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Bile micelle binding of structurally diverse ionized drug molecules

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Abstract

Background and purpose: Predicting the food effect on oral drug absorption by physiologically based biopharmaceutical modelling (PBBM) remains challenging. The bile micelle unbound fraction (f_u) is one of the primary determinants of the negative food effect for high solubility drugs. To calculate the pH- f_u profile for PBBM, the bile micelle partition coefficients of ionized and un-ionized drug species ($K_{bm,z}$, z : charge) are required. The general rules for the ratio of the partition coefficients of ionized and un-ionized drug species have been reported for the octanol/water (P_{oct}) and phosphatidylcholine liposome/water partition coefficients. However, the general rule for the bile micelle partition coefficient has not yet been investigated. The purpose of the present study was to clarify the general rule for $K_{bm,z \neq 0}/K_{bm,0}$. **Experimental approach:** The pH- f_u profiles of 4 monovalent weak acids, 8 monovalent weak bases, 2 divalent weak bases, and 2 zwitterion drugs were measured by dynamic dialysis in the pH range about $pK_a \pm 2$. Bile micelles consisted of taurocholic acid (TC)/egg lecithin (15 mM/ 3.75 mM). $K_{bm,z}$ was calculated from the pH- f_u profiles. **Key results:** $K_{bm,-1}/K_{bm,0}$ was ≤ 0.03 for all monovalent acids. $K_{bm,+1}/K_{bm,0}$ ranged from 0.24 to 2.6. $K_{bm,+2}/K_{bm,0}$ was about 0.3. For the two zwitterionic drugs, $K_{bm,-1}/K_{bm,\pm 0}$ was 1.1 and 2.3, and $K_{bm,+1}/K_{bm,\pm 0}$ was 3.9 and 20, respectively. $K_{bm,0}$ roughly correlated with P_{oct} ($r = 0.68$). **Conclusion:** The bile micelle binding of anionic drug species ($z = -1$) is generally negligible, whereas that of cationic drug species ($z = +1$) can be significant. A general rule for $K_{bm,+1}/K_{bm,0}$ was not found. $K_{bm,+1}/K_{bm,0}$ can be greater than 1 in several cases, suggesting an attractive electrostatic interaction between the positive charge of a drug and the negative charge of TC. These points should be considered in food effect prediction.

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Keywords

Bile micelles; ionizable drug; unbound fraction; dynamic dialysis; intestinal membrane permeation; physiologically-based biopharmaceutics modelling

Introduction

Oral drug absorption is affected by various gastrointestinal (GI) conditions [1]. Physiologically based biopharmaceutics modelling (PBBM) is anticipated to be a powerful tool to predict the effect of GI conditions on oral drug absorption. The GI conditions in the fed state differ from those in the fasted state. For example, the concentration of bile micelles (C_{bm}) increases about 5-fold in the fed state compared to the fasted state [2,3]. It has previously been suggested that the unbound (free) fraction (f_u) of a drug in the intestinal fluid is one of the factors of the negative food effect on the oral absorption of highly soluble weak base drugs [4,5]. An increase in bile micelle binding in the fed state reduces f_u , resulting in a decrease in the effective intestinal

permeability (P_{eff}) and a negative food effect on oral drug absorption (cf. P_{eff} is defined based on the total dissolved drug concentration (= bound + unbound concentrations)) [6-10]. Bile micelle-bound drug molecules diffuse across the unstirred water layer (UWL) adjacent to the epithelial membrane; however, only unbound drug molecules permeate the epithelial membrane [5]. When P_{eff} is rate-limited by the epithelial membrane permeation, $P_{\text{eff}} \propto f_u P_{\text{ep}}$ (P_{ep} : the epithelial membrane permeability of unbound drug molecules [5]). The f_u value of an ionizable drug depends on the pH value, which can vary in the small intestine due to factors such as the intestinal position, postprandial conditions, and intra- and inter-individual variations. Therefore, a pH- f_u profile is required for accurate food effect prediction. To calculate the pH- f_u profile, the bile micelle partition coefficient ($K_{\text{bm},z}$, z = charge) of both un-ionized ($z = 0$) and ionized drug species ($z \neq 0$) is required. K_{bm} is the ratio of drug concentration in the bile micelles to the water phase, normalized by the bile micelle and water concentrations [11].

In the case of the octanol-water partition coefficient (P_{oct}), the ratios of the partition coefficients of cationic species ($P_{\text{oct},+1}$) and anionic species ($P_{\text{oct},-1}$) to un-ionized species ($P_{\text{oct},0}$) are generally approximated to be about $P_{\text{oct},+1}/P_{\text{oct},0} \approx 1/1000$ and $P_{\text{oct},-1}/P_{\text{oct},0} \approx 1/10000$, respectively (in the presence of 0.15 M NaCl) [12]. In the case of the liposome-water partition coefficient of phosphatidylcholine (PC) liposomes, the ratios of the partition coefficients of cationic species ($K_{\text{PC},+1}$) and anionic species ($K_{\text{PC},-1}$) to un-ionized species ($K_{\text{PC},0}$) are generally approximated to be about $K_{\text{PC},+1}/K_{\text{PC},0} \approx 1/10$ and $K_{\text{PC},-1}/K_{\text{PC},0} \approx 1/100$, respectively [12-14]. However, it has been unknown whether there is such a general approximation rule for the bile micelle partition coefficient. Previously, Schwartz et al. investigated the bile micelle binding of several ionizable drugs by electrokinetic capillary chromatography at pH 7.4 and 10 [15]. It was suggested that only hydrophobic weak base drugs, such as quinine and propranolol, can interact with bile micelles. Castro et al. investigated the K_{bm} of atenolol, nadolol, and nitrazepam at pH 7.0 and 10.8 by spectrofluorimetry and derivative spectrophotometry [16]. In those studies, the K_{bm} of protonated molecular species ($K_{\text{bm},+1}$) of atenolol and nadolol was greater than that of un-ionized species ($K_{\text{bm},0}$). On the other hand, the K_{bm} of the deprotonated molecular species of nitrazepam (mono-anion) ($K_{\text{bm},-1}$) was markedly less than $K_{\text{bm},0}$. However, the number of drugs was not sufficient to clarify whether there is a general rule for $K_{\text{bm},z \neq 0}/K_{\text{bm},0}$.

The purpose of the present study was to clarify whether there is a general rule for the ratio of $K_{\text{bm},z \neq 0}/K_{\text{bm},0}$ for structurally diverse ionizable drug molecules. Four monovalent weak acids, 8 monovalent weak bases, 2 divalent weak bases, and 2 zwitterionic drugs were employed as model drugs (Figure 1). The pH- f_u profile was measured by dynamic dialysis [17,18]. The f_u value was measured in the pH range of about $\text{pK}_a \pm 2$. The $K_{\text{bm},z}$ values were calculated from the pH- f_u profile in the fed-state simulated intestinal fluid (FeSSIF) containing taurocholic acid (TC) (15 mM) and egg lecithin (EL) (3.75 mM) [19].

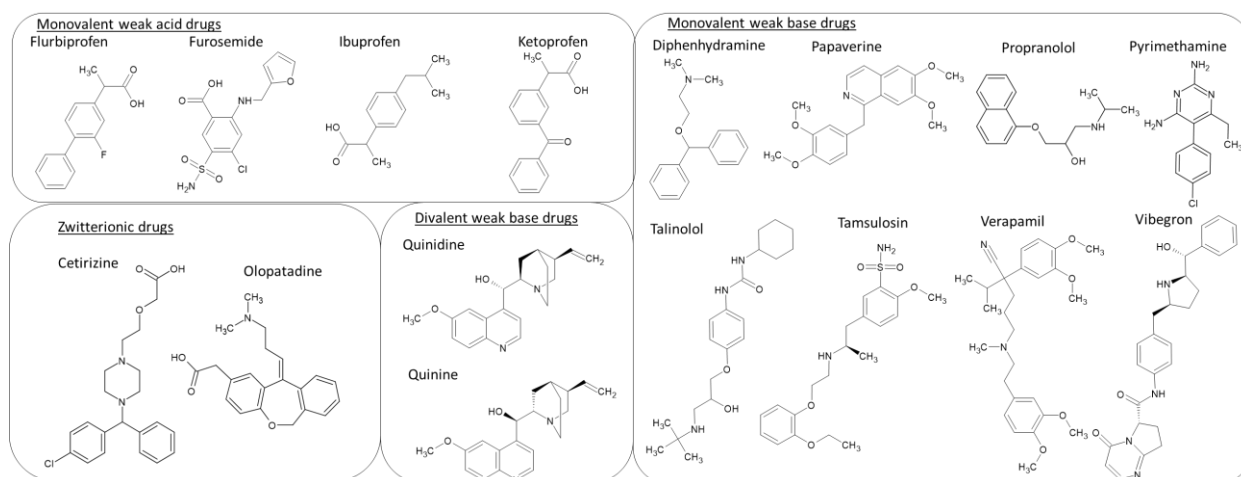


Figure 1. Chemical structures of model drugs

Table 1. Physicochemical properties of model drugs

Drug	MW	pK _a ^a	log P _{oct}	ref
Monovalent weak acid drugs				
Flurbiprofen	244	4.18(A) (25 °C, I = 0.16 M)	4.0	[12]
Furosemide	331	3.53(A) (37 °C, I = 0.15 M)	2.6	[12]
Ibuprofen	206	4.35(A) (25 °C, I = 0.15 M)	4.1	[12]
Ketoprofen	254	4.00(A) (37 °C, I = 0.15 M)	3.2	[12]
Monovalent weak base drugs				
Diphenhydramine	255	8.86(B) (37 °C, I = 0.15 M)	3.2	[12]
Papaverine	339	6.22(B) (37 °C, I = 0.18 M)	3.0	[12]
Propranolol	259	9.16(B) (37 °C, I = 0.15 M)	3.5	[12]
Pyrimethamine	249	7.36(B) ^b	2.7	[20]
Talinolol	363	9.4 (B) ^b	3.1	[21]
Tamsulosin	409	8.37(B) ^b	2.0	[22]
Verapamil	455	8.68(B) (37°C, I = 0.19 M)	4.3	[12]
Vibegron	445	8.9(B) ^b	3.1	[23]
Divalent weak base drugs				
Quinidine	324	4.09(B), 8.55(B) ^b	3.6	[12]
Quinine	324	4.35 (B), 8.57 (B) (26°C, I = 0.15 M)	3.5	[12]
Zwitterionic drugs				
Cetirizine	389	2.12(B), 2.90(A), 7.98(B) (25°C, I = 0.15 M)	1.46 ^c	[12]
Olopatadine	337	4.18 (A), 9.79 (B) ^b	0.34 ^c	[24]

^aA: acid, B: base; ^bTemperature and ionic strength were not reported; ^cOctanol-water distribution coefficient at pH 6.5 [25].

Experimental

Material

Diphenhydramine hydrochloride, ibuprofen, ketoprofen, papaverine hydrochloride, propranolol hydrochloride, quinidine sulphate dihydrate, quinine, sodium taurocholic acid (TC), sodium chloride, sodium dihydrogen phosphate dihydrate, 6 M HCl, and 8 M NaOH were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Cetirizine dihydrochloride, tamsulosin hydrochloride, pyrimethamine, flurbiprofen, furosemide, olopatadine hydrochloride, talinolol, and verapamil hydrochloride were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Vibegron was extracted from the Beova tablet purchased from KYORIN Pharmaceutical Co., Ltd (Tokyo, Japan). Egg yolk lecithin (EL) was purchased from Kewpie Corporation (Tokyo, Japan). A cellulose dialysis membrane (MWCO 3500) was purchased from As-One Corporation (Osaka, Japan).

Methods

Measurement of the unbound fraction by dynamic dialysis

The f_u value was measured by dynamic dialysis using a side-by-side chamber (SANPLATEC Co., Ltd, Osaka, Japan) as previously reported [25,26]. The area of the dialysis membrane was 2.0 cm². The fluid volume was 1.5 mL in both the donor and acceptor chambers. The bile micelle media consisted of TC/EL (15 / 3.75 mM) and phosphate buffer (28.6 mM phosphate, 106 mM NaCl). The pH value was adjusted by NaOH in the range of about pK_a±2 (9615S-10D Standard ToupH electrode, HORIBA Advanced Techno, Co., Ltd., Kyoto, Japan). A drug solution with or without bile micelles at each pH (1.5 mL) was added to the donor chamber. The initial donor concentration of each drug is shown in Supplementary material (SM) Table S1. A blank phosphate buffer solution (same pH) without a drug and bile micelles (1.5 mL) was added to the acceptor chamber. After incubation for 1.0 h at 37 °C, the drug concentration in the acceptor chamber was measured by HPLC (Shimadzu Prominence LC-20 series, column: ZORBAX Eclipse Plus (C18 2.1×50 mm, 3.5 μm) (Agilent Technologies), flow rate: 0.6 mL min⁻¹, column temperature: 40 °C, and injection volume of 10 μL). The mobile phase

composition and the detection wavelength are listed in SM Table S2. The determination coefficient of the standard curves was greater than 0.999 in all cases.

Permeation, % was calculated as the ratio of the concentrations in the acceptor chamber at 1.0 h and the theoretical equilibrium concentration in the absence of bile micelles (1/2 of the initial donor concentration). The unbound fraction (f_u) was calculated as the ratio of permeation in the presence and absence of bile micelles.

K_{bm} calculation

The K_{bm} value of each charge species ($K_{bm,z}$) was calculated from the pH- f_u profile [27]. For monovalent weak acid drugs (HA), the un-ionized fraction (f_0) is defined by Equation (1),

$$f_0 = \frac{[HA]}{[HA] + [A^-]} = \frac{1}{1 + \frac{[A^-]}{[HA]}} = \frac{1}{1 + \frac{K_a}{[H^+]}} \quad (1)$$

The unbound fraction (f_u) is defined by Equation (2),

$$f_u = \frac{[HA] + [A^-]}{[HA] + [A^-] + [HA \cdot BM] + [A^- \cdot BM]} \quad (2)$$

Therefore, the un-ionized unbound fraction ($f_0 f_u$) becomes, Equation (3),

$$\begin{aligned} f_0 f_u &= \frac{[HA]}{[HA] + [A^-]} \times \frac{[HA] + [A^-]}{[HA] + [A^-] + [HA \cdot BM] + [A^- \cdot BM]} = \frac{[HA]}{[HA] + [A^-] + [HA \cdot BM] + [A^- \cdot BM]} = \\ &= \frac{1}{1 + \frac{[A^-]}{[HA]} + \frac{[HA \cdot BM]}{[HA]} + \frac{[A^- \cdot BM]}{[HA] [A^-]}} = \frac{1}{1 + \frac{K_a}{[H^+]} + \frac{C_{bm}}{C_w} \left(K_{bm,0} + \frac{K_a}{[H^+]} K_{bm,-1} \right)} \end{aligned} \quad (3)$$

where BM is bile micelles, C_{bm} is the concentration of BM (mol/L) (in this study, 0.015 mol/L), and C_w is the concentration of water (55.5 mol L⁻¹) [11]. The f_u value can be calculated by dividing Eq. (3) by Eq. (1).

Similarly, for monovalent weak base drugs (B), Equations (4) and (5)

$$f_0 = \frac{[B]}{[B] + [BH^+]} = \frac{1}{1 + \frac{[BH^+]}{[B]}} = \frac{1}{1 + \frac{K_a}{[H^+]}} \quad (4)$$

$$f_0 f_u = \frac{[B]}{[B] + [BH^+] + [B \cdot BM] + [BH^+ \cdot BM]} = \frac{1}{1 + \frac{[H^+]}{K_a} + \frac{C_{bm}}{C_w} \left(K_{bm,0} + \frac{[H^+]}{K_a} K_{bm,+1} \right)} \quad (5)$$

For divalent weak base drugs with pK_{a1} and pK_{a2} (B) ($pK_{a1} < pK_{a2}$), Equations (6) and (7),

$$f_0 = \frac{[B]}{[BH_2^{+}] + [BH^+] + [B]} = \frac{1}{\frac{[BH_2^{+}][BH^+]}{[BH^+][B]} + \frac{[BH^+]}{[B]} + 1} = \frac{1}{\frac{[H^+]^2}{K_{a1}K_{a2}} + \frac{[H^+]}{K_{a2}} + 1} \quad (6)$$

$$\begin{aligned} f_0 f_u &= \frac{[B]}{[BH_2^{+}] + [BH_2] + [B] + [BH_2^{+} \cdot BM] + [BH_2 \cdot BM] + [B \cdot BM]} = \\ &= \frac{1}{\frac{[H^+]^2}{K_{a1}K_{a2}} + \frac{[B^+]}{K_{a2}} + 1 + \frac{C_{bm}}{C_w} \left(\frac{[H^+]^2}{K_{a1}K_{a2}} K_{bm,+2} + \frac{[H^+]}{K_{a2}} K_{bm,+1} + K_{bm,0} \right)} \end{aligned} \quad (7)$$

For zwitterion drugs with pK_{a1} and pK_{a2} (D) ($pK_{a1} < pK_{a2}$), Equations (8) and (9),

$$f_0 = \frac{[D]}{[DH^+] + [D] + [D^-]} = \frac{1}{\frac{[DH^+]}{[D]} + 1 + \frac{[D^-]}{[D]}} = \frac{1}{\frac{[H^+]}{K_{a1}} + 1 + \frac{K_{a2}}{[H^+]}} \quad (8)$$

$$f_0 f_u = \frac{[D]}{[DH^+] + [D] + [D^-] + [DH^+ \cdot BM] + [D \cdot BM] + [D^- \cdot BM]} = \frac{1}{1 + \frac{[H^+]}{K_{a1}} + \frac{K_{a2}}{[H^+]}} + \frac{C_{bm}}{C_w} \left(\frac{[H^+]}{K_{a1}} K_{bm,+1} + K_{bm,0} + \frac{K_{a2}}{[H^+]} K_{bm,-1} \right) \quad (9)$$

The $K_{bm,z}$ values can be obtained by fitting the theoretical pH- f_u curve to experimentally observed data by the least squares method using the Excel solver. Because the f_u was measured at 37 °C, the pK_a value at 37 °C ($pK_{a(37^\circ C)}$) was used for the calculation when available in the literature. The pK_a value at 25 °C can be converted to $pK_{a(37^\circ C)}$ by the Abraham linear free energy relationship [28]. However, the Abraham solute descriptor was not available for some drugs. In addition, the temperature was not reported. Therefore, in these cases, the reported values were used as it is. $[H^+]$ was calculated as 10^{-pH} , neglecting the effect of ionic strength (I) (about 0.1 pH unit) and the electrode factors [12].

Results and discussion

Permeation and f_u values at each pH are summarized in SM Table S1. Since the f_u value becomes sensitive to the variation in permeation at $f_u > 0.9$, they were not used for the following data analysis.

Monovalent weak acid drugs

Figure 2 shows the pH- f_u profiles of monovalent weak acid drugs. The theoretical equations (Eqs. (1) to (3)) appropriately described the pH- f_u profiles. The $K_{bm,z}$ values are summarized in Table 2.

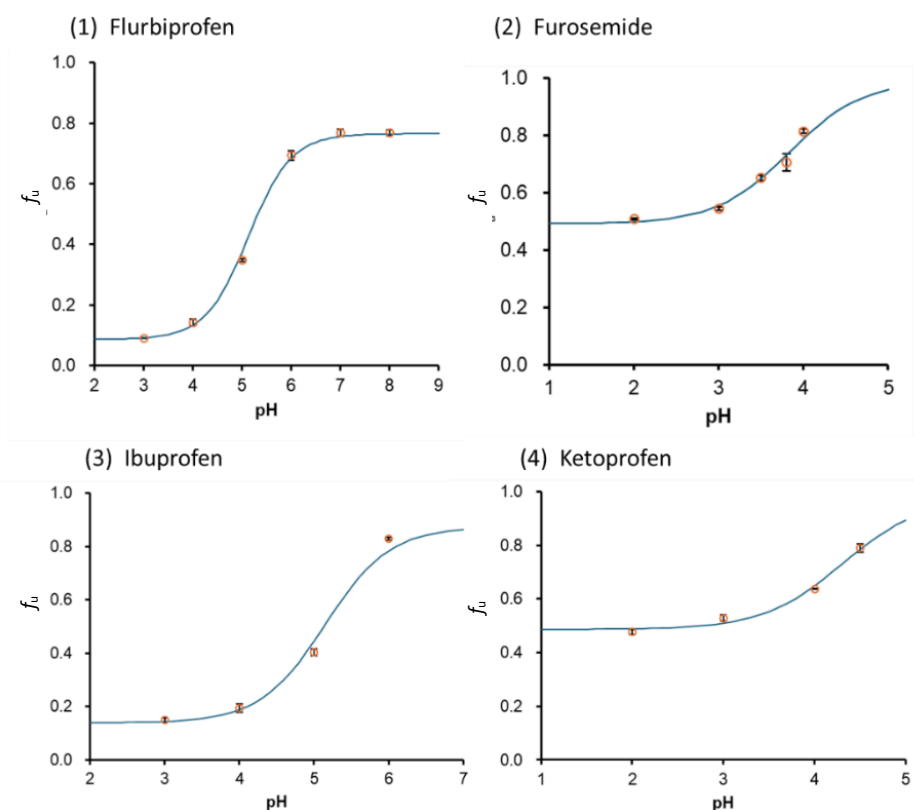


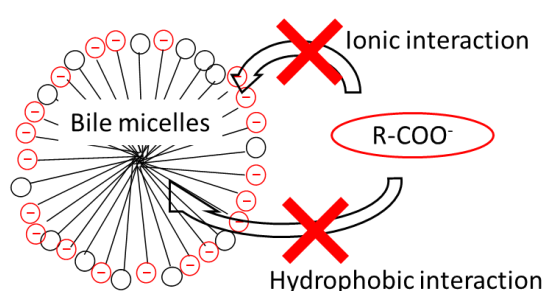
Figure 2. pH- f_u profile of monovalent weak acids in the TC/EL bile micelle media (mean $\pm$ standard deviation (SD), $N = 3$). The solid line is the fitted theoretical curve

Table 2. K_{bm} values of monovalent weak acid drugs in the TC/EL bile micelle media (mean $\pm$ SD, $N = 3$)

Drug	$K_{bm,0}$	$K_{bm,-1}$	$K_{bm,-1} / K_{bm,0}$
Flurbiprofen	$3.93 \pm 0.14 \times 10^4$	$1.14 \pm 0.07 \times 10^3$	0.03
Furosemide	$3.80 \pm 0.04 \times 10^3$	$< 1.00 \times 10^2$	<0.03
Ibuprofen	$2.29 \pm 0.18 \times 10^4$	$5.33 \pm 1.16 \times 10^2$	0.02
Ketoprofen	$3.91 \pm 0.12 \times 10^3$	$< 1.00 \times 10^2$	<0.03

In the case of monovalent weak acid drugs, the bile micelle binding decreased as pH increased (the f_u value increased as a drug became ionized at $pH > pK_a$ (deprotonated)) (Figure 2). The $K_{bm,-1}/K_{bm,0}$ ratio was ≤ 0.03 for all weak acid drugs (Table 2). Like the cases of P_{oct} and K_{pc} , the bile micelle binding of anionic molecular species ($z = -1$) is negligibly small compared to un-ionized molecular species ($z = 0$). The negatively charged moiety of a drug can be energetically unfavourable for partitioning to both the hydrophobic core region and the negatively charged head group region of the bile micelles (Figure 3A).

(A) Negatively charged moiety



(B) Positively charged moiety

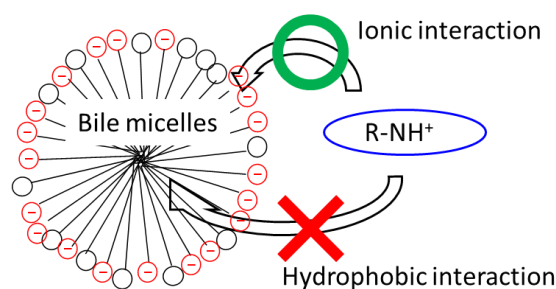


Figure 3. Interactions between the charged moiety of a drug and bile micelles. R-COO⁻: ionized (deprotonated) carboxylic group, R-NH⁺: ionized (protonated) amino group.

Monovalent weak base drugs

Figure 4 shows the pH- f_u profiles of monovalent weak base drugs. The theoretical equations (Eqs. (4) and (5)) appropriately described the pH- f_u profiles. The $K_{bm,z}$ values are summarized in Table 3.

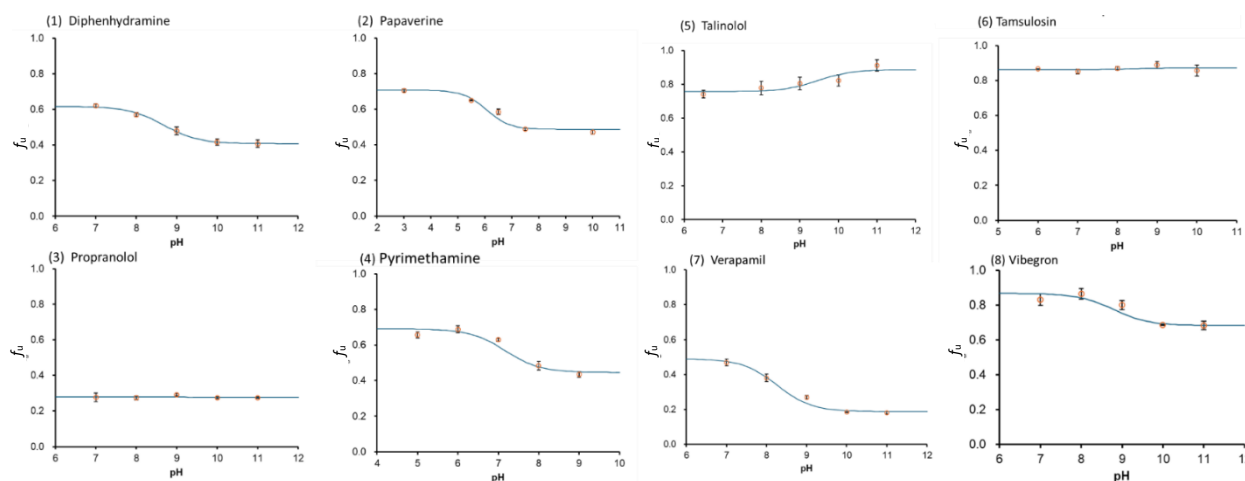


Figure 4. pH- f_u profile of monovalent weak bases in the TC/EL bile micelle media (mean $\pm$ SD, $N = 3$). The solid line is the fitted theoretical curve

In the case of monovalent weak base drugs, the f_u value either increased or decreased as pH decreased (Figure 3). The $K_{bm,+1}/K_{bm,0}$ ratio ranged from 0.24 to 2.6 (Table 3), unlike the cases of octanol and PC liposome partitioning ($P_{oct,+1}/P_{oct,0} \approx 0.001$, $K_{PC,+1}/K_{PC,0} \approx 0.1$) [12–14]. Structurally diverse cationic molecular species ($z = +1$) can bind to bile micelles. However, no general rule was found for $K_{bm,+1}/K_{bm,0}$. Therefore, $K_{bm,+1}/K_{bm,0}$ is not simply explained by ionic interaction. The positively charged moiety of a drug can be energetically unfavourable for partitioning to the hydrophobic core region of bile micelles; however, favourable for partitioning to the negatively charged head group region of bile micelles (Figure 3B).

Table 3. K_{bm} values of monovalent weak base drugs in the TC/EL bile micelle media (mean $\pm$ SD, $N = 3$)

Drug	$K_{bm,0}$	$K_{bm,+1}$	$K_{bm,+1}/K_{bm,0}$
Diphenhydramine	$5.40 \pm 0.34 \times 10^3$	$2.30 \pm 0.04 \times 10^3$	0.43
Papaverine	$3.91 \pm 0.16 \times 10^3$	$1.52 \pm 0.03 \times 10^3$	0.39
Propranolol	$9.66 \pm 0.20 \times 10^3$	$9.64 \pm 0.40 \times 10^3$	1.00
Pyrimethamine	$4.61 \pm 0.27 \times 10^3$	$1.66 \pm 0.16 \times 10^3$	0.36
Talinolol	$4.75 \pm 1.00 \times 10^2$	$1.19 \pm 0.11 \times 10^3$	2.60
Tamsulosin	$5.42 \pm 1.29 \times 10^2$	$5.89 \pm 0.39 \times 10^2$	1.13
Verapamil	$1.60 \pm 0.02 \times 10^4$	$3.87 \pm 0.23 \times 10^3$	0.24
Vibegron	$1.67 \pm 0.06 \times 10^3$	$5.80 \pm 1.80 \times 10^2$	0.35

The balance of these two factors can determine the $K_{bm,+1}/K_{bm,0}$ value. In the case of $K_{bm,+1}/K_{bm,0} > 1.0$, the sulfonate group of taurocholates ($R-SO_3^-$) and the ammonium moiety of a drug (R_3NH^+) might have a strong attractive electrostatic interaction [29]. However, in the cases of $K_{bm,+1}/K_{bm,0} < 1.0$, the cationic charge is less favourable for partitioning into the hydrophobic core region of bile micelles. In the present study, $K_{bm,+1}/K_{bm,0}$ of propranolol and talinolol were ≥ 1.0 (1.0 and 2.6, respectively). Previously, $K_{bm,+1}/K_{bm,0}$ of atenolol and nadolol in deoxycholate/EL micelles were also reported to be > 1.0 (1.3 and 1.7, respectively) [16]. For β -blockers, $K_{bm,+1}/K_{bm,0}$ may be generally ≥ 1.0 .

Divalent weak bases

Figure 5 shows the pH- f_u profiles of divalent weak base drugs. The theoretical equations (Eqs. (6) and (7)) appropriately described the pH- f_u profiles. The $K_{bm,z}$ values are summarized in Table 4.

In the case of the divalent weak base drugs (quinidine and quinine), the bile micelle binding decreased (the f_u value increased) step by step with decreasing pH below each pK_a (Figure 4). The $K_{bm,+1}/K_{bm,0}$ and $K_{bm,+2}/K_{bm,0}$ ratios were about 0.8 to 0.9 and 0.3, respectively (Table 4). In these cases, the first and second positive charges are both unfavourable for bile micelle binding. The K_{bm} values of quinidine and quinine are almost the same, suggesting that diastereomers may show similar K_{bm} values, even though TC and EL are chiral.

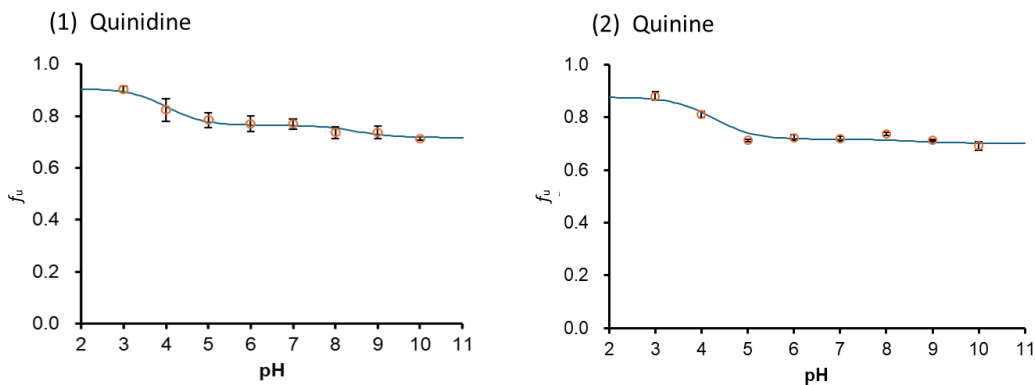


Figure 5. pH- f_u profile of divalent weak base drugs in the TC/EL bile micelle media (mean $\pm$ S.D., $N = 3$). The solid line is the fitted theoretical curve

Table 4. K_{bm} values of divalent base drugs in the TC/EL bile micelle media (mean $\pm$ S.D., $N = 3$)

Drug	$K_{bm,0}$	$K_{bm,+1}$	$K_{bm,+2}$	$K_{bm,+1}/K_{bm,0}$	$K_{bm,+2}/K_{bm,0}$
Quinidine	$1.47 \pm 0.08 \times 10^3$	$1.15 \pm 0.03 \times 10^3$	$3.75 \pm 1.23 \times 10^2$	0.79	0.26
Quinine	$1.58 \pm 0.07 \times 10^3$	$1.47 \pm 0.02 \times 10^3$	$5.27 \pm 0.43 \times 10^2$	0.93	0.34

Zwitterionic drugs

Figure 6 shows the pH- f_u profiles of zwitterionic drugs. The theoretical equations (Eqs. (8) and (9)) appropriately described the pH- f_u profiles. The $K_{bm,z}$ values are summarized in Table 5.

In the case of the zwitterionic drugs (cetirizine and olopatadine), the anionic ($z = -1$) and cationic ($z = +1$) species were bound to bile micelles greater than the zwitterionic species ($z = \pm 0$).

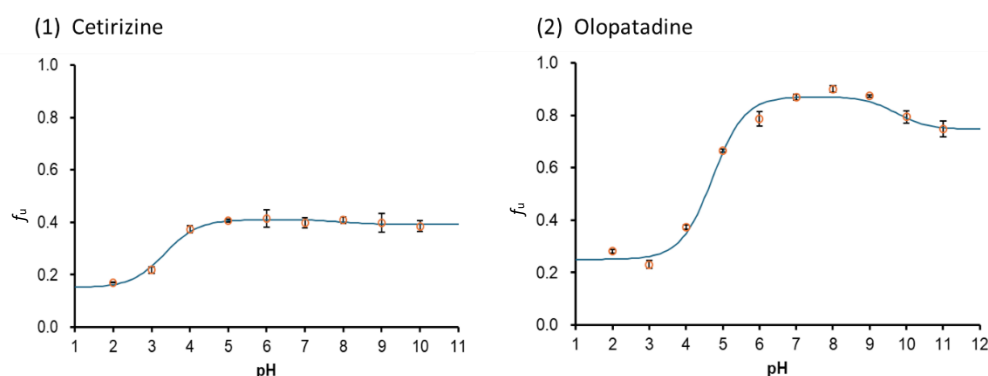


Figure 6. pH- f_u profile of zwitterionic drugs in the TC/EL bile micelle medium (mean $\pm$ SD, $N = 3$). The solid line is the fitted theoretical curve

Table 5. K_{bm} values of zwitterionic drugs in the TC/EL bile micelle media (mean $\pm$ S.D., $N = 3$)

Drug	$K_{bm,\pm 0}$	$K_{bm,-1}$	$K_{bm,+1}$	$K_{bm,-1}/K_{bm,\pm 0}$	$K_{bm,+1}/K_{bm,\pm 0}$
Cetirizine ^a	$5.34 \pm 0.33 \times 10^3$	$5.77 \pm 0.61 \times 10^3$	$2.09 \pm 0.10 \times 10^4$	1.09	3.93
Olopatadine ^a	$5.47 \pm 0.38 \times 10^2$	$1.26 \pm 0.15 \times 10^3$	$1.11 \pm 0.04 \times 10^4$	2.33	20.5

^a See text for the explanation of $z = \pm 0$.

The equilibrium of zwitterionic drugs is expressed by Equation (10),



where COOH is a carboxylic group, and N is an amino group. In the pH region of pK_{a1} (acid) $< \text{pH} < \text{pK}_{a2}$ (base), these drugs can exist as un-ionized ($z = 0$, $\text{COOH} \cdot \text{N}$) and zwitterionic species ($z = \pm 0$, $\text{COO}^- \cdot \text{NH}^+$), the latter being predominant [30,31]. The zwitterionic form contains both negative ($z = -1$) and positive ($z = +1$) charge moieties. In cetirizine and olopatadine, these two moieties are distant from each other and not electrically conjugated. The negatively charged moiety can be energetically unfavourable for partitioning to both the hydrophobic core region and the negatively charged head group region of the bile micelle. On the other hand, the positively charged moiety can be energetically unfavourable for partitioning to the hydrophobic core region; however, favourable for partitioning to the negatively charged head group region. The balance of these factors determines the $K_{bm,z}/K_{bm,\pm 0}$ value.

$K_{bm,+1}/K_{bm,\pm 0}$ was 3.9 and 20 for cetirizine and olopatadine, respectively (Table 4). These values are greater than the $K_{bm,+1}/K_{bm,0}$ value for monovalent weak base drugs (0.24 to 2.6). When changing pH from $\text{pH} < \text{pK}_{a1}$ to $\text{pH} > \text{pK}_{a1}$, a negative charge is added to the cationic species. (*i.e.* $z = +1 + (-1) = \pm 0$). The large $K_{bm,+1}/K_{bm,\pm 0}$ values suggested that the addition of a negative charge is markedly unfavourable for the bile micelle partitioning, like the cases of monovalent weak acids. $K_{bm,+1}/K_{bm,\pm 0}$ can be rearranged as Equation (11)

$$\frac{K_{bm,+1}}{K_{bm,\pm 0}} = \frac{K_{bm,+1}}{K_{bm,0}} \frac{K_{bm,0}}{K_{bm,\pm 0}} \quad (11)$$

Therefore, Equation (12)

$$\frac{K_{bm,0}}{K_{bm,\pm 0}} = \frac{K_{bm,+1}}{K_{bm,0}} \frac{K_{bm,\pm 0}}{K_{bm,+1}} \quad (12)$$

If the average $K_{bm,+1}/K_{bm,0}$ value for monovalent weak base drugs (0.81) is applied to Eq. 12, $K_{bm,0}/K_{bm,\pm 0}$ becomes 0.21 and 0.041 for cetirizine and olopatadine, respectively. This may suggest that, for bile micelle binding, the zwitterionic species ($z = \pm 0$, $\text{COO}^- \cdot \text{NH}^+$) is significantly less favourable than the un-ionized species ($z = 0$, $\text{COOH} \cdot \text{N}$). However, a more detailed investigation is required to conclude this point [13].

The $K_{bm,-1}/K_{bm,\pm 0}$ ratios were also greater than 1.0 (1.1 and 2.3 for cetirizine and olopatadine, respectively). The removal of one positive charge from the zwitterion (*i.e.* $z = \pm 0 - (+1) = -1$) by changing pH from $\text{pH} < \text{pK}_{a2}$ to

pH > pK_{a2} increased the bile micelle partitioning for these drugs. As discussed above, the removal of a positive charge from a drug can be both favourable and unfavourable for bile micelle partitioning, in this case, favourable.

Relationship between $K_{bm,0}$ and P_{oct}

Previously, Glomme *et al.* [11] reported a good correlation between $K_{bm,0}$ and $P_{oct,0}$ ($\log K_{bm,0} = 0.74 \log P_{oct,0} + 2.29$). In that study, $K_{bm,0}$ was obtained from the solubility data in bile micelle media for poorly soluble un-ionizable drugs. However, in the present study, only a poor correlation was found between $K_{bm,0}$ and $P_{oct,0}$ ($\log K_{bm,0} = 0.62 \log P_{oct,0} + 1.55$, $r = 0.68$) (Figure 7). The slope and intercept deviated from the previous report. The reason for this discrepancy is not clear. For high solubility drugs, it is difficult to measure f_u from the solubility values in bile micelle media. Therefore, dynamic dialysis was used in the present study. This might be one of the reasons for the discrepancy.

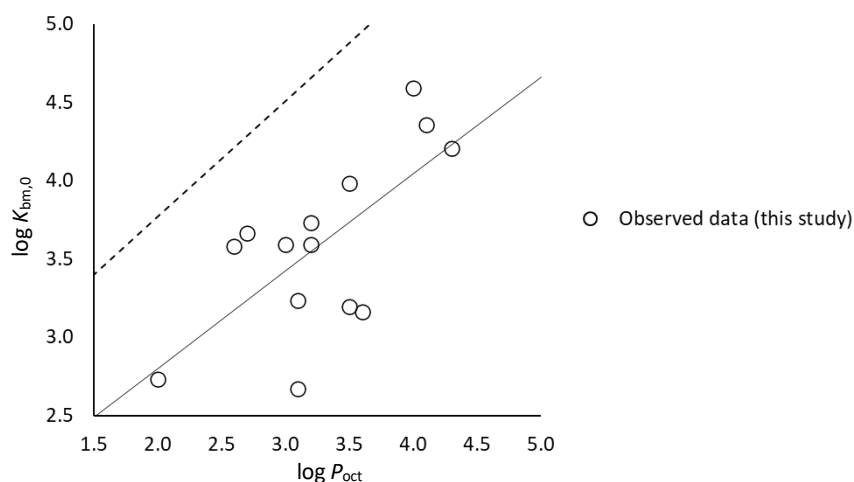


Figure 7. Correlation between $\log P_{oct,0}$ and $\log K_{bm,0}$ for TC/EL bile micelles. Solid line: the fitted line in this study, dotted line: the correlation line previously reported by Glomme *et al.* [11]

Suggestions for food effect prediction by PBBM

The results of this study suggested that, when predicting the food effect for highly soluble weak acid drugs, $K_{bm,-1}$ can be negligible. In contrast, for highly soluble weak base drugs, $K_{bm,+1}$ should be considered. It was previously reported that the f_u value at pH 6.5 correlated with the clinical negative food effect for high solubility weak base drugs [4]. At pH 6.5, a weak base drug with pK_a > 7.5 mainly exists as cationic species (> 90%). It was recently reported that quaternary ammonium compounds (permanent cations) can also bind to bile micelles [25,26]. Since there is no general rule for $K_{bm,+1}/K_{bm,0}$, $K_{bm,+1}$, and $K_{bm,0}$ should be experimentally measured from the pH- f_u profile. The oral absorption of quinine is reduced by bile micelles in vivo [32], in good agreement with the result of this study. At pH 6.5, quinine mainly exists as $z = +1$ species. The f_u values of the zwitterionic drugs ($z = \pm 0$) are less than 1 at pH 6.5, in good agreement with the negative food effect [25], suggesting that $K_{bm,\pm 0}$ should also be considered. Dynamic dialysis would be a suitable tool to measure f_u for high solubility drugs [33].

Conclusions

The bile micelle partitioning of anionic species ($z = -1$) of highly soluble weak acid drugs was negligible. On the other hand, $K_{bm,+1}/K_{bm,0}$ ranged from 0.24 to 2.6 for highly soluble weak base drugs. In about half of the cases, the mono-cationic species ($z = +1$) were bound to bile micelles equal to or greater than the un-ionized species ($K_{bm,+1}/K_{bm,0} \geq 1.0$). Di-cationic ($z = +2$) and zwitterionic species ($z = \pm 0$) also bound to the bile micelles to some extent. Therefore, the bile micelle binding of $z = +1$, $+2$ and ± 0 species should be considered in food effect prediction.

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Author contributions: Material preparation, data collection, and analysis were performed by Mayu Konishi. Kiyohiko Sugano supervised all phases of the study, including the manuscript writing. All authors read and approved the final manuscript.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

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