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The effect of ion pairing on the skin permeation of amlodipine

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The purpose of the present study was to evaluate the effect of ion pairing on the skin permeation of amlodipine. Amlodipine base (AM) was first prepared from amlodipine besilate (AM-B), then amlodipine adipate (AM-A), amlodipine oxalate (AM-O) and amlodipine maleate (AM-M) were prepared using AM and the corresponding organic acids. Differential scanning calorimetry (DSC) thermogram studies demonstrated the formation of complexes between AM and the various acids. *In vitro* percutaneous absorption of AM and its complexes was evaluated through excised rat skin using 2-chamber diffusion cells. The results showed that AM had the greatest steady-state flux and lowest permeability coefficient of the five compounds from the EI system (ethanol : isopropyl myristate (IPM) = 2 : 8), and its four complexes all exhibited a lower flux and higher permeability coefficient than AM.

1. Introduction

Amlodipine belongs to the dihydropyridine class of calcium channel blockers, and it is used to treat hypertension and relieve symptoms of angina pectoris (Haria et al. 1995). Patients suffering from these diseases usually require long-term medication and patient compliance is very important. However, in the case of amlodipine, the only available route is oral administration that requires daily dosing. Transdermal drug delivery systems (TDDS) provide an attractive approach for long-term treatment and have many advantages over traditional therapies (Abraham 1995). The daily dose of amlodipine ranges from 2.5 to 10 mg, which is very suitable for TDDS. Some research has been carried out to verify the feasibility of developing an effective transdermal system for AM-B (Francoeur and Potts 1988). In addition, McDaid and Deasy have discussed the formulation development of a reservoir-type transdermal system containing AM (McDaid and Deasy 1996). However, the steady-state flux they obtained needs further improvement in order to attain the clinical levels required in patients.

Many studies have highlighted the importance of ion pair formation for TDDS (Fang et al. 2003; Cheong and Choi 2002; Kadono et al. 1998). When an ion pair contains both a cation and an anion, it may have a residual degree of hydrophobicity. The ion pair diffuses into the aqueous viable epidermis, then dissociates into its charged species which partition into the epidermis and then diffuse forward (Megwa et al. 2000a, b; Valenta et al. 2000). Accordingly, AM was first prepared from amlodipine besilate (AM-B) in this investigation, and then amlodipine maleate (AM-M), amlodipine oxalate (AM-O) and amlodipine adipate (AM-A) were prepared from AM. Next, the skin permeability of AM and its four complexes through excised rat skin was studied using 2-chamber diffusion cells.

2. Investigations, results and discussion

2.1. Comparison studies among AM and its complexes

Figure 1 shows the DSC curves of AM and its complexes at a heating rate of 5 °C/min over the experimental temperature range 20–300 °C. The DSC curves of AM and its complexes exhibit characteristic sharp endothermic peaks corresponding to their melting points. Table 1 summarizes the melting points and the enthalpies of fusion for AM and its complexes. The melting peaks of the complexes are all shifted to higher temperatures compared with AM. The rank order of the melting points is as follows: AM < AM-A < AM-M < AM-O < AM-B. The changes in the melting point and the enthalpy of fusion confirm the formation of complexes.

Saturated solutions were used in our experiments, because suspensions had the same maximum thermodynamic activity and were able to promote maximum flux in equilibrium systems (Barry 2001). Figure 2 shows the permeation profiles of AM and its complexes from the EI system through excised rat skin. The fluxes of AM and its complexes were in the following order: AM > AM-B > AM-M > AM-A > AM-O. AM has the highest flux, 1.14 ± 0.09 μmol/cm^2/h. It is obvious that complex formation has a negative effect on the flux. The solubilities

Table 1: DSC melting characteristics of AM and its complexes

	m.p. (°C)	Enthalpy of fusion (kJ/mol)
AM	142.32	25.79
AM-B	200.41	33.59
AM-A	170.63	44.84
AM-O	186.71	40.92
AM-M	174.23	20.23

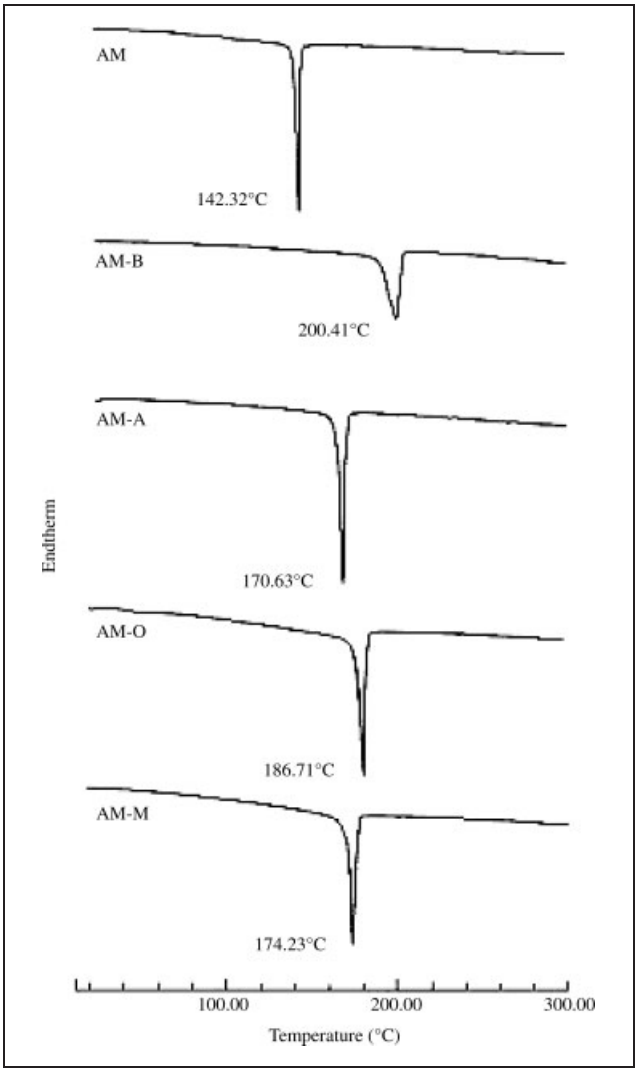


Fig. 1: DSC curves of AM and its complexes at a heating rate of 5 °C /min

in the EI (ethanol : isopropylmyristate) system and the permeation coefficients of AM and its complexes are shown in Tables 2 and 3, respectively. Despite the highest flux of

Table 2: Physicochemical properties relating to the transdermal delivery of AM and its complexes (mean ± S.E., n = 3)

Drug	log $K_{O/W}$	Solubility in the EI system (μmol/ml)	Solubility in water (μmol/ml)
AM	1.50 ± 0.01	154.05 ± 6.95	0.39 ± 0.02
AM-B	0.65 ± 0.03	4.88 ± 0.14	3.37 ± 0.01
AM-A	−0.22 ± 0.02	0.65 ± 0.001	5.57 ± 0.25
AM-O	−0.34 ± 0.04	0.15 ± 0.003	3.56 ± 0.02
AM-M	0.25 ± 0.02	0.59 ± 0.010	3.97 ± 0.01

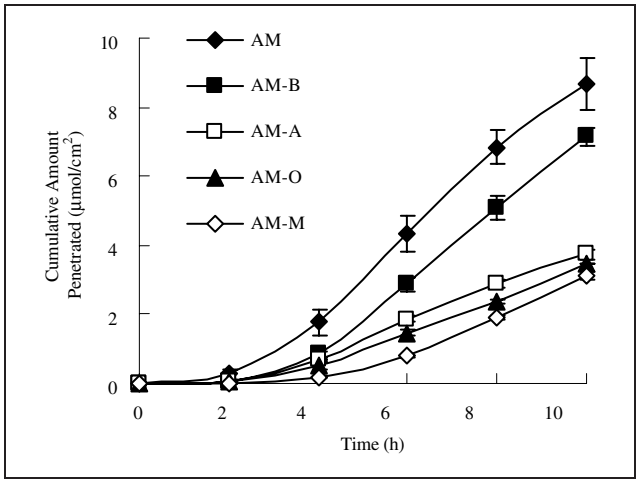


Fig. 2: Permeation profiles of AM and its complexes from the EI system through excised rat skin. Each data point represents the average value of three permeation experiments

AM, its solubility in the EI system is much higher than that of its complexes. Consequently the permeability coefficient of AM is the lowest of the five compounds.

The excised rat skin contains the epidermis (divided into the stratum corneum (SC) and the viable epidermis) and the dermis. The viable epidermis and dermis (ED) are similar and regarded as an aqueous protein gel (Scheuplein 1976). Drugs penetrate the skin according to the following proces. Firstly, drugs partition between the EI system and the SC, then diffuse through the SC. Following this, they partition between the SC and the ED, and finally diffuse through the ED. After drugs reach the dermis, they are absorbed by the blood capillaries within minutes.

The permeability through excised rat skin can be defined by its resistance (Fang et al. 2003).

$$R = R_{SC} + R_{ED} \quad (1)$$

Where R, R_{SC} and R_{ED} are the resistance of excised skin (full thickness), SC and ED, respectively. The resistance can be expressed as the reciprocal of the permeability coefficient, P, which is the product of the partition coefficient and the diffusion coefficient divided by the thickness of the membrane.

$$R = \frac{1}{P} = \frac{1}{P_{SC}} + \frac{1}{P_{ED}} = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h_{ED}}{D_{ED}K_{ED}} \quad (2)$$

Where P, P_{SC} and P_{ED} are the permeability coefficients through excised skin, SC and ED; D_{SC} and D_{ED} are the corresponding diffusion coefficients; K_{SC} and K_{ED} are the partition coefficients between the EI system and SC, SC and ED separately. Also, h_{SC} and h_{ED} are the thickness of SC and ED, and they are assumed to be relatively constant. The cells of the SC are cornified and closely packed, so the SC is the principal barrier in the pene-

Table 3: Permeation parameters, skin contents and partition coefficients between the skin and the EI system (log $K_{S/EI}$) of AM and its complexes (mean ± S.E., n = 3)

Drug	log P	J_s (μmol/cm²/h)	Q_{10} (μmol/cm²)	t_{lag} (h)	Skin content (μmol/g)	log $K_{S/EI}$ (ml/g)
AM	−2.13 ± 0.04	1.14 ± 0.09	8.67 ± 0.73	2.20 ± 0.39	74.72 ± 4.56	0.49 ± 0.03
AM-B	−0.67 ± 0.01	1.05 ± 0.03	7.14 ± 0.24	3.23 ± 0.09	50.01 ± 3.66	10.26 ± 0.75
AM-A	−0.12 ± 0.02	0.49 ± 0.03	3.73 ± 0.14	2.27 ± 0.22	15.10 ± 1.18	23.40 ± 1.83
AM-O	0.49 ± 0.02	0.48 ± 0.02	3.48 ± 0.007	2.84 ± 0.36	4.13 ± 0.48	26.90 ± 3.12
AM-M	−0.01 ± 0.02	0.58 ± 0.03	3.12 ± 0.13	4.66 ± 0.10	10.84 ± 0.95	18.28 ± 1.60

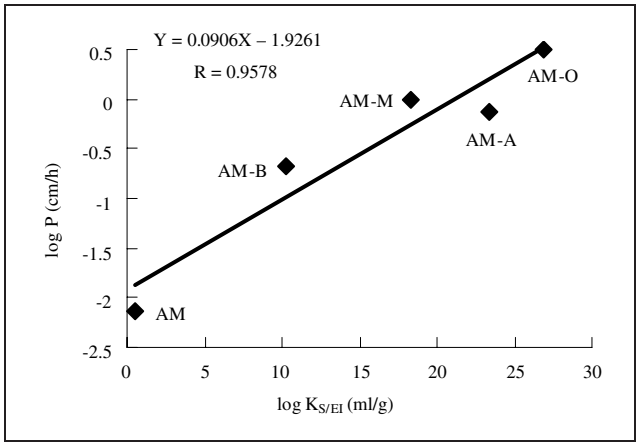
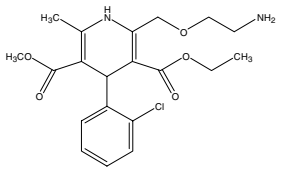


Fig. 3: Relationship between the permeability coefficients (log P) and skin/EI system partition coefficients (log $K_{S/EI}$) of AM and its complexes. Each data point represents the average value of three permeation experiments

tration (Abraham et al. 1995). In contrast, the ED is an aqueous environment for the permeants. Therefore, $D_{SC} \ll D_{ED}$. Three parameters, K_{SC}, D_{SC} and K_{ED}, will be discussed below separately to explain the differences in the fluxes and permeability coefficients of AM and its complexes.

The rank order of the partition coefficients of AM and its complexes between the EI system and the SC is AM-O > AM-A > AM-M > AM-B > AM (Table 3 and Fig. 3), which is almost identical to the rank order of the permeability coefficients of the five compounds. A linear correlation can be obtained between the steady-state fluxes of AM and its complexes and the skin contents of the five compounds (Fig. 4). The flux of AM or each complex through the skin seems to be related directly to the drug amount in the skin. This result strongly suggests that the partition from the EI system into the skin, or the SC, is the main factor governing the permeation of AM and its complexes.

Many studies have shown that the major negative determinant of diffusion across the SC is the H-bonding of the penetrants (e.g. Pugh et al. 1996; Cronin et al. 1998). Based on the theory of functional groups as predictors of diffusion, the order of retardation coefficients of functional groups is acid > alcohol > phenol > ketone > ether. The carboxylic group of adipic acid, oxalic acid and maleic acid is a potent H-bonding group. However, the formation of complexes masks the carboxylic group, which makes H-bonding with the SC impossible. Thus the H-bonding ability of AM and its complexes is almost identical, indicating that $D_{SC\text{-}AM} \approx D_{SC\text{-}complexes}$.



Amlodipine base

For lipophilic drugs, the partition from the SC into the ED becomes a rate-limiting step as far as skin penetration is concerned. The SC is essentially a lipidic layer. Lipophilic drugs pass through the SC, and then they must transfer directly into the aqueous medium – ED. Therefore lipophilic drugs will remain in the SC (Moss et al. 2002). There is an inverse relationship between log P and log $K_{O/W}$ (Fig. 5). This result agrees well with a previous study by Fang et al. (2003). Their investigation came to the conclusion that in the case of isopropyl myristate (IPM), the experimentally determined permeability coefficients generally decreased with the increasing n-octanol/water partition coefficients of the anti-inflammatory drugs having a wide range of lipophilicity (log $K_{O/W} = 0.5 \sim 4.88$).

AM, with a log $K_{O/W} = 1.50$, is lipophilic, so the partition from the SC into the ED becomes a barrier for the transdermal passage of AM. On the other hand, its complexes, having a log $K_{O/W} = -0.34 \sim 0.65$, are hydrophilic. These complexes can partition into the ED much easier than AM after diffusion through the SC. This means that $K_{ED\text{-}AM} < K_{ED\text{-}complexes}$. This could be another factor resulting in a lower permeability coefficient of AM compared with its complexes.

In conclusion, the DSC thermograms provide an evidence of the formation of complexes. The method of ion pairing between AM and organic acids does not increase the flux of AM through excised rat skin. The highest flux is obtained by AM, but it has the lowest permeability coefficient. The four complexes studied all exhibited a lower flux and higher permeability coefficient than AM. The ex-

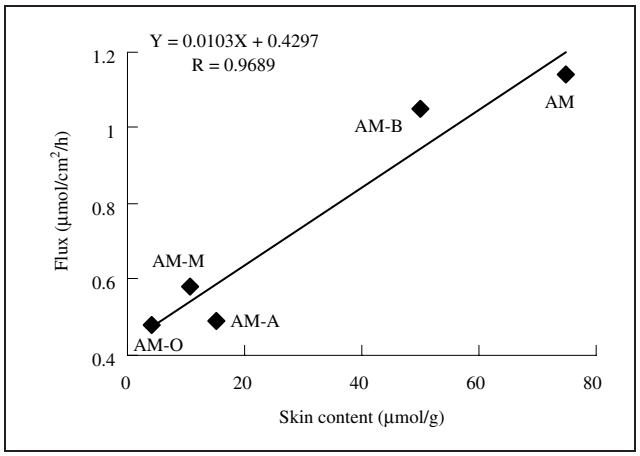


Fig. 4: Relationship between the steady-state fluxes and skin contents of AM and its complexes. Each data point represents the average value of three permeation experiments

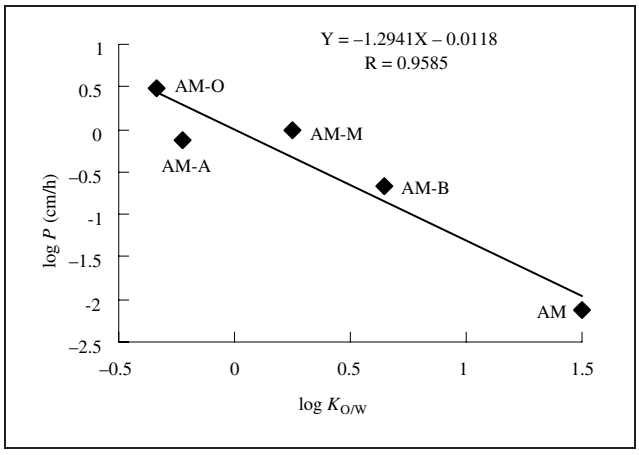


Fig. 5: Relationship between the permeability coefficients (log P) and *n*-octanol/water partition coefficients (log $K_{O/W}$) of AM and its complexes. Each data point represents the average value of three permeation experiments

periment involving skin uptake proves that the partition between the SC and the EI system is the most significant factor governing the permeation of AM and its complexes. In addition, the lipophilicity of the drugs, as indicated by the log $K_{O/W}$ value, influences the partition from the SC into the ED. The highest lipophilicity of AM, among the five compounds tested, is also a negative factor leading to the lowest permeability coefficient.

2.2. Comparison between previous reports and the present study

Many researchers have reported the enhancing effect of ion pairing on the transdermal flux of drug (e.g. Fang et al. 2003; Cheong and Choi 2002). However, an opposite result was obtained in the current study. The increased drug flux induced by ion pairing in the previous investigations can be attributed to the improved permeation properties of drug. Comparing the previous reports with the present study, two points of transport properties are markedly different.

(1) The melting points of complexes are lower than that of the corresponding parent compound in the previous research. In contrast, the melting point of AM (142.32 °C) is the lowest of the five compounds.
(2) The formation of complexes leads to a significant increase in aqueous solubility and minor decrease in liposolubility in the previous investigations. However, the liposolubilities of amlodipine complexes are much lower than that of AM, which is indicated by the solubilities in the EI system and the n-octanol/water partition coefficients of five compounds. Furthermore, the aqueous solubilities of amlodipine complexes are not increased significantly compared with AM (Table 2).

Consequently, the flux of AM is higher than any other ion pairs evaluated, which is different from the previous studies.

3. Experimental

3.1. Materials

3.1.1. Drugs and vehicles

AM-B was kindly donated by Ningxia Kangya Drug Manufacturing Co., Ltd. (Ningxia, China). NaOH and oxalic acid were supplied by Shenyang Zhengxin High-Technologies Research Institute Reagent Department (Shenyang, China). Maleic acid and adipic acid were obtained from Tianjin Standard Chemicals Co., Ltd. (Tianjin, China) and Shenyang Dongxing Chemical Factory (Shenyang, China), respectively. *n*-Octanol, polyethylene glycol 400 (PEG 400) and ethanol absolute were purchased separately from Beijing Fuxing Chemical Factory (Beijing, China), Tianjin Bodi Chemicals Co., Ltd. (Tianjin, China) and Tianjin Baishi Chemicals Co., Ltd. (Tianjin, China). IPM was obtained from China National Medicines Co., Ltd. (Beijing, China). HPLC grade methanol was obtained from Yuwang Chemicals Co., Ltd. (Shandong, China). All the other chemicals and solvents were of analytical reagent grade.

3.1.2. Animals

Male Wistar rats weighing 200 ± 20 g (6–8 weeks old) used in all the experiments were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The experiments were performed in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. The abdominal skin of the rats was used in the transdermal experiments. The hair of the abdominal skin was carefully clipped under anesthesia with urethane (20%, w/w, i.p.) and about 5 cm^2 (circle of 2.5 cm diameter) of full thickness skin was excised from the shaved site. The fat and sub-dermal tissues were removed with surgical scissors. The skin was washed with normal saline and kept frozen at −20 °C. The skin was checked to ensure that no obvious defects were present prior to the experiments.

3.2. Methods

3.2.1. Preparation of amlodipine and its complexes

AM was prepared from AM-B. AM-B was dissolved in methanol at 50 °C, treated with an equal molar amount of aqueous sodium hydroxide solution, stirred and allowed to crystallize out at 4 °C. The precipitated free base was collected by filtration and then dried in an oven for 3 h (McDaid and Deasy 1996). Its complexes were prepared from free base and the corresponding acids by dissolving them in methanol separately at 50 °C, mixing and stirring. The methods of crystallization, filtration and drying were the same as those used for the preparation of free base.

3.2.2. DSC

DSC measurement was performed using a Shimadzu DSC-60 thermal analyzer. Samples of 3–5 mg were placed in a standard aluminum crucible fitted with a perforated lid for scanning. An empty pan was used as a reference. The samples were heated at a rate of $5\ °C \cdot min^{-1}$ over a temperature range of 20–300 °C. The melting points and the enthalpies of fusion are listed in Table 1.

3.2.3. Solubility

The solubilities of AM and its complexes in the EI system and water were determined at 32 °C which reflects the body surface temperature. An excess of AM or each complex was dispersed into approximate 2 ml solution in a sealed glass vial. Each vial was shaken in a water bath for 48 h, and then 1 ml was transferred to a polypropylene micro-vial and centrifuged. The concentration of AM and its complexes in the samples was assayed by HPLC after appropriate dilution with methanol.

3.2.4. n-Octanol/water partition coefficient

The partition coefficients of AM and its complexes were determined in *n*-octanol/water. Initially, *n*-octanol and water were shaken together, the then allowed to stand for 24 h to ensure mutual saturation. A precise amount of AM or each complex was dissolved in *n*-octanol, and then an equal volume of water was added. After that, the system was shaken in a water bath at 32 °C for 48 h. The initial concentration in *n*-octanol was defined as C. The residual concentration in *n*-octanol was C_w measured by HPLC and hence the amount partitioned into aqueous phase was $C - C_w$. The partition coefficient ($K_{O/W}$) could be obtained from the following formula:

$$K_{O/W} = C_w/(C - C_w) \quad (3)$$

3.2.5. In vitro transdermal experiments from the EI system

The experimental equipment consisted of a donor and a receiver compartment of equal volume. The effective diffusion area was 0.95 cm^2. About 5 cm^2 of abdominal skin was mounted between the two diffusion cells which were stirred magnetically and thermoregulated with a water jacket at 32 °C. The donor cell was filled with 2.5 ml drug suspension (an excess of drug was added to the EI system) and the receiver side contained 2.5 ml receiver solution (PEG400 : pH7.4 phosphate buffer = 4 : 6). Two ml samples were withdrawn at regular intervals from the receiver side and an equal volume of receiver medium was immediately added to keep the volume constant. The concentration in each sample was determined by HPLC after centrifugation.

3.2.6. Skin uptake experiments

A procedure similar to that used for the *in vitro* permeation experiment was performed to determine the amount of AM and its complexes in the skin. The excised rat skin was mounted between 2-chamber diffusion cells that connected to a water bath at 32 °C. The dermis side was covered with a plastic sheet, and the SC side was filled with 2.5 ml suspension of AM or each complex. After exposure to the drug solution for 10 h, the excess drug on the skin surface was gently wiped off with methanol swabs. The treated skin site (0.95 cm^2) was punched out, cut, soaked in methanol for 48 h, and homogenized. After centrifugation at $16000 \times g$ for 5 min, the supernatant was analyzed by HPLC. The drug concentration in the treated skin (skin content) was determined as the amount of drug in the skin divided by the weight of skin. The partition coefficient between the skin and the EI system ($K_{S/EI}$) was calculated by dividing the skin content by the solubility of drug in the EI system.

3.2.7. Data analysis

The amount of drug that had penetrated at each sampling interval was obtained from the measured concentration and volume of the receiver phase. The cumulative amount of drug was calculated by the following formula:

$$Q = \sum_{i=2}^{n} (2.5C_i - 0.5C_{i-2})/A \quad i = 2, 4, 6 \ldots \quad (4)$$

where Q is the cumulative amount penetrated; C_i is the concentration in the receiver compartment at the time i; and A is the effective diffusion area (0.95 cm^2). Experiments were performed for 10 h. The cumulative amount penetrated from unit area vs. time was plotted. The steady-state flux (J_s) was calculated from the slope of the linear portion of the plotted curve. The lag time (t_{lag}) was the intercept obtained by extrapolation of the linear portion to the time axis. The permeability coefficient (P) was obtained by dividing J_s by the drug solubility in the EI system.

3.2.8. Analytical method

The concentrations of AM and its complexes were measured by HPLC. The HPLC equipment consisted of a HITACHI L7110 pump, a HITACHI UV–VIS L-7420 detector (both from Hitachi High-Technologies Corporation, Tokyo, Japan), and a HT-220A column temperature controller (Tianjin, China). Analysis was performed on a 5-μm ODS column (200 mm × 4.6 mm, DIKMA technologies, Beijing, China) operated at 40 °C. The mobile phase was methanol-0.03 mol/L KH_2PO_4 buffer solution (7 : 3) at a flow rate of 1 ml/min. The wavelength of the detector was 238 nm and the internal standard was 8 μg/ml propylparaben. Calibration curves were constructed using the peak area ratio between AM-B and propylparaben vs. the molar concentration of AM-B in solution. Comparison of quantity of AM and its complexes permeated was carried out using molar concentrations. The retention times of the drug and the internal standard were approximately 4.3 min and 5.8 min respectively.

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