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RESEARCH PAPER

Relationship Between the Enhancement Effects of Chemical Permeation Enhancers on the Lipoidal Transport Pathway Across Human Skin Under the Symmetric and Asymmetric Conditions *In Vitro*

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ABSTRACT

Purpose Previously, the mechanisms of action of chemical permeation enhancers (CPEs) were studied, and a quantitative structure-enhancement relationship for the lipoidal transport pathway of the stratum corneum was established under symmetric and equilibrium conditions. The present study examined whether the effects of CPEs under the asymmetric conditions could be predicted by those determined using the symmetric transport experimental approach.

Methods Both symmetric (same CPE concentration in both donor and receiver chambers) and asymmetric (CPE in the donor chamber only and phosphate-buffered saline solution in the receiver) transport experiments were carried out in a two-chamber side-by-side diffusion cell with human epidermal membrane (HEM). Corticosterone was the model permeant to probe the effects of CPEs upon the HEM lipoidal pathway under these conditions.

Results A correlation between the experimental enhancement factors under the asymmetric conditions (E_{Asym}) and those under the symmetric conditions (E_{Sym}) was observed. The potencies of CPEs based on their donor concentrations are related to their lipophilicities.

Conclusions The results suggest that the symmetric configuration findings in the previous studies can be used to explain the effects of CPEs under the asymmetric condition likely encountered in practice and to understand drug delivery enhancement in transdermal enhancer formulation development.

KEY WORDS asymmetric · chemical permeation enhancer · human skin · lipoidal pathway · skin transport experiments · symmetric · transdermal

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INTRODUCTION

It is generally realized that stratum corneum (SC), the outermost layer of the skin comprising highly flattened, keratin-filled cells embedded in multiple lipid bilayers, is a major barrier to transdermal drug delivery (1). The common approach to increase transdermal drug absorption is the use of skin permeation enhancers. One of the most widely used enhancers are chemical permeation enhancers (CPEs), the pharmacologically inactive chemicals that can reversibly enhance drug transport across the SC by either increasing drug diffusivity within the membrane and/or by increasing drug-membrane partitioning (2–5). Over the past decades, numerous CPEs have been studied with the aim of gaining better insights into the relationship between the nature of the enhancers and their effectiveness in drug permeation enhancement. In the simple form of *in vitro* studies of CPEs, the investigated enhancer is usually applied with a drug in solution (6,7) or suspension (8–10) to one side of the skin membrane, and the effectiveness of the enhancer compared to a control is determined by the ratio of drug transport with the enhancer to that with the control. Under this approach, the structure/function relationship between CPEs and their effects as skin permeation enhancers have been investigated.

In the past two decades, a number of studies have employed a different approach to establish a quantitative

structure-enhancement relationship for the lipoidal pathway of the SC to understand the mechanisms of action of CPEs (11–16). In these studies, permeation experiments were conducted under symmetric and equilibrium conditions (i.e., aqueous enhancer solution in both the donor and receiver chambers of a side-by-side diffusion cell and the enhancer in equilibrium with the skin membrane). Under these conditions, the complications in data interpretation arising from enhancer concentration (or activity) gradients across the membrane (17,18) were avoided. The permeability enhancement factor, *E*, the ratio of the permeant flux with the enhancer solution to that with the control phosphate-buffered saline solution (PBS), was determined in these experiments with a moderate lipophilic model permeant, corticosterone (CS). The enhancement factor was corrected for any changes in the chemical potential of the permeant in the enhancer solution with respect to that in PBS; this allowed the comparison of enhancement factor at the same permeant thermodynamic activity. CS was chosen as a surrogate permeant as it was found to be appropriate for quantitatively probing the lipoidal pathway of hairless mouse skin (HMS) SC (11–13,15,16). Under the aforementioned conditions, HMS is considered a proper model for the evaluation of the effects of CPEs on the lipoidal pathway of human epidermal membrane (HEM) (19). These studies have gradually improved our understanding of the mechanisms of CPEs and their structure-enhancement relationship.

The purpose of the present study was to examine whether the effects of CPEs in the asymmetric transport experiments where the aqueous enhancer solution was present only in the donor chamber and PBS in the receiver chamber (i.e., conditions similar to those in practice) could be predicted by those determined using the symmetric experimental approach (the aqueous enhancer solution was present in both donor and receiver chambers and the enhancer was in equilibrium with HEM). A correlation between the enhancer effects under the asymmetric and symmetric conditions would support the utility of the mechanistic findings in the previous symmetric transport studies for transdermal delivery in practice.

MATERIALS AND METHODS

Materials

Corticosterone (CS), thymol, 1-butanol, 1-pentanol, 1-hexanol, 1-octanol, 2-phenylethanol (2-PE), and sodium azide (NaN_3) were purchased from Fluka Chemika (Milwaukee, Switzerland). Carvacrol was purchased from Sigma Chemical Co. (St. Louis, MO). 1-Hexyl-2-pyrrolidone (HP) was purchased from ISP Co., Ltd. (Milford, CT). 1-Octyl-2-pyrrolidone (OP) was received as a gift from ISP (Thailand) Co., Ltd. (Wayne, NJ). 1-Octyl-2-azacycloheptanone (OAZ) was synthesized at the Chemical Synthesis Facility, Department of Medicinal Chemistry (University of Utah). Sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) and disodium hydrogenphosphate anhydrous (Na_2HPO_4) were purchased from Ajax Finechem (NSW, Australia). Sodium chloride (NaCl) and high pressure liquid chromatography (HPLC) grade methanol (MeOH) were purchased from Fisher Scientific (Leicestershire, UK). Phosphate-buffered saline solution (PBS) pH 7.4 containing 0.02% NaN_3 was prepared (20). Various concentrations of enhancer solutions were prepared by dissolving the enhancer in PBS (namely enhancer/PBS solutions). The concentrations of enhancers used in the present study were lower than their aqueous solubilities.

Preparation of HEM and Human Stripped Skin

Human skin from abdominoplastic surgical operations of female patients aged between 35–75 years was supplied by the Department of Surgery, Yanhee General Hospital, Thailand. The skin sample was obtained within a few hours after operation. HEM, comprising the SC and viable epidermis, was separated from the dermis by immersing the skin samples free from fatty tissues in water at 60°C for 1 min (21,22). After heat treatment, the epidermis sheet was separated from the underlying dermis and immersed in PBS. The HEM sheet was then patted dry with tissue paper, wrapped in aluminum foil, and subsequently kept in a freezer at −20°C for later use. The Committee on Human Rights Related to Human Experimentation, Mahidol University, Bangkok, Thailand, approved the experimental protocol prior to the study.

Human stripped skin, consisting of only the viable epidermis, was prepared by the removal of the SC via tape-stripping 30–45 times (fresh tape for each stripping) using a 2-inch package sealing tape (3M Co., St. Paul, MN). After tape-stripping, the viable epidermis sheet was separated from the underlying dermis by heat treatment as described above. The efficiency of the tape-stripping was checked by trypsin digestion (0.0005% trypsin solution at 37 ± 1°C for 18 h) of the stripped skin (19) after the transport experiments. Only stripped skin of more than 95 % SC removal (by weight) was used in the asymmetric transport study.

HEM Transport Experiments

Prior to the transport studies, the frozen HEM samples were cut into pieces of the desired size (~2 cm × 2 cm), allowed to thaw at room temperature, and hydrated overnight in PBS. Each hydrated HEM was mounted between the two half-cell of the diffusion cell with a

regenerated cellulose membrane (Spectra/Por® MWCO 12,000–14,000, Spectrum Laboratories Inc., Rancho Dominguez, CA). The cellulose support membrane was previously soaked also in PBS overnight and placed next to the HEM between the viable epidermis side of the HEM sample and the receiver chamber (19,23). Two milliliters of PBS were placed into both donor and receiver chambers. HEM was then equilibrated in the well stirred side-by-side diffusion cells in a circulating water bath at 37±1°C for 12 h (24). The integrity of the HEM was checked by electrical resistance of the membrane. HEM with electrical resistance > 15–20 kΩ cm^2 was shown to demonstrate intact HEM barrier (19,25,26). As a result, only HEM samples with initial resistance ≥ 15 kΩ cm^2 were used in the present study.

Enhancer/PBS solution was prepared as described in the "Materials" section. Saturated CS in the enhancer/PBS solution was prepared by the following procedure. An excess amount of CS was added in the enhancer/PBS solution. The mixture was then equilibrated in a shaking machine for 48 h at room temperature. After equilibration, the supernatant was filtered (first part of filtrate was discarded) through a 0.45-μm Millipore filter (Bioscience, life Science Products). Both symmetric (same CPE concentration in both diffusion cell chambers) and asymmetric (CPE in the donor only and PBS in the receiver) transport experiments were performed in a two-chamber side-by-side diffusion cell (each chamber has a 2-mL volume with an effective diffusional area of around 0.71 cm^2) with HEM and enhancers, i.e., thymol, carvacrol, 1-butanol, 1-pentanol, 1-hexanol, 1-octanol, 2-PE, HP, OP, and OAZ. In the symmetric transport experiments, the enhancer/PBS solutions in both donor and receiver chambers were replaced periodically (9 times of 20 min each) with fresh solution so as to equilibrate HEM with the enhancer (19), and the concentration of enhancer solution was determined at the beginning and the end of the experiments to assure no significant depletion of the enhancer. Saturated CS in the enhancer/PBS solution (filtered saturated solution prepared before the experiments) was then added to the donor chamber after enhancer equilibration. The concentration of CS in the donor chamber was measured and found to remain essentially constant over the duration of the transport experiment. In the asymmetric transport studies, similar to that described in the symmetric transport experiments to attain a steady-state concentration profile of the enhancer in HEM, the enhancer/PBS solution in the donor chamber and PBS in the receiver were replaced periodically (9 times of 20 min each) with fresh solutions. The concentration of the enhancer in the donor chamber was also checked before and after the experiments as in the symmetric transport experiment.

Samples were withdrawn from the donor and receiver chambers at predetermined time intervals in both symmetric and asymmetric transport experiments (e.g., 5, 7, 8 and 9 h). Samples of 10-μL aliquot were withdrawn from the donor chamber and 500-μL aliquot from the receiver chamber. The same volume of the fresh solution was replaced to the receiver chamber after each aliquot removal to maintain a constant volume. The samples were suitably diluted with the mobile phase and then analyzed by HPLC for CS. Experiments were carried out around three to five times longer than the transport lag times, using at least 4 different skin samples for each enhancer tested. In a separate study, transport experiments were performed for 46 h with PBS and a selected enhancer (i.e., 1-octanol) to ensure that the shorter duration (9 h) CS transport experiments attained steady state. The total permeability coefficient (P_T) was determined from the slope of linear region (steady-state) of the plot of cumulative transport amount across the membrane *versus* time and the donor concentration. Experiments conducted without the enhancers but only PBS were the baseline control. To determine the concentration of the enhancer and to calculate the enhancer permeability coefficient, the samples in the asymmetric transport experiments were also analyzed by HPLC or gas chromatography (GC) for the enhancer.

Stripped Skin Transport Experiments

The asymmetric transport experiments with stripped skin were carried out in the same manner as the asymmetric transport experiments with HEM to determine the viable epidermis permeability coefficients for CS, 1-butanol, 1-octanol, OP, and OAZ.

Determination of CS Solubility

As previously described (12,27), approximately 2 mg of CS was added in screw-capped Pyrex culture tubes with 1 mL of PBS or an enhancer/PBS solution. The drug suspension was shaken in a thermostatically controlled water bath at 37±1°C for 72 h. Afterward, the culture tubes were centrifuged at 1500×g for 15 min (Hettich Universal 30F, Tuttlingen, Germany), and the clear supernatants were analyzed for CS by HPLC.

Determination of CPE Solubility

In the present study, the CPEs are liquid except thymol. The aqueous solubility of 1-butanol was not determined due to the miscibility of 1-butanol and PBS. The aqueous solubilities of the CPEs were determined to ensure that the enhancer concentrations in the enhancer/PBS solutions used were below their aqueous solubilities in the present study. To determine the solubility of the CPE in PBS, an excess amount of the enhancer was added to PBS. The mixture was equilibrated in a shaking machine for 48 h at

room temperature. The 48-h equilibration period was chosen in this study because CPE equilibrium in PBS was observed within 24 h. After equilibration, the mixture was centrifuged at 1500×g for 15 min. The clear supernatant was analyzed for the enhancer by GC or HPLC.

GC Analysis

The GC system (Perkin Elmer, Norwalk, CT) consisted of an injector, controller, flame ionization detector (FID), and fused silica capillary column of 0.32-mm column ID, 0.25-μm film thickness, and 30-m length (Supelco, Bellefonte, PA). All the analyses were performed using nitrogen as the carrier gas at a flow rate of 2.5 mL/min. The samples were injected using split mode with a split ratio of 5:1. The injector temperature and FID detector temperature were 230°C and 230°C, respectively. A temperature programming of 60–200°C at 25–45°C/min was used. For all the GC analyses performed, an appropriate internal standard for each alcohol enhancer (1-butanol, 1-pentanol, 1-hexanol, 1-octanol, 2-PE, thymol, and carvacrol) was prepared and added to the standard and sample solutions. The standard solutions to construct the calibration curves were prepared in MeOH. The concentration was calculated based on the peak area ratio of each alcohol and that of the internal standard.

HPLC Analysis

The HPLC system consisted of two Shimadzu pumps (Kyoto, Japan), a variable wavelength UV absorbance detector, and a Sil-10A Shimadzu autoinjector with a 25 cm BDS Hypersil C18 column (Hypersil, Thermo Electron Corporation, Runcorn, UK). The mobile phases, flow rates, detection wavelengths, and retention times for CS, HP, OP, and OAZ were: 65% (v/v) MeOH in water, 1.0 mL/min, 248 nm, 6.5 min; 55% (v/v) MeOH in water, 1.0 mL/min, 220 nm, 13.9 min; 80% (v/v) MeOH in water, 1.0 mL/min, 220 nm, 5.9 min; 85% (v/v) MeOH in water, 1.0 mL/min, 220 nm, and 6.8 min, respectively. The standard solutions to construct the calibration curves were prepared in the mobile phase. The concentration was determined based on peak area measurement.

Permeability Coefficients and Enhancement Factors

The total permeability coefficient (P_T) across HEM can be calculated as follows:

$$P_T = \frac{1}{\frac{1}{P_P+P_L} + \frac{1}{P_{Epi}}} \quad (1)$$

where P_{Epi}, P_P, and P_L are the permeability coefficients across the viable epidermis, the SC pore pathway, and the SC lipoidal pathway, respectively. P_P and P_L represent the parallel transport pathways across SC. For the permeation of a moderate lipophilic compound (i.e., CS) under moderate permeation enhancement ($P_L << 10^{-5}$ cm/s), the lipoidal pathway is the transport rate determining step and this allows the approximation:

$$P_T \approx P_L \quad (2)$$

The enhancement factor for transport across the lipoidal pathway of HEM under the symmetric condition (E_{Sym}) was determined by

$$E_{Sym} = \left(\frac{P_{L,X,Sym}}{P_{L,0}}\right).\left(\frac{S_X}{S_0}\right) \quad (3)$$

where $P_{L,X,Sym}$ is the lipoidal pathway permeability coefficient of CS when the solvent in both chambers is enhancer/PBS, and $P_{L,0}$ is the CS permeability coefficient when both chambers are PBS. S_X and S_0 are the CS solubilities in enhancer/PBS and PBS, respectively. The total permeability coefficients ($P_{T,X,Sym}$ and $P_{T,0,Sym}$) were determined by

$$P_{T,X,Sym} \text{ or } P_{T,0,Sym} = \left(\frac{1}{AC_{D,Sym}}\right) \cdot \left(\frac{dQ_{Sym}}{dt}\right) \quad (4)$$

where A is the effective diffusion area of the diffusion cell, $C_{D,Sym}$ is the concentration of the permeant in the donor chamber, and dQ_{Sym}/dt denotes the slope of the steady-state region of the cumulative amount of permeant transported into the receiver chamber *versus* plot under sink conditions in the symmetric transport experiments.

The enhancement factor for transport across the lipoidal pathway of HEM under the asymmetric condition (E_{Asym}) in the present study was determined by

$$E_{Asym} = \left(\frac{P_{L,X,Asym}}{P_{L,0}}\right).\left(\frac{S_X}{S_0}\right) \quad (5)$$

where $P_{L,X,Asym}$ is the lipoidal pathway permeability coefficient of CS when the solvents in donor chamber and receiver chamber are enhancer/PBS and PBS, respectively.

$P_{T,X,Asym}$ and $P_{T,0,Asym}$ were determined by

$$P_{T,X,Asym} \text{ or } P_{T,0,Asym} = \left(\frac{1}{AC_{D,Asym}}\right).\left(\frac{dQ_{Asym}}{dt}\right) \quad (6)$$

where $C_{D,Asym}$ is the concentration of the permeant in the donor chamber, and dQ_{Asym}/dt is the slope of the steady-state region of the plot of cumulative amount of permeant transported into the receiver chamber *versus* time under sink conditions.

For the CPEs in the asymmetry transport experiments, the enhancer permeability coefficients were determined in a similar manner as that for CS using the slopes of the steady-state regions in the cumulative amount of enhancer transported *versus* time plots and an equation similar to Eq. 6.

RESULTS

Symmetric and Asymmetric Transport Experiments and Enhancement Factors

Fig. 1a shows the typical cumulative amount of CS permeated across HEM *versus* time plot under the symmetric and asymmetric conditions of enhancer/PBS and its PBS control. For all enhancer conditions studied, significant flux enhancement was observed. The average CS transport lag time in PBS was approximately 2 h, consistent with those observed previously (19,23). The average transport lag times in the asymmetric experiments (~ 0.9–1.4 h) were also comparable to those observed previously with HMS (11–16) and HEM (19,23) under the symmetric conditions. In the 46-h transport experiments (Fig. 1b), steady-state fluxes were attained within 2–3 h and maintained over the entire period of transport experiments. This supports the validity of the 9-h symmetric and asymmetric transport experiments for measuring steady-state transport and studying skin penetration in the present study.

Table I summarizes the permeability coefficients of CS across HEM in the symmetric and asymmetric transport experiments (column 3 and column 5, respectively), the enhancement factors for CS transport across the lipoidal pathways of HEM under the symmetric conditions (E_{Sym}) and those under the asymmetric conditions (E_{Asym}) (column 4 and column 6, respectively), and the CS solubility ratios (CS solubility in the enhancer solution divided by CS solubility in PBS) (column 7). E_{Sym} and E_{Asym} were calculated from the ratio of CS permeability coefficients in enhancer/PBS to those in PBS for HEM from each human skin donor according to Eqs. 3 and 5, respectively. Thus, the HEM sample from the same human donor acted as the control to determine the enhancement factor in this experimental design. The E_{Sym} of 1-hexanol, 1-octanol, carvacrol, and thymol from previous HEM transport experiments (19,23) are shown in Table I for comparison. The E_{Sym} data in the present study are consistent with previous results (11–16,19,23). In all symmetric transport experiments, the permeability coefficients of CS were below 5×10^{-6}cm/s, except at 3.5 mM OP, and were significantly lower than the permeability of the human viable epidermis for CS ($13\pm6\times10^{-6}$cm/s). Under the asymmetric conditions, the permeability coefficients of HEM for CS were lower than those under the symmetric conditions and were below 3×10^{-6}cm/s in all cases. The CS solubility ratios ranged from 1.0 to 1.5 under the enhancer conditions in the present study, implying that the corrections for the different activity coefficients of CS in the aqueous enhancer systems and PBS were modest. The 0–50% changes in CS solubility were small compared to E_{Sym} and E_{Asym}, which were always greater than 2 and could be as large as 33.

Comparison of Experimental Enhancement Factors under Asymmetric and Symmetric Conditions

The E_{Sym} and E_{Asym} values *versus* enhancer concentrations in their respective transport experiments are shown in Fig. 2. The figure illustrates a trend of increasing enhancement effects of the enhancer on CS permeation with increasing the concentration of the enhancer under the asymmetric conditions similar to those observed under the symmetric conditions. For carvacrol and thymol, it was not feasible to study and compare the enhancement effects of the enhancers at higher concentrations due to the limitation of the enhancer aqueous solubilities. As expected, the results show that E_{Asym} was lower than E_{Sym}. At the lower enhancer concentrations that induced the lower symmetric

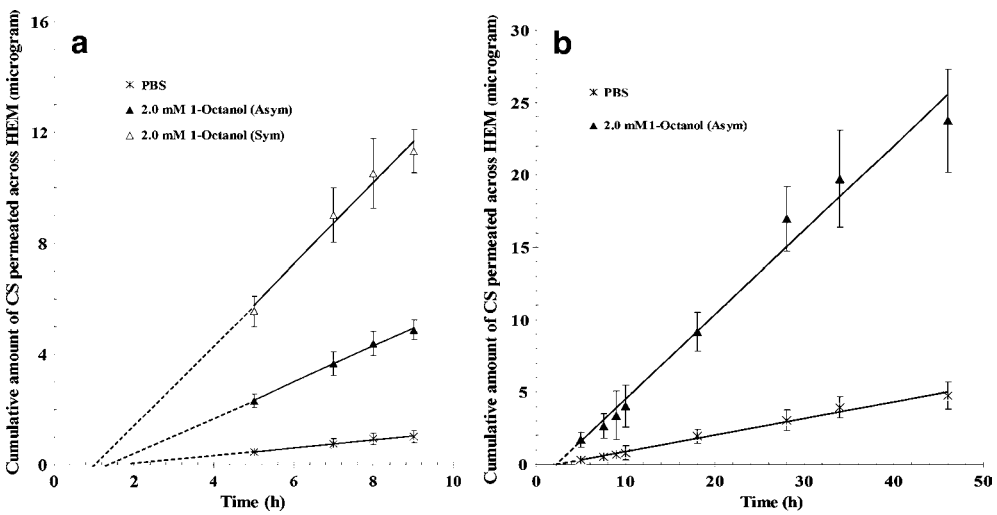

Fig. 1 Representative permeation-time profiles of cumulative amount of CS permeated across HEM. Each point represents mean ± SD ($n \geq 3$). The lag time was obtained by extrapolating the linear regression line (dotted line) to the time axis. (**a**) The transport experiments were performed for 9 h with PBS and 2.0 mM 1-octanol under the symmetric (Sym) and asymmetric (Asym) conditions, (**b**) The transport experiments were performed for 46 h with PBS and 2.0 mM 1-octanol under the asymmetric condition.

Table I HEM Permeability Coefficients of Corticosterone and Enhancement Factors in Symmetric and Asymmetric Transport Experiments and Corticosterone Solubility Ratios in PBS and Enhancer/PBS Solutions

Enhancer	Enhancer Concentration [a] (mM)	Symmetric Transport Experiments [b]		Asymmetric Transport Experiments [b]		CS Solubility Ratio [c]
		Permeability Coefficient of CS (10^{-7}cm/s)	Enhancement Factor [d] (E_{Sym})	Permeability Coefficient of CS (10^{-7}cm/s)	Enhancement Factor [e] (E_{Asym})	
1-Butanol	0	2.8 ± 1.0	–	2.8 ± 1.0	–	–
	218	16.3 ± 3.5	8.9 ± 0.6	9.2 ± 2.9	5.1 ± 0.7	1.51 ± 0.12
1-Pentanol	0	3.5 ± 0.4	–	3.5 ± 0.4	–	–
	93	29.8 ± 7.9	11.5 ± 2.2	12.5 ± 3.1	5.9 ± 1.3	1.33 ± 0.09
1-Hexanol	0		–	2.4 ± 0.9	–	–
	25		11.7 ± 0.4 [f]	10.1 ± 3.9	5.3 ± 0.6	1.23 ± 0.09
1-Octanol	0		–	2.5 ± 0.6	–	–
	1.2		3.6 ± 0.3 [f]	5.4 ± 1.9	2.0 ± 0.6	1.02 ± 0.05
	2.0		9.6 ± 3.1 [f]	11.2 ± 2.1	5.2 ± 1.9	1.02 ± 0.07
	2.4		19.6 ± 2.9 [f]	17.0 ± 2.9	6.2 ± 1.5	1.03 ± 0.04
2-Phenylethanol (2-PE)	0	2.8 ± 0.8	–	2.8 ± 0.8	–	–
	40	10.9 ± 3.5	4.9 ± 0.9	5.2 ± 2.0	2.6 ± 0.7	1.25 ± 0.03
	60	21.4 ± 8.7	10.0 ± 1.8	10.1 ± 2.4	5.4 ± 0.9	1.36 ± 0.08
	75	28.6 ± 9.9	15.3 ± 4.8	12.6 ± 3.8	6.9 ± 1.0	1.43 ± 0.06
1-Hexyl-2-pyrrolidone (HP)	0	3.0 ± 0.2	–	3.0 ± 0.2	–	–
	28.5	24.6 ± 1.9	10.6 ± 0.2	15.3 ± 1.8	6.5 ± 0.6	1.25 ± 0.08
1-Octyl-2-pyrrolidone (OP)	0	2.6 ± 0.9	–	2.6 ± 0.9	–	–
	1.5	20.1 ± 3.1	7.1 ± 0.5	6.8 ± 2.8	2.8 ± 0.9	1.02 ± 0.03
	2.3	34.1 ± 4.3	11.3 ± 1.5	13.0 ± 4.3	5.4 ± 1.5	1.05 ± 0.02
	2.8	45.1 ± 10	16.2 ± 2.0	18.5 ± 4.5	6.3 ± 1.0	1.06 ± 0.03
	3.5	94 ± 24	32.9 ± 4.9	26.1 ± 4.7	10.3 ± 1.8	1.10 ± 0.04
Carvacrol	0		–	2.5 ± 0.8	–	–
	3.0		9.5 ± 1.7 [g]	13.8 ± 3.4	6.0 ± 0.6	1.01 ± 0.05
Thymol	0		–	2.6 ± 0.9	–	–
	3.0		10.9 ± 1.6 [g]	15.8 ± 5.3	6.3 ± 1.3	1.01 ± 0.03
1-Octyl-2-azacycloheptanone (OAZ)	0	2.7 ± 0.8	–	2.7 ± 0.8	–	–
	0.30	12.8 ± 1.7	5.2 ± 0.5	6.0 ± 1.2	2.4 ± 0.7	1.00 ± 0.01
	0.46	28.4 ± 5.2	10.3 ± 0.2	14.2 ± 3.1	5.4 ± 0.3	1.01 ± 0.03
	0.54	44.5 ± 14.2	18.5 ± 2.2	24.3 ± 0.9	7.2 ± 0.8	1.01 ± 0.02

[a] Concentration of enhancer in PBS (enhancer/PBS). PBS alone with no enhancer (0 mM enhancer) was the PBS control.

[b] Mean ± SD ($n \geq 4$).

[c] Solubility ratio = (CS solubility in enhancer/PBS solution)/(CS solubility in PBS).

[d] E_{Sym} was calculated according to Eq. 3.

[e] E_{Asym} was calculated according to Eq. 5.

[f] E_{Sym} data obtained from Chantasart *et al*. (19).

[g] E_{Sym} data obtained from Chantasart *et al*. (23).

enhancement factors, e.g., $E_{Sym}=4$, E_{Asym} is close to the baseline, and higher concentrations of the enhancers are required under the asymmetric conditions to induce the same permeation enhancement as those when the enhancers are in equilibrium with the SC under the symmetric conditions. In general, E_{Sym} is at least 2 times greater than E_{Asym} for all the enhancer concentrations studied, and there is a correlation between E_{Sym} and E_{Asym}. The term isoenhancement concentrations were defined previously as the aqueous concentrations at which different enhancers induced the same extent of transport enhancement of the steroidal model permeants across the SC lipoidal pathway to

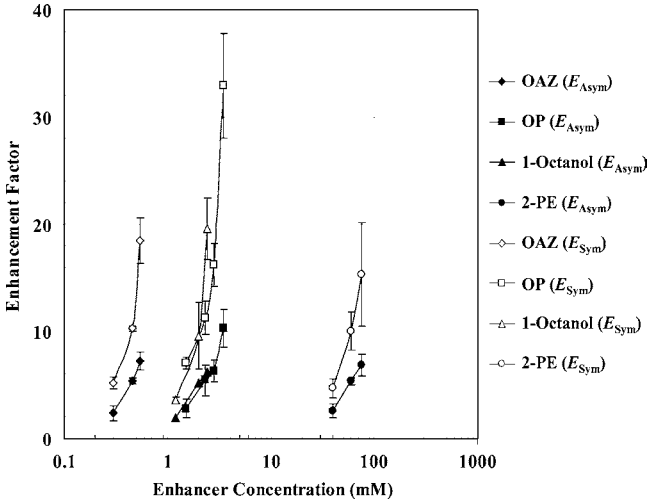

Fig. 2 E_{Sym} and E_{Asym} *versus* the concentration of enhancer in enhancer solution (mean ± SD, $n \geq 4$). E_{Sym} and E_{Asym} were calculated using Eqs. 3 and 5, respectively. Enhancer concentration is expressed in mM enhancer in PBS.

study the potencies of the enhancers (11–16,19,23). The relatively constant E_{Asym} values at the enhancer concentrations of $E_{Sym}=10$ for all the enhancers studied suggest that the enhancement effects on the rate-limiting barrier induced by the different enhancers at the isoenhancement concentrations are essentially the same. As discussed in previous HMS and HEM studies, the E_{sym} data show that the potencies of the CPEs based on their aqueous concentrations in the diffusion cells are related to the enhancer lipophilicities (11–13,15,16,19); higher aqueous enhancer concentrations are required for the less lipophilic enhancers to induce the same permeation enhancement for CS compared to the more lipophilic enhancers. The E_{Asym} data demonstrate a similar CPE potency and lipophilicity relationship as E_{sym}.

Enhancer Permeation across HEM

Table II lists the physicochemical properties of the enhancers used in the present study and their HEM and viable epidermis permeability coefficients. As shown in the table, the enhancers employed in the present study included a range of different classes of enhancers (i.e., alkyl alcohols, alkyl pyrrolidones, alkyl azacycloheptanone, and terpenes alcohols) with different molecular weight (from 74 to 225) and lipophilicities ($K_{O/W}$ from 7.6 to 6354). The permeability coefficients of these enhancers across HEM were found to range from 3.3 to 14.9×10^{-6}cm/s (column 4, Table II), suggesting that the SC is relatively permeable for these enhancers. To examine the situation further, the permeability coefficients of human stripped skin for four enhancers of different lipophilicities (1-butanol, 1-octanol, OP, and OAZ) were determined. The permeability coefficients of the enhancers across human stripped skin (i.e., viable epidermis) are in the range around $15–21\times10^{-6}$cm/s (column 5, Table II). The viable epidermis does not discriminate the transport of the enhancers of different molecular weight and lipophilicities possibly due to the barrier nature of the viable epidermis and the unstirred aqueous boundary layer.

Table II The Octanol-Water Partition Coefficients and Molecular Weight of Enhancers and the Permeability Coefficients of Enhancers across HEM and Stripped Skin in the Asymmetric Transport Experiments

Enhancer	$K_{O/W}$ (Log $K_{O/W}$)	Molecular Weight	Permeability Coefficient of Enhancer across HEM [e] (10^{-6}cm/s)	Permeability Coefficient of Enhancer across Viable Epidermis [e] (10^{-6}cm/s)
218 mM 1-Butanol	7.6 (0.88) [a]	74	3.3 ± 1.1	19.8 ± 6.5
93 mM 1-Pentanol	32 (1.51) [a]	88	7.5 ± 0.1	–
25 mM 1-Hexanol	121 (2.08) [b]	102	5.2 ± 0.2	–
2.0 mM 1-Octanol	1336 (3.12) [b]	130	13.5 ± 0.7	14.7 ± 3.7
60 mM 2-Phenylethanol (2-PE)	37 (1.57) [a]	122	5.3 ± 1.2	–
28.5 mM 1-Hexyl-2-pyrrolidone (HP)	80 (1.90) [c]	169	6.2 ± 1.9	–
2.3 mM 1-Octyl-2-pyrrolidone (OP)	884 (2.94) [c]	197	14.9 ± 1.1	15.5 ± 2.1
3.0 mM Thymol	3311 (3.52) [d]	150	13.8 ± 2.3	–
3.0 mM Carvacrol	3311 (3.52) [d]	150	14.4 ± 4.8	–
0.46 mM 1-Octyl-2-azacycloheptanone (OAZ)	6354 (3.80) [c]	225	14.6 ± 1.3	21.2 ± 1.8

[a] Octanol-water partition coefficients (Chapter 16). In: Lide DR, editor. CRC Handbook of Chemistry and Physics, 78th edition. New York: CRC Press; 1997, p. 39–43.

[b] Data obtained from Chantasart *et al.* (11).

[c] Data obtained from Warner *et al.* (15).

[d] Data obtained from Chantasart *et al.* (23).

[e] Mean ± SD ($n \geq 4$).

DISCUSSION

Prediction of Enhancement Factors under Asymmetric Conditions using Symmetric Enhancement Factors

The CPEs examined in the present study were from different classes of enhancers. Terpenes (i.e., thymol and carvacrol) are good CPE candidates because they are safe and effective skin permeation enhancers derived from plant essential oils. The US Food and Drug Administration classified them as "Generally Recognized As Safe" (GRAS) (28). They were reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (1–5%) (29,30). An azone derivative 1-octyl-2-azacycloheptanone was selected because azone derivatives were reported to be effective CPEs for both hydrophilic and lipophilic drugs and for peptides. They have low irritating potential and low toxicity when used at low concentrations (1–5%) (5). However, the clinical use of some CPEs is limited because of their toxicity/irritation potential. Erythema and other irritant cutaneous reactions were observed after pyrrolidone use on human skin (31). In addition, aliphatic alcohol of C6-C11 (e.g., 1-hexanol and 1-octanol) was reported to be potential skin irritant when applied undiluted for 4–24 h. Application of diluted alcohols resulted in a lower grade irritation (32). Short-chain alcohols (i.e., 1-butanol and 1-pentanol) were found to be used at relative high concentrations (10–40%) (33). In general, increasing the concentration of CPEs increases their enhancing effects, and an optimum balance should therefore be chosen between the enhancing and irritant effects.

Fig. 3 summarizes the E_{Asym} and E_{Sym} results and examines a possible correlation between E_{Asym} and E_{Sym} at the enhancer concentrations studied in the present experiments. Note that the concentrations of the enhancers studied were over two orders of magnitudes from 0.3 to 200 mM. Although there is experimental variability, the E_{Asym} *vs.* E_{Sym} correlation in Fig. 3 demonstrates the feasibility of using E_{Sym} to estimate E_{Asym}. In addition, the E_{Asym} values are consistent with model predictions within the data scattering (e.g., at $E_{Sym}=10$, $E_{Asym}=3.9–5.5$). These empirical model predictions (as shown in Appendix) were derived based on the results from previous studies on permeation enhancement under the symmetric condition (11–16,19,23). Together, these findings suggest that transdermal permeation enhancement commonly encountered in practice under asymmetric conditions (E_{Asym}) can be estimated using the E_{Sym} data obtained in previous studies (11–16,19,23). The E_{Asym} *vs.* E_{Sym} relationship allows the utilization of the E_{Sym} database from these previous studies in transdermal permeation enhancer development and in studying the quantitative structure enhancement relationship under the asymmetric conditions.

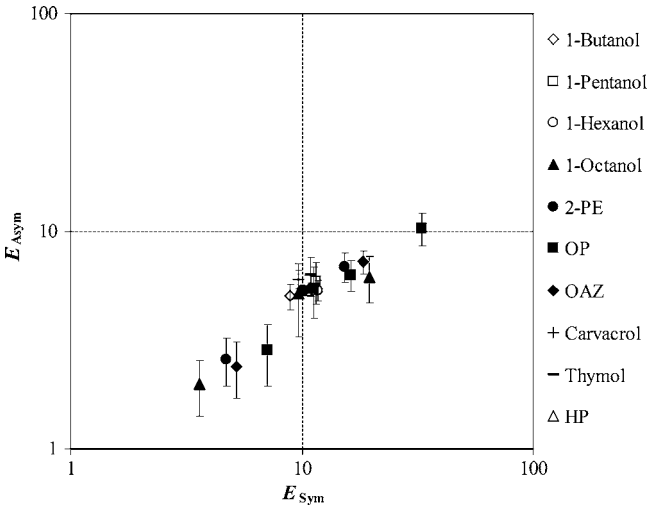

Fig. 3 Relationship between the experimental enhancement factors under the asymmetric conditions (E_{Asym}) and those under the symmetric conditions (E_{Sym}) (mean ± SD, $n \geq 4$).

A number of factors could contribute to the data scattering in the E_{Asym} *vs.* E_{Sym} correlation in Fig. 3. First, as the SC was the transport rate-limiting barrier for CS permeation under the conditions in the present study, different enhancer concentration profiles (concentration gradients) in the SC due to different enhancer physicochemical properties could lead to deviation from the E_{Asym} *vs.* E_{Sym} correlation. Particularly, the molecular weight and lipophilicities of the CPEs could affect the E_{Asym} and E_{Sym} correlation as the concentration profiles (and amounts) of the enhancers in the SC are related to the permeation of the enhancers and hence their molecular weight and lipophilicities. The studied enhancers have molecular weight ranging from 74 to 225 (column 3, Table II) and lipophilicities measured by $K_{O/W}$ from 7.6 to 6354 (column 2, Table II), and for some of these enhancers, the transport of the enhancers across HEM was not SC barrier-controlled. When the transport of the enhancers is not SC-controlled, higher concentrations of the enhancers in the SC are expected, and E_{Asym} would approach E_{Sym}. The enhancers with the viable epidermis as the transport rate-limiting barrier (viable epidermis-controlled permeation), therefore, are likely to induce higher E_{Asym} than those of SC-controlled. According to the data in Table II, the permeability coefficients of HEM for the more lipophilic enhancers (e.g., OP) approached the viable epidermis barrier limit, and the permeation of these enhancers across HEM was likely not to be SC-controlled. Despite this, the E_{Asym} data of these enhancers do not significantly deviate from the correlation. No significant difference was observed among the E_{Asym} *vs.* E_{Sym} correlation of the low and high molecular weight and low and high

lipophilic enhancers. One explanation is that the experimental variability encountered in the present study (e.g., skin-to-skin variability) does not allow the examination of small discrepancies in the E_{Asym} and E_{Sym} correlation.

Permeation Enhancement Mechanisms under Asymmetric Conditions and Transdermal Permeation

A number of studies that employed a symmetric and equilibrium approach to investigate CPEs have provided several important insights into the mechanism of action of the enhancers (11–16,19,23). In these studies, the enhancer is present at equal concentrations in both the donor and receiver and in equilibrium with the SC. This configuration avoids the complications arising from enhancer concentration (or activity) gradients across the SC in which the local permeation enhancement varies with the position across the SC for direct comparison of the effectiveness of the enhancers (34,35). However, a major disadvantage of this approach is the difference between the concentration profiles of the enhancers in SC under the symmetric configuration and those generally encountered in transdermal drug delivery in practice.

Unlike the symmetric equilibrium enhancer setup in the previous studies, the experimental setup in the present study provided a system more closely resembling those encountered in transdermal drug delivery in practice. It should be noted that the present work did not examine the effects of co-solvents or any potential synergistic effects among different enhancers as these topics were beyond the scope of the study. The interpretation of the results in the present study was therefore limited to aqueous-based systems such as in transdermal hydrogel without a co-solvent. In addition, since the concentration gradients of the enhancers across SC were established in a pre-equilibration step before the start of the transport run in the present study, the transport lag times observed in this study would be different from those encountered in transdermal delivery in reality; whereas steady-state was established for the enhancers in the present study, so only the lag times of CS transport contributed to the apparent transport lag times, the transport lag times in practice (or in other studies in the literature (36,37)) are a result of both the transport lag times of the enhancers and CS. This lag time difference should not affect the conclusion based on the steady-state transport results in the present study.

The significance of the present study is the correlation between the CPE effects under the asymmetric and symmetric conditions to "bridge the gap" between the previous symmetric transport studies and transdermal delivery in practice. The results in the present study suggest that the findings in the previous studies under the symmetric conditions such as the proposed quantitative structure enhancement relationship (11–13,15,19) would likely hold in the asymmetric systems. The findings in these previous studies include the hypotheses that (a) the potencies of the CPEs based on their aqueous concentrations in contact with the SC on permeation enhancement are related to enhancer lipophilicities, (b) the intrinsic potencies of the CPEs based on their concentrations in the SC intercellular lipids are relatively the same (for all the tested enhancers) and independent of the enhancer physiochemical properties such as enhancer lipophilicities and molecular weight, and (c) the enhancer site of action in the SC lipid domain can be mimicked by liquid *n*-octanol (11–16,19,23). With the data obtained in the present study, Fig. 4 presents a plot of the logarithm of the aqueous enhancer concentration in the donor chamber to induce $E_{Asym}=6\pm1$ *vs.* the logarithm of enhancer octanol/water partition coefficient, similar to those presented previously for the symmetric conditions. The correlation between $E_{Asym}=6\pm1$ concentration and enhancer octanol/water partition coefficient in the figure is consistent with the hypothesis that the CPE potencies based on their aqueous concentrations are related to their lipophilicities. The similar correlations between enhancer E_{Asym} *vs.* enhancer lipophilicity in the present study and E_{Sym} *vs.* enhancer lipophilicity observed in previous studies further support our conclusion that the mechanistic findings in the previous studies can be extrapolated to the asymmetric configuration. The enhancer membrane concentration, rather than its aqueous concentration, is the major factor in determining permeation enhancement. The validation of the previous findings in the present

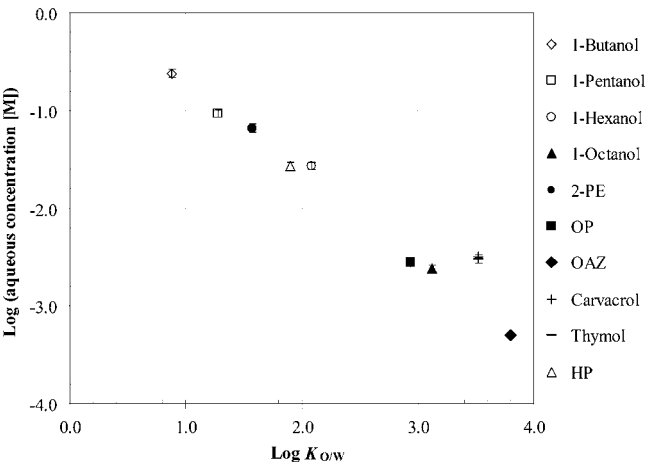

Fig. 4 Relationship between the enhancer concentration in donor chamber to induce $E_{Asym}=6\pm1$ and its octanol-water partition coefficient ($K_{O/W}$). The aqueous OAZ, OP, 2-PE and 1-octanol concentrations were determined by interpolation in the E_{Asym} *versus* the concentration of enhancer in Fig. 2. The aqueous carvacrol, thymol, 1-butanol, 1-pentanol, 1-hexanol, and HP concentrations are estimations obtained from the experimental data in Table I.

asymmetric configuration study will allow scientists to utilize the symmetric configuration results in these previous studies to explain the enhancement effects of CPEs under the asymmetric conditions and to predict drug delivery enhancement in transdermal enhancer formulation development before enhancer screening.

Despite that a correlation between the enhancer effects under the asymmetric and symmetric conditions was found and that the mechanistic findings in the previous symmetric transport studies can now be utilized, caution must be exercised to extrapolate the present asymmetric results to *in vivo* transdermal delivery in practice. For example, the asymmetric condition in the present study is not representative of transdermal systems that do not contain water or lead to fully hydrated skin. The present results are also likely not to be predictive of transdermal enhancement of polar permeants that utilize the SC polar pathway and very lipophilic permeants that their permeation is viable epidermis- or dermis-controlled because the SC lipoidal pathway is not the transport rate-determining pathway for these permeants. It should also be noted that an important factor in the evaluation of CPEs is the assessment of the toxicity and irritation potential of the enhancers. This is difficult to assess with the present *in vitro* transport setup and is beyond the scope of the present study.

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APPENDIX

Theoretical Considerations of Permeation Enhancement under Symmetric and Asymmetric Conditions

Based on the results from previous studies on permeation enhancement under the symmetric conditions (11–16, 19, 23), some enhancers exhibit linear relationships between E_{Sym} and enhancer aqueous concentration (C_{aq}) and some enhancers show exponential relationships between E_{Sym} and C_{aq}. Thus, the permeation enhancement effects under the symmetric condition can be modeled empirically by the following:

$$E_{Sym} = kC_{aq} + 1 \tag{A1}$$

for the linear E_{Sym} vs. C_{aq} relationship where k is a constant or:

$$E_{Sym} = e^{mC_{aq}} \tag{A2}$$

for the exponential E_{Sym} vs. C_{aq} relationship where m is a constant. Assuming that the permeation enhancement induced by the enhancer on CS and on the enhancer itself are the same, which is a reasonable assumption when both the enhancer and permeant utilize the same lipoidal transport pathway in HEM and have similar molecular weight, E_{Asym} in the linear and exponential relationship models can be quantified.

In general, the flux (J) of a permeant in a membrane can be described by the first Fick's law:

$$J = -D\frac{dC_{m,p}}{dx} = -DK\frac{dC_{aq,p}}{dx} \tag{A3}$$

where D is the diffusion coefficient in the membrane, $C_{m,p}$ is the concentration in the membrane, and x is the position in the membrane for the permeant. To express Eq. A3 in the form of aqueous concentration related to the concentration in the donor chamber, K represents the membrane-to-solution partition coefficient, and $C_{aq,p,}$ is the respective aqueous concentration of the permeant.

Under the symmetric condition where the solutions in donor and receiver chambers are the same, as those in the symmetric transport experiments with SC in the present study, the enhancement factor can be written as:

$$E_{Sym} = \frac{D_E K_E}{D_0 K_0} \tag{A4}$$

where D_0 and D_E are the diffusion coefficients of the permeant in the SC when the solutions are PBS and enhancer/PBS, respectively, and K_0 and K_E are the SC lipid-to-solvent partition coefficients for the permeant when the solutions are PBS and enhancer/PBS, respectively. In this case, D_0 and K_0 are constant and D_E and K_E are related to the concentration of the enhancer in the membrane C_m and hence the respective aqueous enhancer concentration in the diffusion chamber C_{aq}. Because C_m is constant across the membrane under the symmetric condition, D_E and K_E are constant independent of the position in the membrane under this condition. Therefore, the linear relationship Eq. A1 obtained under the symmetric condition can be used to determine E_{Asym} under the asymmetric condition as follows.

For a heterogeneous membrane under the asymmetric condition in which the donor solution is enhancer/PBS and the receiver solution is PBS, Eq. A3 can be rewritten as

$$J = -D_E K_E \frac{dC_{aq,p}}{dx} \tag{A5}$$

In this case, the diffusion and partition coefficients D_E and K_E are not constant but vary with position x: D_E and K_E

increase or decrease with enhancer concentration C_m at position x. Since C_m is related to C_{aq}:

$$C_m = C_{aq}K_E \tag{A6}$$

By combining Eqs. A1, A4, and A5,

$$J = -D_0K_0(kC_{aq} + 1)\frac{dC_{aq,p}}{dx} \tag{A7}$$

It should be pointed out that enhancer concentration C_{aq} (the hypothetical aqueous enhancer concentration in the membrane related to C_m through Eq. A6) here is a function of position x.

Now, consider the permeation of a CPE across the SC under the asymmetric condition in the present study. The enhancer is the permeant (i.e., $C_{m,p}=C_m$ and $C_{aq,p}=C_{aq}$) and enhances its own permeation. The flux of the enhancer in the membrane J_E can be expressed similar to Eq. A5 as

$$J_E = -D_E\frac{dC_m}{dx} = -D_EK_E\frac{dC_{aq}}{dx} \tag{A8}$$

At steady state, J_E is constant and independent of position x. Note that the steady-state requirement is satisfied at all locations in the membrane via the relationships of C_m, D_E, and dC_m/dx in Eq. A8. When C_m is high in the membrane close to the donor chamber, D_E is high, and the concentration gradient dC_m/dx is low. When C_m is low in the membrane near the receiver (sink condition), D_E is low, and a higher concentration gradient dC_m/dx offsets this to maintain a constant steady-state flux. Eq. A8 holds at all position x under steady state.

From the linear relationship model of Eq. A1, Eq. A8 becomes

$$J_E = -D_0K_0(kC_{aq} + 1)\frac{dC_{aq}}{dx} \tag{A9}$$

Because J_E is independent of x at steady state, Eq. A9 is a separable differential equation (in the form of $G = f(y)\frac{dy}{dx}$, where G is a constant independent of x and y) that can be solved for J_E by simple integration. Integrating Eq. A9 from $x=0$ to $x=h$ and $C_{aq}=C_{D,Asym}$ to $C_{aq}=0$, where h is the effective thickness of SC and $C_{D,Asym}$ is the concentration in the donor chamber, J_E can be determined:

$$\int_0^h J_E dx = \int_{C_{D,Asym}}^0 -D_0K_0(kC_{aq} + 1)dC_{aq} \tag{A10}$$

$$J_Eh = \left(\frac{kC_{D,Asym}^2}{2} + C_{D,Asym}\right)D_0K_0 \tag{A11}$$

$$J_E = \left(\frac{kC_{D,Asym}^2}{2} + C_{D,Asym}\right)\frac{D_0K_0}{h} \tag{A12}$$

As shown above, the steady-state flux J_E can be solved without solving the equation for concentration C_{aq} or for the non-steady state condition.

The flux of the baseline control (in PBS or PBS with trace amount of the enhancer) is

$$J_0 = K_0D_0\frac{C_{D,Asym}}{h} \tag{A13}$$

Dividing Eq. A12 by Eq. A13, E_{Asym} for the enhancer in the linear relationship model becomes

$$E_{Asym} = \frac{1}{2}kC_{D,Asym} + 1 \tag{A14}$$

Similar to the derivation of E_{Asym} in the linear relationship above, the steady-state flux of the enhancer J_E under the asymmetric condition in the exponential relationship model can be determined based on the exponential relationship of E_{Sym} in Eq. A2 and the following:

$$J_E = -D_0K_0e^{mC_{aq}}\frac{dC_{aq}}{dx} \tag{A15}$$

$$\int_0^h J_E dx = \int_{C_{D,Asym}}^0 -D_0K_0e^{mC_{aq}}dC_{aq} \tag{A16}$$

$$J_Eh = D_0K_0\frac{e^{mC_{D,Asym}}}{m} - \frac{D_0K_0}{m} \tag{A17}$$

$$J_E = \frac{D_0K_0}{mh}\left(e^{mC_{D,Asym}} - 1\right) \tag{A18}$$

and dividing Eq. A18 by Eq. A13, E_{Asym} for the enhancer in the exponential relationship model becomes

$$E_{Asym} = \frac{e^{mC_{D,Asym}} - 1}{mC_{D,Asym}} \tag{A19}$$

Assuming that the permeation enhancement induced by the enhancer on CS and on the enhancer itself are the same, E_{Asym} for the permeant can be described by Eqs. A14 and A19.

According to Eqs. A14 and A19, at $E_{Sym}=10$, $E_{Asym}=5.5$ and 3.9 for the linear and exponential models, respectively. These values are consistent with the experimental data for the enhancers studied within the data scattering ($E_{Asym}\approx5$–6 at $E_{Sym}\approx10$).

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