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Characterization of solvation properties of lipid bilayer membranes in liposome electrokinetic chromatography

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Abstract

The nature of solute interactions with biomembrane-like liposomes, made of naturally occurring phospholipids and cholesterol, was characterized using electrokinetic chromatography (EKC). Liposomes were used as a pseudo-stationary phase in EKC that provided sites of interactions for uncharged solutes. The retention factors of uncharged solutes in liposome EKC are directly proportional to their liposome–water partition coefficients. Linear solvation energy relationship (LSER) models were developed to unravel the contributions from various types of interactions for solute partitioning into liposomes. Size and hydrogen bond acceptor strength of solutes are the main factors that determine partitioning into lipid bilayers. This falls within the general behavior of solute partitioning from an aqueous into organic phases such as octanol and micelles. However, there exist subtle differences in the solvation properties of liposomes as compared to those of octanol and various micellar pseudo-phases such as aggregates of sodium dodecyl sulfate (SDS), sodium cholate (SC), and tetradecylammonium bromide (TTAB). Among these phases, the SDS micelles are the least similar to the liposomes, while octanol, SC, and TTAB micelles exhibit closer solvation properties. Subsequently, higher correlations are observed between partitioning into liposomes and the latter three phases than that into SDS.

Keywords: Electrokinetic chromatography; Solvation; Membranes; Lipid bilayer; Linear solvation energy relationships; Liposome electrokinetic chromatography; Lipids; Liposomes; Phospholipids; Cholesterol

1. Introduction

Solute partitioning between water and *n*-octanol has been used as a simple chemical model for drug interactions with biomembranes [1]. A number of researchers have indicated that an isotropic solvent such as *n*-octanol provides a simplistic view of the complex, anisotropic environments of lipid bilayers [2–4]. The thermodynamics of solute partitioning into a lipid bilayer was first thought to follow models established in bulk phases, where the hydrophobic effect was the dominant feature. However, in light of recent results concerning membrane molecular dynamics, a picture of a more heterogeneous interfacial phase is being established. Improved thermodynamic models that consider the bilayer effect have been

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proposed [5]. Lipid bilayer pseudo-phases such as liposomes have emerged as suitable models for biomembranes over the past two decades [6–8]. Nevertheless, the determinations of thermodynamic parameters that measure the extent of solute interactions with liposomes (e.g. partition coefficients, Gibbs free energy) have been a major obstacle. The classical methods are time consuming, tedious, require pure solutes, and large sample size.

An on-going investigation in this laboratory focuses on the use of biomembrane-mimetic pseudo-phases in electrokinetic chromatography (EKC) for investigating the physico-chemical foundation of solute partitioning into lipid bilayers. The technique is termed vesicle electrokinetic chromatography (VEKC) or liposome electrokinetic chromatography (LEKC) and has been reported by a number of groups [9–13]. EKC greatly facilitates physico-chemical studies of solute partitioning from a purely aqueous phase into vesicles (made of double chain synthetic surfactants) or liposomes (made of phospholipids) [13,14]. The combination of a biomembrane mimetic pseudo-phase and EKC provides a powerful tool for investigating drug partitioning into lipid bilayers. Like LC methods, LEKC offers the advantages of small sample size, lack of sample purity requirements, and automation in physico-chemical studies. In contrast to LC methods, however, LEKC is a *solution*-based separation technique, i.e. no solid stationary phase particles. As a result, it provides greater flexibility and versatility over immobilized artificial membrane (IAM) chromatography and immobilized liposome chromatography. The composition of the liposomes can be readily adjusted and a different vesicular solution can be easily incorporated.

The focus of this work is to examine the solvation properties of liposomes as determined by linear solvation energy relationship (LSER) modeling of solute partitioning into $DPPG_{24}/DPPC_{46}/Chol_{30}$ and $DPPG_{30}/DPPC_{70}$ liposomes. These LSER models were compared to those for octanol–water and various micellar systems that had been investigated previously [15–18]. The subscripts after the phospholipids represent the percent molar compositions of the phospholipids dipalmitoyl-L-α-phosphatidylglycerol (DPPG), dipalmitoyl-L-α-phosphatidylcholine (DPPC), and cholesterol (Chol). Recently, solute interactions with unilamellar vesicles made from synthetic surfactants of dihexadecyl phosphate (DHP) were characterized [13].

2. Experimental

2.1. Chemicals

Dipalmitoyl-L-α-phosphatidylcholine (DPPC), dipalmitoyl-L-α-phosphatidylglycerol (sodium salt) (DPPG), and cholesterol (Chol) were purchased from Sigma (St. Louis, MO, USA) (Fig. 1). The acidic form of HEPES [*N*-(2-hydroxyethyl)piperazine-*N′*-(2-ethanesulfonic acid)] was purchased from Sigma, while chloroform and the basic form of HEPES [*N*-(2-hydroxyethyl)piperazine-*N′*-(2-ethanesulfonic acid)(Na salt)) were purchased from Fluka (Buchs, Switzerland). Sodium phosphate monobasic $NaH_2PO_4 \cdot H_2O$ was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All solutes listed in Table 1 were of highest purity, did not need further purification, and were purchased from Aldrich (Milwaukee, WI, USA). Methanol (HPLC grade) was purchased from Fisher Scientific.

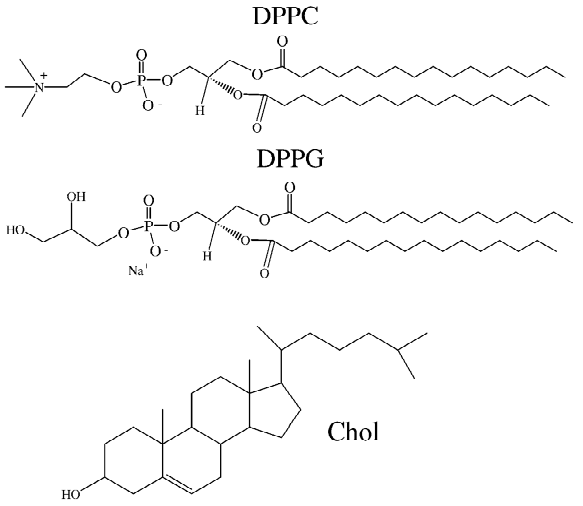

Fig. 1. Structures of the phospholipids and cholesterol.

Table 1
List of solutes along with their descriptor values used for LSER modeling (Ref. [19])

	Solutes	*V*	*E*	*S*	*B*	*A*
1	Benzene	0.716	0.610	0.52	0.14	0.00
2	Toluene	0.857	0.601	0.52	0.14	0.00
3	Ethylbenzene	0.998	0.613	0.51	0.15	0.00
4	Propylbenzene	1.139	0.604	0.50	0.15	0.00
5	*p*-Xylene	0.998	0.613	0.52	0.16	0.00
6	Acetophenone	1.014	0.818	1.01	0.48	0.00
7	Nitrobenzene	0.891	0.871	1.11	0.28	0.00
8	Methyl benzoate	1.073	0.733	0.85	0.46	0.00
9	Ethyl benzoate	1.214	0.689	0.85	0.46	0.00
10	Chlorobenzene	0.839	0.718	0.65	0.07	0.00
11	Bromobenzene	0.891	0.882	0.73	0.09	0.00
12	Iodobenzene	0.975	1.188	0.82	0.12	0.00
13	*p*-Dichlorobenzene	0.961	0.825	0.75	0.02	0.00
14	*o*-Dichlorobenzene	0.961	0.872	0.78	0.04	0.00
15	4-Nitrotoluene	0.98	0.705	0.67	0.07	0.00
16	Biphenyl	1.324	1.36	0.99	0.22	0.00
17	4-Chlorotoluene	1.032	0.87	1.11	0.28	0.00
18	4-Chloroacetophenone	1.136	0.955	1.05	0.44	0.00
19	4-Methylbenzyl alcohol	1.057	0.81	0.88	0.6	0.33
20	4-Ethylphenol	1.057	0.80	0.90	0.36	0.55
21	1-Chloro-4-nitrobenzene	1.013	0.98	1.17	0.25	0.00
22	3-Methylbenzyl alcohol	1.057	0.815	0.9	0.59	0.33
23	Phenethyl alcohol	1.057	0.784	0.83	0.66	0.3
24	3-Phenyl-1-propanol	1.198	0.821	0.9	0.67	0.3
25	3,5-Dimethylphenol	1.057	0.82	0.84	0.36	0.57
26	Butylbenzene	1.28	0.60	0.51	0.15	0.00
27	Naphthalene	1.085	1.34	0.92	0.20	0.00
28	1-Methylnaphthalene	1.226	1.344	0.90	0.20	0.00
29	2-Methylnaphthalene	1.226	1.34	0.88	0.20	0.00

2.2. Liposome preparation

All liposome mixtures were made in the same manner. Firstly, the desired amount of each phospholipid and cholesterol were weighed out and transferred to a 50-ml round bottom flask. A 10-ml chloroform–methanol (90:10, v/v) mixture was used to dissolve the phospholipids and cholesterol. The round bottom flask was placed on a rotary evaporator that was thermostated at 65 °C and allowed to rotate on the highest rotation rate for 2 min. This was done in order to ensure complete dissolution of the phospholipids and cholesterol in the organic mixture. Then vacuum was applied and the phospholipid cholesterol mixture was allowed to dry and adhere to the glass surface. An additional 10 min drying time was included in the procedure to ensure that no trace amounts of organic solvent were present. After the drying process was complete, a 3-ml solution of 25 m*M* HEPES (pH 7.5) was added and the round bottom flask was placed in a hot water bath. The flask was swirled around to form multilamellar vesicles (MLVs). The contents were emptied into a 20-ml scintillation flask and the process was repeated two more times with an additional 3-ml and 2-ml portion. This resulted in a total volume of 8 ml. Finally, the opaque mixture of MLVs was placed in a water bath thermostated at 65 °C and a sonication probe was placed several millimetres in the aqueous mixture. The total sonication time was 30 min with an intermittent cycle of 3-s on with a 2-s pause. The final product was a relatively clear homogeneous

solution of small unilamellar vesicles (SUV). All liposome solutions were used on the day they were prepared.

2.3. Retention factor

The retention factor (k) of each solute was calculated as reported previously for LEKC [13]:

$$k = (t_r - t_{eo})/\{t_{eo} \cdot [1 - (t_r/t_{lip})]\} \quad (1)$$

where t_{eo} is the retention time of the unretained marker (methanol), t_r is the retention time of the solute, and t_{lip} is the retention time of the liposome marker (decanophenone).

2.4. CE apparatus

The capillary electrophoresis system was a home-built system composed of a 0–30 kV power supply with a positive voltage (Spellman SL30); an interlock box (for safety reasons); a variable wavelength UV–Vis detector (Vestec); analog to digital converter (National Instruments); a Pentium computer (PC Innovations, Raleigh, NC, USA); an untreated fused-silica capillary (O.D. = 367 μm, I.D. = 50 μm, Polymicro Technologies Phoenix, AZ, USA); two 5-ml buffer reservoirs (Ace Glass Vineland, NJ, USA) and two 200-ml jacketed beakers (Ace Glass). The data were collected at a rate of 10 Hz by CE data acquisition software written in Lab View (Austin, TX, USA).

The system was set up such that each buffer reservoir and the capillary were thermostated by circulating mineral oil. The system was kept at 36 °C so that the liposomes would not fall below their transition temperature, and therefore would not undergo a phase transition during the chromatographic experiments.

2.5. Procedures

The capillary was rinsed in the following manner prior to use each day: a 10-min rinse with Milli-Q water; a 20-min rinse with 1.0 *M* NaOH; a 10-min rinse with Milli-Q water; a 10-min rinse with methanol; another 10-min rinse with Milli-Q water; and a 20-min rinse with the liposome solution. Furthermore, the Milli-Q water, methanol, 1.0 *M* NaOH, and liposome solution were all filtered through a 0.45 μm filter disk (Scientific Resources) prior to use. The capillary was vacuum-rinsed with the upstream liposome solution for 2–3 min in between injections. However, the downstream reservoir that contained the liposome solution was removed and replaced with a buffer reservoir containing water. This was done to prevent the temperature of the liposomes in the downstream reservoir from falling below their transition temperature while the vacuum was being applied. Once the 2–3-min rinse cycle was complete, the downstream buffer reservoir containing water was replaced with the liposome solution. Finally, solutes dissolved in methanol were injected for 1–2 s by gravimetric injection and a 25 kV voltage was applied that resulted in a current between 10 and 11 μA.

2.6. Data analysis

Microsoft Excel 2000 was used for fitting the LSER models and all other calculations. Retention of each solute was measured in triplicate. The log K_{lw} values (K_{lw} = liposome–water partition coefficient) for $DPPG_{24}/DPPC_{46}/Chol_{30}$ liposomes were between 1.13 and 3.41 with a standard deviation of 0.03. A paired *t*-test with a 95% confidence interval also was used to analyze the log K_{lw} values. The *t*-test showed that there was no significant difference between the observed K_{lw} values for different batches of liposome solutions.

3. Results and discussion

3.1. LSER modeling of LEKC retention

Using the recently revised symbols for solute descriptors, the LSER model for retention factor in EKC can be written as [19]:

$$\log k = vV + bB + aA + sS + eE + C \quad (2)$$

where k is the retention factor in EKC at a given liposome or micelle concentration. This model is essentially the same as that reported previously [14–19], except that new symbols are used for four of the solute descriptors. V is McGowan's volume, B

(formerly $\Sigma\beta$) is effective hydrogen-bond acceptor basicity, A (formerly $\Sigma\alpha$) is effective hydrogen-bond donor acidity, S (formerly π^*) is dipolarity/polarizability, and E (formerly R) is the excess molar refraction [19]. The coefficients v, b, a, s, and e are relative measures of the interactive nature of the pseudo-phase as compared to the bulk aqueous media. The v coefficient is related to the difference in cohesive energy between the aqueous and liposome phases as well as dispersion interactions, the b coefficient is a measure of the H-bond donor strength while the a coefficient represents the H-bond acceptor strength. The coefficient s describes the dipolarity/polarizability, and e is a measure of the interaction of the pseudo-phase with solute's n- or π-electrons. The regression constant, C depends on the phase ratio, which is determined by the amphiphile concentration.

Since retention factors in MEKC and LEKC are directly related to solute partition coefficient into micellar and liposome pseudo-phases, the LSER models of retention factor essentially provide information about the contributions of hydrophobic, hydrogen bonding, and dipolar interactions for solute partitioning from the bulk aqueous phase into these organized assemblies. In this study, the LSER models were developed for solute retention in LEKC to better understand solvation properties of liposomes as compared to those for the bulk solvent octanol as well as micelles of sodium dodecyl sulfate (SDS), sodium cholate (SC), and tetradecyltrimethylammonium bromide (TTAB). The solvation properties of the latter systems have been previously characterized by LSER [15–19]. The octanol–water partition coefficient is a widely used scale for solute hydrophobicity and is often used as a measure of solute affinity for the lipid bilayer of biological cell membranes.

The liposomes used in this study are unilamellar vesicles, which are composed of a mixture of two phospholipids, the anionic DPPG (at mole percentages of either 24% or 30%) and zwitterionic DPPC (at mole percentages of either 46% or 70%), with (at 30%) or without cholesterol. The liposomes are referred to as $DPPG_{24}/DPPC_{46}/Chol_{30}$ or $DPPG_{30}/DPPC_{70}$ in this paper. In general, cell biomembranes are constituted of mixtures of phospholipids, especially DPPC, as well as cholesterol. A set of aromatic solutes was used for building the LSER models. The solute descriptor values are listed in Table 1 [20]. In Table 2, the cross-correlation analysis shows that the descriptors are orthogonal and do not contain redundant information for the solute set.

The LSER models for retention in LEKC at different total phospholipid concentrations in the absence and presence of two buffer solutions, HEPES and phosphate, were obtained. Initially, addition of buffer to the liposomes causes some notable differences in the v, s, and b coefficients (Table 3). This is probably due to the modification of the head group region of the liposomes by the buffer which influences hydration of this region. However, the type and concentration of buffer had little effects on the LSER coefficients. Likewise, the LSER model (Eq. (2)) did not change with phospholipid concentration. This is expected as retention factor, k, in LEKC is directly related to the liposome–water partition coefficient, K_{lw}, and phase ratio (V_l/V_{aq}) that is proportional to the phospholipid concentration [P] as [14]:

$$k = K_{lw}\left(\frac{V_l}{V_{aq}}\right) = K_{lw}\cdot\bar{v}([P] - \mathrm{CAC}) \quad (3)$$

where V_l is the volume occupied by the liposomes, V_{aq} is the aqueous volume, $\bar{v}$ is the partial specific molar volume of the amphiphile, and CAC is the

Table 2
Cross-correlation analysis of LSER solute parameters

Cross-correlation analysis (R^2)	V	E	S	B	A
V	1.00				
E	0.18	1.00			
S	0.072	0.33	1.00		
B	0.11	0.03	0.21	1.00	
A	0.01	0.012	0.02	0.33	1.00

Table 3
The LSER models of log k in LEKC and MEKC; liposomes: $DPPG_{24}/DPPC_{46}/Chol_{30}$ and $DPPG_{30}/DPPC_{70}$ at specified concentration in the absence and presence of buffer (25 m*M* HEPES at pH 7.5). For the SDS, SC, and TTAB micelles, buffer was sodium phosphate at pH 7.0, $n=27$

System	v	e	s	b	a	c	R^2	SE
$DPPG_{24}/DPPC_{46}/Chol_{30}$	2.97	0.61	−0.69	−2.38	0.16	−2.52	0.99	0.07
6 m*M*/no buffer	(0.11)	(0.08)	(0.10)	(0.11)	(0.09)	(0.11)		
$DPPG_{24}/DPPC_{46}/Chol_{30}$	3.01	0.60	−0.69	−3.12	0.32	−1.74	0.99	0.07
30 m*M*/HEPES	(0.12)	(0.09)	(0.11)	(0.11)	(0.09)	(0.11)		
$DPPG_{24}/DPPC_{46}/Chol_{30}$	3.01	0.54	−0.55	−3.12	0.32	−2.30	0.98	0.08
10 m*M*/HEPES	(0.16)	(0.12)	(0.12)	(0.14)	(0.07)	(0.13)		
$DPPG_{30}/DPPC_{70}$	3.13	0.45	−0.44	−3.23	0.71	−2.21	0.99	0.07
12 m*M*/HEPES	(0.14)	(0.10)	(0.11)	(0.13)	(0.06)	(0.12)		
SDS micelles	2.98	0.26	−0.30	−1.76	−0.14	−1.94	0.99	0.06
40 m*M*	(0.12)	(0.09)	(0.09)	(0.10)	(0.05)	(0.10)		
SC[a]	2.73	0.60	−0.72	−2.46	0.14	−1.82	0.99	0.06
60 m*M*	(0.13)	(0.09)	(0.10)	(0.11)	(0.06)	(0.11)		
TTAB[a]	2.98	0.36	−0.30	−2.68	0.90	−2.24	0.98	0.07
10 m*M*	(0.13)	(0.10)	(0.10)	(0.12)	(0.06)	(0.11)		
Octanol–water	3.84	0.47	−0.94	−3.69	0.10	0.11	0.99	0.07
	(0.15)	(0.11)	(0.11)	(0.13)	(0.06)	(0.12)		

[a] Observed retention factors (log k) taken from Ref. [17].

critical aggregation concentration. Fig. 2 illustrates the effect of phospholipid concentration on the retention factor for five typical aromatic compounds. Note that all lines intersect over a very narrow range on the x-axis, which is equivalent to the CAC. For the double chain phospholipids, the CAC is very small (8.31×10^{-5} M) as evident from the near origin intersection point for the lines in Fig. 2. The liposome–water partition coefficient, K_{lw}, can be readily determined by LEKC from the slope of Eq. (3) [14].

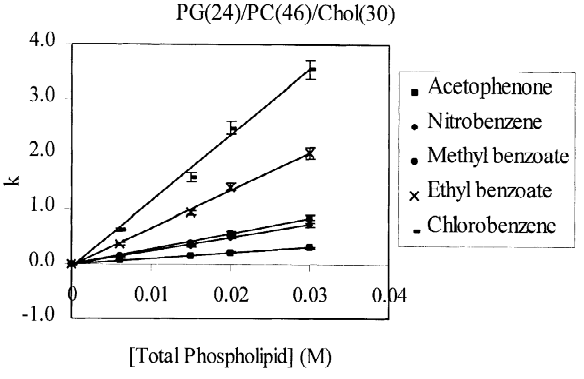

Fig. 2. Dependence of retention factor (k) on [total phospholipid] for DPPG–DPPC–Chol (24:46:30) in 25 m*M* HEPES, pH 7.5. The standard deviation for these solutes ranged between 0.014 and 0.028.

3.2. Comparison of solvation properties

The LSER models for two types of liposomes ($DPPG_{24}/DPPC_{46}/Chol_{30}$ and $DPPG_{30}/DPPC_{70}$), octanol–water, and three types of micelles (SDS, TTAB, and SC) are listed in Table 3. The large positive v values imply that bulkier solutes have stronger interactions with the liposomes, micelles, and octanol. As mentioned previously, the v coefficient is related to the difference in cohesive energy between the aqueous and liposome phases. In addition, the v coefficient contains information about dispersion forces [21]. The larger the v coefficient, the less cohesive or ''more hydrocarbon-like'' the solute microenvironment in the liposome pseudophase is [14–21]. The liposome and micellar systems are more cohesive (or less ''hydrocarbon-like'') than octanol.

The negative b coefficients mean that the pseudophases have substantially smaller H-bond donor

ability as compared to the aqueous phase. The liposome pseudo-phases are weaker hydrogen bond donors than the micellar pseudo-phases, and only slightly stronger than octanol. Like micelles, liposomes are strongly hydrated in the head-group region [22]. The water molecules that are located in between the head-groups are bound to one or more polar moieties in the head group. The water in this region does not have bulk-like properties; thus, the ability of these water molecules to form a H-bond with a solute is drastically reduced. As mentioned earlier, the H-bond donor ability of the pseudo-phase stems from water molecules that hydrate the surface and the interface region. The stronger interaction between the water and the head-groups and/or counter-ions of the pseudo-phase would lead to weaker H-bond donating ability of water in the hydration layer. This results in larger negative *b* coefficients. The effect of head group and counter-ion on solvation properties of micellar systems has already been examined [15–18]. For the anionic micelles, the *b* coefficients became systematically more negative as the hydrogen bond acceptor strength of the head groups increased [15,16]. The large negative *b* coefficients for the liposomes and micellar systems of TTAB, and SC indicate strong hydration of polar, H-bond acceptor moieties in the head-group region.

The *a* coefficient is usually small for the majority of micellar pseudo-phases and for octanol. Cationic micelles such as TTAB, however, have large positive *a* coefficients that means their hydrogen bond acceptor strengths are greater than the bulk aqueous and the other pseudo-phases. This can be attributed to the presence of the cationic head group. In this respect, the liposomes behave similarly to the cationic micelles. The positive *a* coefficient for the liposomes can also be attributed to the cationic center of the zwitterionic phospholipid, DPPC. Table 3 shows that upon replacement of some of the zwitterionic phospholipid with cholesterol, the *a* coefficient decreases from 0.71 to 0.32, while the other coefficients remain constant. This might be mainly due to the lower concentration of the DPPC, rather than presence of cholesterol. It is then expected that solutes with hydrogen bond donor groups would interact more strongly with the stronger H-bond acceptor liposomes that have larger concentration of DPPC. This is illustrated in Table 4 where the liposome–water partition coefficient, K_{lw}, of several hydrogen bond donor solutes, a non-hydrogen bond solute, and a hydrogen bond acceptor solute in two liposomes with different DPPC content (with and without cholesterol) are compared. Larger K_{lw} are observed for the hydrogen bond donor compounds in the liposome with higher concentration of DPPC (without cholesterol). The K_{lw} values of non-hydrogen bond donor compounds were essentially the same in both types of liposomes. This is also further illustrated in Fig. 3 that shows two hydrogen bond donor solutes shift to longer retention times for the liposome without cholesterol.

The liposome pseudo-phases have lower dipolarity/polarizability coefficients than the aqueous phase. The magnitudes of the *s* coefficients for both liposome systems are greater (less negative) than the one recorded in octanol. The dipolar character of the pseudo-phase stems from the presence of water

Table 4
Comparison of the partition coefficients of hydrogen bond donor solutes and non-hydrogen bond donors into liposomes with different compositions

Solute	log K_{lw} $DPPG_{30}/DPPC_{70}$	*s*	log K_{lw} $DPPG_{24}/DPPC_{46}/Chol_{30}$	*s*	Δlog K_{lw}	Classification
Toluene	2.09	0.03	2.09	0.02	0.00	NHB
4-Ethylphenol	2.40	0.02	2.23	0.03	0.17	HBD
3,5-Dimethylphenol	2.33	0.02	2.15	0.03	0.18	HBD
4-Chloroacetophenone	2.03	0.03	1.97	0.02	0.06	HBA

The *s* represents the standard deviation and Δlog K_{lw} is the difference between log K_{lw} $DPPG_{24}/DPPC_{46}/Chol_{30}$ and log K_{lw} $DPPG_{30}/DPPC_{70}$. The solutes are classified as either nonhydrogen bond donors (NHB), hydrogen bond donors (HBD), or hydrogen bond acceptors (HBA).

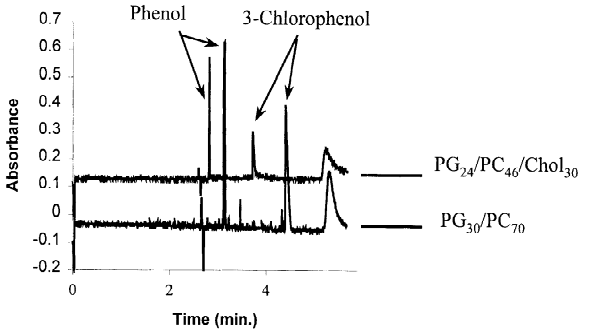

Fig. 3. Comparison of the retention behavior of hydrogen bond donor solutes in liposomes with different compositions. The liposome with higher DPPC concentration and without cholesterol ($DPPG_{30}/DPPC_{70}$) is a stronger hydrogen bond acceptor and has greater interaction with the hydrogen bond donor solutes than the liposome with lower DPPC content that contain cholesterol (i.e. $DPPG_{24}/DPPC_{46}/Chol_{30}$).

molecules at the polar region of the liposome and the nature of the head-group, as well as the buffer components that are present in the interfacial region.

The *e* coefficient is a polarizability correction term; it accounts for the residual factors not considered by the *s* coefficient. The more positive *e*, the more polarizable the pseudo-phase is. It is also a reflection of the ability of the pseudo-phase to adjust its electron cloud density to accommodate solutes with π- and/or n-electrons. This term is positive in all cases.

In order to better distinguish the similarities and differences between different pseudo-phases, the ratios of the LSER coefficients relative to the *v* coefficient are listed in Table 5. The correlations between partitioning into liposomes to those of micelles and octanol–water systems are also given. Partitioning into the liposome with cholesterol has higher correlations (R^2_{lw}) to those in SC, TTAB, and octanol–water systems (Fig. 4). This is mainly due to the similar hydrogen bonding characteristics between these phases and those of the liposome with cholesterol (a/v and b/v ratios). The LSER model for the liposome is also similar to that reported for microemulsion pseudo-phases that have been examined; thus high correlations in partitioning are also expected [23,24]. The liposome without cholesterol, however, correlates well with partitioning into the cationic micelles, mainly due to the similarity in hydrogen bonding accepting strengths (a/v).

It is quite intriguing that better correlations are observed between partitioning behavior in phases with diverse chemical and structural properties than those that have closer resemblance in some respect. For example, micelles and liposomes share common characteristics of interfacial systems that are structurally heterogeneous and are quite different from an isotropic solvent such as *n*-octanol. It is known that the physical and chemical properties (such as polarity, viscosity, etc.) of the interfacial systems change with distance from the ''surface'' of the pseudo-phase. It has been demonstrated that solute partitioning into interfacial systems depends on factors such as chain density [4]. This is quite different from partitioning into isotropic, homogeneous liquids such as *n*-octanol, where the phase chain density is not a relevant issue [4]. Nevertheless, better correlation is observed between the partition coefficients into liposomes and *n*-octanol than that between liposomes and SDS micelles. These results indicate that correlation analysis of partition coefficients in different systems mainly illustrate similarities in solvation

Table 5
LSER coefficient ratios and correlation coefficients between octanol–water partition coefficients ($R^2_{o/w}$), the retention factors of 10 m*M* $DPPG_{24}/DPPC_{46}/Chol_{30}$ liposomes (R^2_{lw1}), and the retention factors of 12 m*M* $DPPG_{30}/DPPC_{70}$ liposomes (R^2_{lw12}) with various pseudo-stationary phases

System	e/v	s/v	b/v	a/v	$R^2_{o/w}$	R^2_{lw1}	R^2_{lw2}	n
$DPPG_{24}/DPPC_{46}/Chol_{30}$	0.18	−0.18	−1.04	0.11	0.95	1.00	0.95	27
$DPPG_{30}/DPPC_{70}$	0.14	−0.14	−1.03	0.23	0.84	0.95	1.00	27
SDS	0.09	−0.10	−0.59	−0.05	0.86	0.79	0.67	27
SC	0.22	−0.26	−0.90	0.05	0.98	0.97	0.87	27
TTAB	0.12	−0.10	−0.90	0.30	0.70	0.84	0.96	27
Octanol–water	0.11	−0.24	−0.96	0.03	1.00	0.95	0.84	27

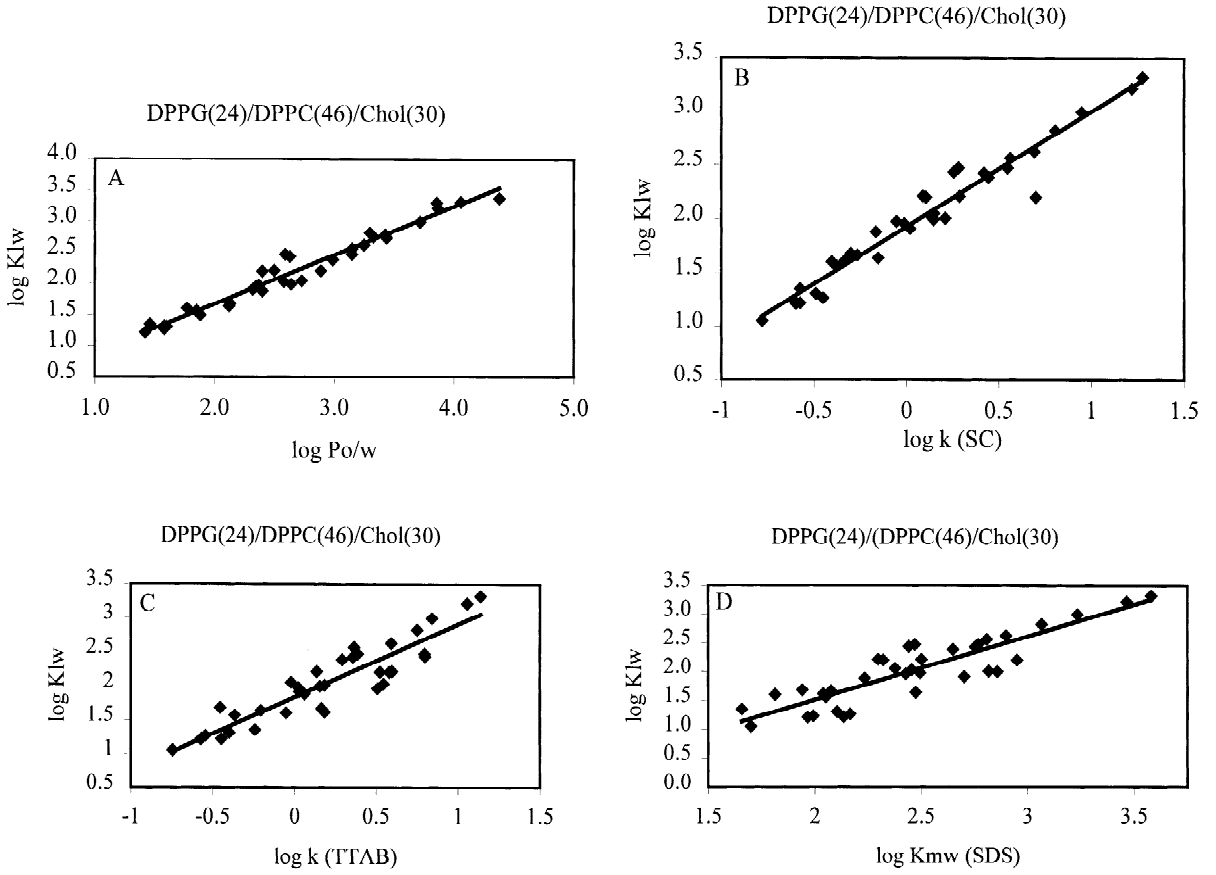

Fig. 4. The relationship between $DPPG_{24}DPPC_{46}Chol_{30}$ liposome–water partition coefficients and: (A) octanol–water partition coefficients: $\log K_{lw} = 0.78 \log P_{o/w} + 0.10$, $n = 35$, $R^2 = 0.96$, (B) SC micelle–water retention factors: $\log K_{lw} = 1.07 \log k + 1.93$; $n = 36$, $R^2 = 0.95$, (C) TTAB micelle–water retention factors: $\log K_{lw} = 1.07 \log k + 1.83$; $n = 36$, $R^2 = 0.85$; (D) SDS micelle–water partition coefficients: $\log K_{lw} = 1.10 \log K_{mw} - 1.69$; $n = 36$, $R^2 = 0.79$.

properties and do not reflect the differences in organization or structural properties of the phases.

4. Conclusion

LEKC provides a convenient method for investigation of physico-chemical properties of liposomes. In spite of great differences in the organization between liposomes, micelles, and octanol, high correlations were observed between partitioning behavior in these phases. Such behavior can be attributed to similarities in solvation properties of these various phases. Water content and hydration of the ''organic media'' in these pseudo-phases play a significant role in the partitioning schemes.

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