Oil-Water Interface Transfer of Cefoperazone Pivaloyloxymethyl Ester

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세포페라존피바로일옥시메칠에스텔의 유-수 계면 이행에 관한 연구

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Kinetic and thermodynamic aspects of the interface transfer of cefoperazone and its pivaloyloxymethyl ester were studied in a two-phase system composed of aqueous buffers and *n*-octanol by using the absolute reaction rate theory. In terms of the net thermodynamic parameters for the process, ΔS increased and ΔF decreased as the lipophilicity increased. With the increased ratio of forward (k_f) to backward rate constants (k_b), the ester was more lipophilic than cefoperazone, but the aqueous solubility was reduced.

Keywords—cefoperazone, prodrug, cefoperazone pivaloyloxymethyl ester, interface transfer kinetics, thermodynamic parameters

Gastrointestinal absorption of drugs usually depends upon their ability to penetrate lipid barriers. Based on the pH-partition hypothesis, the degree of absorption of weak acidic and basic drugs depends on their lipid solubility and degree of ionization.[1,2]

In vitro experimental techniques for the gastrointestinal absorption to mimic *in vivo* absorption behavior have been described by Robertson, *et al.*[3]. In these methods, compounds examined are in a buffered aqueous phase, which is overlaid by an organic phase to simulate a lipid barrier.[4-7]

The present investigation was to elucidate the pH dependency of the absorption rate and its mechanism at the GI tract for cefoperazone pivaloyloxymethyl ester. The *in vitro* interface transfer kinetics of cefoperazone and its ester was also studied.

EXPERIMENTAL

Materials and Apparatus

Cefoperazone dihydrate was used as supplied by Samsung Pharm. Co., and cefoperazone pivaloyloxymethyl ester was synthesized in this laboratory. *n*-Octanol and the other solvents were reagent grade. Interface transfer experiments were performed in a rotating cell apparatus (Fig. 1) equipped with a thermostatic circulator (Teche model TE 7, U.K.), and analyzed by high performance liquid chromatography (HPLC; Waters model 510, U.S.A.).

Solubility Determination

The solubilities of cefoperazone and its ester in

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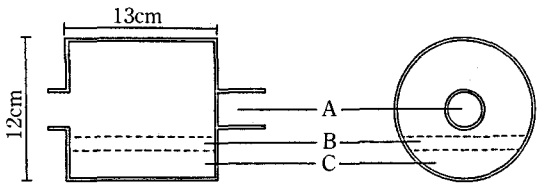

Figure 1—Apparatus for rotating cell used for the *in vitro* interface transfer study.
Key: A, rotating shaft; B, *n*-octanol phase; C, aqueous phase.

various pH buffers were determined by the KP IV method. The samples were diluted with methanol and assayed by HPLC (Fig. 2).

***In Vitro* Interface Transfer Studies**[4-9]

A rotating cell, similar to that described by Robertson *et al.*[3], was used for the *in vitro* transfer study of cefoperazone and its pivaloyloxymethyl ester. The apparatus consisted of a glass cell was slowly rotated by a shaft in an water bath thermostated at a constant temperature of 36.5 ± 1 °C.

n-Octanol and the various pH buffers of 1.2, 2.3, 3.0, 4.0, and 5.6 were mutually saturated prior to the experiment. 50 m*l* of buffer solution containing the drugs at a concentration of 1 ± 10^{-4}M was placed, and the aqueous phase was carefully overlaid with 50 m*l* of organic phase. The cell was rotated at 50rpm, and the samples were removed by microsyringe at appropriate time intervals. The final sample was taken after 3-6hr to measure the concentration at equilibrium. All samples were diluted with methanol, filtered through 0.5μm Millipore filter, and analyzed by HPLC.[10-12]

Additional experiments were carried out at 25 ± 1 °C and 30 ± 1 °C to observe the temperature effects.

RESULTS AND DISCUSSION

pH-Solubility Profile

Both cefoperazone and its ester showed similar solubilities in a low pH range, but the solubility of cefoperazone increased gradually with increase of pH (Fig. 3).

Kinetic and Thermodynamic Aspects of *In Vitro* Interface Transfer

Partitioning of a drug at the oil/water two phase system depends on the hydrophobic char-

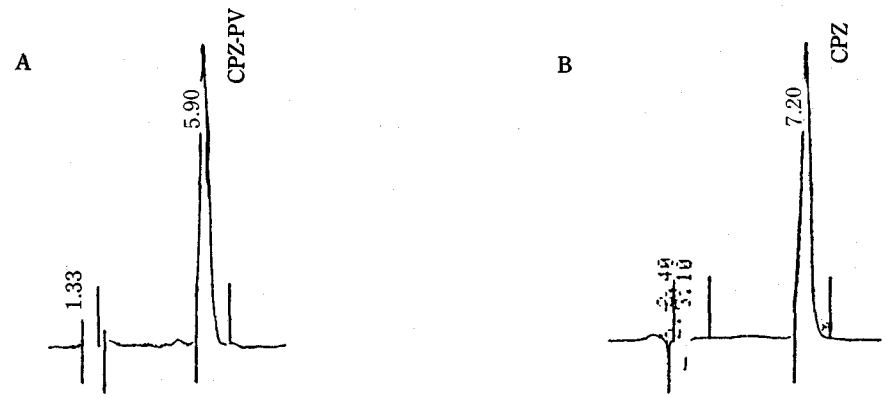

Figure 2—Typical chromatograms of cefoperazone pivaloyloxymethyl ester(CPZ-PV) and cefoperazone(CPZ) in methanol.
HPLC conditions were as follows:
for A; column: radial-Pak C_{18} cartridge, mobile phase: methanol/water=60/40, detector: Waters model 480 absorbance detector, 254nm, 0.01 AUFS, flow rate: 2 m*l*/min.
for B; column: radial-Pak C_{18} cartridge, mobile phase: mixture/1N acetic Acid/acetonitrile/water=1.2/2.8/180/816, (mixture-triethylamine/glacial acetic acid/water=14/5.7/80.3), detector: Waters model 480 absorbance detector, 254nm, 0.01 AUFS, flow rate: 2m*l*/min.

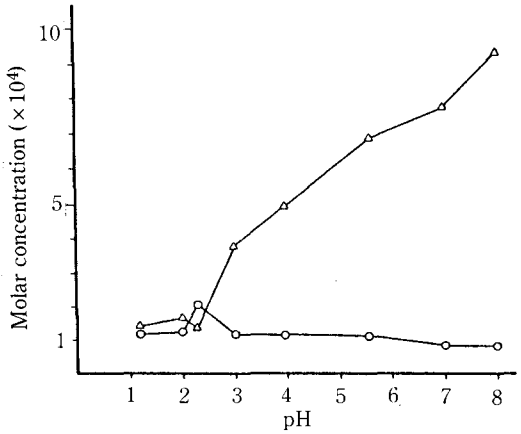

Figure 3—pH-Solubility profile of free cefoperazone and cefoperazone pivaloyloxymethyl ester at 25 °C.
Key; △, free CPZ; ○, CPZ-PV.

acteristics of the drug molecule and it is also affected by the unionized drug molecule existing in aqueous phase. It can be suggested that an exchange of aqueous and organic solvate molecules occurs during formation of the activated complex. Therefore, the oil/water interface transfer of the molecule may be interpreted by the absolute rate theory and can be written as Scheme 1,

$$C_w + X \rightleftharpoons [C_w - X]^* \rightarrow C_o$$

Scheme 1

where C_w and C_o are the drug molecule in aqueous and organic phases, respectively; X represents the organic solvent molecule in *n*-octanol phase; and $[C_w-X]^*$ represents the activated complex in transition state. If formation of the activated complex is the rate-limiting step, overall reaction rate depends on the concentration of the activated complex formed. And the rate constant of the reaction, k, is given by:

$$k = \frac{RT}{Nh} \cdot e^{-\Delta F^*/RT} \quad (1)$$

$$= \frac{RT}{Nh} \cdot e^{\Delta S^*/R} \cdot e^{-\Delta H^*/RT} \quad (2)$$

$$= \frac{RT}{Nh} \cdot e^{\Delta S^*/R} \cdot e^{-\Delta E^*/RT} \quad (3)$$

$$= \frac{RT}{Nh} \cdot e^{\Delta S^*/R} \cdot e^{-(Ea-RT)/RT} \quad (4)$$

where R is the molar gas constant, T is the absolute temperature, N is the Avogadro's number, h is the Planck's constant, and F, S, H, E and E_a are the thermodynamic parameters.

In consideration of Scheme 1, the activated complex formed for the forward or backward transfer involves hydrogen bonding with organic or aqueous molecules, respectively, which may re-

Table I—*In Vitro Transfer Rate-pH Profile for Free Cefoperazone and Cefoperazone Pivaloyloxymethyl Ester in a Two-Phase System at 36.5±1 °C.*

Material	pH Value	Time (min)							Equilibrium*
		10	20	30	45	60	90	120	
Free CPZ	1.2	0.18	0.38	0.50	0.78	0.93	1.25	1.53	2.55
	2.3	0.18	0.33	0.48	0.60	0.75	1.08	1.30	2.38
	3.0	—**	—	—	0.13	0.18	0.23	0.35	1.30
	4.0	—	—	—	—	—	—	—	—
	5.6	—	—	—	—	—	—	—	—
CPZ-PV	1.2	14.5	25.8	33.5	41.9	46.9	51.8	53.8	55.0
	2.3	18.5	31.5	40.4	49.2	54.5	59.7	61.5	62.5
	3.0	22.5	39.1	50.5	60.8	66.5	71.9	73.7	74.5
	4.0	17.0	29.0	38.0	47.2	52.8	58.4	60.6	62.0
	5.6	13.4	23.0	30.5	38.6	43.8	49.2	51.4	53.0

Data are the average of four experiments and expressed in μg per m*l* of *n*-octanol phase.
*Required 3-6 hr depending on the rate of transfer. **Not observed.

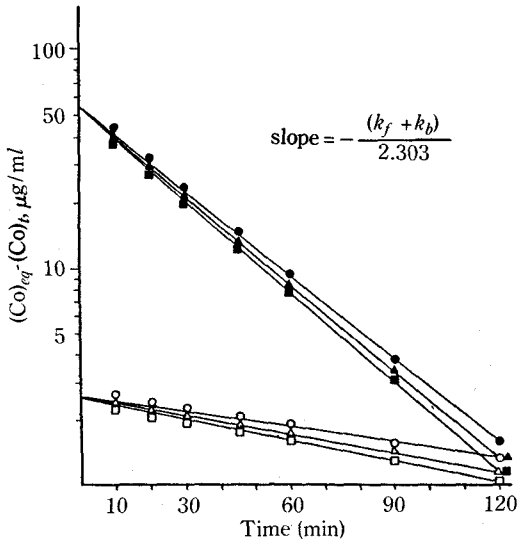

Figure 4—Transfer of free cefoperazone and cefoperazone pivaloyloxymethyl ester from an aqueous pH 1.2 buffer to *n*-octanol at various temperatures.
Key; ●, 25 °C, CPZ-PV; ▲, 30 °C, CPZ-PV; ■, 36.5 °C, CPZ-PV; ○, 25 °C, free CPZ; △, 30 °C, free CPZ; □, 36.5 °C, free CPZ

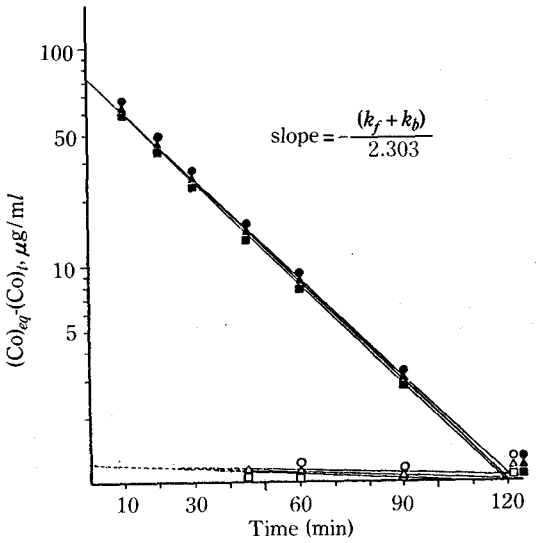

Figure 5—Transfer of free cefoperazone and cefoperazone pivaloyloxymethyl ester from an aqueous pH 3.0 buffer to *n*-octanol at various temperatures.
Key; ●, 25 °C, CPZ-PV; ▲, 30 °C, CPZ-PV; ■, 36.5 °C, CPZ-PV; ○, 25 °C, free CPZ; △, 30 °C, free CPZ; □, 36.5 °C, free CPZ

sult in displacing some water or octanol molecules. Hence these steps can be depicted as Schemes 2 and 3.

$$C_{HW} \rightleftharpoons C_{PHW}$$
$$C_{PHW} + ROH \rightleftharpoons [C_{PHW} - ROH] \rightarrow C_{HO}$$

Scheme 2

$$C_{HO} \rightleftharpoons C_{PHO}$$
$$C_{PHO} + HOH \rightleftharpoons [C_{PHO} - HOH] \rightarrow C_{HW}$$

Scheme 3

In these schemes, C_{HW} and C_{HO} represent the hydrogen-bonded drug molecule with water and octanol, respectively; C_{PHW} and C_{PHO} represent partially dehydrogen-bonded molecule in the aqueous and organic phases, respectively; ROH and HOH represent alcohol and water molecules, respectively; and [P_{PHW}-ROH] and [C_{PHO}-HOH] represent the activated complex formed at the interface for the forward and backward transfer, respectively.

Based on these considerations, the oil/water interface transfer of the drug molecule can be a reversible process as shown in Scheme 4:

$$C_W \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Co$$

Scheme 4

where k_f and k_b are the rate constants of forward and backward reactions, respectively. Hence,

$$(Co)_t = \frac{(C_w)_i \cdot k_f}{k_f + k_b}(1 - e^{-(k_f + k_b)t}) \quad (5)$$

$$\log[(Co)_{eq} - (Co)_t] = -\frac{k_f + k_b}{2.303}t + \log(Co)_{eq} \quad (6)$$

in which the subscripts *i*, *eq*, and *t* indicate initial, equilibrium and time, respectively. The value of $(k_f + k_b)$ can be obtained from the slope of Eq. 6, and then k_f and k_b are calculated by the following equations:

$$k_f = \frac{(Co)_{eq}}{(C_w)_i} \cdot (k_f + k_b) \quad (7)$$

$$k_b = (k_f + k_b) - k_f \quad (8)$$

In addition, the partition coefficient (PC) at equilibrium state can be obtained as follows:

$$PC = k_f / k_b \quad (9)$$
$$= e^{-\Delta F/RT} \quad (10)$$

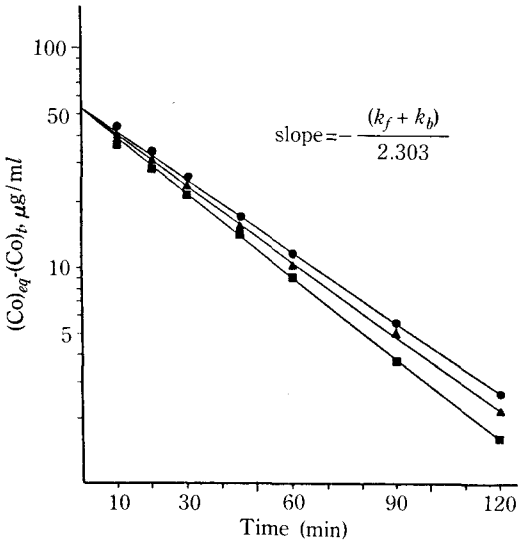

Figure 6—Transfer of cefoperazone pivaloyloxymethyl ester from an aqueous pH 5.6 buffer to *n*-octanol at various temperatures.
Key; ●, 25 ℃; ▲, 30 ℃; ■, 36.5 ℃

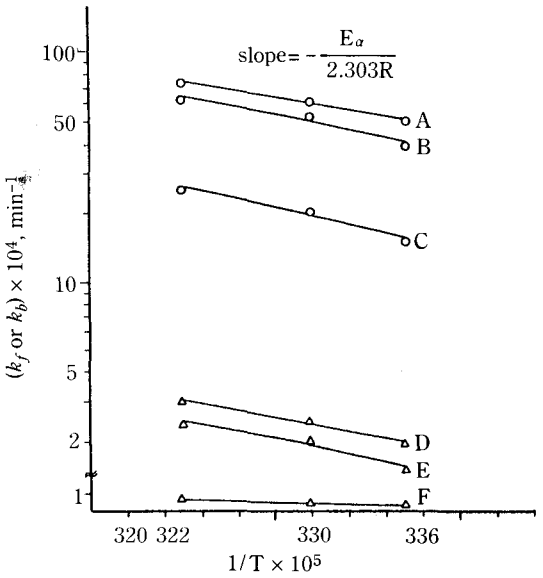

Figure 7—Arrhenius plots of the forward and backward transfer of free cefoperazone at the aqueous pH buffers/*n*-octanol interface.
Key; A, k_b, pH 1.2; B, k_b, pH 2.3; C, k_b, pH 3.0; D, k_f, pH 1.2; E, k_f, pH 2.3; F, k_f, pH 3.0

As shown in Table I, cefoperazone pivaloyloxymethyl ester was easily transferred into *n*-octanol phase at the interface than cefoperazone. And it is postulated that the transfer rate of (free) cefoperazone at the interface is pH-dependent.

The semilog plotting of these data based on Eq. 6 gave straight lines (Figs. 4-6), and k_f and k_b were obtained by Eqs. 7 and 8. The transfer of cefoperazone to the octanol phase was difficult because k_b was greater than k_f. In contrast, the ester revealed greater k_f in all pH ranges. Both rate constants increased as the temperature increased, and the experimental activation energy was obtained by the Arrhenius plot (Figs. 7 and 8).

Thermodynamic parameters such as ΔH^*, ΔF^* and ΔS^* were calculated by Eqs. 1-4 and listed in Table II. The ΔF^* values over 20 kcal/mole suggest that the energy barrier for interface transfer is great. ΔH, ΔS, ΔF, and partition coefficients are listed in Table III. The ester was more lipophilic than cefoperazone with an high increase of partition coefficient, and ΔS increased and ΔF decreased as the lipophilicity increased. It is considered that remarkable difference of both cefoperazone and its ester in the interface transfer to

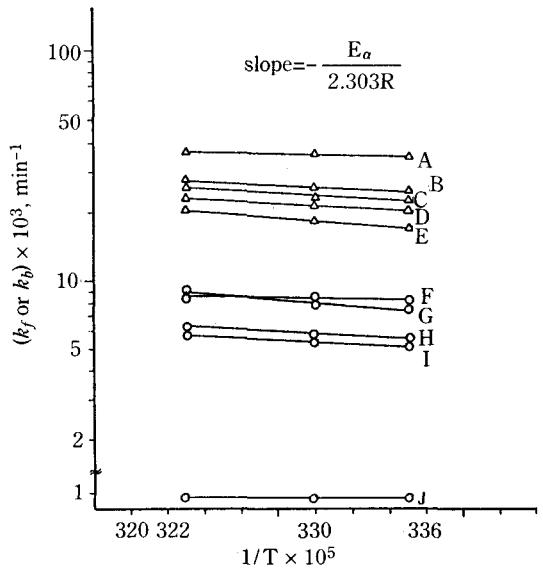

Figure 8—Arrhenius plots of the forward and backward transfer of cefoperazone pivaloyloxymethyl ester at the aqueous pH buffers/*n*-octanol interface.
Key; A, k_f, pH 3.0; B, k_f, pH 2.3; C, k_f, pH 4.0; D, k_f, pH 1.2; E, k_f, pH 5.6; F, k_b, pH 1.2; G, k_b, pH 5.6; H, k_b, pH 2.3; I, k_b, pH 4.0; J, k_b, pH 3.0

Table II—*Kinetic and Thermodynamic Parameters of Activation for the Interface Transfer of Free Cefoperazone and Cefoperazone Pivaloyloxymethyl Ester in a Two-Phase System at 36.5 ± 1 °C.*

Material	pH Value	k(min^{-1})		ΔH^*(kcal/mole)		ΔS^*(cal/mole·deg)		ΔF^*(kcal/mole)		Ea(kcal/mole)	
		$k_f(\times 10^4)$	$k_b(\times 10^4)$	ΔH_f^*	ΔH_b^*	ΔS_f^*	ΔS_b^*	ΔF_f^*	ΔF_b^*	Ea_f	Ea_b
Free CPZ	1.2	2.99	72.77	6.286	6.170	−62.58	−56.60	25.660	23.696	6.901	6.785
	2.3	2.40	62.77	7.333	7.148	−59.62	−53.73	25.795	23.787	7.948	7.764
	3.0	0.55	26.85	8.425	8.445	−59.02	−51.23	26.702	24.309	9.040	9.061
CPZ-PV	1.2	230.6	88.1	0.714	0.446	−71.93	−74.71	22.986	23.578	1.329	1.061
	2.3	282.9	61.1	0.729	0.737	−71.47	−74.49	22.861	23.804	1.344	1.352
	3.0	369.3	7.5	0.624	0.156	−71.28	−80.54	22.697	25.094	1.240	0.772
	4.0	257.7	58.2	1.043	1.025	−70.65	−73.66	22.918	23.833	1.658	1.640
	5.6	204.1	88.6	2.114	2.123	−67.65	−69.28	23.061	23.575	2.730	2.738

Table III—*Partition Coefficients and Net Thermodynamic Parameters for the Interface Transfer for Free Cefoperazone and Cefoperazone Pivaloyloxymethyl Ester in a Two-Phase System at 36.5 ± 1 °C.*

Material	pH Value	k_f/k_b, PC	ΔH, ΔE(cal/mole)	ΔS(cal/mole·deg)	ΔF(kcal/mole)
Free CPZ	1.2	0.041	116.0	−5.975	1.964
	2.3	0.038	184.1	−5.891	2.008
	3.0	0.021	−20.4	−7.792	2.392
CPZ-PV	1.2	2.618	268.0	2.778	−0.592
	2.3	4.630	−8.0	3.019	−0.943
	3.0	49.240	468.0	9.255	−2.397
	4.0	4.427	17.7	3.013	−0.916
	5.6	2.304	−8.7	1.630	−0.513

octanol phase is due to the pivaloyloxymethyl group of the ester, which is responsible for the increased lipophilicity.

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