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Rapid estimation of octanol–water partition coefficients using synthesized vesicles in electrokinetic chromatography

W.L. Klotz[a,b,*], M.R. Schure[b,c], J.P. Foley[d]

[a]*Department of Chemistry, Villanova University, Villanova, PA 19085, USA*
[b]*Analytical and Computational Technology Center, Rohm and Haas Company, Spring House, PA 19477-0904, USA*
[c]*Theoretical Separation Science Laboratory, Rohm and Haas Company, Spring House, PA 19477-0904, USA*
[d]*Department of Chemistry, Drexel University, Philadelphia, PA 19104, USA*

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Abstract

Vesicle electrokinetic chromatography (VEKC) using vesicles synthesized from the oppositely charged surfactants cetyltrimethylammonium bromide (CTAB) and sodium octyl sulfate (SOS) and from the double-chained anionic surfactant bis(2-ethylhexyl)sodium sulfosuccinate (AOT) was applied to the indirect measurement of octanol–water partition coefficients (log $P_{o/w}$). A variety of small organic molecules with varying functional groups, pesticides, and organic acids were evaluated by correlating log $P_{o/w}$ and the logarithm of the retention factor (log k') and comparing the calibrations. A linear solvation energy relationship (LSER) analysis was conducted to describe the retention behavior of the vesicle systems and compared to that of octanol–water partitioning. The solute hydrogen bond donating behavior is slightly different with the vesicle interactions using CTAB–SOS vesicles as compared to the octanol–water partitioning model. The AOT vesicle and octanol–water partitioning systems showed similar partitioning characteristics. VEKC provides rapid separations for determinations of log $P_{o/w}$ in the range of 0.5 to 5 using CTAB–SOS vesicles and 0 to 5.5 using AOT vesicles.

Keywords: Vesicles; Partition coefficients; Electrokinetic chromatography; Linear solvation energy relationships; Pesticides; Cetyltrimethylammonium bromide; Bis(2-ethylhexyl) sodium sulfosuccinate

1. Introduction

Micellar electrokinetic chromatography (MEKC) combines the high efficiency and speed of capillary electrophoresis with the features of conventional chromatographic separation utilizing a retentive phase. Anionic micelles of sodium dodecyl sulfate (SDS) were first used to demonstrate the separation of some neutral phenolic compounds in an aqueous capillary experiment [1,2]. Since then, a variety of retentive phases and additives have been used in electrokinetic separations for a number of applications which have been conducted with anionic, cationic, nonionic, and zwitterionic surfactants [3–6], mixed micelles [7–9], cyclodextrins [10], bile salts [11], gels [11–13], polymers [14], microemulsions [15,16], and vesicles [17–19].

Vesicles are self-assembling, organized structures that contain a continuous bilayer of monomers and

*Corresponding author. Rohm and Haas Company, 727 Norristown Road, P.O. Box 0904, Spring House, PA 19477-0904, USA. Tel.: +1-215-641-7438; fax: +1-215-641-7254.
E-mail address: wklotz@rohmhaas.com (W.L. Klotz).

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enclose an aqueous core region. The formations occur in nature through the aggregation of phospholipids to form liposomes. Vesicles can also be synthesized using surfactants with structures containing two alkyl chain groups and a polar head group or using single chain amphiphilic molecules or mixtures of single chain cationic and anionic surfactants under appropriate conditions.

Bis(2-ethylhexyl)sodium sulfosuccinate, also known as Aerosol OT or AOT, is an anionic, double-chain surfactant which can form vesicles as described below. This hydrophobic ionic surfactant is highly soluble in hydrocarbons and is most often used to form three-component microemulsions. When forming microemulsions with AOT surfactant, the water diffusion is slow, resulting in the water-in-oil type of microemulsion in this situation. Because AOT–water–oil microemulsions can be formed without the use of a cosurfactant, it is a popular model for the study of the phase behavior of microemulsions due to its simplicity [20]. It has been observed that with the addition of salt to AOT solutions, the anionic, double-chain surfactant may aggregate to form vesicular phases [21,22]. The use of AOT vesicles as a retentive phase in electrokinetic chromatography (EKC) was demonstrated for the separation of neutral and charged synthetic antioxidants [23]. Since synthetic vesicles formed by AOT mimic lipid bilayers, the resulting structures may serve as an alternative model of biopartitioning in cell membranes.

The octanol–water partition coefficient (log $P_{o/w}$) has been the standard measure of hydrophobicity used to predict bioaccumulation and biotransport in living systems [24,25]. MEKC has been explored as an alternative technique for the indirect measurement of hydrophobicity, since micellar structures contain hydrophobic and hydrophilic sites for interaction in separate regions of their structures [26–29]. MEKC using micelles modified with alcohols has also provided high correlations of log $P_{o/w}$ and retention factor [30–32]. Microemulsion EKC has been used successfully for the analysis of log $P_{o/w}$ of small neutral and charged organic molecules [33–37], and for a wide hydrophobicity range of pesticide compounds [38]. A simplified study using vesicles formed from oppositely charged surfactants showed good correlation of hydrophobicity and retention for substituted benzene solutes [19], and immobilized liposome chromatography has also been used to evaluate neutral and charged species [39].

The focus of the study reported in this paper was to evaluate the use of vesicles synthesized from oppositely charged surfactants and from an anionic double-chained surfactant as retentive phases in electrokinetic chromatography for the application of hydrophobicity measurements. The retention factor and octanol–water partition coefficients were correlated for the calibration of over 100 small neutral organic molecules and pesticides compounds that vary in functionality, size and shape. The effects of hydrogen bonding on retention were examined, and the interactive nature of the retentive phases were studied and compared to that of octanol–water partitioning through linear solvation energy relationships (LSERs).

2. Experimental

2.1. Instrumental

A Waters Quanta 4000 (Waters, Milford, MA, USA) capillary electrophoresis instrument equipped with a fixed-wavelength UV detection system at 254 nm was employed for the standard and pesticide compound separations. Electrokinetic separations on the Waters Quanta 4000 were performed using a fused-silica capillary of 37.5 cm×50 μm I.D.×375 μm O.D. (Polymicro Technologies, Tucson, AZ, USA). Injections for the cetyltrimethylammonium bromide–sodium octyl sulfate (CTAB–SOS) vesicle experiments were made hydrostatically for 2 s at a height of 9.8 cm. Injections for the AOT vesicle experiments were made hydrostatically for 5 s at a height of 9.8 cm. The applied voltage for the CTAB–SOS studies was 10 kV and for the AOT studies was 7.5 kV. The data were collected at a rate of 10 Hz and processed on a PC using ChemStation software (Hewlett-Packard, Wilmington, DE, USA). All experiments were performed at ambient temperature (≈25 °C).

A Hewlett-Packard (3D)CE system was used for the CTAB–SOS organic acid compound separation experiments. Electrokinetic separations were performed on the Hewlett-Packard CE system using a

fused-silica capillary of 38.5 cm×50 μm I.D.×375 μm O.D. (Polymicro Technologies). Injections were made hydrodynamically with a pressure of 50 mbar for 2 s. The applied voltage for the acid compound separations was 10 kV. The data were collected at a rate of 10 Hz and processed on a PC using ChemStation software (Hewlett-Packard). All experiments performed with the HP CE system were conducted at 25 °C with active temperature control.

2.2. Materials

All organic acid and standard test analytes were purchased from Aldrich (Milwaukee, WI, USA). The pesticide compounds were purchased from Chem Service (West Chester, PA, USA). SOS was purchased from Lancaster Synthesis (Windham, NH, USA), and CTAB was purchased from Aldrich. AOT was obtained from Fluka (Milwaukee, WI, USA). The sodium dihydrogen phosphate and HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffers were obtained from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile and methanol solvents were purchased from J.T. Baker. The water used for the preparation of the buffer solutions was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA).

Stock buffer solutions for the CTAB–SOS experiments were prepared with sodium dihydrogen phosphate at a concentration of 50 m*M* in Milli-Q water and adjusted to pH 7.0 with 1.0 *M* NaOH or pH 2.0 with phosphoric acid. A thermodynamically stable vesicle composed of 1.8% total mass of CTAB–SOS at a 30–70 mole ratio was chosen as the model system for study in these investigations. This retentive phase composition provided a reasonably wide migration window with sufficiently short analysis times. The CTAB–SOS vesicles also have a negative charge at this surfactant composition [40].

The CTAB–SOS vesicle solutions were prepared by weighing appropriate amounts of CTAB and SOS surfactants into a 25 ml volumetric flask. A 5 ml volume of 50 m*M* sodium phosphate buffer solution was added and diluted with Milli-Q water to 25 ml to provide a final buffer concentration of 10 m*M*. The vesicle solution was mixed by ultrasonication for 40 min and was allowed to equilibrate at ambient temperature for a minimum of 4 h prior to use in electrokinetic separations. These solutions are stable and able to be used for separation purposes for up to 4 days after preparation.

The test solutes for the CTAB–SOS vesicle studies were dissolved in acetonitrile or methanol and then diluted in buffer solution at a buffer-to-solvent ratio of 80:20. The solute concentrations ranged from 0.2 to 0.5 mg/ml. The presence of solvent in the injection solutions posed no effect on the retention behavior of the analytes, as the volume of sample injected onto the capillary was small (ca. 0.1–0.3% of the capillary volume).

The AOT vesicles were prepared in 10 m*M* sodium dihydrogen phosphate buffer in Milli-Q water adjusted to pH 7.0 with 1 *M* sodium hydroxide. A composition of 40 m*M* AOT with 10% methanol in 10 m*M* sodium phosphate buffer at pH 7.0 was selected for use as the experimental vesicle separation system for the log $P_{o/w}$ studies. This surfactant concentration combined with the organic modifier content allowed for good resolution and a moderate migration window to accommodate the separation and survey of several compounds in a run. The vesicle solutions were prepared by weighing appropriate amounts of AOT, methanol, and buffer solution and mixing by ultrasonication for 30 min. The compounds were dissolved directly in the vesicle solution for injection onto the separation capillary. The solute concentrations ranged from 0.2 to 0.5 mg/ml. All solutions were filtered through 0.45 μm GHP Acrodisc filters (Pall Gelman, Ann Arbor, MI, USA) prior to use.

2.3. Methods

The capillary was conditioned by purging with 1.0 *M* NaOH for 15 min, followed by a purge of 0.1 *M* NaOH for 15 mm. The capillary was then rinsed with water for 10 min, followed by the vesicle solution for 10 min. Purges with 0.1 *M* NaOH and water were performed periodically to remove contaminants from the capillary walls.

2.4. Calculations

The retention factor for a neutral solute in VEKC can be calculated using the equation:

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_v)} \quad (1)$$

where t_r, t_0, and t_v are the migration times of the solute, the electroosmotic flow, and the vesicle, respectively. The use of a marker compound to verify the elution of CTAB–SOS vesicles through the capillary was not successful due to solubility issues. Therefore, the migration time of the CTAB–SOS vesicles through the capillary, t_v, was calculated for each separation using an alkylphenone homologous series with an iterative computational method [41]. The migration time of the AOT vesicles through the capillary, t_v, was determined by the elution time of dodecylbenzene which was fully retained by the retentive phase.

The relationship between retention factor and octanol–water partitioning may be expressed using the functional form [42,43]:

$$\log P_{o/w} = a \log k' + b \quad (2)$$

where a and b are constants that represent the slope and intercept of a linear calibration line.

3. Results and discussion

One hundred and fifteen compounds varying in structure, size and functionality were evaluated to test the feasibility of using CTAB–SOS for the indirect measurement of hydrophobicity. One hundred and eight compounds were evaluated with the AOT vesicle system. The relationship between octanol–water partitioning and chromatographic partitioning was investigated by plotting $\log P_{o/w}$ versus $\log k'$. The compounds were divided into three general categories for further evaluation: (1) common organic standard compounds with varying functionality that are not dissociated under the conditions utilized here, (2) pesticide compounds varying in type, and (3) organic acids, analyzed at pH 2. The test solutes and the $\log P_{o/w}$ values used are listed in Table 1. Chromatograms showing the separation of some organic standard compounds for each vesicle phase are given in Figs. 1 and 2.

Figs. 3 and 4 show plots of the $\log P_{o/w}$ versus $\log k'$ correlations for the compound groups investigated for each retentive phase. Linear regression analysis provided the following equations for the CTAB–SOS and AOT correlations.

CTAB–SOS vesicle linear regression results:

Organic standards:

$$\log P_{o/w} = (1.291 \pm 0.032) \log k' + 1.928 \pm 0.032$$

$$r^2 = 0.970,\ n = 51,\ \mathrm{SE} = 0.203 \quad (3)$$

Pesticides:

$$\log P_{o/w} = (1.238 \pm 0.035) \log k' + 2.199 \pm 0.036$$

$$r^2 = 0.968,\ n = 43,\ \mathrm{SE} = 0.200 \quad (4)$$

Organic acids:

$$\log P_{o/w} = (1.226 \pm 0.053) \log k' + 1.903 \pm 0.053$$

$$r^2 = 0.971,\ n = 18,\ \mathrm{SE} = 0.192 \quad (5)$$

AOT vesicle linear regression results:

Organic standards:

$$\log P_{o/w} = (1.249 \pm 0.025) \log k' + 2.053 \pm 0.028$$

$$r^2 = 0.978,\ n = 58,\ \mathrm{SE} = 0.182 \quad (6)$$

Pesticides:

$$\log P_{o/w} = (1.360 \pm 0.040) \log k' + 2.041 \pm 0.053$$

$$r^2 = 0.960,\ n = 50,\ \mathrm{SE} = 0.249 \quad (7)$$

The r^2 value is the correlation coefficient, n is the number of compounds employed in the regression, and SE is the standard error.

Upon visual inspection, the calibration lines of both the CTAB–SOS and AOT vesicle phases appear to compare well in regards to slope and intercept. If the calibration curves of a retentive phase are similar, it may be feasible to use a universal set of standard compounds to establish the calibration for the prediction of $\log P_{o/w}$ for a wide variety of compound types with certainty, regardless of structure, size and functionality. The calibration

Table 1
Test solutes and their literature log $P_{o/w}$ values

Organic standard compounds	log $P_{o/w}$ [24,44]	Compound	log $P_{o/w}$ [24,44]
acetanilide	1.16	*N*-Methylbenzamide[a]	0.90
aniline	0.94	Methylbenzoate	2.12
anisole	2.11	Methyl-2-fuorate	1.00
anthracene[b]	4.45	1-Methylindole	2.64
benzaldehyde	1.48	1-Methylnaphthalene	3.95
benzamide[a]	0.64	1-Methylpyrrole[a]	1.15
2,3-benzofuran[a]	2.67	Naphthalene	3.37
benzene[b]	2.13	2-Naphthol[b]	2.84
benzonitrile	1.56	4-Nitroanisole	2.03
benzophenone[b]	3.18	Nitrobenzene	1.86
benzyl alcohol	0.87	2-Nitrotoluene[b]	2.30
bibenzyl	4.60	3-Nitrotoluene[b]	2.42
biphenyl	3.95	4-Nitrotoluene	2.37
bromobenzene	2.99	Pentachlorophenol[a]	3.81
butyl benzene	4.44	Phenol[a]	1.46
tert.-butyl benzene	4.11	Propyl benzene	3.69
carbazole[b]	3.72	4-Propylphenol	3.20
catechol[b]	1.01	Pyrrole	0.75
chlorobenzene[a]	2.84	Quinoline[b]	2.06
1,4-chloronitrobenzene	2.39	Quinoxaline	1.30
1,3-chloronitrobenzene	2.46	Salicylaldehyde	1.70
4-chlorophenol	3.33	1,2,4,5-Tetrabromobenzene[b]	5.10
m-cresol	1.96	1,2,3,4-Tetrachlorobenzene	4.50
o-cresol[a]	1.95	2,3,5,6-Tetrachloronitrobenzene	3.90
4-cyanophenol	1.63	1,2,4,5-Tetramethylbenzene	4.00
dibenzothiozine[b]	4.38	1,3,5-Tribromobenzene[b]	4.50
1,3-dichlorobenzene	3.52	2,4,6-Tribromophenol[b]	5.07
ethylbenzene	3.13	1,2,4-Trichlorobenzene	3.98
2-ethylfuran[b]	2.40	1,3,5-Trichlorobenzene[b]	4.49
ethyl-2-fuorate[b]	1.50	1,3,5-Trimethylbenzene[b]	3.42
9-fluorenone	3.58	Toluene	2.73
hydroquinone	0.55	*m*-Xylene	3.20
indole	2.14	*o*-Xylene	3.12
4-methoxyphenol[a]	1.34	*p*-Xylene	3.15

Pesticide compounds	log $P_{o/w}$ [45,46]	Compound	log $P_{o/w}$ [45,46]
Aldicarb	1.13	Linuron[a]	3.20
Aminocarb	1.73	Metalaxyl[a]	1.27
Atrazine	2.66	Methiocarb	3.34
Benfluralin[b]	5.29	Metribuzin	1.70
Bensulide	4.20	Napropamide	3.30
Bromacil	2.11	Oxadiazon[a]	4.09
Carbaryl	2.34	Paclobutrazol	3.20
Carbendazim[b]	1.52	Paraoxon	1.98
Carbofuran[a]	1.52	Parathion	3.83
Carbophenothion[b]	4.82	Pendimethalin[b]	5.18
Chlorpyrifos[b]	5.27	Phenothiazine[a]	4.15

Table 1. Continued

Pesticide compounds	log $P_{o/w}$ [45,46]	Compound	log $P_{o/w}$ [45,46]
Pesticide compounds	log $P_{o/w}$	Compound	log $P_{o/w}$
Chlortoluron	2.50	Phosalone[a]	4.30
Coumaphos[b]	4.13	Pirimiphos-ethyl[b]	4.85
Dicapthon	3.72	Pirimiphos-methyl[a]	4.20
Dichlofenthion[b]	5.14	Profenfos	4.44
Dieldrin[a]	4.54	Propanil[b]	3.07
Dimethoate[a]	0.704	Propoxur	1.56
Dipropetryn[a]	3.81	Propyzamide[b]	3.09
Diuron	2.80	Pyrazon	1.50
EPN[b]	5.02	Simazine[b]	2.10
Fenbuconazole[b]	3.23	Simetryn	2.66
Fenchlorphos[b]	5.07	Terbutryne	3.74
Fenobucarb	2.79	Tetrachlorvinphos	3.53
Fensulfothion	2.33	Tetramethrin[b]	4.70
Fenuron	0.96	Thiram	1.73
Fluorodifen	4.40	Tolylfluanid	3.90
Iprodione	3.00	Triadimefon	3.11
Isofenphos[b]	4.04	Tricyclazole[a]	1.40
Isoprocarb	2.30	Trietazine	3.35
Kelthane	4.28	Trifluralin[b]	5.34
Organic acid compounds at pH 2	log $P_{o/w}$ [44]	Compound	log $P_{o/w}$ [44]
acetylsalicylic acid[a]	1.19	Flurbiprofen[a]	4.16
3-chlorobenzoic acid[a]	2.68	3-hydroxybenzoic acid[a]	1.50
4-chlorobenzoic acid[a]	2.65	Naproxen[a]	3.34
3,4-dichlorophenoxyacetic acid[a]	2.81	2-phenoxybenzoic acid[a]	3.11
3,5-diiodosalicylic acid[a]	4.56	3-phenoxybenzoic acid[a]	3.91
2,4-dihydroxybenzoic acid[a]	1.63	4-propylbenzoic acid[a]	3.42
3,4-dihydroxybenzoic acid[a]	1.15	*m*-toluic acid[a]	2.37
2,6-dimethoxybenzoic acid[a]	0.66	*o*-toluic acid[a]	2.46
3,4-dimethoxybenzoic acid[a]	1.61	*p*-toluic acid[a]	2.27

[a] Compound used only for CTAB–SOS studies.
[b] Compound used only for AOT studies.

curves of the individual vesicle phases were compared statistically for differences using SAS JMP statistical software (SAS Institute, Cary, NC, USA).

No statistical differences were found to exist among the slopes of the lines for the CTAB–SOS vesicle calibration lines at 95% confidence; the lines are statistically parallel. The intercept of the CTAB–SOS vesicle pesticides calibration line is, however, statistically different (higher) than the intercepts of the organic standard and acid calibration lines. The pesticide compounds appear to be less retained by the vesicle formations as compared to the organic standard and neutral acid compounds. This is evidenced by the higher *y*-intercept and similar slope of the pesticides calibration line as compared to the organic standards and acids calibration lines. The pesticide compounds are generally larger in size than the organic standards and acids analyzed in this study. This suggests that there may be some degree of size and or shape selectivity with the CTAB–SOS vesicle technique.

The results of the comparison of the organic standard and pesticide compound calibrations of the AOT vesicle phase showed that the lines are statistically similar in intercept but are statistically different in slope at 95% confidence. In order to achieve the

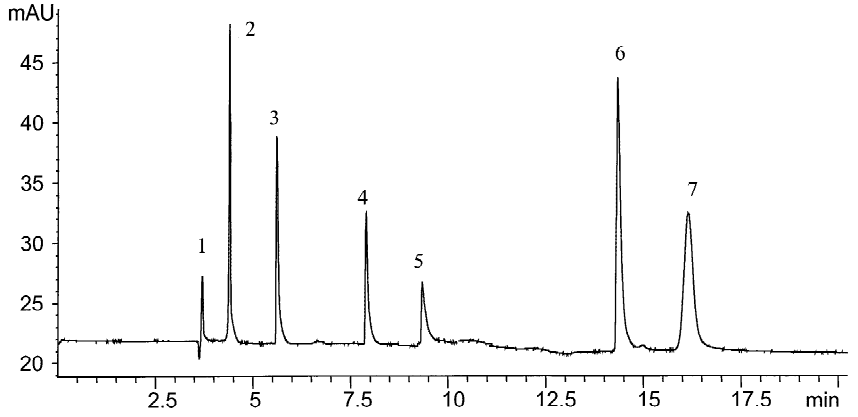

Fig. 1. Separation of representative organic standard compounds by VEKC using CTAB–SOS vesicles: (1) hydroquinone, (2) benzaldehyde, (3) nitrobenzene, (4) 1,4-chloronitrobenzene, (5) 1,3-chloronitrobenzene, (6) propyl benzene, and (7) biphenyl. Separation solution: 1.8% total mass CTAB–SOS vesicle, 30–70 mole ratio, in 10 m*M* sodium phosphate buffer, pH 7.0. Capillary dimensions: 37.5 cm×50 μm I.D. The injection was for 2 s hydrostatically at a height of 9.8 cm, and the operating voltage was 10 kV with detection at 254 nm.

best accuracy of measurement with the AOT vesicle system, compounds similar in character to the unknown molecules being analyzed should be used for the calibration of log $P_{o/w}$ versus log k'.

Out of convenience, some analysts prefer to use a single, universal calibration for the screening of a variety of compound types. Since the calibration lines for the standard and pesticide groups were statistically different with each of the vesicle phases, the data of the *individual* vesicle phases were pooled

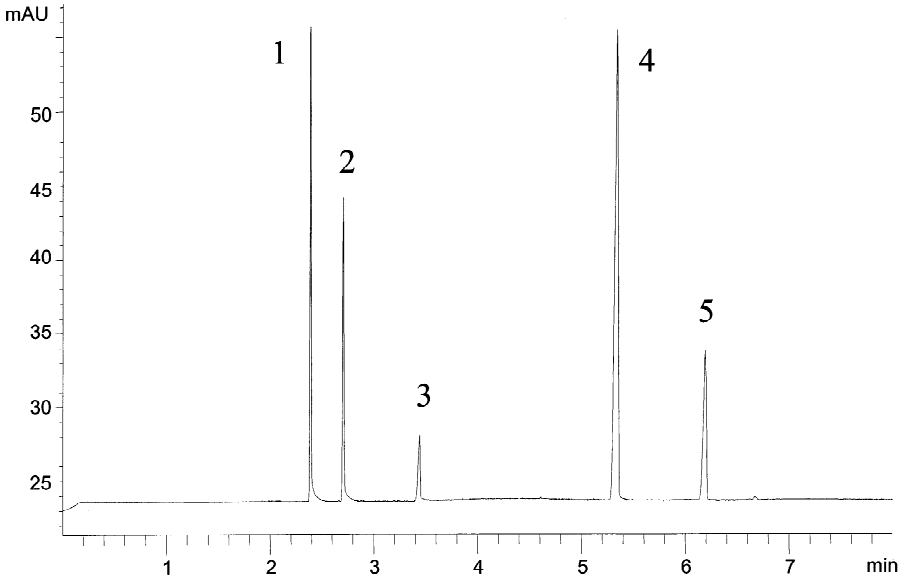

Fig. 2. Separation of representative organic standard compounds by VEKC using AOT vesicles: (1) methyl-2-fuorate, (2) salicylaldehyde, (3) 4-nitrotoluene, (4) 9-fluorenone, and (5) biphenyl. Separation solution: 40 m*M* AOT with 10% methanol in 10 m*M* sodium phosphate buffer, pH 7.0. Capillary dimensions: 37.5 cm×50 μm I.D. The injection was for 5 s hydrostatically at a height of 9.8 cm, and the operating voltage was 17.5 kV with detection at 254 nm.

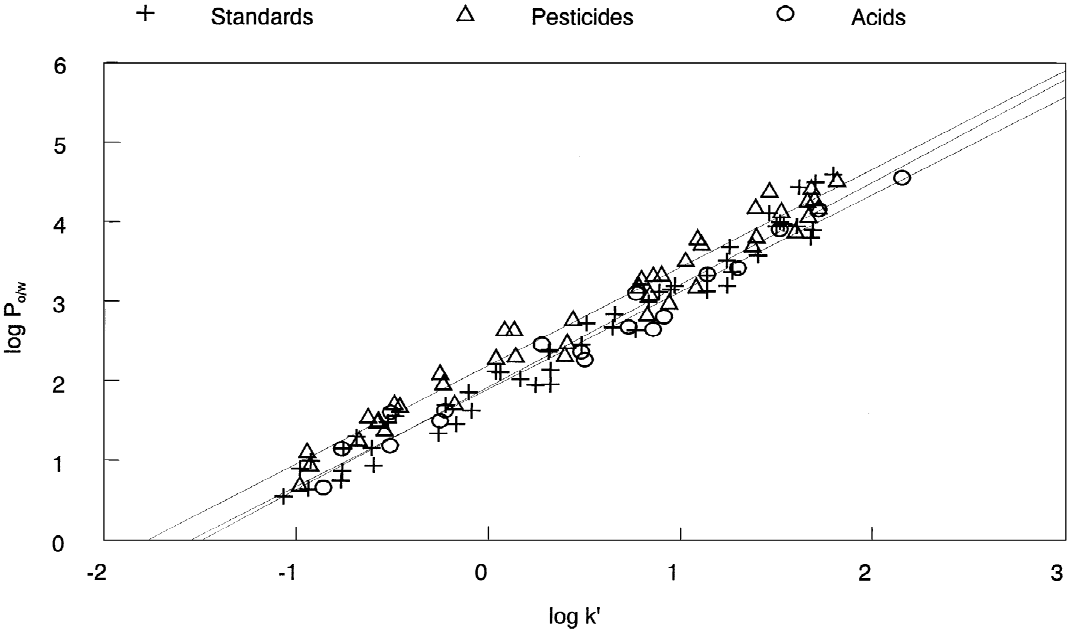

Fig. 3. Plot of log $P_{o/w}$ versus log k' for the CTAB–SOS vesicle system. Conditions as in Fig. 1.

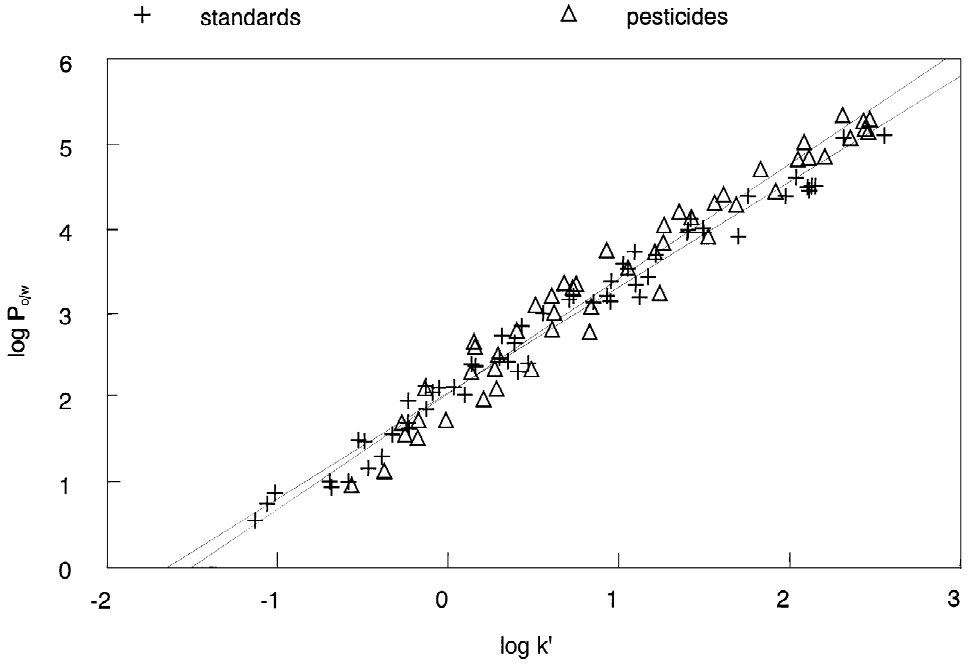

Fig. 4. Plot of log $P_{o/w}$ versus log k' for the AOT vesicle system. An operating voltage of 7.5 kV was used in the measurement of the retention factors. Other conditions as in Fig. 1.

to evaluate the error in bias that may be introduced if a general calibration curve were used for the log $P_{o/w}$ determinations with this technique. The following equations were obtained from the linear regression analysis of each pooled set of data:

CTAB–SOS vesicle phase pooled data correlation:

$$\log P_{o/w} = (1.266 \pm 0.026) \log k' + 2.024 \pm 0.026$$
$$r^2 = 0.957, n = 112, \text{SE} = 0.236 \quad (8)$$

AOT vesicle phase pooled data correlation:

$$\log P_{o/w} = (1.305 \pm 0.023) \log k' + 2.054 \pm 0.028$$
$$r^2 = 0.968, n = 108, \text{SE} = 0.223 \quad (9)$$

Upon observation of the residuals versus predicted log $P_{o/w}$, for the pooled data of each vesicle phase (not shown), there are no apparent outliers present. Therefore, first order linear regression models are valid for the relationships of retention factor and log $P_{o/w}$. If the entire CTAB–SOS data set was used to predict the log $P_{o/w}$ value of a compound, and the organic standard and organic acid correlations are simplified by assigning a common slope and intercept, the value of an organic standard or acid compound may be consistently over estimated by an average of 0.06 units. Pesticide values may be consistently under estimated by an average of 0.19 units. If the pooled calibration data were employed for the AOT vesicle log $P_{o/w}$ determinations, the resulting value of an organic standard or a pesticide compound may be in error of bias from using the pooled calibration by an average of 0.1 log $P_{o/w}$ units. The level of bias error demonstrated with both techniques may be more than adequate for most applications.

The organic standard and acid compounds were divided into two classes, hydrogen bonding and non-hydrogen bonding compounds. This delineation is made by assessing a compound's functional groups and its acidity and basicity solute descriptors from quantitative structure–activity relationship (QSAR) measurements [44,47]. The effect of hydrogen bonding on retention was studied to determine whether any biases exist with the use of the CTAB–SOS and AOT vesicle systems as retentive phases. By analyzing these two groups separately, a plot of the log $P_{o/w}$ values versus log k' resulted in two linear calibrations for each vesicle phase. The linear regression results for both vesicle phases are given below.

CTAB–SOS vesicle hydrogen bonding comparison:

hydrogen bonding:

$$\log P_{o/w} = (1.218 \pm 0.023) \log k' + 1.871 \pm 0.027$$
$$r^2 = 0.971, n = 44, \text{SE} = 0.175 \quad (10)$$

non-hydrogen bonding:

$$\log P_{o/w} = (1.245 \pm 0.052) \log k' + 2.053 \pm 0.063$$
$$r^2 = 0.962, n = 25, \text{SE} = 0.194 \quad (11)$$

AOT vesicle hydrogen bonding comparison:

hydrogen bonding:

$$\log P_{o/w} = (1.270 \pm 0.038) \log k' + 2.016 \pm 0.033$$
$$r^2 = 0.978, n = 27, \text{SE} = 0.169 \quad (12)$$

non-hydrogen bonding:

$$\log P_{o/w} = (1.203 \pm 0.038) \log k' + 2.130 \pm 0.050$$
$$r^2 = 0.971, n = 31, \text{SE} = 0.186 \quad (13)$$

Although the CTAB–SOS data appear to overlay fairly closely, a comparison of the above two calibration lines using SAS JMP statistical software shows that they are statistically different in intercept. The higher *y*-intercept of the non-hydrogen bonding compounds may be explained by these solutes interacting less with the retentive phase. The hydrogen bonding compounds may be interacting more with the hydrophilic surfactant head groups on the outside of the vesicle structure, causing slightly greater retention of these compounds. The two linear calibration curves for the AOT vesicle data also overlay fairly closely. Statistical comparison of the two lines shows that they are similar in slope and intercept. Therefore, one may infer that there are no significant differences in retention between hydrogen bonding and non-hydrogen bonding small organic

solutes using the AOT vesicle phase under the conditions given.

LSERs quantitatively describe solvation effects in terms of the interaction between the solutes and the solvent of a given system and are often used to describe the solvation effects in biological and physicochemical systems [48–52]. This method also facilitates the comparison of different partitioning phases to determine similarities and differences in the interactions of the test solutes between systems.

A model equation commonly used in LSER is [50,51]:

$$\log SP = c + rR_2 + s\pi_2^* + a\sum\alpha_2 + b\sum\beta_2 + vV_x \quad (14)$$

The dependent variable, log *SP*, is regressed against the known parameters of the solutes. In our study, log *SP* represents log k' or log $P_{o/w}$ for comparison purposes. In this equation R_2 represents the excess molar refraction, π_2^* is the solute dipolarity/polarizability, $\Sigma\alpha_2$ and $\Sigma\beta_2$ are the hydrogen bond acidity and basicity, and V_x is the McGowan characteristic volume of the solute [50,51]. The coefficients *r*, *s*, *a*, *b*, and *v* represent relative measures of excess molar refraction, dipolarity/polarizability, hydrogen bond accepting (basicity), hydrogen bond donating (acidity), and cohesive energy density and dispersion interactions. They are *relative* measures because they represent the differences of a given property (e.g., H-bond acidity) between the two phases (octanol/water or vesicle/buffer). Finally, the *c* term is the intercept or regression constant [50,51] and is not relevant to the above chemical properties.

The organic standard compounds of Table 1 for which reliable LSER coefficients were available were employed in a comparison of the partitioning behavior of the octanol–water and the 1.8% total mass, 30–70 mole ratio CTAB–SOS vesicle–water systems as well as the 40 m*M* AOT–10% methanol–10 m*M* sodium phosphate buffer vesicle system and octanol–water. The subset of compounds and the solute descriptors are listed in Table 2 [50,51]. The V_x and R_2 terms are divided by 100 and 10, respectively, to bring the values on the same scale as the other parameters. Because the AOT vesicle phase contains 10% organic solvent and the CTAB–SOS

Table 2
LSER organic standard test solutes and their solvation descriptors [51]

Compound	R_2	π_2^*	$\Sigma\alpha_2^*$	$\Sigma\beta_2$	V_x
Acetanilide	0.870	1.40	0.50	0.67	1.113
Aniline	0.955	0.96	0.26	0.41	0.816
Anisole	0.708	0.75	0.00	0.29	0.916
Anthracene[b]	2.290	1.34	0.00	0.26	1.454
Benzaldehyde	0.820	1.00	0.00	0.39	0.83
Benzamide[a]	0.990	1.50	0.49	0.67	0.973
Benzene[b]	0.61	0.52	0.00	0.14	0.716
2,3-Benzofuran[a]	0.888	0.83	0.00	0.15	0.905
Benzonitrile	0.742	1.11	0.00	0.33	0.871
Benzophenone[b]	1.447	1.50	0.00	0.50	1.481
Benzyl alcohol	0.803	0.87	0.39	0.56	0.916
Biphenyl	1.360	0.99	0.00	0.22	1.324
Bromobenzene	0.882	0.73	0.00	0.09	0.891
Butylbenzene	0.600	0.51	0.00	0.15	1.280
tert.-Butylbenzene	0.619	0.49	0.00	0.16	1.280
Catechol[b]	0.970	1.07	0.85	0.52	0.834
Chlorobenzene[a]	0.718	0.65	0.00	0.07	0.839
m-Cresol	0.822	0.88	0.57	0.34	0.916
o-Cresol[a]	0.840	0.86	0.52	0.30	0.916
4-Cyanophenol	0.940	1.63	0.79	0.29	0.930
Dibenzothiophene[b]	1.959	1.31	0.00	0.18	1.379
1,3-Dichlorobenzene	0.847	0.73	0.00	0.02	0.961
Ethylbenzene	0.613	0.51	0.00	0.15	0.998
Ethyl-2-fuorate[b]	0.560	1.00	0.00	0.50	1.033
2-Ethylfuran[b]	0.361	0.50	0.00	0.14	0.818
Hydroquinone	1.000	1.00	1.16	0.60	0.834
Indole[a]	1.200	1.12	0.44	0.31	0.946
4-Methoxyphenol[a]	0.900	1.17	0.57	0.48	0.975
N-Methylbenzamide[a]	0.950	1.44	0.35	0.73	1.114
Methyl benzoate	0.733	0.85	0.00	0.46	1.073
1-Methylindole	1.206	1.03	0.00	0.37	1.087
1-Methylnaphthalene	1.344	0.90	0.00	0.20	1.226
1-Methylpyrrole[a]	0.559	0.79	0.00	0.31	0.718
Naphthalene	1.340	0.92	0.00	0.20	1.085
2-Naphthol[b]	1.520	1.08	0.61	0.40	1.144
4-Nitroanisole	0.970	1.29	0.00	0.40	1.090
Nitrobenzene	0.871	1.11	0.00	0.28	0.891
2-Nitrotoluene[b]	0.866	1.11	0.00	0.27	1.032
3-Nitrotoluene[b]	0.874	1.10	0.00	0.25	1.032
4-Nitrotoluene	0.870	1.11	0.00	0.28	1.032
Phenol[a]	0.805	0.89	0.60	0.30	0.775
Propylbenzene	0.604	0.50	0.00	0.15	1.139
Pyrrole	0.613	0.73	0.41	0.29	0.577
Quinoline[b]	1.268	0.97	0.00	0.54	1.044
Quinoxaline	1.300	1.22	0.00	0.59	1.003
Toluene	0.601	0.52	0.00	0.14	0.857
1,2,4-Trichlorobenzene	1.030	0.86	0.00	0.00	1.084
1,3,5-Trichlorobenzene[b]	0.980	0.73	0.00	0.00	1.084
1,3,5-Trimethylbenzene[b]	0.649	0.52	0.00	0.19	1.139
m-Xylene	0.623	0.52	0.00	0.16	0.998
o-Xylene	0.663	0.56	0.00	0.16	0.998
p-Xylene	0.613	0.52	0.00	0.16	0.998

[a] Compound used only for CTAB–SOS studies.
[b] Compound used only for AOT studies.

phase is totally aqueous, it is not feasible to make direct comparisons of the LSER data between the two vesicle phases.

The following LSER parameter estimates were obtained for the vesicle system retention and octanol–water partition coefficients for the solutes tested. The standard error for each coefficient is given in parentheses below the coefficient values.

LSER comparison of CTAB–SOS vesicles–buffer and octanol–water partitioning:

$$\text{CTAB–SOS:}\quad \log k' = \underset{(0.13)}{-1.52} + \underset{(0.12)}{0.56R_2} - \underset{(0.09)}{0.57\pi_2^*} + \underset{(0.18)}{0.23\sum\alpha_2} - \underset{(0.16)}{3.25\sum\beta_2} + 2.85V_x$$

$$r^2 = 0.978,\ n = 39 \qquad (15)$$

$$\text{Octanol–Water:}\quad \log P_{o/w} = \underset{(0.10)}{-0.16} + \underset{(0.10)}{0.38R_2} - \underset{(0.08)}{0.70\pi_2^*} + \underset{(0.14)}{0.04\sum\alpha_2} - \underset{(0.13)}{3.86\sum\beta_2} + 4.03V_x$$

$$r^2 = 0.992,\ n = 39 \qquad (16)$$

LSER comparison of AOT vesicles–buffer and octanol–water partitioning:

$$\text{AOT:}\quad \log k' = \underset{(0.10)}{-1.82} + \underset{(0.13)}{0.34R_2} - \underset{(0.10)}{0.43\pi_2^*} + \underset{(0.19)}{0.02\sum\alpha_2} - \underset{(0.18)}{3.02\sum\beta_2} + 3.09V_x$$

$$r^2 = 0.972,\ n = 43 \qquad (17)$$

$$\text{Octanol–water:}\quad \log P_{o/w} = \underset{(0.09)}{-0.10} + \underset{(0.11)}{0.49R_2} - \underset{(0.10)}{0.89\pi_2^*} + \underset{(0.17)}{0.05\sum\alpha_2} - \underset{(0.16)}{3.85\sum\beta_2} + 3.83V_x$$

$$r^2 = 0.987,\ n = 43 \qquad (18)$$

The interactive behavior of the synthesized vesicle models were compared to octanol–water partitioning through the LSER model. Both the CTAB–SOS and AOT vesicles show large positive values for the v coefficient, indicating that the cohesive energy density and dispersion interaction term has a great amount of influence on retention. The v coefficient values suggest that the hydrophobic molecules prefer to interact highly with the vesicle and octanol phases. The CTAB–SOS vesicles, AOT vesicles, and octanol–water show a large, negative b coefficient, suggesting that hydrogen bond donating ability plays an important role in partitioning.

The large negative b term shows that the octanol and vesicle phases have poor hydrogen bond donating behavior as compared to the aqueous phase and methanol-modified aqueous phase, and the solutes will tend to have more hydrogen bond accepting interactions with the aqueous phase. The a term, representing hydrogen bond accepting ability, is slightly larger and positive for the CTAB–SOS model as compared to the octanol–water model. This may imply that the CTAB–SOS vesicle phase has some minor hydrogen bond accepting ability.

The s coefficient, representing the dipolarity/polarizability of the solvent, is less negative for the AOT vesicle phase as compared to the octanol–water system. The negative value indicates that polar molecules prefer to be in the aqueous portion of the model system. The AOT vesicle phase may show more selectivity for hydrophilic compounds in this case.

Abraham et al. suggests that the absolute values of the coefficients are not as descriptive as their relative values [53]. If the processes are similar, the ratios should be very similar. Therefore, the r, s, a, and b coefficients have been normalized against v for comparison, since hydrophobic interaction is a maj or factor in partitioning. The results are summarized in Table 3.

The differences between the coefficient ratios were compared, and the ratios are similar between the CTAB–SOS vesicle partitioning and the octanol–water partitioning models for each coefficient, except for the b/v ratios which differ by 0.18. Due to the moderate uncertainties in these ratios, however, a difference of 0.18 is not statistically different using a t-test at the 90% confidence level. Thus, our results suggest but do not prove that the solute hydrogen bond donating behavior is slightly different with the vesicle interactions than with the octanol–water partitioning model.

The ratios are similar between the AOT vesicle and the octanol–water partitioning models for each coefficient. The coefficient showing the most difference, although not statistically significant, is the s/v ratio. This suggests that the dipole–dipole and

Table 3
LSER coefficient ratios

	r/v	s/v	a/v	b/v
CTAB–SOS VEKC and log $P_{o/w}$				
1.8% CTAB–SOS, 30–70 mass–10 m*M* sodium phosphate	0.19	−0.20	0.08	−1.14
Log $P_{o/w}$	0.09	−0.17	−0.01	−0.96
Difference between ratios	0.10	0.03	0.09	0.18
AOT VEKC and log $P_{o/w}$				
40 m*M* AOT–10% MeOH–10 m/*M* sodium phosphate	0.11	−0.14	0.00	−0.98
Log $P_{o/w}$	0.13	−0.23	0.01	−1.01
Difference between ratios	0.02	0.09	0.01	0.03

dipole–induced dipole interactions may be slightly different for the solute with the AOT vesicle phase than with the octanol phase. However, it is difficult with semi-empirical models of the LSER type to make definitive statements when molecular interactions are subtle, as is often found in separation methods based on phase equilibria.

4. Conclusions

VEKC with vesicles formed from oppositely charged surfactants and a double-chained anionic surfactant has shown its effectiveness as an alternative for the rapid prediction of log $P_{o/w}$ of small organic compounds and pesticides. The vesicle model systems chosen for study provides good correlation of log k' to log $P_{o/w}$ for over 100 neutral solutes with varying functional groups, shapes and sizes. Similar correlations have been observed using microemulsions [33–37] and micelles modified with alcohols [30–32]. The range of hydrophobicity able to be examined with the CTAB–SOS model vesicle system was 0.5 to 5 log $P_{o/w}$ units and for the AOT vesicle model was 0 to 5.5 log $P_{o/w}$. The analyses may be optimized by extending or decreasing the migration window by adjusting the composition of the vesicle formations. A comparison of the hydrogen-bonding and non-hydrogen bonding compound retention was statistically different for the CTAB–SOS experiments. For the AOT vesicle studies, the partitioning behavior of the hydrogen bonding and non-hydrogen bonding compounds was statistically similar, indicating that there are no appreciable biases in the retention character caused by hydrogen bonding effects. LSER analysis of the CTAB–SOS/buffer and octanol–water data suggests that the hydrogen bond acidity of the CTAB–SOS vesicles and octanol may differ slightly. LSER analysis of the AOT vesicle data shows that the AOT vesicle and octanol–water partitioning models portray similar partitioning behavior, except possibly for some minor differences in dipole–dipole and dipole–induced dipole interactions.

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