995$, where Y was the concentration (mol/l) of DP, X was the concentration (mol/l) of HP- β -CD. The obtained diagram was classified as A_L type, which suggested the inclusion complexes were formed in the stoichiometric ratio of 1:1 [24]. The inclusion complexes were stable in water because bonding constant (K) of DP/HP- β -CD inclusion complexes was 3658.91 M^{-1} according to the Eq. 2 which was classified as a moderate binding [27]. For the medium of 0.1 mol/l HCl HP- β -CD solution, the regression equation was $Y = 0.1516X + 0.0372$, $r^2 = 0.9991$, and the binding constant was 4.80 M^{-1} , which was significant low. Generally, larger binding constant (K) indicated more stable complex [27]. The dramatic change of K from 3658.91 M^{-1} to 4.80 M^{-1} indicated a decrease in stability in the presence of HCl.

The FT-IR spectra of DP, HP- β -CD, the physical mixture and DP/HP- β -CD inclusion complexes were shown in Fig. 6. The intense sharp absorption band in the FT-IR spectrum of DP at 1685.6 cm^{-1} and 1308.5 cm^{-1} were assigned to the characteristic peak of C=O and the C-N stretching vibration [26]. The intense broad absorption band at 3416.6 cm^{-1} in the spectrum of HP- β -CD was attributed to the stretching vibration of O-H [10]. These characteristic bands showed a simple sum in the spectrum of the physical mixtures, which indicated that HP- β -CD and DP just mixed with each other. However, in the spectrum of DP/HP- β -CD inclusion complexes, the characteristic absorption of C=O at 1685.6 cm^{-1} disappeared, and

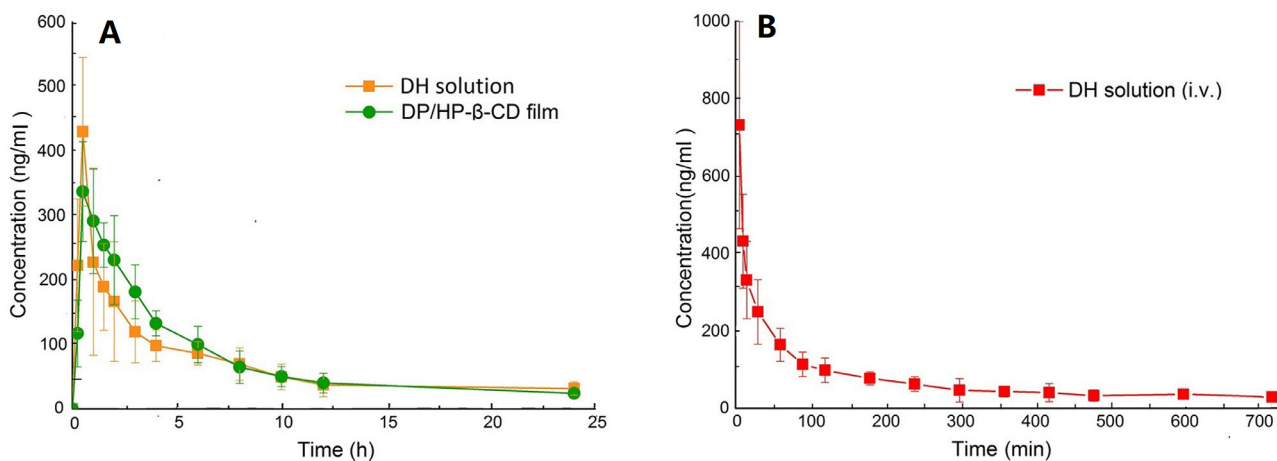


Fig. 5 – Pharmacokinetic profiles of DH solution after intragastrical administration and DP/HP-β-CD ODF after oral cavity administration (A); Pharmacokinetic profile of DP after injection (B).

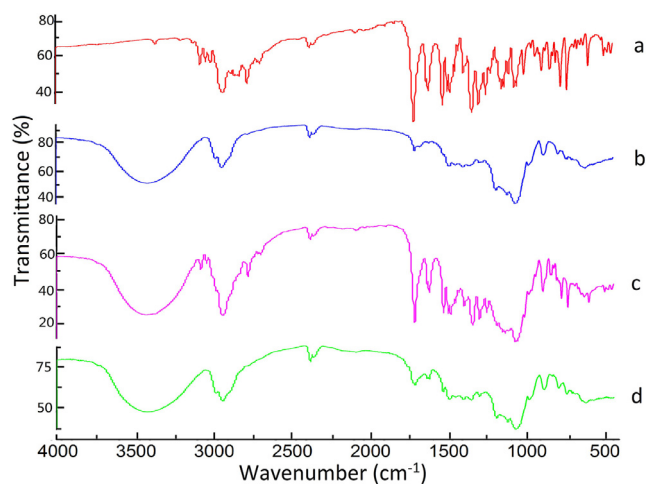


Fig. 6 – The FT-IR spectra of samples: DP (a), HP-β-CD (b), the physical mixtures (c) and DP/HP-β-CD inclusion complexes (d).

the absorption of C–N at 1308.5 cm⁻¹ diminished greatly. It demonstrated that some groups of DP were embedded into the cavity of HP-β-CD successfully. Additionally, it showed about 2.0 cm⁻¹ wavenumber variations of O–H absorption of HP-β-CD, which indicated the existence of interaction between DP and HP-β-CD.

The thermograms of DP, HP-β-CD, physical mixtures and DP/HP-β-CD inclusion complexes were shown in Fig. 7. The endothermic peak starting from 94.33 °C in the profile of DP was assigned to the melting point of DP [17]. The peak in the profile of HP-β-CD indicated that HP-β-CD was amorphous. The thermal peak from 60 °C to 110 °C was attributed to the evaporation of water molecules. The physical mixtures of DP and HP-β-CD showed a simple superposition of the endothermic peaks of DP and HP-β-CD, which suggested that there was no interaction between DP and HP-β-CD. In the curve of DP/HP-β-CD inclusion complexes, the endothermic peak of DP disappeared. It was reported that melting point

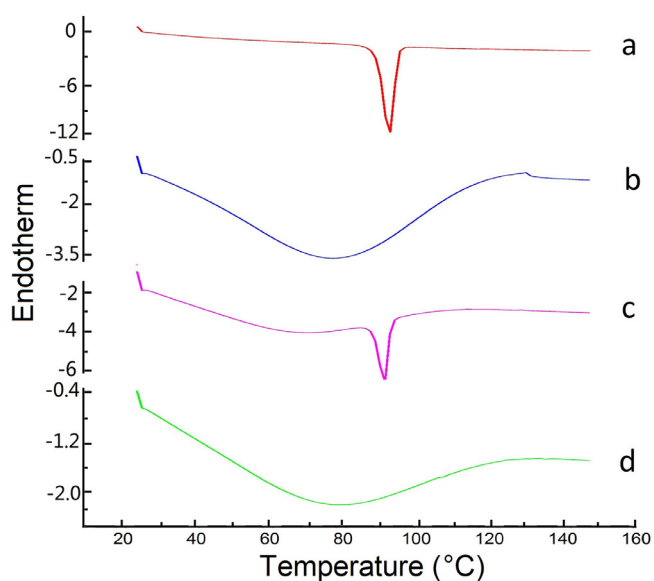


Fig. 7 – The DSC curves of samples: DP (a), HP-β-CD (b), the physical mixtures (c) and DP/HP-β-CD inclusion complexes (d).

of guest molecules shifted to different temperature or disappeared when they inserted into cavities or crystal lattice of CD [28]. Therefore, the results mentioned above indicated the formation of DP/HP-β-CD complexes.

The XRD profiles of DP, HP-β-CD, the physical mixtures and DP/HP-β-CD inclusion complexes were shown in Fig. 8. Again, DP alone presented the typical XRD pattern of crystalline state, i.e., sharp characteristic diffraction peaks, while HP-β-CD existed as amorphous form. The disappearance of diffraction peaks of DP in the pattern of DP/HP-β-CD inclusion complexes, which were still detectable in the pattern of physical mixtures, indicated the complete amorphization of DP after it inserted into the cavity of HP-β-CD.

The DP/HP-β-CD interaction was further explored by molecular modeling. As shown in Fig. 9, the most prob-

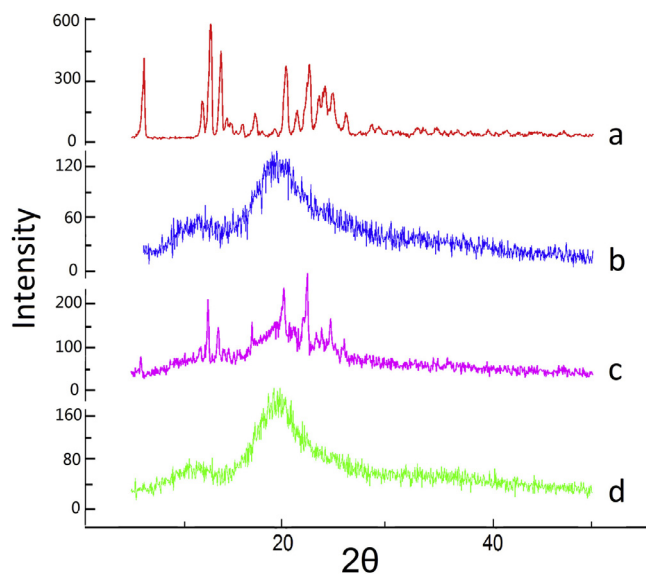


Fig. 8 – The XRD patterns of samples: DP (a), HP- β -CD (b), the physical mixtures (c) and DP/HP- β -CD inclusion complexes (d).

able modes of DP/HP- β -CD inclusion complexes were determined by the program of Autodock. Hydrogen bonding was found between the carbonyl, tertiary nitrogen groups of the guest molecule and the hydroxyl group of host molecule, respectively, which was in accordance with the results of FT-IR. Additionally, the minimum energy DP/HP- β -CD was -6.51 kcal/mol. However, the minimum energy of DP/HCl was -23.46 kcal/mol, which was far less than DP/HP- β -CD inclusion complexes. There is no definite regulation about the minimum energy value in molecular modeling. Generally, lower energy value indicated stronger stability [29]. These results suggested that DP/HCl interaction was much stronger than DP/HP- β -CD interaction, which verified the results of phase solubility study. Therefore, the reason for successful taste-masking was that DP molecule with unpleasant taste entered into the cavity of HP- β -CD in whole or in part, and the complexes was relatively stable in mouth due to moderate DP/HP- β -CD interaction.

After the DP/HP- β -CD ODF was applied to the tongue of rat, a series of changes occurred in DP/HP- β -CD inclusion complexes and the dynamic process was closely related to drug absorption. For the optimized DP/HP- β -CD ODF, the pharmacokinetic profile was similar to the DH solution according to *in vivo* study. The reason was explained from the following two aspects. On one hand, the environment had changed dramatically from mouth to stomach, which led to a variation of DP/HP- β -CD inclusion complexes. As shown in phase solubility study, the binding constant (K) changed from 3658.91 M^{-1} to 4.80 M^{-1} in the presence of HCl. From the results of molecule modeling, DP was easier to interact with HCl than HP- β -CD. Meanwhile, the film exhibited better dissolution behavior in 0.1 mol/l HCl solution according to the results of *in vitro* dissolution test (Fig. 2), indicating that most of hydrogen bonding between DP and HP- β -CD may be broken due to the acidic environment of stomach, which facilitated the

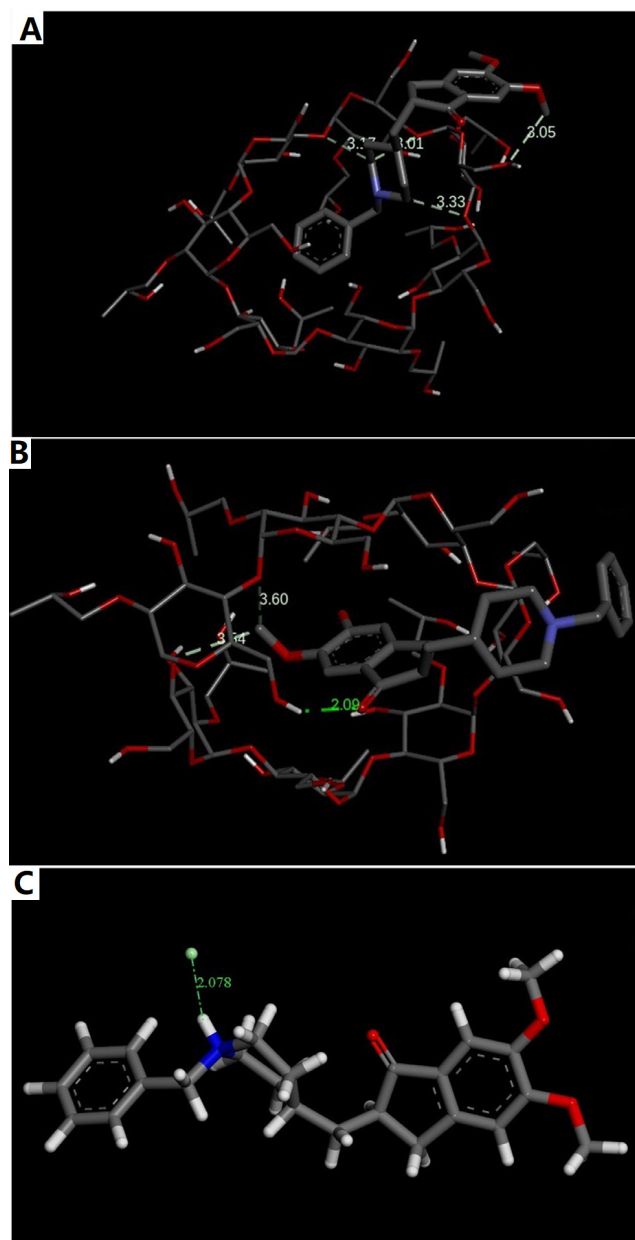


Fig. 9 – Models of the DP/HP- β -CD inclusion complexes and DP/HCl from docking calculation: DP/HP- β -CD inclusion complexes (A) and DP/HCl (B).

DP absorption. On the other hand, the absorption of cyclodextrin occurred in the colon where cyclodextrin was metabolized by colonic bacteria [27]. DP/HP- β -CD inclusion complexes dissolved quickly from film due to the amorphous form of DP after it inserted into the cavity of HP- β -CD. After that, the DP/HP- β -CD inclusion complexes quickly passed through the static water layer with the help of HP- β -CD and reached to the surface of biofilm. Since cyclodextrins could not pass through the lipid biofilm, there was a dynamic balance between the inclusion and the free DP. A small binding constant ($K = 4.80$ M^{-1}) was beneficial for DP across the biofilm [27]. Therefore, rapid dissolution, and the decreased stability of DP/HP- β -CD inclu-

sion complexes in stomach, explained why the DP/HP- β -CD ODF was bioequivalent with DH solution while taste-masking.

In summary, taste-masking was achieved by moderate interaction between DP and HP- β -CD, which made DP/HP- β -CD stable in oral cavity. Moreover, DP/HP- β -CD ODF showed favorable pharmacokinetic behavior in rat, which was mainly attributed to the decrease of stability of complexes in stomach based on DP/HP- β -CD dynamic process.

4. Conclusions

A rapid dissolving DP/HP- β -CD ODF was developed, and the taste was masked successfully. Apart from fulfilling the original aim of improving the bitter taste and solving the swallowing difficulties, the effect of cyclodextrin on taste-masking was illustrated at molecular level based on dynamic process and drug absorption *in vivo*. It was found that taste-masking was achieved successfully by the moderate DP/HP- β -CD interaction. Additionally, the varied stability of DP/HP- β -CD inclusion complexes from oral cavity to stomach facilitated drug absorption. These conclusions extended our understanding about the application of cyclodextrin complexation in the development of ODF and provided guidance for the design of ODF, especially for the drugs needed taste-masking.

Conflict of Interest

The authors declare that there is no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ajps.2018.05.001](https://doi.org/10.1016/j.ajps.2018.05.001).

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