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Study of solute partitioning into cationic vesicles of dihexadecyldimethylammonium bromide using electrokinetic chromatography

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Abstract

A cationic vesicular pseudo-stationary phase was used in electrokinetic chromatography. The stable cationic lipid bilayer, composed of a double chain cationic surfactant, dihexadecyldimethylammonium bromide was prepared in deionized water, with no buffer additive. The chromatographic characteristics of this novel pseudo-phase are presented. A linear solvation energy relationship was successfully used to characterize the interaction of neutral solutes with the cationic vesicles. This study was complemented by the determination of changes in the free energy for transfer of various groups from the aqueous phase into the bilayer.

Keywords: Pseudo-stationary phases; Linear solvation energy relationships; Dihexadecyldimethylammonium bromide

1. Introduction

Double-chain surfactants when dispersed in aqueous phase are known to form well a defined bilayer structure [1,2]. Small unilamellar vesicles (SUVs) made of double-chain synthetic surfactants or phospholipids have received much attention in various areas of biological and pharmaceutical research. Cationic vesicles are increasingly used as delivery devices in gene therapy. The usage of cationic carrier enhances the interactions with the negatively charged cell membranes, which in turn increases the probability of transfer of the encapsulated materials into the cell [3]. Recently the efficacy of cationic lipids for delivery of DNA-based materials in different cell lines was evaluated using capillary electrophoresis–laser-induced fluorescence detection (CE–LIF) [4,5].

There exists a great deal of interest in understanding solute partitioning into lipid bilayer of cell membranes. Liposomes that are made of phospholipids are suitable models for biological membranes. Previous reports from this laboratory focused on characterization of the interactions of neutral solutes with anionic vesicles [6,7] and liposomes [8,9], using linear solvation energy relationship (LSER) methodology. The earlier studies indicated that the surfactant head group plays a significant role on the solvation properties of micellar aggregates [10–12]. For example, the hydrogen bonding properties of micelles made of anionic surfactants are distinctly different from those of cationic micelles. The parameters that control the distribution of sol-

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utes between the cationic surface of a bilayer and the aqueous bulk phase are not clearly established. As a continuation of previous studies of anionic vesicles, the focus of this paper is to unravel the solvation properties of cationic vesicles. Cationic vesicles made of a double chain surfactant with quaternary ammonium head-group are used as pseudo stationary phase in electrokinetic chromatography (EKC). The EKC approach is suitable for modeling, and for gaining insight into the factors that are relevant in the partitioning process between the analytes and the bilayer surface. A similar approach is used to probe the nature of the interphase boundary between the aqueous phase and the cationic bilayer surface. The LSER model quantitatively describes the solvation properties of the vesicles in terms of hydrogen bonding, dipolarity/polarizability, dispersion, as well as hydrophobic interactions. This multiparameter approach deconvolutes retention data in EKC into useful information about the contributions of various types of interactions that influence solute partitioning into the bilayer.

2. Experimental

2.1. Vesicle preparation and characterization

The cationic surfactant dihexadecyldimethylammonium bromide (DHAB) was obtained from Fluka (Buchs, Switzerland). Small unilamellar vesicles were prepared from DHAB. An appropriate amount (mass ranging from 15 to 60 mg depending on the desired vesicles' concentration) of the double-chain surfactant was dissolved in 8 ml water. The heterogeneous solution was maintained in a temperature-controlled water bath at 64 °C and sonicated with a titanium-tipped probe sonicator. The total sonication time was 30 min. The ultrasound energy input was intermittent, with 3 s of sonication followed by a 2 s pause. The final product was a clear translucent solution containing unilamellar vesicles. The solution was filtered through a 0.2 μm Acrodisc filter (STRI, Eatontown, NJ, USA) to remove particles and undispersed lamellar aggregates of the surfactant before use. The vesicular solutions were stored at room temperature and remained stable for 3–4 weeks before any sign of decomposition (appearance of turbidity) was observed. The sonicated solutions however, were used within a week following preparation.

The size of the vesicles was determined at 25 °C by quasi-elastic light scattering (QLS). The laser source was INNOVA 70-3 argon (Coherent, Palo Alto, CA, USA). The filtered samples were placed in a temperature-controlled cuvette holder with decalene index-matching bath positioned in a BI-200 (Brookhaven, Holtsville, NY, USA) goniometer system. Measurements were done at a 90° scattering angle, using diluted solutions to ensure a condition of non-interacting Brownian particles. The time-dependent fluctuation of scattered light was collected with a 9863/350 photomultiplier tube. The signal analysis was performed with a BI 2030AT digital correlator equipped with 128 real-time data channels. The data were analyzed by the methods of cumulants.

2.1.1. CE apparatus

The CE apparatus was a laboratory-made instrument comprised of a high-voltage power supply (Series EH, Glassman High Voltage, Whitehouse Station, NJ, USA). The temperature of the two buffer reservoirs and the capillary were all controlled at 35 °C by circulating mineral oil, both in the tubing holding the capillary and around the 200 ml jacketed beakers (Ace Glass, Vineland, NJ, USA). This modification was warranted to maintain the vesicles above their transition temperature and to avoid the occurrence of a phase transition during the chromatographic experiments. The cationic vesicles modify the walls of the fused-silica capillary that leads to the reversal of the electroosmotic flow. As a result, a negative voltage was applied to the upstream electrode. The silica capillary had an O.D of 367 μm and I.D of 50 μ and was purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length of the untreated fused-silica capillary was 67 cm, and the distance from the injection point to the detection window was 50 cm.

The output of the variable wavelength UV–visible detector (Model 200, Scientific System, State College, PA, USA) was connected to an analog-to-digital converter from National Instruments on a serial port

of a Pentium computer (PC Innovation, Raleigh, NC, USA). The data was sampled at a rate of 5 Hz, by CE data acquisition software written in LabView.

2.2. Procedures

The conditioning of the untreated capillary before any injection was performed in six steps. The initial 10-min rinsing of the newly installed capillary with deionized water was followed by a 20-min rinse with 1.0 *M* NaOH solution. Another 10-min rinse by deionized water was followed by a 10-min methanol rinse for the removal of any trace of organic residues. The last step was a 10-min rinse with water, followed by 20-min conditioning of the capillary with the vesicular solution. The conditioning solvents were vacuum-pumped, no electrical field was applied at the conditioning stage. The capillary was vacuum-rinsed with the vesicular phase between each injection.

2.3. Retention factor

The analytes with the retention time of t_R elute in a time window bracketed by the solvent front (t_0) and the retention time of the vesicles (t_{vs}). The t_0 marker used in this case is methanol. The t_{vs} marker is often a very hydrophobic compound that interacts strongly with the pseudo-stationary phase, thus migrating with the vesicles, dodecanophenone was used in this case. The retention factors were calculated using Eq. (1) [7,9,13]:

$$k = \frac{t_R - t_0}{t_0 \cdot \left(1 - \frac{t_R}{t_{vs}}\right)} \quad (1)$$

3. Results and discussion

3.1. Vesicle characteristics

The DHAB (Fig. 1) vesicles were most stable for their usage in EKC when prepared in water in the absence of any buffer additives. The vesicle's physical properties were determined through the application of dynamic light scattering method and the measurements of the electrophoretic mobilities. The

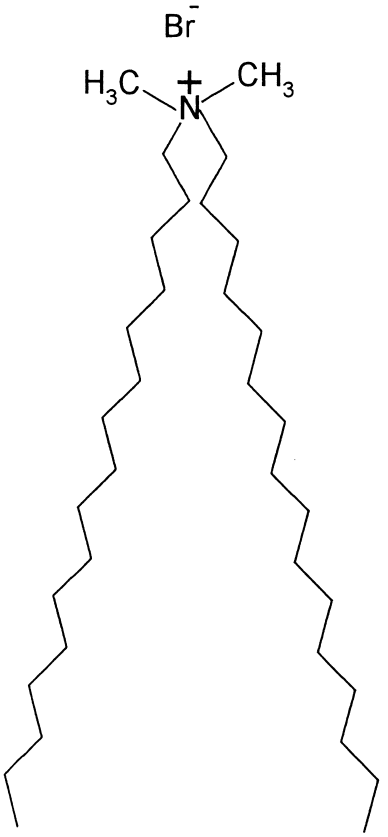

Fig. 1. Dihexadecyldimethylammonium bromide (DHAB) monomer structure.

average vesicle diameter is 50 nm. In the case of DHAB, the Br^- ions remain tightly bound to the surface of the vesicles (less than 1% dissociation), and neutralize the ionic sites. The cationic vesicles have a Stokes charge of 118 that is considerably lower than 17 523, which is the calculated number of surfactant monomers present at the surface.

3.2. Chromatographic behavior

As expected, linear relationships between retention factor, *k*, and amphiphile concentration was observed for various compounds (data not shown). The elution window (t_{vs}/t_0) was around 2.5, and did not vary significantly with changes in vesicle concentration.

The chromatograms for different classes of compounds (alkylphenones, and steroids) illustrating the

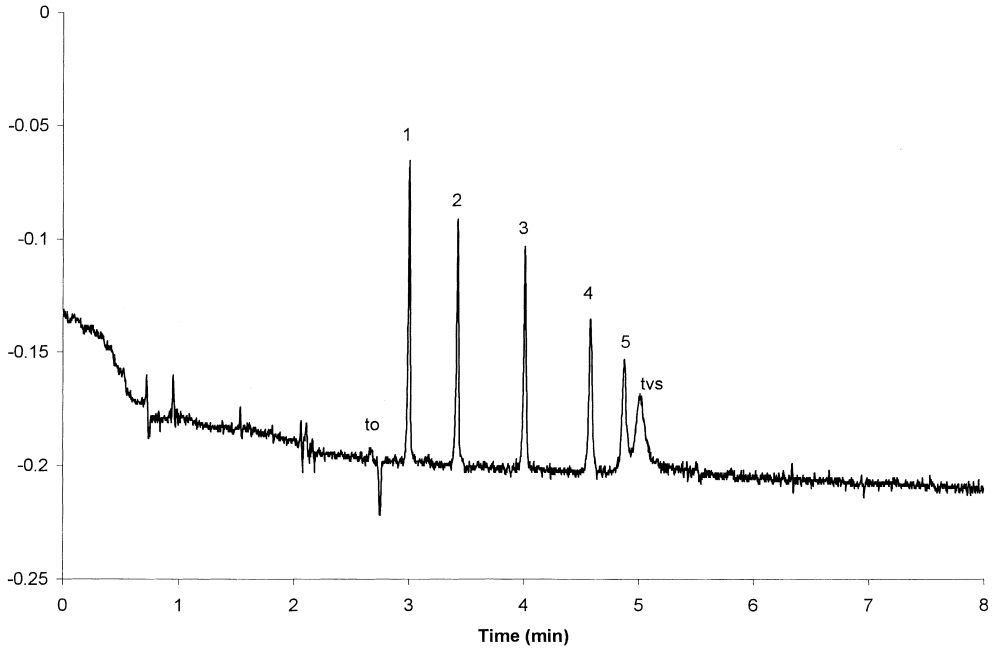

Fig. 2. Cationic vesicles EKC elution pattern for alkylphenones, 10.0 m*M* DHAB in deionized water, UV detection at $\lambda = 254$ nm, -25 kV. (1) Acetophenone, (2) propiophenone, (3) butyrophenone, (4) valerophenone, (5) hexanophenone, octanophenone coelutes with t_{vs}.

chromatographic ability of this separation media are presented in Figs. 2 and 3.

3.3. LSER study

Abraham's LSER model describes the contribution of various types of interactions to retention in EKC as:

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

where k is the retention factor in EKC at a given vesicle concentration. This model is essentially the same as that reported previously [5–7], 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 [14]. 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 e term is also considered as a correction term that explained the residual polarizability unaccounted for by the s coefficients. The regression constant, C depends on the phase ratio, which is determined by the amphiphile concentration.

Since retention factor in EKC is directly related to solute partition coefficient into the pseudo-phase, the LSER model of retention factor essentially provides information about the contributions of hydrophobic, hydrogen bonding, and dipolar interactions for solute partitioning from the bulk aqueous phase into these organized assemblies.

The solvation properties of the cationic DHAB vesicles are compared to those obtained for the

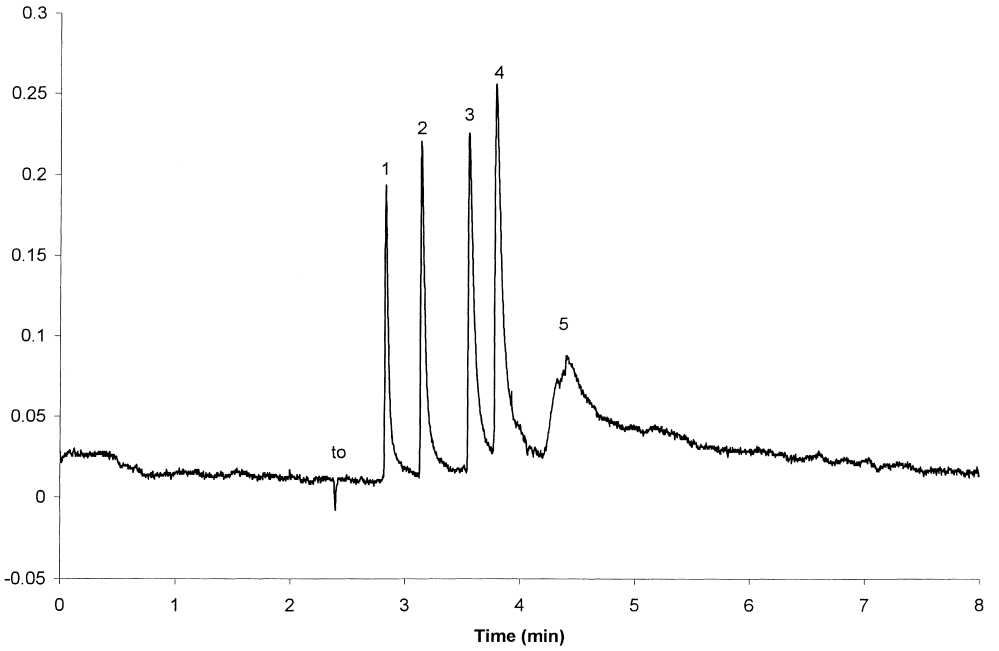

Fig. 3. Cationic vesicles EKC elution pattern for steroids, 10 m*M* DHAB in deionized water, UV detection at $\lambda = 254$ nm, -25 kV. (1) Cortisone, (2) hydrocortisone, (3) corticosterone, (4) deoxycorticosterone, (5) andosterone coelutes with t_{vs}.

cationic C_{16}TAB micelles, anionic vesicles of dihexadecyl phosphate (DHP), and for the octanol–water partitioning systems.

As shown in Table 1, the hydrophobic interaction is the most important contributor to retention, which is consistent with the overall partitioning behavior in the organized assemblies. The large v value indicates that the solutes are subjected to a pseudo-phase that is less cohesive (more ''hydrocarbon-like'') relative to the environment provided by the aqueous medium. The DHAB vesicles are considerably ''more hydrocarbon-like'' than the CTAB micelles with similar head group and identical hydrocarbon lengths. This seems to be the consistent trend among other lipid bilayer assemblies such as the anionic DHP vesicles and liposomes made of phospholipids [6,7].

The b term measures the hydrogen bond donor ability (acidity) of the organized media surface relative to that of the bulk aqueous phase. The large negative b coefficient for the DHAB vesicles points to a much weaker hydrogen bond donor environment than the bulk aqueous phase as well as the other three pseudo-phases. The H-bond acidity of the pseudo-phase is related to its water content, noting that the water molecules present in the interphase region of the aggregates have preferential orienta-

Table 1
Linear solvation energy relationship coefficient in vesicular and micellar media

	v	b	a	s	e	c	R^2
18 m*M* DHAB	4.01 (±0.36)	−4.38 (±0.31)	1.34 (±0.17)	−0.59 (±0.28)	1.46 (±0.33)	−2.96	0.92
10 m*M* C_{16}TAB	2.99 (±0.11)	−2.71 (±0.10)	0.87 (±0.05)	−0.20 (±0.08)	0.30 (±0.08)	−2.26	0.99
10 m*M* DHP in Tris buffer	3.59 (±0.10)	−3.27 (±0.15)	0.47 (±0.17)	−0.65 (±0.12)	0.42 (±0.09)	−2.68	0.99
Octanol–water	3.83 (±0.07)	−3.65 (±0.10)	0.04 (±0.11)	−1.04 (±0.08)	0.48 (±0.06)	0.19	0.99

tion, relative to the water molecules in the bulk phase. Water molecules are not only attached to the surface, but they are present in the interstices of the head groups of the bilayer. The water layer at the surface and its depth of penetration in the hydrocarbon chain constitutes the polar layer of the aggregates, thus establishes a polarity gradient at the interface between the vesicle and the bulk aqueous phase.

The weaker H-bond donor strength of the DHAB vesicles indicates that the water molecules are more tightly bound to the surface head groups. The water–cationic head group interactions are more pronounced than those with the anionic groups. The weaker H-bond donor acidity of the vesicles as compared to micelles could also be attributed to the smaller water content of the more tightly packed vesicles.

The opposite trend is observed for the H-bond acceptor strength as evident from the large positive *a* coefficient that shows the cationic vesicular phase has significantly higher tendency of accepting H-bond from a solute than the cationic micelle and the anionic DHP vesicles. The three vesicular and micellar pseudo-phases are stronger H-bond acceptors than octanol that has nearly the same strength as the bulk aqueous phase. The *s* term is an estimate of the dipolarity. Both of the vesicular phases have less dipolar characters than the micelles. As often observed in the LSER analysis of organized media, the dipolarity term is a minor contributor to the regression model. The measure of the ability to interact with solutes with available *n*- or π-electrons is quantified by the *e* coefficient. It favors the vesicular phase, and is larger than the ''*a*'', coefficient. According to some authors, it accounts for the residual factors not incorporated into the *s* coefficient [15,16]. The polarizability term for the cationic vesicle is considerably much larger than those for the micellar phases, which shows that the vesicular pseudo-phase has stronger interactions with the *n*-, or π-electrons of solutes. The unusually large *e* coefficient could also be partly ascribed to the presence of bromide counterions. The bromide anion extensively binds to the quaternary ammonium head group. The presence of strongly bound Br^- ions reduces the cohesion within the bilayer polar region as well as the number of water molecules attached to the surface of the vesicles by allowing greater exposure of the cationic head groups [17]. The outcome of this head group exposure is the formation of vesicle surface that is less dipolar than in the micellar phase and has considerable fewer water molecules attached at it surface than should have been otherwise.

The plot of log *k* vs. log P_{ow} in Fig. 4 displays a strong congeneric behavior [18]. The solutes are clearly segregated into non-hydrogen bonding and hydrogen bonding solutes. The strong hydrogen bond acceptor and weak hydrogen bond donor strengths of the pseudo phase selectively differentiate between solutes with different hydrogen bonding characteristics. The clustering into two distinct classes of hydrogen bond acceptors (HBAs) and donors (HBDs) is attributed to the difference in hydrogen bonding pattern between the cationic vesicular phase and the octanol. The existence of a higher correlation between the NHB (non-hydrogen bond donor) solutes supports the earlier conclusion of the dominant role of the cavity formation term, as well as the similarity in the hydrophobic interaction with the octanol phase.

3.4. Functional groups Gibbs free energy of transfer

The free energies for transfer of functional groups between the aqueous and the vesicular phases pro-

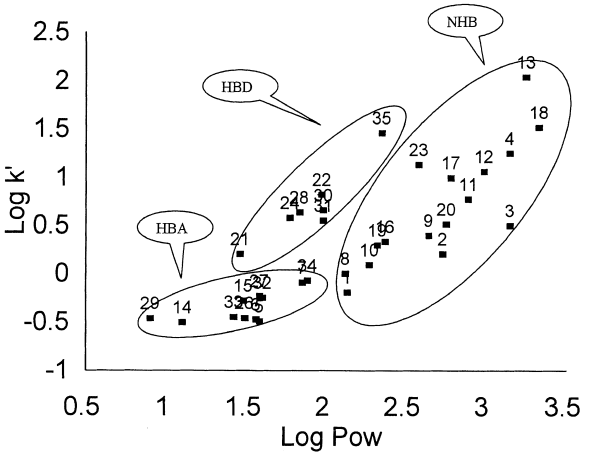

Fig. 4. Correlation of the logarithm of the retention factors (log *k*) in cationic vesicular medium with partitioning coefficients in octanol–water (log P_{ow}).

vide useful information about the interactions in the interfacial system. The free energy of transfer of a functional group $\Delta\Delta G$ is defined as:

$$\Delta\Delta G = -RT \cdot \ln\left(\frac{k'_{\phi\text{-R}}}{k'_{\phi\text{-H}}}\right) \quad (3)$$

where $k'_{\phi\text{-R}}$ is the retention factor for substituted benzene (-R), $k'_{\phi\text{-H}}$ the retention factor of benzene (-H). T is the temperature of the capillary and R is the gas constant. The information about the change in free energy can be coupled to the LSER results by substituting Eq. (2) (for $k'_{\phi\text{-R}}$ and $k'_{\phi\text{-H}}$) into Eq. (3). This combination results in the following equation for a given pseudo-stationary phase:

$$\Delta\Delta G = -2.303RT \cdot \left\{ \begin{array}{l} v(V_{\phi\text{-R}} - V_{\phi\text{-H}}) + b(B_{\phi\text{-R}} - B_{\phi\text{-H}}) + \\ a(A_{\phi\text{-R}} - A_{\phi\text{-H}}) + s(S_{\phi\text{-R}} - S_{\phi\text{-H}}) + e(E_{\phi\text{-R}} - E_{\phi\text{-H}}) \end{array} \right\} \quad (4)$$

In a sense, Eqs. (3) and (4) indicate that the change in free energy of transfer for a functional group is the sum of changes in free energies due to the cavity formation:

$$\Delta\Delta G_{(\text{cavity})} = -2.303RT \cdot \left[v(V_{X_{\phi\text{-X}}} - V_{X_{\phi\text{-H}}}) \right] \quad (5a)$$

type A hydrogen bonding:

$$\Delta\Delta G_{(\text{type A, H-bond})} = -2.303RT \cdot \left[b(B_{\phi\text{-X}} - B_{\phi\text{-H}}) \right] \quad (5b)$$

type B hydrogen bonding:

$$\Delta\Delta G_{(\text{type B, H-bond})} = -2.303RT \cdot \left[a(A_{\phi\text{-X}} - A_{\phi\text{-H}}) \right] \quad (5c)$$

dipolarity:

$$\Delta\Delta G_{(\text{dipolarity})} = -2.303RT \cdot \left[s(S_{\phi\text{-X}} - S_{\phi\text{-H}}) \right] \quad (5d)$$

and polarizability:

$$\Delta\Delta G_{(\text{polarizability})} = -2.303RT \cdot \left[e(E_{\phi\text{-X}} - E_{\phi\text{-H}}) \right] \quad (5e)$$

The contributions of each type of interaction to the free energies of transfer for various functional groups are listed in Tables 2 and 3 for the DHAB vesicular and CTAB micellar pseudo-phases, respectively. In all cases, the $\Delta\Delta G$ (cavity formation) is the major contributor to the total change in the Gibbs free energy of interaction. The net free energies associated with the transfer of the halogen substituents (solutes 1–3) are substantially more pronounced in the vesicular phase. The affinity of the halogen-substituted benzene for the DHAB phase is accentuated in the series from chlorine to iodine. The selectivity associated with these electronegative substituents is mostly due to the cavity formation, the polarizability, and the H-bond donating ability, with the exception of ''Cl'' for which H-bond (type A) contribution is greater than the $\Delta\Delta G$ (polarizability).

Table 2
Contributions of various types of interactions the total energy of transfer (Eqs. (5a)–(5e)) for different functional groups: DHAB vesicle

No.	Functional group	$\Delta\Delta G$ (cavity)	$\Delta\Delta G$ (polarizability)	$\Delta\Delta G$ (dipolarity)	$\Delta\Delta G$ (H-bond, type A)	$\Delta\Delta G$ (H-bond, type B)
1	Cl	−2.91	−0.93	0.45	−1.81	0.00
2	Br	−4.14	−2.34	0.73	−1.29	0.00
3	I	−6.12	−4.98	1.04	−0.52	0.00
4	CH_3	−3.33	0.08	0.00	0.00	0.00
5	CH_2CH_3	−6.67	−0.03	−0.03	0.26	0.00
6	$CH_2CH_2CH_3$	−10.00	0.05	−0.07	0.26	0.00
7	NH_2	−5.27	−3.87	2.12	4.39	−2.37
8	CN	−3.67	−1.14	2.05	4.91	0.00
9	NO_2	−4.14	−2.25	2.05	3.62	0.00
10	$COCH_3$	−7.05	−1.79	1.70	8.78	0.00
11	CO_2CH_3	−8.44	−1.06	1.15	8.26	0.00
12	$CO_2CH_2CH_3$	−11.78	−0.68	1.15	8.26	0.00
13	CH_2OH	−4.73	−1.66	1.22	10.85	−2.61

Table 3
Contributions of various types of interactions the total energy of transfer (Eqs. (5a)–(5e)) for different functional groups: CTAB micelles

No.	Functional group	$\Delta\Delta G$ (cavity)	$\Delta\Delta G$ (polarizability)	$\Delta\Delta G$ (dipolarity)	$\Delta\Delta G$ (H-bond, type A)	$\Delta\Delta G$ (H-bond, type B)
1	Cl	−2.17	−0.19	0.15	−1.12	0.00
2	Br	−3.09	−0.48	0.25	−0.80	0.00
3	I	−4.57	−1.02	0.35	−0.32	0.00
4	CH_3	−2.49	0.02	0.00	0.00	0.00
5	CH_2CH_3	−4.97	−0.01	−0.01	0.16	0.00
6	$CH_2CH_2CH_3$	−7.46	0.01	−0.02	0.16	0.00
7	NH_2	−3.93	−0.80	0.72	2.72	−1.54
8	CN	−2.73	−0.23	0.70	3.04	0.00
9	NO_2	−3.09	−0.46	0.70	2.24	0.00
10	$COCH_3$	−5.25	−0.37	0.58	5.43	0.00
14	CO_2CH_3	−6.29	−0.22	0.39	5.11	0.00
15	$CO_2CH_2CH_3$	−8.78	−0.14	0.39	5.11	0.00
16	CH_2OH	−3.53	−0.34	0.41	6.71	−1.69

The strong interaction involving, and π-electrons results in a larger *e* coefficient.

The net free energy of transfer of the short alkyl substituents (Nos. 4–6) on the benzene ring indicates more favorable interactions with the DHAB vesicles relative to the cationic micelles. The energies of interactions of the alkyl groups are exclusively due to the hydrophobic effect (i.e., cavity formation term). The stronger hydrogen bond acceptor functional groups have very large unfavorable hydrogen bond type A and dipolar terms that largely cancel the favorable hydrophobic effects. However, as the carbon chain is extended, the McGowan's volume of the substituents increases, and the hydrophobic energy augments accordingly (e.g., Nos. 11 and 12). The increment in the energy of transfer associated with the addition of a methylene group to the alkylphenone substituents is on average more negative for the vesicular pseudo-phase (−3.0 vs. −2.2 kJ/mol for the micellar phase). The bilayer offers a more hydrocarbon-like environment for the transfer of the hydrophobic portion of the molecules.

In summary, the results clearly show just like micellar aggregates, the head group charge has a significant impact on the solvation properties of the vesicles. Cationic vesicles have distinctly different interactive characteristics from those of anionic vesicles and liposomes, as well as micelles and octanol. The combination of LSER analysis and EKC is quite useful in characterizing solute partitioning into such pseudo-phases.

Acknowledgements

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