

# Application of Dissolution/Permeation System for Evaluation of Formulation Effect on Oral Absorption of Poorly Water-Soluble Drugs in Drug Development

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## ABSTRACT

**Purpose** The aim of the present study is to evaluate the formulation effect on the oral absorption of poorly water-soluble drugs using a dissolution/permeation system (D/P system).

**Methods** This D/P system, consisting of apical and basal chambers and a Caco-2 cell monolayer mounted between chambers, can be used to perform simultaneous analysis of drug dissolution and permeation process of drugs applied as various dosage forms. Oral administration study with rats was also performed for both drugs as the same dosage forms.

**Results** When danazol, a low-soluble and high-permeable drug, was applied to the D/P system as various formulations, dissolved and permeated amounts were significantly high compared with those from a suspension form. On the other hand, whereas the dissolved amount of pranlukast, a low-soluble and low-permeable drug, was significantly increased by formulations, there were no significant changes observed in the permeated amount between suspension and formulation. The oral availability of danazol was significantly increased by formulations but not pranlukast, which corresponded well to *in vitro* evaluations.

**Conclusion** These results indicated that the D/P system might be applicable for selection of formulation on the basis of physicochemical drug properties.

**KEY WORDS** absorption · dissolution · permeability · solubility · supersaturation

## INTRODUCTION

The number of poorly water-soluble drug candidates in drug discovery has recently increased (1). Their limited water solubility often causes low and variable oral absorption because poor dissolution of drugs in the gastrointestinal (GI) fluid is insufficient for drug absorption to show a pharmacological effect. Except for first-pass metabolism, the rate and amount of drug absorption are expressed by the following equations:

$$\text{Absorption rate} = P_{\text{eff}} \times S \times C_{\text{GI}} \quad (1)$$

$$\begin{aligned} \text{Absorption amount} &= P_{\text{eff}} \times S \times \int_0^t C_{\text{GI}} dt \quad (2) \\ &= P_{\text{eff}} \times S \times \text{AUC}_{\text{GI}} \end{aligned}$$

where  $P_{\text{eff}}$  is the effective permeability of drugs,  $S$  is the surface area of the intestinal membrane, and  $C_{\text{GI}}$  is the luminal concentration of drugs.  $\text{AUC}_{\text{GI}}$  expresses the area under the drug concentration-time curve in the GI tract during the intestinal transit time,  $t$ . In order to estimate the fraction of absorbed drugs ( $F_a$ ) after oral administration, these parameters in the equations should be assessed. In particular, in the case of poorly water-soluble drugs, solubility and dissolution rate of a drug to the gastrointestinal fluid are key factors to consider the extent of oral absorption. Maintenance of a high concentration of dissolved drugs in the gastrointestinal fluid results in high

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Fa. Hence, various techniques to enhance drug dissolution have been used to improve the oral absorption of poorly water-soluble drugs (2,3).

Recently, many researchers have focused on supersaturation-generating formulations to improve the oral absorption of poorly water-soluble drugs. Drugs in a state of supersaturation are kinetically soluble in solution at a concentration above their thermodynamic equilibrium solubility. If a supersaturated drug solution exists in the intestinal fluid for a sufficient length of time to be absorbed, it may result in an enhanced flux across the intestinal wall and thus improve the absorption. Several formulations that induce supersaturation *in vitro* and enhance oral absorption *in vivo* include amorphous ones, such as solid dispersion (4–6), crystalline salts (7), cocrystals (8,9), higher-energy polymorphic forms (10), and self-emulsified drug delivery system (SEDDS) (11). Moreover, the existence of several polymers in SEDDS (supersaturable SEDDS, S-SEDDS) such as hydroxypropyl methylcellulose (HPMC) at a supersaturable state of drugs can enhance the oral absorption of poorly water-soluble drugs (12,13).

In order to achieve successful drug development for oral use, the effect of such techniques on the oral absorption of poorly water-soluble drug candidates in humans should be assessed in advance of pre-clinical and clinical studies. In particular, drug concentration at supersaturable state and its sustained period in the intestinal fluid are important factors to determine the oral absorption of drugs from a supersaturation-generating formulation. However, it is still difficult to evaluate quantitatively what effect supersaturation has on the enhanced absorption because these formulations often increase not only the dissolved concentration but also the dissolution rate of drugs. In addition, the *in vitro/in vivo* correlation of supersaturable formulation has not yet been understood clearly.

We have developed an *in vitro* system for simultaneous evaluation of drug dissolution and permeation in oral drug absorption (14). This system (dissolution/permeation system, D/P system) consists of apical and basal chambers with a Caco-2 cell monolayer mounted between the two chambers as a model membrane of human intestine. Previous studies with the D/P system indicate that it enables evaluation of the effect of various factors such as food intake (15,16), solid dosage forms (17), and dose strength (16) in terms of their influence on the oral absorption of poorly water-soluble drugs.

In this study, we focused on evaluation of the effect of SEDDS and co-solvent formulations with or without water-soluble polymers on the oral absorption of poorly water-soluble drugs using the D/P system. In addition, animal study with rats was also performed to compare the results with *in vitro* estimation.

## MATERIALS AND METHODS

### Materials

The human colorectal adenocarcinoma cell line Caco-2 (passage: 17 times) was purchased from American Type Culture Collection (Rockville, MD). Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Sigma-Aldrich (St. Louis, MO). Non-essential amino acids (10 mM), fetal bovine serum (FBS), L-glutamine (200 mM), trypsin-EDTA (trypsin: 0.25%, EDTA: 1 mM), and antibiotic-antimycotic (penicillin: 10,000 U/mL, streptomycin: 10 mg/mL, amphotericin B: 25 µg/mL; dissolved in 0.85% (w/v) sodium chloride aqueous solution) were purchased from Gibco Laboratories (Lenexa, KS). Cell culture inserts with polyethylene terephthalate filters (pore size: 3.0 µm, growth area: 4.20 cm<sup>2</sup>) were obtained from Becton Dickinson Bioscience (Bedford, MA).

Bovine serum albumin (BSA), egg-phosphatidylcholine (lecithin), ethanol, polyethyleneglycol 400 (PEG400), pranlukast, sodium taurocholate (NaTC), and Tween 80 were obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Polyoxyl 35 hydrogenated castor oil (Cremophor® EL), danazol, and HPMC were purchased from Sigma-Aldrich (St. Louis, MO). Mono/diglyceride of capric acid (Capmul® MCM) was obtained from ABITEC Corp. (Janesville, WI). Polyoxyl 40 hydrogenated castor oil (Cremophor® RH40) was purchased from BASF SE (Ludwigshafen, Germany). Hydroxypropylcellulose (HPC) and methylcellulose were obtained from Acros Organics (Morris Plains, NJ). Glyceryl monolinoleate (Maisine 35–1) was purchased from GATTEFOSSÉ SAS (Saint Priest, France).

### Preparation of Various Formulations

The standard suspension of danazol and pranlukast was prepared as follows. Danazol (20 mg/mL) and pranlukast (10 mg/mL) were suspended into purified water containing 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80. Various formulations of danazol and pranlukast were prepared according to the composition of each formulation shown in Tables I and II, respectively. Danazol and pranlukast were completely dissolved into vehicle of all formulations.

### In Vitro Experiments Using the D/P System

#### Preparation of the Caco-2 Cell Monolayer

Caco-2 cells were grown in D-MEM supplemented with 10% FBS, 1% L-glutamate, 1% NEAA, and 5% antibiotic-

**Table I** Vehicle Composition of Danazol Formulations (20 mg/mL) for *In Vitro* and *In Vivo* Studies

Formulations	Vehicle composition (ratio by weight)
Standard suspension	Methylcellulose/Tween 80/water = 0.5/0.1/99.4
SEDDS	Sesame oil/Cremophor EL/Maisine 35-1 = 30/40/30
S-SEDDS/HPC	Sesame oil/Cremophor RH40/Maisine 35-1/Ethanol/HPC = 28/30/28/10/4
S-SEDDS/HPMC	Sesame oil/Cremophor RH40/Maisine 35-1/Ethanol/HPMC = 28/30/28/10/4
Co-solvent	Ethanol/PEG400 = 10/90
S-Co-solvent/HPC	Ethanol/PEG400/HPC = 10/86/4
S-Co-solvent/HPMC	Ethanol/PEG400/HPMC = 10/86/4

antimycotic solution as culture medium at 37°C in culture flasks (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) in humidified air with a 5% CO<sub>2</sub> atmosphere. Cells were harvested with trypsin-EDTA and seeded onto polycarbonate filters (3.0 µm pores, 4.20 cm<sup>2</sup> growth area) inside a cell culture insert (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) at a density of 3 × 10<sup>5</sup> cells/filter. The culture medium (1.5 mL in the insert and 2.6 mL in the well) was replaced every 48 h for the first 6 days and every 24 h thereafter. After 18–21 days in culture, the Caco-2 cell monolayer was utilized for the following experiments.

#### Chambers for the D/P System

In the D/P system, the Caco-2 cell monolayers are mounted in side-by-side chambers. The effective surface area of the Caco-2 cell monolayer in the D/P system is 1.77 cm<sup>2</sup>. Both sides of the Caco-2 cell monolayer are consistently stirred at 200 rpm with magnetic stirrers. The volumes of apical and basal sides are set to 8 mL and 5.5 mL, respectively. As a buffer solution in this study, Hank's balanced salt solution (HBSS), containing 5.36 mM KCl, 136.89 mM NaCl, 0.34 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.44 mM KH<sub>2</sub>PO<sub>4</sub>, 4.17 mM NaHCO<sub>3</sub>, 1.26 mM CaCl<sub>2</sub>, 0.49 mM MgCl<sub>2</sub>, 0.41 mM MgSO<sub>4</sub>, and 25 mM glucose, was used (transport medium, TM). Simulated intestinal medium as the apical side of the D/P system was prepared, which was based on TM with the addition of NaTC (3 mM) and lecithin (0.75 mM) for FaSSIF<sub>mod</sub>. The pH of FaSSIF<sub>mod</sub>

was adjusted to 6.5 with HEPES. As a basal medium, TM containing BSA (4.5% w/v) with a pH adjusted to 7.4 with HEPES was used in the D/P system.

#### Dissolution and Permeation Study with the D/P System

A solution (TM, pH adjusted to 6.5) and the basal medium were introduced to the apical and basal sides of the Caco-2 monolayer in the well, respectively. After preincubation for 20 min, the Caco-2 monolayer with support filter was mounted between the chambers of the D/P system. Then, the apical side of the Caco-2 monolayer was filled with the apical medium, FaSSIF<sub>mod</sub>. The basal side was filled with the basal medium in all experiments. Drugs were applied to the apical side as a standard suspension or formulation. The applied volume of all forms was set to 50 µL for danazol and 225 µL for pranlukast which contains 1 mg and 2.25 mg (1% of the clinical dose), respectively. Then, aliquots of samples were routinely taken from the basal solution for 2 h. The volume of the basal solution was maintained by adding fresh medium. After completion of the experiment, the apical solution was immediately collected and filtered through a cellulose acetate filter. All apical samples (0.2 mL) were mixed with 0.2 mL of the solution consisting of 50 mM phosphate buffer (pH 2.5) and acetonitrile (50/50) to prevent the dissolved drug from precipitating in the apical solution. The transepithelial electric resistance (TEER) of the Caco-2 cell monolayer was checked before and after the experiment. All experiments were performed at 37°C.

**Table II** Vehicle Composition of Pranlukast Formulations (10 mg/mL) for *In Vitro* and *In Vivo* Studies

Formulations	Vehicle composition (ratio by weight)
Standard suspension	Methylcellulose/Tween 80/water = 0.5/0.1/99.4
SEDDS	Capmul MCM/Tween 80/PEG400/Ethanol = 10.5/47.4/31.6/10.5
S-SEDDS/HPMC	Capmul MCM/Tween 80/PEG400/Ethanol/HPMC = 10/45/30/10/5
Co-solvent	Ethanol/PEG400 = 10/90
S-Co-solvent/HPMC	Ethanol/PEG400/HPMC = 9.5/85.7/4.8

### Prediction of the Oral Absorption of Drugs from *In Vitro* Data

In order to predict the fraction of dose absorbed ( $F_a$  values) of each drug in fasted human from *in vitro* data, the following equation was used:

$$F_a(\%) = \frac{\text{Abs}_{\max} * \text{PA}^\gamma}{\text{PA}_{50}^\gamma + \text{PA}^\gamma} \quad (3)$$

where  $\text{Abs}_{\max}$  is the maximum absorption (defined as 100%),  $\text{PA}$  is the *in vitro* permeated amount in the D/P system (% of dose/2 h),  $\text{PA}_{50}$  is the permeated amount, which corresponds to 50% of the absorption *in vivo*, and  $\gamma$  is Hill's coefficient. In a previous study,  $\text{PA}_{50}$  and  $\gamma$  were obtained by fitting the permeated amount ( $\text{PA}$ ) of non-P-gp substrate drugs in the D/P system and their oral absorption in humans; there was a good correlation between oral absorption in humans and % of dose/2 h with these substitutions (Kataoka et al., 2006).  $\text{PA}_{50}$  and  $\gamma$  of 0.334 and 0.883, respectively, that were obtained in a previous study were used for prediction of the oral absorption in fasted humans.

### Animal Study

All animal experiments were approved by the Ethical Review Committee of Setsunan University and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85–23, revised 1985). Wistar male rats weighing 250–300 g were deprived of food but given free access to water for 18 h before the experiments. The doses of danazol and pranlukast were set to 1.5 mg/kg and 4.0 mg/kg, respectively, on the basis of their clinical doses. The volume of each formulation was set to 0.075 mL/kg for danazol and 0.4 mL/kg for pranlukast. Standard suspensions and supersaturation-generating formulations of danazol and pranlukast were immediately administered to rats after suspensions and formulations were mixed with appropriate volumes of purified water. The total volume of administered solution with drugs was set to 2 mL/kg. At pre-determined time points, blood samples were collected from the jugular vein. Blood samples were centrifuged and plasma was kept at  $-30^\circ\text{C}$  before quantification of drugs.

### Assay

The sample solutions from *in vitro* study were pre-treated as follows. All basal samples (0.1 mL) were mixed with 0.9 mL of acetonitrile. The mixture was shaken and the supernatant was used for the assay after centrifugation at

15,000 rpm for 20 min. The plasma samples from *in vivo* study were pre-treated as follows. All plasma samples (0.1 mL) were mixed with 0.9 mL of acetonitrile. The mixture was shaken and the supernatant (0.7 mL) was collected after centrifugation at 15,000 rpm for 20 min. After removal of the solvent under a vacuum at  $40^\circ\text{C}$ , the residue was dissolved in 0.1 mL of the solution consisting of 50 mM phosphate buffer (pH 2.5) and acetonitrile (50/50). These solutions were used for the assay.

The amounts of danazol and pranlukast in the solutions were determined using a UPLC system (ACQUITY® UPLC, Waters, Milford, MA) equipped with a tandem mass spectrometer (ACQUITY® TQD, Waters, Milford, MA). A reversed-phase Waters ACQUITY® UPLC BEH C18 analytical column of  $50 \times 2.1$  mm and  $1.7 \mu\text{m}$  particle size (Waters, Milford, MA) was used with a mobile phase consisting of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile containing 0.1% (v/v) formic acid (solvent B) with gradient time period. The initial mobile phase was 98% solvent A and 2% solvent B pumped at a flow rate of 0.3 mL/min. Between 0 and 1.0 min, the percentage of solvent B was increased linearly to 95%, where it was held for 1.0 min. Between 2.01 and 2.5 min, the percentage of solvent B was decreased linearly to 2%. This condition was maintained until 3 min, at which time the next sample was injected into the UPLC system. All treated samples were injected at 5  $\mu\text{L}$  into the UPLC system. Ionization conditions for analysis of danazol were as follows: electrospray ionization, positive mode; source temperature,  $150^\circ\text{C}$ ; desolvation temperature,  $400^\circ\text{C}$ ; cone voltage, 50 V; and collision energy, 50 eV. Precursor and production ions ( $m/z$ ) of danazol were 338.19 and 91.02, respectively. Ionization conditions for analysis of pranlukast were as follows: electrospray ionization, positive mode; source temperature,  $150^\circ\text{C}$ ; desolvation temperature,  $400^\circ\text{C}$ ; cone voltage, 40 V; and collision energy, 30 eV. Precursor and production ions ( $m/z$ ) of pranlukast were 482.52 and 121.13, respectively.

### Data Analysis

*In vitro* data obtained from experiments using the D/P system are expressed as a fraction of the dose (% of dose); the dose is the drug amount applied to the apical side of the Caco-2 cell monolayer attached to the D/P system. In *in vivo* study, the area under the plasma concentration-time curve (AUC) from 0 to the time of final sampling point was calculated using a linear trapezoidal rule. Data are presented as means and standard deviations (s.d.) for individual groups. Statistical significance was assessed with the unpaired Student's *t*-test and *p* values of 0.05 or less were considered significant.

## RESULTS

### *In Vitro* Estimation of the Effect of Various Formulations on the Dissolution and Permeation of Poorly Water-Soluble Drugs Using the D/P System

Figure 1 shows the effect of various supersaturation-generating formulations on the danazol dissolution (Fig. 1a) and permeation (Fig. 1b) in the D/P system. The dissolved and permeated amounts of danazol for 2 h are summarized in Table III. The PEG400-based formulation (Co-solvent) enhanced danazol dissolution in the apical medium and then the amount that permeated to the basal solution was increased compared with that from standard suspension. Moreover, when HPC or HPMC was added to PEG400-based formulation (S-Co-solvent/HPC, S-Co-solvent/HPMC), both dissolution and permeation of danazol were significantly increased, indicating that these polymers could maintain a supersaturated state in the apical solution. This effect by HPMC was stronger than that by HPC. As lipid-based formulation, SEDDS was used and significantly enhanced danazol dissolution and permeation. In comparison with Co-solvent, there was no observed prolonged effect by HPC or HPMC on danazol dissolution. In addition, no significant difference in the permeated amount of danazol was observed between SEDDS and SEDDS containing HPC or HPMC (S-SEDDS/HPC, S-SEDDS/HPMC). Significant change in the TEER value of the monolayers was not observed.

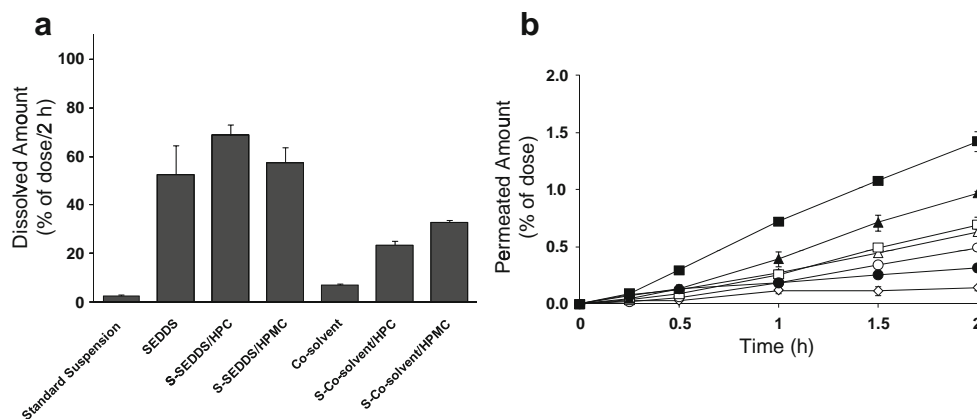
Dissolution and permeation study with the D/P system was performed for pranlukast (Table IV). When SEDDS and S-SEDDS formulations were applied to the apical side of the D/P system, pranlukast was completely dissolved for 2 h but decrease in the TEER value (30% of initial value at 2 h) across the Caco-2 cell monolayer during the

experiment was observed. In case of other dosing conditions, no significant change in the TEER value of the monolayers was observed. However, the permeated amount of pranlukast was significantly lower in spite of complete dissolution. In the case of co-solvent formulations, whereas the dissolved amount was significantly increased compared with that from standard suspension and an inhibitory effect on the precipitation by HPMC was observed, permeation to the basal compartment was not enhanced.

### Prediction of Oral Absorption from *In Vitro* Data

The *in vitro* data (% of dose/2 h) were substituted for PA in Eq. 3 in order to predict the *in vivo* oral absorption ( $F_a\%$ ) of each drug from various dosage forms. As shown in Table III, the oral absorption of danazol was estimated to be 32% when 100 mg of danazol was administered as standard suspension to fasted human. Although the oral absorption of danazol from Co-solvent (49%) could be enhanced by addition of polymer, the oral absorption was predicted not to be affected by the difference in the addition of HPC (72%) and HPMC (78%). In the case of lipid-based formulations, the oral absorption of danazol was predicted to be high compared with that from standard suspension and the predicted absorption of danazol, ranging from 58% to 65%, was not affected by the presence of polymer in SEDDS.

The prediction of the oral absorption of pranlukast from various dosage forms was performed in the same manner as for danazol and is shown in Table IV. The oral absorption of pranlukast from standard suspension and PEG400-based formulation (Co-solvent and S-Co-solvent/HPMC) was predicted to be lower than that from lipid-based formulation (SEDDS and S-SEDDS/HPMC). There were no differences in the predicted oral absorption of pranlukast



**Fig. 1** Effect of various dosage forms on the dissolution (a) and permeation (b) of danazol in the D/P system. The dissolved amount of danazol at 2 h and the time-courses of danazol permeation were observed for 2 h in the D/P system. As formulations of danazol, standard suspension (◇), SEDDS (○), S-SEDDS/HPC (△), S-SEDDS/HPMC (□), Co-solvent (●), S-Co-solvent/HPC (▲), and S-Co-solvent/HPMC (■) were applied to the D/P system. The data are expressed as the mean  $\pm$  s.d. of three independent experiments.



**Table III** Dissolved and Permeated Amount of Danazol from Various Dosage Forms at 2 h in the D/P System and Estimated Absorption from the *In Vitro* Data

Dosage forms	Dissolved amount (% of dose/2 h)	Permeated amount (% of dose/2 h)	Estimated absorption (%)
Standard suspension	2.777 ± 0.338	0.140 ± 0.022	32
SED DS	52.349 ± 12.102*	0.492 ± 0.022*	58
S-SED DS/HPC	68.612 ± 4.476*	0.630 ± 0.026*	64
S-SED DS/HPMC	57.317 ± 6.562*	0.691 ± 0.068*	65
Co-solvent	7.182 ± 0.385*	0.316 ± 0.032*	49
S-Co-solvent/HPC	23.374 ± 1.788*	0.989 ± 0.051*	72
S-Co-solvent/HPMC	32.979 ± 0.859*	1.422 ± 0.088*	78

\*Statistically significant difference from standard suspension ( $p < 0.05$ )

between SED DS and S-SED DS/HPMC. The rank order of formulation efficacy on the oral absorption of pranlukast was SED DS = S-SED DS/HPMC > Co-solvent/HPMC > standard suspension > Co-solvent.

### **In Vivo Evaluation of the Effect of Various Formulations on the Oral Absorption of Poorly Water-Soluble Drugs by Rats**

The plasma concentration *versus* time profiles of danazol after oral administration as various dosage forms are shown in Fig. 2. The pharmacokinetic parameters are listed in Table V. The oral absorption of danazol ( $AUC_{0-8h}$ ) from standard suspension was the lowest of all values. The rank order of the PEG400-based formulation on the  $AUC_{0-8h}$  values was S-Co-solvent/HPMC > S-Co-solvent/HPC > Co-solvent, corresponding to the present *in vitro* results. In the case of lipid-based formulations, although the rank order of the  $AUC_{0-8h}$  values did not agree with that of *in vitro* results, the oral absorption of danazol was significantly increased by SED DSs.

Figure 3 shows the plasma concentration-time profile of pranlukast after oral administration as various dosage forms; pharmacokinetic parameters are summarized in Table VI. Although significant change in the oral availability ( $AUC_{0-24h}$ ) of pranlukast could not be observed between all formulations used in this study, the oral availability from co-solvent formulations seemed to be lower than that from other formulations. Maximum drug

concentration times ( $T_{max}$ ) when pranlukast was administered as SED DS formulations delayed compared to that after administration as other formulations.

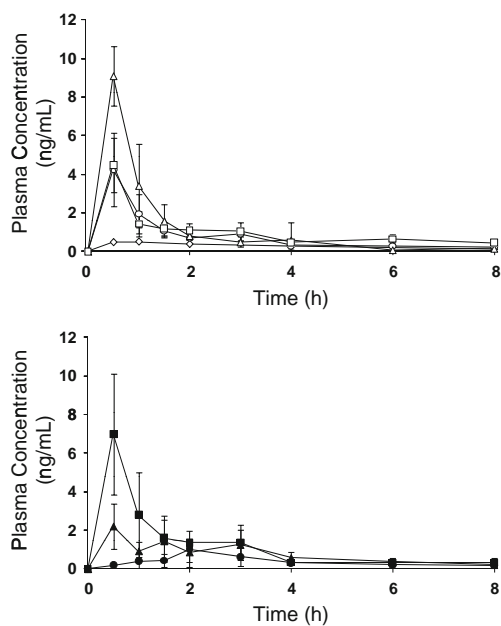
### **DISCUSSION**

Self-emulsifying drug delivery systems (SED DS) to improve the oral bioavailability of poorly water-soluble drugs are well known and have been demonstrated with various drugs (18–20). SED DS formulation is composed of drug, oil, surfactant, and co-surfactant, which are emulsified in aqueous media under conditions of gentle agitation (21). SED DS formulation increases dissolution rate and solubility of poorly water-soluble drugs. Gao *et al.* have revealed that supersaturable SED DS (S-SED DS) that was developed employing HPMC significantly improved oral availability of poorly water-soluble drugs (12,13). For example, paclitaxel rapidly precipitated after dispersal into buffer solution as SED DS formulation. On the other hand, precipitation of paclitaxel was significantly inhibited by the presence of HPMC. *In vivo* oral availability of paclitaxel from S-SED DS was 10-fold higher than that from SED DS, corresponding to the findings of *in vitro* study (12). However, although similar correspondences were observed between *in vitro* and *in vivo* studies, absolute values of inhibition effect on precipitation observed by *in vitro* study did not necessarily correspond to those calculated from *in vivo* results.

**Table IV** Dissolved and Permeated Amount of Pranlukast from Various Dosage Forms at 2 h in the D/P System and Estimated Absorption from the *In Vitro* Data

Dosage forms	Dissolved amount (% of dose/2 h)	Permeated amount (% of dose/2 h)	Estimated absorption (%)
Standard suspension	3.213 ± 0.631	0.009 ± 0.001	4
SED DS	116.167 ± 1.975*	0.032 ± 0.001*	11
S-SED DS/HPMC	119.368 ± 14.928*	0.032 ± 0.003*	11
Co-solvent	8.045 ± 2.419*	0.006 ± 0.000	3
S-Co-solvent/HPMC	17.626 ± 1.059*	0.014 ± 0.006	6

\*Statistically significant difference from standard suspension ( $p < 0.05$ )



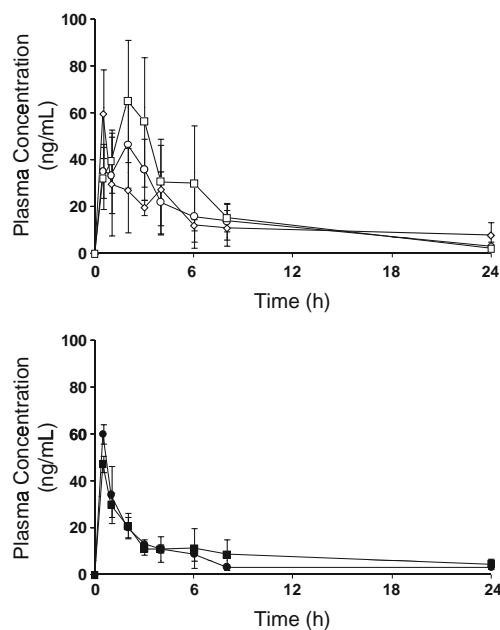
**Fig. 2** The time-profile of plasma concentration of danazol after oral administration as various dosage forms. As formulations of danazol, standard suspension ( $\diamond$ ), SEDDS ( $\circ$ ), S-SEDDS/HPC ( $\Delta$ ), S-SEDDS/HPMC ( $\square$ ), Co-solvent ( $\bullet$ ), S-Co-solvent/HPC ( $\blacktriangle$ ), and S-Co-solvent/HPMC ( $\blacksquare$ ) were administered to rats (1.5 mg/kg). The data are expressed as the mean  $\pm$  s.d. of three or four independent experiments.

Miller *et al.* have reported the impact of micellar solubilization on the intestinal membrane permeability (22). They demonstrated that a trade-off exists between micellar apparent solubility increase and permeability decrease that must take into account to consider the oral absorption of drugs. Moreover, some surfactants affect the membrane permeability of drug due to inhibition of transporters such as P-gp (23). In case of co-solvent formulations, these effects, effect on solubility and permeability, should be considered. It was reported that co-solvent agents such as PEG400 had no impact on the membrane permeation of drugs (24,25); however, several observations that co-solvent agents decrease membrane

**Table V** Pharmacokinetic Parameters of Danazol after Oral Administration as Various Dosage Forms to Rats

Formulations	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC (ng·h/mL)
Standard suspension	0.74 $\pm$ 0.35	1.3 $\pm$ 1.1	2.87 $\pm$ 0.97
SEDDS	4.23 $\pm$ 1.91*	0.5	6.26 $\pm$ 2.50*
S-SEDDS/HPC	9.09 $\pm$ 1.55*	0.5	9.31 $\pm$ 3.27*
S-SEDDS/HPMC	4.49 $\pm$ 1.41*	0.5	7.79 $\pm$ 0.29*
Co-solvent	1.18 $\pm$ 0.86	3.0 $\pm$ 1.0	3.72 $\pm$ 2.02
S-Co-solvent/HPC	2.20 $\pm$ 1.17	0.5	7.01 $\pm$ 3.18*
S-Co-solvent/HPMC	6.98 $\pm$ 3.12*	0.5	10.86 $\pm$ 3.56*

\* Statistically significant difference from standard suspension ( $p < 0.05$ )



**Fig. 3** The time-profile of plasma concentration of pranlukast after oral administration as various dosage forms. As formulations of pranlukast, standard suspension ( $\diamond$ ), SEDDS ( $\circ$ ), S-SEDDS/HPMC ( $\square$ ), Co-solvent ( $\bullet$ ), and S-Co-solvent/HPMC ( $\blacksquare$ ) were administered to rats (4.0 mg/kg). The data are expressed as the mean  $\pm$  s.d. of three or four independent experiments.

permeability of drugs were shown (26). In these cases, drug concentration related to permeation across a membrane could be important to determine the absorption. The permeated amount of drug in the D/P system could reflect the various phenomena which affected *in vivo* drug absorption because the system could evaluate drug dissolution and permeation, simultaneously.

In order to assess the food effect by micelles, the limiting process of oral drug absorption ( $Fa < 95\%$ ) can be considered as three categories, permeability limited (PL), dissolution rate limited (DRL), and solubility limited (SL) oral absorption (27). The permeability limited case can be further categorized as epithelial membrane permeation limited (PL-E) and unstirred water layer limited (PL-U). In addition, the solubility limited case can be further categorized as epithelial membrane permeation limited

**Table VI** Pharmacokinetic Parameters of Pranlukast after Oral Administration as Various Dosage Forms to Rats

Formulations	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC (ng·h/mL)
Standard suspension	59.71 $\pm$ 18.75	0.5	326.35 $\pm$ 159.66
SEDDS	47.87 $\pm$ 18.13	1.88 $\pm$ 1.03	338.49 $\pm$ 116.70
S-SEDDS/HPMC	69.07 $\pm$ 25.74	2.25 $\pm$ 0.50	426.06 $\pm$ 184.70
Co-solvent	60.17 $\pm$ 4.24	0.5	179.44 $\pm$ 5.95
S-Co-solvent/HPMC	47.26 $\pm$ 3.50	0.5	230.82 $\pm$ 104.60

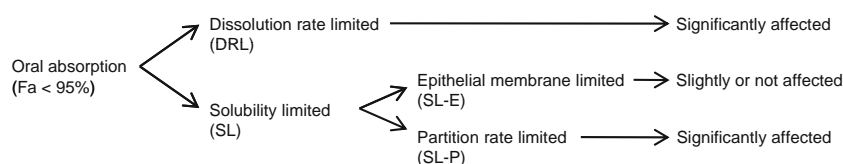
(SL-E) and unstirred water layer (UWL) limited (SL-U). In drugs categorized into SL-E, the increase of solubility by bile micelles would be cancelled out by the decrease of effective permeability due to decreasing free drug as a fraction of the total dissolved amount. This would result in slightly positive food effect on the oral absorption. On the other hand, in drugs categorized into DRL and SL-U, a positive food effect by bile micelles was anticipated. The extent of positive food effect would depend on the balance of change of effective diffusion coefficient in the UWL and an increase of solubility. This categorizing could be applicable to consider not only the food effect on the oral absorption of poorly water-soluble drugs by bile micelles but also the formulation effect on it.

Figure 4 shows a scheme of the relationship between the limiting steps of oral absorption of poorly water-soluble drugs and the formulation effect based on the report by Sugano *et al.* (27). In the case of liquid formulation, UWL limited can be translated into partition rate limited (SL-P) from micelles and emulsions to water phase. In low-soluble compounds with high permeability (DRL and/or SL-P), significant increase in the oral absorption by formulation techniques was anticipated. The extent of the formulation effect would depend on the balance of free fraction and bound fraction with micelles or emulsions and an increase of solubility. In low-soluble compounds with low permeability (SL-E), slight or no formulation effect on the oral absorption was anticipated. Danazol (28) and pranlukast (29) used in this study are high-permeability and low-permeability compounds with low solubility, respectively. Therefore, danazol could be classified into the DRL and/or SL-P category and pranlukast could be classified into the SL-E category. In addition, it can be mainly considered that drug permeation from a solution with the micelle and emulsion depends on free drug concentration (unbound and unenclosed into micelle and emulsion). The free drug concentration is decreased by permeation across the membrane. In case of DRL and/or SL-P categorized drugs, the free concentration of drug in a solution significantly decreases and then drug is released from the micelle and emulsion on the basis of the partition ratio which results in the maintaining high drug permeation. On the other hand, SL-E categorized drugs do not lead to such permeation cycle due to low membrane permeation. According to this categorizing, a significant effect of formulation on the oral absorption of danazol was anticipated but not of pranlukast.

Two types of formulation, SEDDS and simple co-solvent formulations, were used in this study. Moreover, a water-soluble cellulosic polymer, HPC or HPMC, was added to each formulation to generate a supersaturated state in a solution. In the apical side of the D/P system, formulation effect on the dissolution and inhibition effect on precipitation by polymers could be clearly detected (Tables III and IV). In danazol, the high dissolution from various formulations led to high permeation to the basal solution compared with that for a standard suspension (Table III). Whereas supersaturated state could be generated in the apical solution of the D/P system because significant increase in the dissolved amount after applying pranlukast as co-solvent formulation with HPMC was observed compared to that as co-solvent formulation, permeated amount of pranlukast was slightly increased (Table IV). It was considered that the flux of drug across the membrane depended upon the product of the drug concentration related to permeation, the surface area and permeation rate. If the detectability of the D/P system was increased (e.g. by extension of the surface area of the monolayer), the effect of supersaturation from co-solvent with HPMC on the permeated amount of pranlukast could be detected, clearly. When SEDDS and S-SEDDS formulations were applied to the apical side of the D/P system, pranlukast was completely dissolved for 2 h but a decrease in the TEER value across the Caco-2 cell monolayer during the experiment was observed. The decrease in the TEER value of the monolayer indicated disruption of the monolayer stricture which led to enhancing drug permeation through paracellular route. Accordingly, the observed permeated amount and estimated oral absorption of pranlukast from SEDDS formulations should be used as references. These results supported the scheme of the relationship between the limiting steps of oral absorption of poorly water-soluble drugs and the formulation effect shown in Fig. 4.

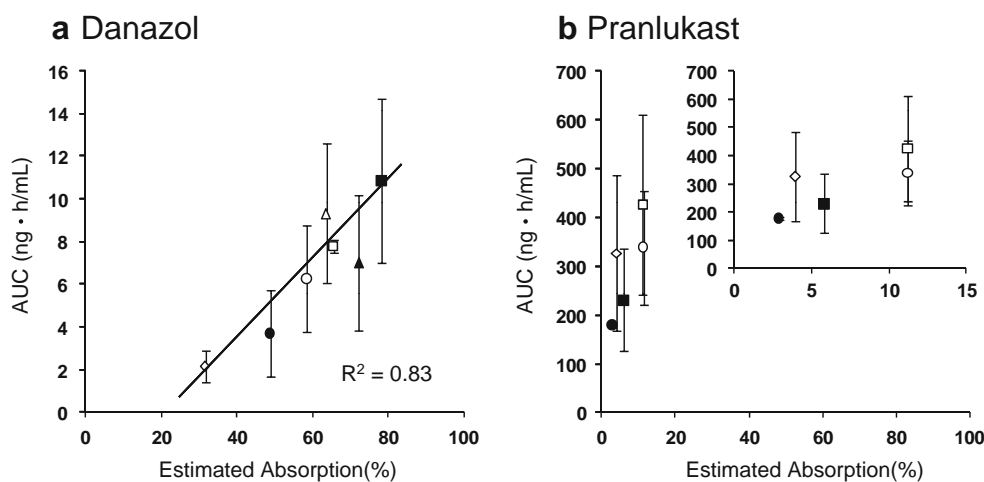
In animal study with rats, all formulations improved the oral availability of danazol (Fig. 2, Table V). In SEDDS formulations, the oral availabilities in rats were similar between three formulations. In co-solvent formulations, *in vivo* rank order of the formulation effect corresponded well to that from oral absorption estimated using the D/P system, indicating that a similar inhibition effect by polymers observed *in vitro* occurred in the *in vivo* gastrointestinal absorption process. Significant difference in the oral availability of danazol between SEDDS and S-SEDDS forms was not observed. In contrast, each formulation

**Fig. 4** A schematic presentation of the relationship between the limiting steps of oral absorption of poorly water-soluble drugs and the formulation effect.





**Fig. 5** Correlation for the various formulations on the oral absorption of danazol (**a**) and pranlukast (**b**) between *in vitro* and *in vivo* experiments. The same symbols defined in Figs. 2 and 3 are used for each formulation.



performance could be evaluated from *in vitro* experiment with the D/P system. This discrepancy in the results could be caused by the difference in the sensitivity between the *in vitro* and *in vivo* studies. *In vitro* study provides repeatable data with small deviation. In contrast, *in vivo* study provides data with large deviation due to intra-species difference. From Fig. 5 a, significant correlation ( $r^2=0.83$ ) between *in vivo* oral availability ( $AUC_{0-8h}$ ) and estimated absorption ( $F_a\%$ ) from the permeated amount in the D/P system was obtained for danazol. In the case of pranlukast, because significant formulation effect on the oral availability was not observed, irrespective of formulation type (Table VI), we did not obtain a correlation coefficient (Fig. 5 b). However, when pranlukast was administered as SEDDS formulations, the maximum drug concentration time ( $T_{max}$ ) was seemed to be delayed compared to that after administration as standard suspension and co-solvent formulations (Table VI). As the reason for the change in  $T_{max}$ , several possibilities were considerable as follows: due to decrease in the free fraction of drugs in the luminal solution and due to change in the gastrointestinal movement of drug solution. Although the reason for the change in  $T_{max}$  was not clarified, the oral availability of pranlukast was not affected by any formulations used in this study. The estimated absorption from the permeated amount in the D/P system indicated that the oral absorption of pranlukast was not increased by co-solvent formulations, irrespective of the presence of HPMC, corresponding well to the *in vivo* observations. The increase of solubility by SEDDS formulations would be cancelled out by the decrease of effective permeability, because free fraction of drugs in the luminal solution might be the same between drug only and lipid-based formulation. In the case of co-solvent formulation, PEG400 in a solution enhances drug solubility but reduces effective permeability with an increase in the concentration of PEG400 (26). PEG400 is regarded as a safe and non-toxic agent and there is no positive evidence for direct interaction of PEG400 with

passive permeation process. Thus, the indirect effects of PEG400, such as the increased viscosity of the apical solution and interaction between PEG400 and pranlukast, are considered to influence effective permeability of pranlukast. In order to clarify the effect, more detailed studies are necessary. However, these effects of PEG400 could be observed both *in vitro* and *in vivo* studies, suggesting that the D/P system detects the similar effect observed *in vivo*.

These *in vitro/in vivo* correlations in the oral absorption indicated that limiting steps in the *in vivo* gastrointestinal absorption of poorly water-soluble drugs followed the scheme shown in Fig. 4. Therefore, the D/P system may be a useful tool not only for the estimation of drug absorption from various formulations but also for understanding of the formulation effect on oral absorption of poorly water-soluble drugs. In addition, the D/P system may provide valuable information to select the oral dosage form in drug development.

In this paper, the effect of lipolysis on the oral absorption of poorly water-soluble drugs after administration as lipid-based formulation was not discussed since the formulation effect on the drug dissolution and permeation was focused on. Whereas lipolysis is also the key factor to determine the oral absorption of drugs from lipid-based formulation, how lipolysis impacts on the absorption has not been reported. By using the D/P system, the effect of lipolysis on the drug absorption from lipid-based formulation may be estimated and is a subject for future study.

## CONCLUSION

In conclusion, we have successfully demonstrated that the D/P system enables evaluation of the formulation effect on oral drug absorption and have indicated that the use of supersaturation-generated formulation could be a favorable

strategy to improve the oral absorption of poorly water-soluble drugs with high permeability. The D/P system may be a useful tool for formulation selection in the early stage of drug development.

## ACKNOWLEDGMENTS & DISCLOSURES

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