Distribution of Protease Inhibitors in Lipid Emulsions: Gabexate Mesilate and Camostat Mesilate

Jiahui YIN, Yasuhiro NODA, Norio HAZEMOTO, and Toshihisa YOTSUYANAGI*

Department of Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University; 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan.

Received November 22, 2004; accepted May 26, 2005; published online June 29, 2005

Gabexate mesilate (GM) and camostat mesilate (CM) are protease inhibitors used for the treatment of pancreatitis, and have been reported to show anticancer effects *in vivo*. Lipid emulsions (20% fractionated soybean oil) were investigated in terms of physicochemical interaction between the drugs and lipid emulsions as a possible drug carrier. The result showed that the drugs did not distribute in the oil phase but were adsorbed at the phospholipid interface of oil droplets. With increasing concentration of the drugs, the adsorption amount at the interface rose steeply to around 2.2×10^{-11} mol/cm^2 for GM and 1.2×10^{-11} mol/cm^2 for CM, respectively, followed by further adsorption deviated from the Langmuir adsorption manner after the inflection. To interpret this two-stage adsorption of the drugs, surface potential and fluorescence changes were examined in addition to thermodynamics for their interaction with the interfacial lipid layer. The primary adsorption was exothermic and was due to electrostatic interaction and van der Waals interaction between drug molecules and phospholipid molecules. Both acidic and neutral phospholipids in the lipid were involved in the adsorption of GM, while acidic phospholipids were mainly involved in the adsorption of CM. On the other hand, the secondary adsorption was endothermic and was entropy-driven most probably due to hydrophobic interaction for GM and CM in common, including peripheral penetration of drug molecules into the interfacial lipid layer.

Key words distribution; lipid emulsion; adsorption; gabexate mesilate; camostat mesilate

Lipid emulsions are a parenteral product containing vegetable oil that is emulsified with lecithin, and the droplet size generally ranges from 200—300 nm. They were originally introduced to provide a source of calories for patients unable to ingest food. Over the 20 years, extensive researches on their use for drug delivery systems have been reported.[1,2] Reasons for using lipid emulsions as a drug carrier include: solubilization of low water-solubility drugs; stabilization of hydrolytically susceptible compounds; reduction of toxicity of intravenously administered drugs; potential for sustained release dosage forms; possible directed drug delivery to various organs and *etc.* Examples of marked formulations are intravenous emulsions with diazepam,[3] prostaglandin E_1[4] or α-tocopherol.[5]

Many workers have mainly chosen oil-soluble drugs for lipid emulsions formulation, while little has been paid attention for ionized drugs. Ionized drugs will possibly be adsorbed, at least partly, to the droplet interface which may contribute to the stability of the drugs. Physostigmine salicylate may be one example where the drug was adsorbed at the interface in the emulsions and was protected from the aqueous decomposition.[6] The stability of ionized drugs and salts in lipid emulsions has been reported recently.[7] However, studies mainly concerned about destablization of the mixture under the autoclaved condition.

Gabexate mesilate (GM) and camostat mesilate (CM) are chemically ionized drugs and are clinically protease inhibitors currently available to inhibit the biological activities of plasma kallikrein, thrombin, plasmin and trypsin. They have been used for the treatment of pancreatitis.[8,9] Ohkoshi *et al.* have been engaging in the research of anticancer effect of these protease inhibitors since the middle of the 1970's.[10—12] The drugs are thought to inhibit cell-surface enzymatic activity and the cell-to-cell contact, and prevent metastasis of cancer cells. Different from conventional anticancer agents, they are found to be non-cytotoxic. However due to their ionization property at physiological pH, these inhibitors suffer from low membrane permeability, high protein binding in blood, rapid elimination from plasma and *etc.* In their clinical anticancer trial, Ohkoshi *et al.* reported that frequent and long-term administration (at least for 6 weeks) was necessary for CM to maintain therapeutic blood level and to show its effect.[11]

With the aim of improving the therapeutic efficiency of GM and CM, we investigated the lipid emulsions (20% fractionated soybean oil) as a possible drug carrier for these protease inhibitors in terms of physicochemical interaction because of their structural characteristics: a relatively long hydrophobic moiety with a cationic guanidino group at the end. The distribution of GM and CM in the lipid emulsions was first examined. Subsequently, to interpret the distribution behavior of the two drugs at the oil droplet interface, surface potential changes and fluorescence changes were examined in addition to thermodynamics of the interaction.

Experimental

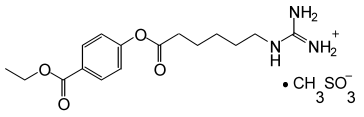
Materials Gabexate mesilate (GM, *p*-hydroxybenzoic acid ethyl ester 6-guanidinohexanoate mono-methanesulfonate), ethyl *p*-hydroxybenzoate and methyl *p*-hydroxybenzoate, dipalmytoylphosphatidylcholine (DPPC) and dipalmytoylphosphatidylglycerol (DPPG), 2-(9-anthroyloxy)stearic acid (2-AS), 6-(9-anthroyloxy)stearic acid (6-AS), 12-(9-anthroyloxy)stearic acid (12-AS) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Soybean oil was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Camostat mesilate (CM, *N*,*N*-dimethylcarbamoylmethyl-*p*-(*p*-guanidinobenzoloxy)phenyl-acetatemethanesufonate) was extracted from commercially available tablets (Ono Pharmaceutical Co., Osaka, Japan): Tablets were ground into powder and dissolved into methanol and then filtered. To the filtrate, ether was added and then it was stored in a refrigerator overnight to obtain white precipitate. The pure CM was obtained by recrystallization from methanol and ether (2 : 3 v/v). Analytical data, calculated for CM=$C_{20}H_{22}N_4O_5\cdot CH_4O_3S$ (mol wt=494.53): C, 51.00; H, 5.30; N, 11.33 and found: C, 50.96; H, 5.40; N, 11.03; mp 194 °C. Lipid emulsions for intravenous injection, Intralipos® 20% (Otsuka Pharmaceutical Co., Tokyo,

* To whom correspondence should be addressed. e-mail: yotsuyng@phar.nagoya-cu.ac.jp

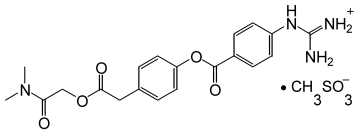
Table 1. Formulation of Lipid Emulsions[a)]

Ingredient	Ratio (%, w/v)
Fractionated soybean oil	20
Fractionated egg lecithin	1.2
Glycerol	2.2
NaOH (for pH adjustment)	Appropriate
Water for injection	To specified volume

a) Intralipos® 20%.



Gabexate Mesilate



Camostat Mesilate

Fig. 1. Chemical Structures of Gabexate Mesilate and Camostat Mesilate

Japan) was used (see Table 1). All other chemicals were of reagent grade. The chemical structures of GM and CM are shown in Fig. 1.

Determination When the drug concentration in lipid emulsions was low, the assay was performed by HPLC. The HPLC method for GM described by Nishijima *et al.*[13)] was modified as follows: Samples were diluted appropriately with pH 3.5 phosphate buffer and then injected into HPLC (Column, COSMOSIL $5C_{18}$-MS/$5C_{18}$, Nacalai Tesque; UV detector, SPD-6A, Shimadzu, detected at 245 nm; mobile phase, acetonitrile/0.05 M pH 3.5 phosphate buffer (32 : 68, v/v); flow rate, 0.8 ml/min; injection volume, 20 µl). Methyl *p*-hydroxybenzoate was used as an internal standard substance. For CM, samples were diluted with mobile phase and then injected into HPLC (Column, Inertsil ODS-80A, GL Sciences; UV detector, SPD-6A, Shimadzu, detected at 256 nm; mobile phase, methanol/sodium 1-heptansulfonate (1 : 500)/sodium lauryl sulfate (1 : 1000)/acetic acid (200 : 100 : 50 : 1, v/v); flow rate, 0.4 ml/min; injection volume, 20 µl). Ethyl *p*-hydroxybenzoate was used as an internal standard substance. At high drug concentrations, the assay was performed by UV. Samples were appropriately diluted with pH 3.5 phosphate buffer and then detected at 235 nm for GM and 265 nm for CM.

Drug Distribution in Lipid Emulsions and Thermodynamics Study At low drug concentrations, an aliquot (0.1 ml) of drug solution was added in lipid emulsions (10 ml). At the high concentrations, accurately weighed powder of drug was directly added in the lipid emulsions to maintain the volume change of lipid emulsions as small as possible. After uniformly agitated for 30 min, they were centrifuged at 85000×***g*** for 90 min and an aliquot of the water phase was withdrawn for determination of the drug concentrations.[14)] The agitation time was determined according to a pre-experiment in which the system was found to reach equilibrium within this period. After the ultracentrifugation, phase-separated samples were shaked again to retrieve the lipid emulsions, and no coalescence of drpoplets was found by microscopy. The experiment was conducted at 4 °C, 15 °C and 25 °C respectively for the thermodynamics study.

According to a three-phase model,[15)] the total amount of drug in the system M_{total} can be expressed as follows:

$$M_{total}=C_oV_o+C_wV_w+M_i \quad (1)$$

where M_i is the amount of drug adsorbed at the interface, C_o and C_w are the drug concentrations in the oil phase with the volume of V_o and in the water phase with the volume of V_w, respectively. According to a cryo-electron microscopic observation of lipid emulsions (Intralipid® 20%), liposomes (mostly unilamellar) formed from excess phospholipids were found in the infranatant even after ultracentrifugation.[16)] Therefore, C_w in fact represents the total concentration of free and liposomally-associated drug in the water phase. A three-phase model was therefore applied to the drug distribution, focusing on the adsorption of these drugs at the interface of oil droplets.

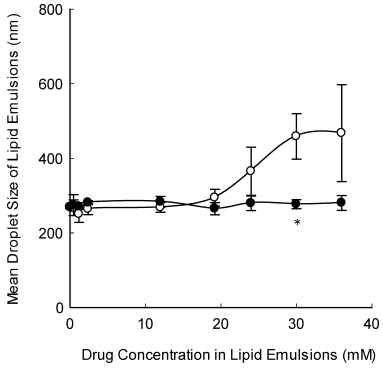


Fig. 2. Effects of Drug Concentration on the Mean Droplet Size of Lipid Emulsions

Each point represents the mean±S.D. (n=4, $*p<0.05$). ○, GM; ●, CM. Temperature, 25 °C.

Measurement of Droplet Size of Lipid Emulsions The average droplet size of lipid emulsions after the addition of GM and CM was measured by dynamic light scattering (Model DLS-7000, Otsuka Electronic Co., Osaka). Before the measurement, samples were appropriate diluted with purified water.

Zeta Potential Measurement Before the measurement, the samples were diluted for 1000 times with 2.2% glycerin aqueous solution which was filtrated with 0.22 µm filter in advance. Zeta potential of lipid emulsions after the addition of GM and CM was measured at 25 °C by electrophoretic light scattering (Model ELS-800, Otsuka Electronic Co., Osaka).

Fluorescence Study of Drug–Lipid Membrane Interaction The interaction of GM or CM with neutral and acidic phospholipids was investigated using liposomes as a mimic of the interfacial lipid layer. DPPC and DPPG liposomes containing the fluorescent probes, 2-AS, 6-AS and 12-AS, of which the anthracene group was reported to be well located at graded depths in the membrane, *i.e.* near polar head, middle and edge of hydrophobic chain side, respectively.[17)] Stock solutions (1.6 mM) of fluorescent probes in tetrahydrofuran were prepared. Dispersions of the probes were prepared at 32 µM in PBS (pH 7.4, 0.15 M NaCl) immediately before use. DPPC or DPPG solution in $CHCl_3$ was evaporated to dryness to obtain a dry film to which 3.9 ml of PBS was added and the whole was vortex-stirred. The liposome suspensions was incubated for 20 min and sonicated at 25 °C after 100 µl of the probe dispersion was added in. The final lipid concentration was 40 µM. After addition of concentrated drug solution to desired concentrations, the fluorescence intensity of the suspensions was measured at 450 nm using a fluorescence spectrometer (F-4500, Hitachi, Tokyo).

Results and Discussion

Distribution of GM and CM in Lipid Emulsions We first investigated the effect of the drug concentration on the droplet size of the lipid emulsions. Figure 2 shows that the mean droplet size of the lipid emulsions alone was found approximately 260 nm in diameter. It remained unchanged with the addition of GM until the drug concentration reached about 20 mM. After that concentration, the mean droplet size increased to about 400—500 nm with increasing drug concentration, suggesting the aggregation of oil droplets due to the drug. On the other hand, CM had almost no effect on the mean droplet size in the range of drug concentration examined up to 36 mM. Except for the GM concentration more than about 20 mM, the polydispersity index of the lipid emulsions fell in the range of 0.06—0.16 by DLS, suggesting the narrowly distributed property of oil droplet and similar droplet size distribution in the CM- and GM-lipid emulsions.

To clarify whether GM and CM were distributed in the oil phase, the distribution of the drugs in the soybean oil–water system without emulsifier was examined. The drug concentrations in the water phase remained equivalent to the con-

centration of the original solution added to the oil, even in the system with large amounts of oil (1 ml of drug solution (0.48 mM, GM and CM) in 30, 40, 50 g of oil). It was therefore concluded that GM and CM were not transferred into the oil phase and also not practically distributed at the interface.

Subsequently, we focused on the amount adsorbed at the interface of oil droplets. Shown below are the physical dimensions calculated for 10 ml of the lipid emulsions (20% (w/v) soybean oil; mean droplet size, 260 nm) used, from which the interfacial surface area of oil droplet was estimated. The physical dimensions are assumed as follows:

Total volume of oil:

$V_o = 2.17\ (\mathrm{cm}^3)$

Mean diameter of oil droplet:

$d = 2.60\times10^{-5}\ (\mathrm{cm})$

Surface area of each oil droplet:

$S_d = 4\pi(d/2)^2 = 2.12\times10^{-9}\ (\mathrm{cm}^2)$

Volume of each oil droplet:

$V_d = (4/3)\pi(d/2)^3 = 9.19\times10^{-15}\ (\mathrm{cm}^3)$

Number of oil droplet:

$N_d = (V_o)/(V_d) = 2.36\times10^{14}$

Total surface area of oil droplet:

$(N_d)(S_d) = 5.00\times10^{5}\ (\mathrm{cm}^2)$

Figure 3 shows a typical example of the drug amount adsorbed at the interface of oil droplet expressed in mol per cm^2 against the equilibrium concentration in the water phase at 25 °C in addition to hypothetical Langmuir adsorptions simulated from adsorption at low drug concentrations. Since lipid emulsions consist of three phases was assumed, *i.e.* the water bulk phase, oil phase and interfacial lipid layer in terms of drug loading,[15] a fraction of the drug was transferred only onto the phospholipid interface. It should be noticed that the drugs were adsorbed in a two-stage manner with increasing drug concentration. The adsorption amount of the drugs rose steeply to around 2.2×10^{-11} mol/cm^2 for GM and 1.2×10^{-11} mol/cm^2 for CM, respectively, followed by a deviated increase from the Langmuir adsorption manner after the inflection with further increase of the equilibrium concentration. There were found more amounts of GM adsorbed than that of CM. The plot was carried out within the concentration range where no droplet size change occurred (Fig. 2).

The Langmuir isotherm equation is based on the theory that the molecules of adsorbate are adsorbed on the surface of adsorbent to form a layer one molecule thick. The equation is written for the adsorption in the lipid emulsions as

$$a = \frac{a_m bc}{1+bc} \tag{2}$$

where a is the mol of drug molecules adsorbed per unit area of the interface of oil droplet at constant temperature and at the equilibrium concentration of drug, c. a_m is the maximum moles of drug adsorbed per unit area of the interface and b is the adsorption constant. Then, to overcome disadvantage of a

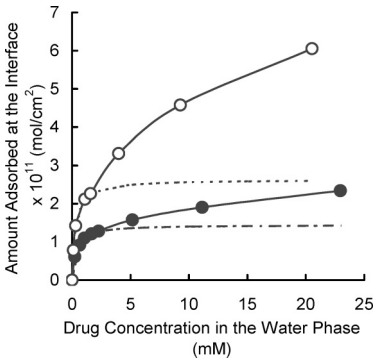


Fig. 3. Amounts of Gabexate Mesilate and Camostat Mesilate Adsorbed at the Interface against Drug Concentrations in the Water Phase

○, GM; ●, CM. Temperature, 25 °C. Dotted line and blocked line indicate Langmuir adsorption of GM and CM simulated from adsorption at low concentration, respectively.

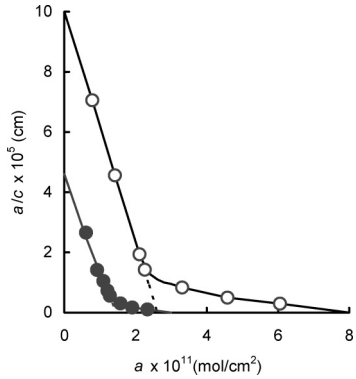


Fig. 4. Scatchard-Type Plots of a/c against a

a, the moles of molecules adsorbed per cm^2; c, equilibrium concentration. ○, GM; ●, CM. Temperature, 25 °C.

conventional linear plot where the experimental points obtained at lower concentrations of free drug are heavily weighed, Eq. 2 is converted to

$$\frac{a}{c} = a_m b - ba \tag{3}$$

Figure 4 shows a plot of a/c against a obtained at 25 °C. The inflection of this plot indicates the existence of two kinds of sites for the drug adsorption, the primary and secondary sites. The intercepts at $a = 2.62\times10^{-11}$ and 1.44×10^{-11} on the abscissa could be considered to represent the hypothetical completion of adsorption at the primary adsorption site for GM and CM, respectively (Fig. 3).

Zeta Potential Changes in Drug–Lipid Emulsions Figure 5 shows the changes of the zeta potential of the lipid emulsions with increasing GM and CM concentrations. The zeta potential of the lipid emulsions in which no drug was incorporated was about −44 mV. Egg lecithin is a mixture of phospholipids of which the composition slightly depends on the source.[18] The major constituents of egg lecithin are phosphatidylcholine and phosphatidyl-ethanolamine that are electrically neutral at physiological pH. Additionally, it comprises somewhat minor constituents that are anionic phospholipids such as phosphatidylserine, phosphatidic acid, and phosphatidylglycerol and they are negatively charged at pH 7. The content of acidic phospholipids in egg lecithin by percentage was reported about 2—5%.[18] The negative potential

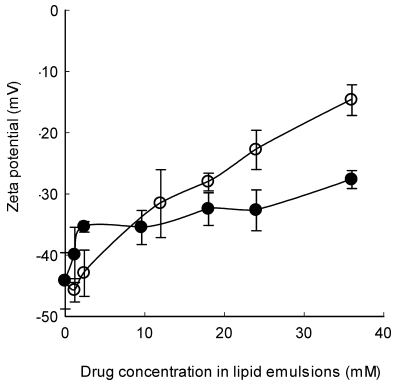


Fig. 5. Zeta Potential Changes as a Function of Drug Concentrations in Lipid Emulsions

Samples were diluted with 2.2% glycerin aqueous solution for 1000 times before the measurement. Each point represents the mean±S.D. (n=3). ○, GM; ●, CM. Temperature, 25 °C.

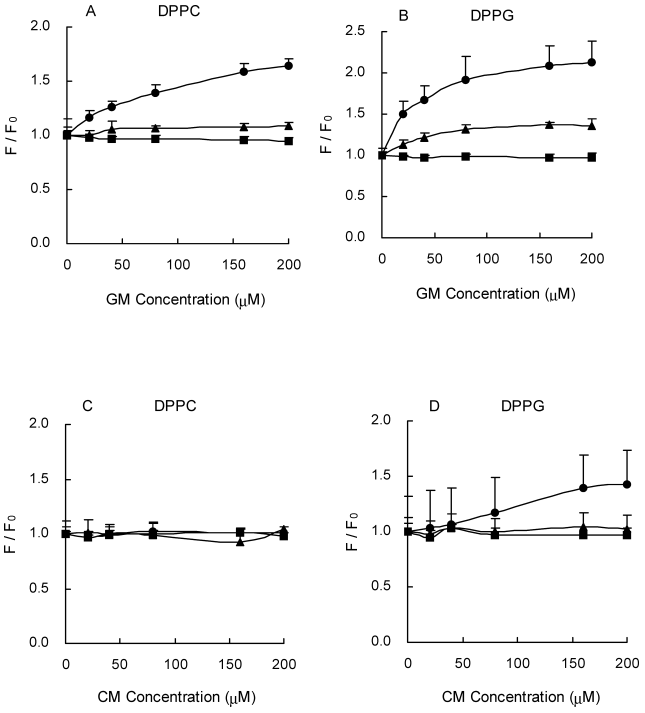


Fig. 6. Relative Fluorescence Intensity as a Function of Drug Concentration in Phospholipid Dispersions

A, GM-DPPC; B, GM-DPPG; C, CM-DPPC; D, CM-DPPG. Each point represents the mean±S.D. (n=3). ●, 2-AS; ▲, 6-AS; ■, 12-AS. Temperature, 25 °C. F_0 and F are the fluorescence intensities in the absence and presence of the drugs, respectively.

of lipid emulsion is considered to arise from the negatively charged phospholipids of egg lecithin existing at the interface.

With increasing GM concentration up to 36 mM, the negative potential was electrically neutralized. On the other hand, CM reached to about −30 mV in the same range of the drug concentration. The titration tendencies show that the positively charged GM and CM molecules were adsorbed on the negatively charged phospholipids spreading at the oil droplet interface. It also shows that there were more adsorption sites for GM than for CM, which is phenomenologically consistent with the results of adsorption amounts before dilution. At higher concentration of GM, the decrease of the negative interface charge due to the neutralization effect of adsorbed GM molecules could result in the aggregation of oil droplets in the lipid emulsions.

Normally, for zeta potential measurement, it is not necessary to rigorously control the dilution of dispersed systems, since its surface property does not change notably on dilution. However, it was reported that in the case of emopamil (an analogue of verapamil), the partition of the partially ionized drug at the high dilution of the electrophoresis experiment depleted the loading of the emulsion droplet, and the measured zeta potential decreased.[19] The dilution used might also contribute to the depletion of the loading of drugs at the interface. So the present results should be regarded as a qualitatively limited tendency of electrical neutralization process of the surface potential due to drug adsorption.

Fluorescence Intensity Changes in Drug–Lipid Membrane The interaction of pharmacologically active compounds with membrane lipids has been studied by the measurement of fluorescence intensity change of probes embedded in the lipid layer where liposomes have been often used as a model membrane.[20] The enhancement in fluorescence intensity of probes after the incorporation of such compounds is considered to be due to changes of the molecular environment of the probe, the affinity of the lipid-binding sites for probes or in the number of lipid-binding sites available.[20] Therefore appropriate measurements allow information to be inferred about the localization and interaction of these compounds with lipid membrane.

GM or CM itself does not exhibit any fluorescence. No wavelength shift was observed after the addition of GM or CM but the fluorescence intensities changed depending on the probes used, as shown in Fig. 6. The intensities of 2-AS and 6-AS were dependent on the GM concentration both in DPPG and DPPC. By contrast, little dependency was found on CM concentration in DPPG and no dependency was found in DPPC. The intensity of 12-AS did not change with the GM or CM concentration, whether in DPPG or in DPPC, indicating that the drugs did not penetrate into deep interior. For 2-AS, of which the intensity increased most among the three probes, its intensity increase in DPPG suspensions was more than in DPPC suspensions. It should be noticed that the drugs had a preference of acidic DPPG to neutral DPPC, which was consistent with electrical neutralization shown in Fig. 5. In the lipid emulsions, the alkyl chain of phospholipids interacts with the oil core of droplets, and the drugs did not distribute to the oil phase, as described earlier. From these results, it is likely that GM and CM interacted with the methylene groups near the carboxyl region of the phospholipids while GM penetrated into slightly deeper interior of the hydrophobic phase than CM. Also, these results suggest that even very peripheral invagination of CM into the phospholipid layer was rather unfavorable compared with that of GM and therefore fewer adsorption sites were occupied.

Thermodynamics of the Adsorption at the Phospholipid Interface To obtain more insight into the adsorption process, thermodynamics study was performed at three temperatures 4 °C, 15 °C and 25 °C. The free energy change of adsorption is related to the adsorption constant b by the relationship $\Delta G=\Delta G^{\circ}+RT\ln b$. At equilibrium, $\Delta G=0$ and the standard free energy change is $\Delta G^{\circ}=-RT\ln b$. The values of b_1 and b_2 were obtained from the slopes in the range of lower

Table 2. Adsorption Constants and the Thermodynamic Functions for GM and CM at the Interface in Lipid Emulsions

Drug	T (°C)	Primary site				Secondary site			
		b_1 ($\times 10^3$ M^{-1})	ΔG_1° (kJ)	ΔH_1° (kJ)	ΔS_1° (J/(mol deg))	b_2 ($\times 10^2$ M^{-1})	ΔG_2° (kJ)	ΔH_2° (kJ)	ΔS_2° (J/(mol deg))
GM	4	4.7	−19.5	−6.8	45.7	1.4	−11.4	9.7	76.3
	15	4.5	−20.1	−6.8	45.7	1.8	−12.4	9.7	76.3
	25	3.8	−20.4	−6.8	45.7	1.9	−12.6	9.7	76.3
CM	4	4.7	−19.5	−14.0	20.1	2.0	−12.2	9.0	76.4
	15	4.3	−20.0	−14.0	20.1	2.3	−13.0	9.0	76.4
	25	3.1	−19.9	−14.0	20.1	2.6	−13.8	9.0	76.4

concentrations and higher concentrations, respectively (Fig. 4). Assuming no significant temperature dependence of enthalpy change occurring within the temperature range examined, the standard enthalpy change ΔH° was estimated from the van't Hoff equation, namely

$$\log b = -[\Delta H^\circ/(2.303R)]1/T + \Delta S^\circ/(2.303R) \quad (4)$$

in which $\Delta S^\circ/2.303R$ is the intercept on the $\log b$ axis for a plot of $\log b$ *versus* $1/T$. The thermodynamics parameters obtained from the above plots were shown in Table 2.

The positive and negative thermodynamic functions resulting from several kinds of interactions have been explained by Martin.[21] Electrostatic interaction exhibits ΔH° near zero and positive ΔS° in aqueous solution. Negative ΔG° is favored by positive ΔS°. Positive ΔH° and ΔS° are the characteristic signs of the hydrophobic interaction, where large positive ΔS° contributes to the negative ΔG°. Van der Waals and hydrogen bond formation always show both negative ΔH° and negative ΔS°. Negative ΔG° is favored by negative ΔH°. The similar conclusions based upon a large body of thermodynamic results have been also reported.[22]

For the adsorption at the primary site, no pattern in the magnitude of ΔG° was discernible and the thermodynamic functions showed negative ΔH_1° and positive ΔS_1° for both GM and CM. Then, the absolute value of ΔH_1° for CM was about twice of that for GM and the absolute value of ΔS_1° was about half of that for GM. Because the zeta potential of lipid emulsions was neutralized with increasing concentration of the drug and ΔS_1° was positive, it was considered that the electrostatic interaction between the positively charged moiety of these drugs and the anionic phospholipids was involved. However, the negative ΔH_1°, especially the larger one for CM, showed that the adsorption might not be exclusively due to the electrostatic interaction because the ΔH° of a typical electrostatic interaction usually exhibits slightly positive or negative value. The exothermic interaction such as van der Waals interaction may also be involved. This kind interaction, together with the rehydration of the bound system was considered to overwhelm the dehydration of the drug and phospholipids molecules, thus leading to the negative ΔH°. It was also reported that the interactions involving delocalized electrons of aromatic ring system make significant contribution of negative sign of both ΔH° and ΔS°.[22] The van der Waals interaction between CM and the phospholipids may be more significant than that that of GM probably because one benzene ring connects with the positively charged guanidine group. This structural difference of drug molecules is also assumed to be one reason why fewer adsorption sites were available for CM than for GM, *i.e.* steric hindrance caused by the benzene ring would be a significant factor.

The secondary adsorption observed at higher drug concentrations was characterized by the results that there are little difference of ΔG_2°, ΔH_2° and ΔS_2° between GM and CM and the whole process was endothermic, suggesting the same type of interaction was involved. The large positive values of ΔS_2° of adsorption contribute to yield negative ΔG_2° for GM and CM. Thus, this adsorption is likely to follow the pattern of the classical hydrophobic effect, which is an entropy-driven phenomenon and the 'squeezing out' effect of water molecules from hydrophobic portion of GM and CM is not different from each other. Because of the structural characteristics of GM and CM, the interaction mode is possibly due to hydrophobic interactions including drug's peripheral penetration into the interfacial lipid layer, as demonstrated by the fluorescence experiment.

Conclusions

GM and CM did not distribute in the oil phase in lipid emulsions but were adsorbed at the phospholipid interface of oil droplets. These drugs were adsorbed in a two-stage manner as the drug concentration increased, which was deviated from the Langmuir adsorption. The primary adsorption was exothermic and was due to electrostatic interaction and van der Waals interaction between drug molecules and phospholipid molecules. Both acidic and neutral phospholipids in the lipid were involved in the adsorption of GM, while acidic phospholipids were mainly involved in the adsorption of CM. On the other hand, the secondary adsorption was endothermic and was entropy-driven most probably due to hydrophobic interaction for GM and CM in common, including peripheral penetration of drug molecules into the interfacial lipid layer.

Refrences

1) Singh M., Ravin L. J., *J. Parenter. Sci. Technol.*, **40**, 34—41 (1986).
2) Prankerd R. J., Stella V. J., *J. Parenter. Sci. Technol.*, **44**, 139—149 (1990).
3) Von Dardel O., Mebius C., Mossberg T., *Anaesth. Scand.*, **20**, 221—224 (1976).
4) Otomo S., Mizushima Y., Aihara H., Yokoyama K., Watanabe M., Yanagawa A., *Drugs Exp. Clin. Res.*, **11**, 627—631 (1985).
5) Ezra R., Benita S., Ginsburg I., Kohen R., *Eur. J. Pharm. Biopharm.*, **42**, 291—298 (1996).
6) Benita S., Friedman D., Weinstock M., *Int. J. Pharm.*, **30**, 47—55 (1986).
7) Sznitowska M., Janicki S., Dabrowska E., Zurowska-Pryczkowska K., *Eur. J. Pharm. Sci.*, **12**, 175—179 (2001).
8) Nakahara M., *Arzneim. Forsch./Drug Res.*, **33**, 969—971 (1983).

9) Muramatsu M., Fuji S., *Biochim. Biophys. Acta*, **268**, 221—224 (1972).
10) Ohkoshi M., *Gann To Kagaku Ryoho*, **22**, 417—429 (1995) (in Japanese).
11) Ohkoshi M., *Chiryogaku*, **32**, 1066—1071 (1998) (in Japanese).
12) Hisawa T., Kondo K., Nakagawa A., Ohkoshi M., *Cancer Lett.*, **126**, 221—225 (1998).
13) Nishijima M. K., Takezawa N., Shimada Y., Yoshiya I., *Thromb. Res.*, **31**, 279—284 (1983).
14) Benita S., Levy M. Y., *J. Pharm. Sci.*, **82**, 1069—1079 (1993).
15) Yamamura K., Nakao M., Yano Y., Miyamoto K., Yotsuyanagi T., *Chem. Pharm. Bull.*, **39**, 1032—1034 (1991).
16) Rotenberg M., Rubin M., Bor A., Meyuhas D., Talmon Y., Lichtenberg D., *Biochim. Biophys. Acta*, **1086**, 265—272 (1991).
17) Terce F., Tocanne J. F., Laneelle G., *Eur. J. Biochem.*, **133**, 349—354 (1983).
18) Washington C., Chawla A., Christy N., Davis S. S., *Int. J. Pharm.*, **54**, 191—197 (1989).
19) Washington C., *Adv. Drug Deliv. Rev.*, **20**, 131—145 (1996).
20) Au S., Schacht J., Weiner N., *Biochim. Biophys. Acta*, **862**, 205—210 (1986).
21) Martin A., "Physical Pharmacy," 4th ed., Lea & Febiger, Philadelphia, 1993, pp. 274—277.
22) Ross P. D., Subramanian S., *Biochemistry*, **20**, 3096—3102 (1981).