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Effect of HPβCD on solubility and transdermal delivery of capsaicin through rat skin

Peng Zi[a], Xinghao Yang[a,*], Huifen Kuang[a], Yanshuang Yang[a], Lili Yu[b]

[a] *Laboratory of Pharmaceutics, Jiangsu Key Laboratory for Molecular and Medical Biotechnology, College of Life Science, Nanjing Normal University, Nanjing 210046, People's Republic of China*
[b] *Nanjing Chang'ao Pharmaceutical Science & Technology Co. Ltd., Nanjing 210022, People's Republic of China*

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ABSTRACT

We evaluated the ability of hydroxypropyl-β-cyclodextrin (HPβCD) to influence the percutaneous absorption of capsaicin (CP) through isolated rat skin. Phase solubility analysis and phase distribution studies suggested the potential of HPβCD as a solubilizer and permeation enhancer for CP. In vitro permeation studies showed the trend that, the penetration flux (J_s) of CP increased with the increasing concentration of HPβCD from 0 to 2.20% (w/v), and then decreased dramatically when the concentration of HPβCD kept on increasing up to 15% (w/v). 2.20% (w/v) of HPβCD provided both just adequate solubilization and preferred J_s for the permeation of CP (0.075%, w/v). Similar change patterns of the permeation parameters were also observed in the hydrogels, but the J_s of CP was reduced significantly along with the increasing concentration of Carbopol U21. Histological analysis showed an invasive action of HPβCD on the stratum corneum (SC) of rat skin, which could only reduce the lag time (T_L) but could not increase the J_s of CP. On the other hand, the complexation of HPβCD with CP could attenuate this invasive action. It is inferred that excess of HPβCD could not only disturb the percutaneous absorption of CP but also disrupt the structure of SC.

1. Introduction

Capsaicin (CP, Fig. 1), the active ingredient of hot peppers of the genus capsicum, exhibits broad bioactivity, including antinociception, antihypertension and lipid-lowering activities (Hayes et al., 1981; Wang et al., 1984; Monsereenusorn et al., 1982) and has been employed topically to treat various diseases such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy and post-therapy neuralgia (Fusco and Giacovazzo, 1997). The high degree of first-pass metabolism of capsaicinoids in rats and mice was observed (Sietsema et al., 1988; Donnerer et al., 1990) and the half-life of CP by intravenous administration from rats was also detected very short (7.06 min) (Kawada et al., 1985). Therefore, topical delivery should be applied for this drug to circumvent the hepatic metabolism and achieve better bioavailability. It is well known that vehicles of external preparations may greatly influence the flux and extent of drug permeating through the skin. Some investigators prepared the topically applied formulations of CP, including hydrogel, solution, ointment and cream and in vitro percutaneous absorption experiments were also performed with these formulations (Wang et al., 2001; Magnusson and Koskinen, 2000; Fang et al., 1996a,b). However, most of these formulations employed organic solvents or surfactants, such as ethanol, propylene glycol and sodium laurylsulfate as the solubilizer of CP, which often disturbed or interacted with the intercellular lipids or keratin of human skin (Williams and Barry, 2004). Furthermore, it was observed that the percutaneous effect of CP was reduced with increasing concentrations of ethanol and isopropanol (Fang et al., 1996b). Consequently, further studies are still necessary to exploit a new solubilizer for CP with permeation enhancement.

Hydroxypropyl-β-cyclodextrin (HPβCD) is able to form hydrophilic inclusion complexes with many lipophilic compounds in aqueous solution, which can enhance the aqueous solubility of the lipophilic drugs without changing their intrinsic ability to permeate lipophilic membranes (Loftsson and Masson, 2001). HPβCD has exhibited the potential as a solubilizer and penetrating enhancer for topically applied delivery by increasing the availability of dissolved drug molecules immediate to the biological membrane surface or by direct action on the stratum corneum (Loftsson et al., 2006; Ventura et al., 2006). However, some other studies considered that HPβCD had no effect on drug transport through human skin or through mouse skin with any manners (Simeoni et al., 2004; Shaker et al., 2003). To our knowledge, there is not any report to date concerning the influence of HPβCD on the percutaneous absorption of CP.

* Corresponding author. Tel.: +86 25 85891871; fax: +86 25 85891526.
E-mail address: yangxinh@jlonline.com (X. Yang).

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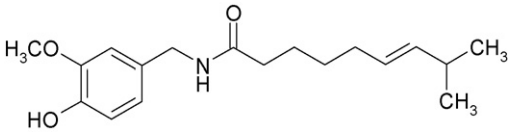

Fig. 1. Chemical structure of capsaicin.

This study aimed at exploring the effect of HPβCD on the permeation behavior of CP in solution and hydrogel. We characterized the interaction of CP with HPβCD in solution by phase solubility studies and in solid state by X-ray diffractometry (XRD) and differential scanning calorimetry (DSC) analyses. Phase distribution studies were performed to evaluate the effect of HPβCD on the observed distribution coefficients (D_{obs}) of CP at either pH 7.0 and 5.5 which was close to the acidity of human skin. The change patterns of in vitro permeation parameters of CP through excised rat skin were investigated in the presence of HPβCD at different concentrations. The permeation parameters of solutions were further compared with that of Carbopol U21 hydrogels. The rat skin samples pretreated with free CP, HPβCD alone and their complex were examined with transmission electron microscope (TEM) to explore the enhancement mechanism of HPβCD on the percutaneous absorption of CP.

2. Materials and methods

2.1. Materials

Capsaicin was purchased from Wuhan Yuancheng Technology Development Co., Ltd. (Hubei, PR China) and the purity was 99.15%. Hydroxypropyl-β-cyclodextrin (HPβCD, MW 1380, MS 5.9) was a gift from Xinxin Excipients Inc. (Jiangsu, PR China). *N*-Octanol (99%) was purchased from Shanghai Lingfeng Chemical Co., Ltd. (Shanghai, PR China). Carbopol U21 was a gift from Goodrich (Charlotte, USA). All other chemicals and solvents were of at analytical reagent grade or HPLC reagent grade. Double-distilled water was used throughout the study.

2.2. Preparation of the CP/HPβCD solid samples

The freeze-dried product of CP and HPβCD was prepared. 0.5 g of CP and 2.26 g of HPβCD (1.64×10^{-3} mol each of CP and HPβCD) were dissolved in 8 ml of ethanol/water solution (6/2, v/v) to obtain a solution containing CP and HPβCD in 1:1 molar ratio. After stirring for 48 h at room temperature, the solution was freeze-dried using the Modulyo 4 K system (Edwards, Crawley, U.K.). The obtained white powder was stored in sealed glass container at 25 °C for further investigations. The CP content of the complex was determined by dissolving an accurately weighed quantity in ethanol/water solution (50/50, v/v) followed by HPLC assay.

The physical mixture was prepared by simple mixing CP/HPβCD in 0.5:2.26 (w/w) ratio in a mortar for 15 min.

2.3. X-ray diffractometry

The X-ray diffraction pattern of the solid sample was recorded using Philips X-ray diffractometer (PW-1710) equipped with graphite monochromator, under the following operating conditions: Ni filtered Cu Kα radiation, 30 kV voltage, 20 mA current and scan speed 1° 2θ min^{-1}.

2.4. Differential scanning calorimetry

DSC scans of the solid sample were recorded on a PerkinElmer instrument equipped with a low temperature cell. The sample weight was 3.5 mg (approximately) and the heating rate was 10 °C/min.

2.5. Phase solubility studies

Phase solubility diagram of CP/HPβCD system was obtained according to Higuchi and Connors' method (1965). CP in excess of its solubility was weighed into a series of screw-capped vials containing aqueous solutions of HPβCD at concentrations ranging from 0 to 0.10 M. The sealed vials were agitated on a rotary shaker for 48 h under 27 °C and equilibrated for further 24 h. The clear supernatant was passed through 0.45 μm Millipore filter. The filtrates were immediately diluted with methanol followed by HPLC assay.

2.6. Phase distribution studies

The procedure for determining the observed distribution coefficients (D_{obs}) of drug distributing between *n*-octanol and aqueous solutions containing HPβCD at different concentrations was described previously (Másson et al., 2005). Solutions of CP were prepared at a concentration of 1 mg/ml in *n*-octanol that had been saturated with aqueous solutions containing 0, 1.0, 2.20, 3.0, 10.0, or 15.0% (w/v) of HPβCD at pH 7.0 (new distilled water) and 5.5 (phosphate buffer solution (PBS)). 3 ml aliquot of the CP *n*-octanol solution were transferred to 10 ml of vials, respectively, then 3 ml aqueous HPβCD solutions saturated with octanol were also added into the corresponding vials. The vials were shaken with a mechanical shaker for 24 h at room temperature. The phases were then separated with separating funnels and samples from each phase were analyzed by HPLC. The partition coefficient was calculated as the ratio between the concentrations in the octanol phase and the aqueous phase.

2.7. Preparation of solutions/suspensions

HPβCD was added to water at 0, 1, 2.20, 3, 10 and 15% (w/v) concentrations, respectively to obtain clear aqueous solutions. CP was added at 0.075% (w/v) concentration to these solutions equivalent to the strength of commercial topical preparations of CP, and the mixtures were stirred at room temperature for 12 h before the permeation experiments. Then the uniform suspensions (free CP, the sample prepared in the presence of 1% (w/v) of HPβCD) and solutions (the samples prepared in the presence of HPβCD at 2.20, 3, 10 and 15% (w/v) concentrations) were obtained.

2.8. Preparation of hydrogels

Various hydrogels with CP at 0.075% (w/v) concentration were prepared equivalent to the strength of commercial topical preparations of CP.

To prepare CP/HPβCD hydrogels containing 0.3–0.8% (w/v) of Carbopol U21 in the presence of HPβCD at 2.20% (w/v) concentration, 0.3, 0.5 and 0.8 g of Carbopol U21 was dissolved in 50 ml of water, respectively with continuous stirring for 2 h, and then these solutions were adjusted to pH 5.5 with 10 M of NaOH solution stirred for 1 h at room temperature. 2.20 g of HPβCD and 0.075 g of CP were dissolved in 20 ml of water with continuous stirring for 2 h; three parallel solutions were prepared with this method. Then the Carbopol U21 solutions and CP/HPβCD solutions were mixed together, respectively. At last, sufficient quantity of water was added to these three solutions to obtain 100 g of hydrogels. These hydrogels were stirred at room temperature for 2 h before the permeation experiments.

CP/HPβCD hydrogels containing 3, 10 and 15% (w/v) of HPβCD in the presence of 0.3% (w/v) of Carbopol U21 were also prepared with the method mentioned above.

2.9. Preparation of skin samples for in vitro studies

Sprague Dawley rats of either sex (150–180 g, Southeast University Zoological Animal Center, Nanjing, PR China) were used in the study. The rats were sacrificed by an overdose of diethyl ether anesthesia. Hair on the abdominal area of the rats was removed by trimming with a clipper followed by shaving with an electrical shaver. Care was taken not to damage the stratum corneum. The hair-free abdominal skin was excised with a surgical blade and a pair of scissors and the adhering subcutaneous fat, tissue and capillaries were removed. The skin was cut into 1 cm × 1 cm samples for permeation studies.

2.10. In vitro percutaneous studies

The excised skin was mounted between the donor and receptor chambers of Franz-type diffusion cells (Nanjing Red & Blue Inc., Nanjing, China) with the stratum corneum (SC) side up facing the donor fluid. The area of skin available for diffusion was 0.785 cm^2. The donor compartment was covered with Parafilm® (American National Can, Greenwich, CT, USA) in order to achieve occlusive conditions. The receptor was filled with 5 ml of water/ethanol (80/20, v/v) to ensure pseudo-sink condition by increasing CP solubility in the receptor phase. The receptor fluid was constantly stirred at 300 rev/min with a small magnetic stirring bar to ensure homogeneity. The apparatus was immersed in a water bath maintained at 37 ± 0.5 °C throughout the experiment. Samples (3 ml) were withdrawn from the receptor chamber at predetermined time intervals (1, 2, 3, 4, 5, 6, 7 and 8 h) and filtered with 0.45 μm Millipore filter. The CP concentration was determined by HPLC. The fluid remaining in the receptor cell was drained off and the receptor cells were rinsed thrice quickly and filled with the fresh warmed (37 °C) and degassed water/ethanol (80/20, v/v) solution at every sampling interval. Sink condition was maintained by inclusion of ethanol (20%, v/v) in the receptor and also by replacing the fluid at every sampling interval.

2.11. TEM observation

TEM observation was performed on scrap of excised rat skin coming from the same batch used in the percutaneous studies, which was pretreated with free CP suspension (0.075%, w/v), HPβCD solution (3%, w/v) and CP/HPβCD solution (0.075 and 3%, w/v) prepared as described above. 2 ml of these samples were applied in the donor compartment, respectively. The receptor compartment was filled with saline. Samples were withdrawn at short time intervals (20 min) and immediately replaced with an equal volume of saline to relieve the supersaturation of CP. The Franz cell was maintained under 37 °C for 24 h in a water bath.

At the end of the experiment, the rat skin samples were fixed with glutaral solution (4%, w/v in PBS, pH 7.4) under 4 °C for 2 h. Then the samples were washed with PBS thrice and stayed overnight under 4 °C. The samples were fixed again with osmium tetroxide (1%, w/v in PBS, pH 7.4) under 4 °C for 2 h. Rinse the samples twice with distilled water and dehydrate for 15 min with acetone/water solutions (30, 50, 70, 80, 90 and 100%, v/v), respectively. Then the samples were embedded in Spurr's resin and polymerized for 24 h under 30, 45 and 60 °C, respectively. The 5 μm thick sections obtained using a Reichert-Jung 2050 microtome (2050 Super-cut Reichert-Jung, Leica Instruments, Wetzlar, Germany) were stained with uranyl acetate and lead citrate. Sections were examined in a Zeiss EM10 (Germany) electron microscope.

2.12. HPLC analyses

HPLC analyses were performed at room temperature (25 °C) using a Shimadzu 20A apparatus (Shimadzu, Japan) on a 5 μm Dikma ODS cartridge (250 mm × 4.6 mm i.d.) (Dikma Technologies, Beijing, PR China) and eluted isocratically with methanol/phosphoric acid solution (0.1%, w/v) (80/20, v/v). The flow rate was fixed at 0.8 ml/min and UV light at 280 nm was used for detection.

2.13. Data calculation

The concentration of CP in the receptor compartment was diluted to the linear range of HPLC analysis. Data were analyzed by linear regression of the cumulative amount of the drug permeated through skin surface unity (Q_s) versus time in the steady-state range. Flux (J_s) is represented by the slope of the regression lines. The lag time (T_L) was determined from the *x*-intercept values of the regression lines. All the obtained permeation profiles were determined six times in different experiments and the mean values ± standard deviations were calculated. The D_{obs} and percutaneous absorption data were subjected to Tukey's test to determine level of significance between various groups. The data were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Complexation characterization in the solid state

The freeze-dried product and physical mixture of CP and HPβCD were prepared and the interaction between them was characterized in the solid state by X-ray diffractometry and differential scanning calorimetry. The XRD patterns of powder samples are shown in Fig. 2. The diffraction peaks of CP indicated the crystalline nature of the drug, whereas the HPβCD was amorphous as evidenced from

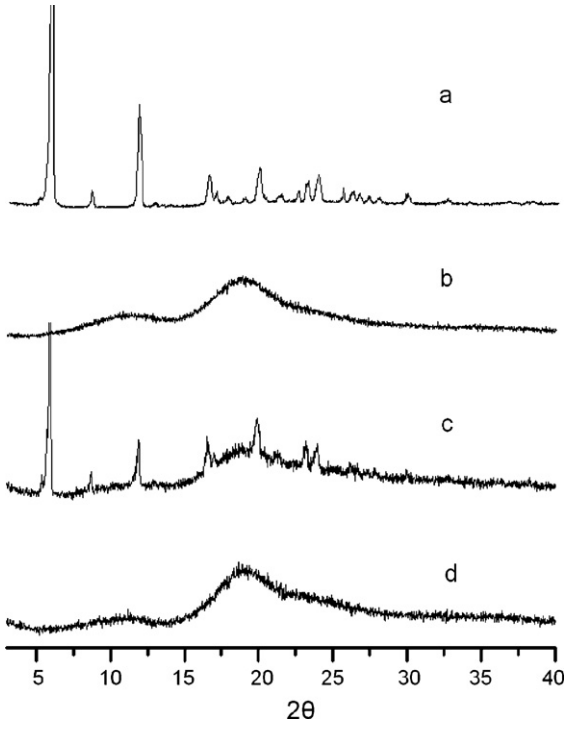

Fig. 2. X-ray diffraction pattern of capsaicin (a), HPβCD (b), physical mixture of capsaicin and HPβCD (c) and inclusion complex of capsaicin and HPβCD (d).

the absence of diffraction peaks in Fig. 2. The characteristic diffraction peaks of CP completely disappeared in the freeze-dried product of CP with HPβCD; however, most of these peaks were evident in the physical mixture of the two components. This indicated that the complex constituted a new solid state.

More direct evidence of complexation formation was obtained from DSC thermograms as shown in Fig. 3. CP showed a characteristic endothermic peak corresponding to its melting point (65.7 °C). The DSC trace of HPβCD showed a broad endothermic peak at 93.1 °C representing a loss of water molecule, a dehydration process as described by Miro et al. (2004). The characteristic endothermic peak of CP also was evident in the graph of the physical mixture of CP and HPβCD. This suggested that there was no interaction between CP and HPβCD on simple mixing. In case of freeze-dried product, the characteristic melting peak of CP almost completely disappeared, showing the interaction of CP with HPβCD. These results showed that an intensive interaction of CP and HPβCD existed in the freeze-dried product. The complexation characterization will be discussed in more detail in other paper.

3.2. Phase solubility studies

The phase solubility diagram of CP as a function of HPβCD concentration under 27 °C is shown in Fig. 4. The solubility of CP with increase of HPβCD concentration indicates an A_L type of phase solubility diagram (Higuchi and Connors, 1965). The apparent 1:1 stability constant (K_s) of the CP/HPβCD complex was calculated to be 1822 M^{-1} from the slope and intercept (S_0) of the phase solubility diagram according to the equation:

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

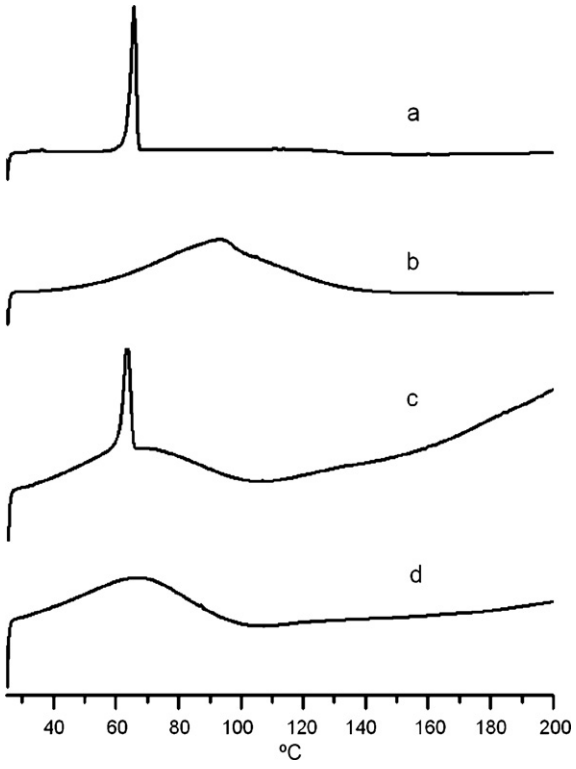

Fig. 3. Differential scanning calorimetric thermograms of capsaicin (a), HPβCD (b), physical mixture of capsaicin and HPβCD (c) and inclusion complex of capsaicin and HPβCD (d).

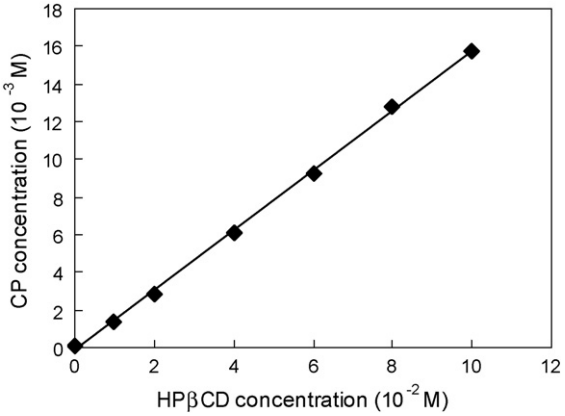

Fig. 4. Phase solubility diagram of capsaicin with HPβCD in water under 27 °C.

According to the theory of Rao and Stella (2003), cyclodextrins may provide adequate solubilization for the drug by complexation if the utility number of cyclodextrin (U_{CD}) is greater than or equal to 1. U_{CD} is expressed as the following equation:

$$U_{CD} = \left[\frac{K_s S_0}{1 + K_s S_0}\right] \frac{CD_t}{D_t}$$

where CD_t is the total amount of workable cyclodextrin (M) and D_t is the dose of drug (M). In this case, D_t of CP was 2.46×10^{-3} M (equivalent to 0.075%, w/v), S_0 was 1.04×10^{-4} M and K_s was 1822 M^{-1} constantly. Therefore, the unique variable in this equation was CD_t, the concentration of HPβCD (M). If we fixed U_{CD} as 1, then CD_t could be calculated to be 1.59×10^{-2} M (equivalent to 2.20% approximately, w/v) according to this equation, under which HPβCD could just solubilize CP at the constant concentration of 2.46×10^{-3} M (0.075%, w/v).

Actually, the concentration of HPβCD in our phase solubility studies had increased up to 0.1 M (equivalent to 13.80%, w/v), under which the U_{CD} could be calculated to be 6.48, far greater than 1. These results indicated that HPβCD was a potential solubilizer for CP and CP/HPβCD complex was suitable for practical application in formulations.

3.3. Phase distribution studies

The observed distribution coefficients (D_{obs}) for CP in the two-phase system of *n*-octanol and an aqueous HPβCD solution (pH 5.5 or 7) are listed in Table 1. Generally, drugs with $\log D_{obs}$ values within the range of 1–4 are considered suitable for topically applied delivery. It could be found clearly that the $\log D_{obs}$ of CP under pH 7.0 always decreased, but was among the suitable range (3.61–2.37) along with the increase of HPβCD concentration from 0 to 15% (w/v). In pH 5.5 of PBS solution, D_{obs} of CP had no significant difference ($P > 0.05$) compared with that in new distilled water (pH 7.0). These results indicated that pH 5.5 close to the acidity of human skin had no significant influence on the distribution coefficient of CP between *n*-octanol and aqueous HPβCD solutions. Therefore, it could be inferred from these results that HPβCD was

Table 1
The observed distribution coefficients (D_{obs}) for capsaicin in various HPβCD solutions

	HPβCD concentration (%, w/v)					
	0	1	2.20	3	10	15
$\log D_{obs}$ of capsaicin (pH 7.0)	3.61	3.41	3.15	3.04	2.67	2.37
$\log D_{obs}$ of capsaicin (pH 5.5)	3.64	3.39	3.22	3.01	2.65	2.42

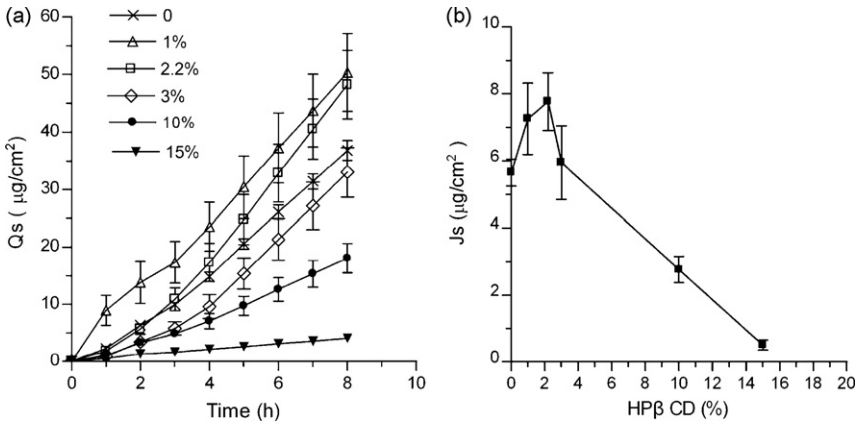

Fig. 5. Permeation profiles through rat skin of capsaicin solutions/suspensions containing HPβCD at different concentrations (w/v) under pH 7.0 (a) and J_s of capsaicin permeated through rat skin in the presence of HPβCD at different concentrations (w/v) under pH 7.0 (b).

potential to be used as a penetration enhancer in topical formulations of CP, but the concentration of HPβCD should be as low as possible to obtain a greater D_{obs} of CP.

3.4. In vitro permeation studies

First, the influence of HPβCD on the percutaneous absorption of CP was investigated in solutions based on the change patterns of the parameters Q_s, J_s and T_L. Previous studies indicated that the penetration flux of CP through pig skin was closest to that of human skin; rat skin was inferior to pig and rabbit skin but better than mouse skin and cellophane membrane (Fang et al., 1995); considering better availability, the excised rat skin was employed for our permeation studies. The Q_s and J_s of CP permeated through rat skin in the presence of HPβCD at 0, 1, 2.20, 3, 10 and 15% (w/v) concentrations are shown in Fig. 5 and Table 2. The two parameters showed the similar change patterns. It was observed that the J_s of CP increased significantly before the concentration of HPβCD increased to 2.20% (w/v), and then it dramatically decreased with the increase of HPβCD concentration up to 15% (w/v). This fact meant that the maximum J_s of CP could be achieved at 2.20% (w/v) of HPβCD concentration, under which the U_{CD} was 1. This U_{CD} could provide just adequate solubilization for CP (0.075%, w/v), as described in phase solubility studies. Increase of HPβCD concentration above 2.20% (w/v) would decrease the J_s of CP. These results confirmed the conclusion of previous literature that HPβCD might act as permeation enhancers by transferring the drug from the solution towards the lipophilic surface of biological membranes, where the drug molecules distributed from the complex into the membrane; however, excess of HPβCD would decrease the amount of free drug and then reduce the penetration flux of drug through skin (Másson et al., 1999). In short, when the HPβCD concentration was lower than 2.20% (w/v), it failed to dissolve total amount of CP. The suspensions (CP alone, the sample prepared in the presence of 1% (w/v) of HPβCD) could not facilitate a greater J_s of CP compared to the solution (the sample prepared in the presence of 2.20% (w/v) of HPβCD), although the Q_s after 8 h reached the maximum value in the presence of HPβCD at 1.0% (w/v). Therefore, it could be inferred that 2.20% (w/v) of HPβCD provided both adequate solubilization and greater D_{obs} for CP and it should be the preferred concentration for enhancing the J_s of CP through skin.

Table 2
Percutaneous permeation parameters[a] of free capsaicin (CP) or in the presence of HPβCD at different concentrations (%, w/v) in solution or in Carbopol U21 (CB) gels at 37 ± 0.5 °C

Compounds	Q_s[b] (μg/cm²)	T_L (h)	J_s (μg/cm² h)
CP alone	36.78 ± 1.71	1.36 ± 0.23	5.643 ± 0.395
CP/HPβCD (1%)	50.36 ± 6.77	0.78 ± 0.26	7.248 ± 1.072
CP/HPβCD (2.20%)	48.25 ± 5.96	1.80 ± 0.23	7.766 ± 0.862
CP/HPβCD (3%)	32.99 ± 4.32	2.36 ± 0.27	5.941 ± 1.100
CP/HPβCD (10%)	18.05 ± 2.59	1.46 ± 0.19	2.756 ± 0.388
CP/HPβCD (15%)	4.08 ± 0.77	−0.65 ± 0.22	0.494 ± 0.151
CP/HPβCD (2.2%)/CB (0.3%) gel	26.42 ± 4.18	1.71 ± 0.17	4.218 ± 0.692
CP/HPβCD (2.2%)/CB (0.5%) gel	23.55 ± 4.25	1.84 ± 0.27	3.801 ± 0.743
CP/HPβCD (2.2%)/CB (0.8%) gel	20.11 ± 3.67	1.72 ± 0.11	3.227 ± 0.429

[a] Each value is the average ± S.D. of six different experiments.
[b] Cumulative concentration for unity of surface after 8 h.

The change of T_L showed a complicated tendency along with the increase of HPβCD concentration as shown in Table 2. The T_L value fluctuated when the HPβCD concentration was below 3% (w/v), and then decreased when HPβCD concentration progressively increased from 3 to 15% (w/v). When HPβCD concentration was lower than 3% (w/v), the change pattern of T_L could be explained with the formation or dissociation equilibrium of CP/HPβCD complex. When the concentration of HPβCD increased from 0 to 1% (w/v), the amount of free CP available for permeation was also increased, as a result, the permeation of CP was both faster (T_L reduction) and greater (Q_s increase). When HPβCD concentration increased higher (3%, w/v), the dynamic equilibrium of free drug molecules and inclusion complex shifted towards the formation of the complex and this would reduce the amount of free CP available for penetration, considering cyclodextrins molecules with hydrated outer surface are too large to permeate through the lipophilic biomembranes under normal conditions as described by Loftsson and Masson (2001). Then the J_s of CP was decreased accordingly because slower dissolution of free drug would lead to slower penetration (T_L increase and Q_s reduction) as described previously (Ventura et al., 2006).

Unfortunately, the dynamic equilibrium theory could not interpret the decreasing trend of T_L reasonably when HPβCD concentration progressively increased from 3 to 15% (w/v). We performed histological analysis on rat skin samples treated with free CP (0.075%, w/v) suspension, free HPβCD (3%, w/v) solution and CP/HPβCD (0.075 and 3%, w/v) solution. The obtained electron micrographs are shown in Fig. 6. It was recognized that CP had the similar chemical structure with Azone and could be used as permeation enhancer for naproxen by inserting itself into the lipid bilayers within the intercellular channels and disrupting their stacking (Degim et al., 1999). It was also reported that 3% CP in ethanol/water (1:1, v/v) could enhance the skin permeation of indomethacin significantly (Fang et al., 2001). In this study, the concentration of CP (0.075%, w/v) was relatively lower, and the rat

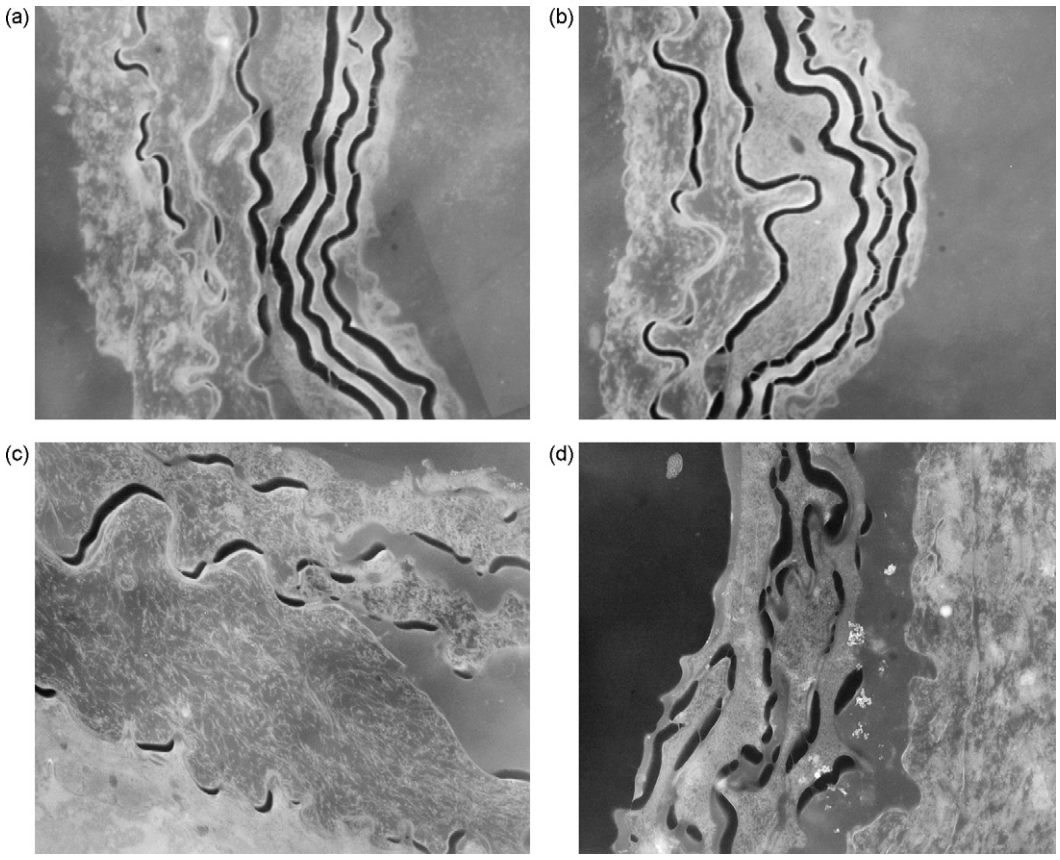

Fig. 6. Transmission electron micrographs of rat skin stratum corneum treated with capsaicin/HPβCD systems for 24 h: saline (a), free capsaicin (0.075%, w/v) (b), free HPβCD (3%, w/v) (c) and capsaicin/HPβCD (0.075 and 3%, w/v) (d).

skin pretreated with CP (photo b) did not show significant change compared with the control (photo a). Interestingly, we observed an invasive action of HPβCD alone (3%, w/v) that caused the blur of the horny thin plates; the distance between the horny thin plates was also enlarged (photo c). A less invasive effect was exerted by CP/HPβCD solution, even if the horny thin plates were broken into segments (photo d).

To date, literatures concerning the mechanism of HPβCD enhancing transdermal drug delivery are still conflicting. Some papers suggested that HPβCD had no effect on the skin barrier but enhanced the percutaneous absorption of drugs by increasing the availability of dissolved drug molecules immediate to the skin surface (Loftsson et al., 2006; Babu and Pandit, 2004). In contrast, others argued that HPβCD could enhance drug flux through isolated human or hairless mouse skin by means of increasing dissolution rate of the drug and exerting a direct action on the stratum corneum simultaneously (Ventura et al., 2006; Bentley et al., 1997). Specifically, HPβCD could extract trivial amount of some specific cholesterol, sterolesters and triglycerides from hairless rat skin but could not cause destabilization of the liposomes of skin completely (Legendre et al., 1995). These results suggested that the CP/HPβCD solution only had a less invasive action on SC of rat skin than the HPβCD alone because CP could form inclusion complex with HPβCD and the skin lipids had to compete the cyclodextrin cavity with CP. Therefore, our studies are in agreement with the second viewpoint. The presence of CP may, to some extent, impede the inclusion of the skin lipids with HPβCD.

Consequently, we can propose a hypothesis about the decreasing trend of T_L as following: When the concentration of HPβCD increased from 3 to 15% (w/v), excess of HPβCD began to extract lipids from skin and the SC of skin was disrupted to some extent, thereby the permeation could reach the pseudo-steady-state faster (T_L reduction), but the Q_s and J_s were still very low because of the lack of free CP. The conclusion implied that the presence of excessive HPβCD in the solution would not only disturb the percutaneous absorption of CP but also disrupt the SC of skin.

Subsequently, CP hydrogels containing various concentrations of HPβCD in the presence of Carbopol U21 at 0.3% (w/v) concentration were also prepared and in vitro percutaneous experiments were performed with these formulations to comprehend the work-

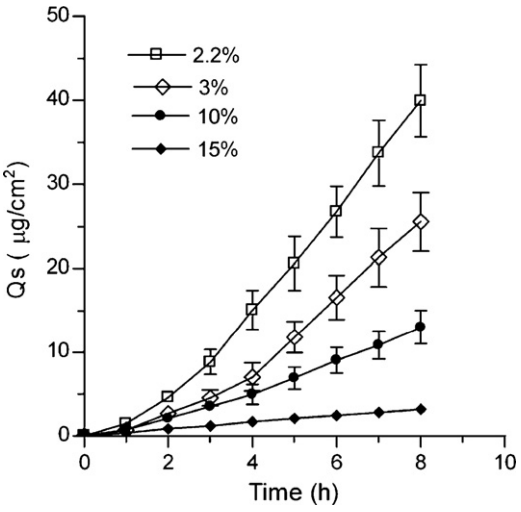

Fig. 7. Permeation profiles through rat skin of capsaicin in the presence of Carbopol U21 (0.3%, w/v) and HPβCD at various concentrations (w/v) under pH 5.5.

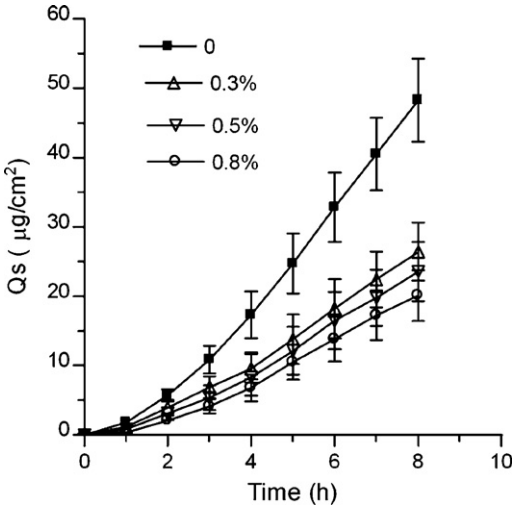

Fig. 8. Permeation profiles through rat skin of capsaicin in the presence of HPβCD (2.20%, w/v) and Carbopol U21 at different concentrations (w/v) under pH 5.5.

ing patterns of HPβCD on the percutaneous absorption of CP in hydrogels. The results (Fig. 7) showed the similar change patterns to that of the solutions. The T_L values of these hydrogels were close to that of the solutions without significant difference ($P > 0.05$, data were not shown). And the hydrogel containing 2.20% (w/v) of HPβCD also exhibited the greatest J_s for percutaneous absorption and this J_s decreased when the concentration of HPβCD kept on increasing up to 15% (w/v). However, the J_s data of the hydrogels decreased significantly ($P < 0.05$) compared with that of the solutions (data were not shown). It could be concluded from these results that HPβCD had the same action patterns in Carbopol U21 hydrogels, but the Carbopol formulation might attenuate the enhancement of HPβCD on the permeation of CP to some extent.

Afterwards, we performed in vitro percutaneous studies with CP hydrogels (0.075%, w/v) containing Carbopol U21 at 0.3, 0.5 and 0.8% (w/v) concentrations in the presence of HPβCD (2.20%, w/v) to evaluate the effect of Carbopol U21 on the permeation of CP. The results are shown in Fig. 8 and Table 2. It was clear that the Q_s and J_s of these hydrogels decreased with the increase of Carbopol U21 concentration. Since the results of phase distribution studies had shown that the pH value of hydrogels did not influence the D_{obs} of CP, it should be the viscosity of Carbopol U21 that reduced the percutaneous effect of CP. The T_L values of permeation had no significant changes compared with that of CP/HPβCD solution (0.075 and 2.20%, w/v) ($P > 0.05$). These results indicated that the presence of Carbopol in the formulation could inhibit the permeation of CP. Therefore, it could be inferred that the amount of Carbopol in hydrogel should be as little as possible to get high penetration flux through skin.

Extended investigations would be carried out including further exploration for the permeation mechanism of CP in presence of HPβCD and the interaction of HPβCD with skin described in this paper. Until now the investigation was focused on permeation behavior of CP in solution and hydrogel, other topical dosage forms remained uninvestigated. Notwithstanding its limitation, this study does suggest the permeation trend of CP in presence of HPβCD.

4. Conclusions

These findings demonstrate that HPβCD is able to complex with CP both in solid state and in solution, significantly increasing its water solubility. The phase distribution and the phase solubility studies showed that HPβCD could serve as a potential solubilizer and permeation enhancer for CP. In vitro permeation studies indicated that the J_s of CP increased before the concentration of HPβCD increased to 2.20% (w/v) and then decreased with excess of HPβCD. Under the experimental condition, 2.20% (w/v) of HPβCD provided both adequate solubilization and greater D_{obs} for CP and it was the optimum HPβCD concentration for the permeation of CP. The similar change patterns of J_s were also observed in the Carbopol U21 hydrogels. We also found that the viscosity of the hydrogels could inhibit the permeation of CP and the amount of Carbopol should be as little as possible to get a higher penetration flux for CP. The TEM observation indicated that the presence of CP could modify an invasive action of HPβCD on the stratum corneum of rat skin. This invasive action could only reduce the T_L but could not increase the J_s of CP permeation through rat skin. It implied that excess of HPβCD was not beneficial to improve the percutaneous absorption of CP and would disrupt the stratum corneum structure of skin.

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