Correlation of the Capacity Factor in Vesicular Electrokinetic Chromatography with the Octanol:Water Partition Coefficient for Charged and Neutral Analytes

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Purpose. The aim of this study was to develop a method based upon electrokinetic chromatography (EKC) using oppositely charged surfactant vesicles as a buffer modifier to estimate hydrophobicity (log P) for a range of neutral and charged compounds.
Methods. Vesicles were formed from cetyltrimethylammonium bromide (CTAB) and sodium *n*-octyl sulfate (SOS). The size and polydispersity of the vesicles were characterized by electron microscopy, dynamic light scattering, and pulsed-field gradient NMR (PFG-NMR). PFG-NMR was also used to determine if ion-pairing between cationic analytes and free SOS monomer occurred. The CTAB/SOS vesicles were used as a buffer modifier in capillary electrophoresis (CE). The capacity factor (log k′) was calculated by determining the mobility of the analytes both in the presence and absence of vesicles. Log k′ was determined for 29 neutral and charged analytes.
Results. There was a linear relationship between the log of capacity factor (log k′) and octanol/water partition coefficient (log P) for both neutral and basic species at pH 6.0, 7.3, and 10.2. This indicated that interaction between the cation and vesicle was dominated by hydrophobic forces. At pH 4.3, the log k′ values for the least hydrophobic basic analytes were higher than expected, indicating that electrostatic attraction as well as hydrophobic forces contributed to the overall interaction between the cation and vesicle. Anionic compounds could not be evaluated using this system.
Conclusion. Vesicular electrokinetic chromatography (VEKC) using surfactant vesicles as buffer modifiers is a promising method for the estimation of hydrophobicity.

KEY WORDS: vesicular electrokinetic chromatography; hydrophobicity; capacity factor; octanol-water partition coefficient; surfactants.

INTRODUCTION

Transport of a drug across cell membranes is usually required to ensure its bioavailability. Consequently, during drug development the determination of cell membrane transport properties of the compound is required. Determination of physicochemical properties, which include solubility, hydrophobicity, and membrane partitioning, is a useful way of estimating the passive absorption of drugs across membranes (1).

Hydrophobicity is one parameter often used to predict passive membrane transport. The 1-octanol-water partition coefficient (log P), proposed initially by Fujita et al. in 1964 (2), is the standard scale for hydrophobicity. Log P is defined as the affinity of a solute for a bulk hydrophobic phase (octanol) compared to a hydrophilic phase (water). The shake-flask method is the most common technique used to measure log P (3), however it is time-consuming and laborious, has a limited dynamic range, and requires pure compounds.

Recently, capillary electrophoretic (CE) methods such as micellar electrokinetic chromatography (MEKC) have been used for the estimation of hydrophobicity (4–13). Compared to the shake-flask method, MEKC has advantages of speed, high sample throughput, wide dynamic range, small sample size, and tolerance of sample impurities. In addition, experimental conditions such as pH, temperature, and ionic strength can be controlled to mimic physiological conditions and the log P values of several analytes can be estimated simultaneously. The behavior of analytes in MEKC has been described extensively (14,15), and the hydrophobicity of solutes has been estimated using electrokinetic chromatography with different surfactant systems, including various types of micelles (4–9), microemulsions (10–12), and cyclodextrin modified micelles (13).

An alternative method for the determination of hydrophobicities is vesicular electrokinetic chromatography (VEKC). This technique utilizes vesicles as a buffer modifier. Vesicles are larger than micelles and have a bilayer composition with an internal cavity. The hydrophobic nature of vesicles is expected to provide the desired hydrophobic-hydrophilic discriminating power.

Vesicles can be formed from oppositely charged surfactants. Electrostatic interactions between the oppositely charged head groups allow for the formation of vesicles, rod-like micelles, and mixed micelles, depending on the composition of the mixture (16,17). The formation of vesicles from oppositely charged surfactant molecules is spontaneous under the appropriate conditions. Foley et al. have shown that capacity factor (log k′) in a VEKC system using vesicles made from *n*-dodecyltrimethylammonium bromide (DTAB) and sodium dodecyl sulfate (SDS) correlates well with log P for neutral compounds (18).

The focus of this work was to study the relationship between log k′ in VEKC and log P for a wide range of analytes. Surfactant vesicles were chosen because they are easy to prepare. For this study, vesicles made from cetyltrimethylammonium bromide (CTAB) and sodium *n*-octyl sulfate (SOS) were used as a buffer modifier. This system was selected over DTAB/SDS because vesicles form over a broader range of concentrations. To date, only neutral analytes have been studied by VEKC. However, many biologically important compounds are charged at physiological pH, and therefore this work investigated the behavior of both neutral and charged analytes in a VEKC system.

EXPERIMENTAL

Chemicals

The following compounds were purchased from Sigma (St. Louis, MO): acetylsalicylic acid, anisole, alprenolol, antipyrine, atenolol, bupivacaine, caffeine, corticosterone, doxepin, ephedrine, hydrocortisone, ibuprofen, imipramine, lidocaine, metoprolol, prilocaine, procainamide, procaine, pro-

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pranolol, salicylic acid, tetracaine, warfarin, sodium deuteroxide (NaOD), 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS), *N*-[2-hydroxyethyl]piperazine-*N*´-[2-ethanesulfonic acid] (HEPES), and CTAB. SOS was purchased from Lancaster (Windham, NH).

Benzaldehyde, benzylalcohol, bromobenzene, toluene, and sodium acetate were obtained from Fisher Scientific (St. Louis, MO). *p*-Nitrotoluene was obtained from Eastman Laboratory Chemicals (Rochester, NY). Dexamethasone was obtained from Purdue Research Center (Yonkers, NY). The alkylphenone homologues (C_8–C_{24}) were purchased as a kit from Aldrich (Milwaukee, WI). Deuterium oxide (D_2O) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Capillary Electrophoresis

Separations were performed either on an ISCO 3850 electropherograph (Lincoln, NE) or a Beckman P/ACE MDQ (Palo Alto, CA) with UV detection at 210 nm.

Separation capillaries were 50 μm i.d. (368 μm o.d.) fused-silica (Polymicro Technologies, Tucson, AZ) with lengths from 60–80 cm. Data from the ISCO 3850 was collected and processed using Turbochrom software (Perkin Elmer, San Jose, CA). The applied voltages were 20 kV (pH 4.3 and 6.0 experiments) and 22 kV (pH 7.3 and 10.2 experiments).

Stock buffer solutions of 25 mM acetate, pH 4.3; 25 mM MES, pH 6.0; 25 mM HEPES, pH 7.3; and 50 mM CAPS, pH 10.2 were prepared. The pH was adjusted using 5 M NaOH. Run buffers (10 mM, without vesicles) were prepared by dilution of the stock buffer solutions in water. Stock sample solutions with an approximate concentration of 15 mM were prepared in 50:50 (v/v) acetonitrile/10 mM run buffer. Vesicle buffer solutions were prepared in 10 mM acetate, pH 4.3; 10 mM MES, pH 6.0; 10 mM HEPES, pH 7.3; or 10 mM CAPS, pH 10.2 as described previously (18). The weight ratio of CTAB/SOS in the prepared vesicle solution was 30:70 and the total surfactant concentration was 1% (w/v). Nanopure water (18.2 MΩ) was used for the preparation of all solutions.

The separation capillary was activated daily by flushing sequentially with 0.1 M NaOH, water, and run buffer. Prior to use, buffer solutions were filtered through either 0.2 μm (buffer only) or 0.45 μm (buffer with vesicles) membrane filters (Millipore, Bedford, MA). When performing runs using vesicles as buffer modifiers, the capillary was flushed between runs with buffer only (no vesicles) followed by a flush of run buffer with vesicles. The system was flushed periodically with 0.1 M NaOH to minimize surfactant adsorption on the capillary wall and to maintain uniform charge density of the silanol groups. When using vesicles in either 10 mM acetate, pH 4.3 or 10 mM MES, pH 6.0 as the run buffer, the system was flushed between each run with 0.1 M NaOH, buffer, and run buffer. Once used for VEKC, a capillary was never used for CE experiments with buffer only. This was done to eliminate the possibility of carry over of the vesicle surfactants on the capillary surface, which would alter the calculation of mobility.

Light Scattering

Light scattering experiments were performed using a Brookhaven BI-200SM goniometer (Holtsville, NY) with a 50 mW HeNe diode laser (Uniphase, San Jose, CA) at a wavelength of 532 nm. The autocorrelation functions were compiled using a BI-9000AT card from Brookhaven. All measurements were made at a scattering angle of 90°. Vesicle solutions were used as prepared for the CE experiments. The vesicle solutions were filtered through 0.45 μm membrane filters prior to analysis.

Pulsed-Field Gradient (PFG) NMR

Vesicles were prepared by first making stock HEPES buffer in D_2O. The pD, calculated by adding 0.40 to the pH meter readings to correct for the deuterium isotope effect (19), was adjusted to 7.3 with NaOD. The appropriate amounts of solid CTAB and SOS were added to the buffer solution. This solution was diluted to the desired volume with D_2O to result in 30:70 CTAB/SOS vesicles (1% w/v total surfactant concentration). The vesicle solution was analyzed by NMR on the same day as preparation.

For ion-pair analysis, stock solutions (10 mM) of lidocaine, alprenolol, and SOS were prepared in 10 mM HEPES, pD 7.3. These stock solutions were diluted in HEPES buffer, resulting in 5 mM lidocaine, 5 mM alprenolol, and 5 mM SOS.

Standards of SOS/alprenolol (each 5 mM) and SOS/lidocaine (each 5 mM) were prepared by mixing equimolar amounts of 10 mM stock solutions.

PFG-NMR experiments were performed using a Bruker AM-360 MHz spectrometer using the longitudinal encode-decode (LED) or the bipolar pulse pair longitudinal encode-decode (BPPLED) pulse sequences (20,21). A Nalorac 5-mm actively shielded gradient probe (coil constant, 0.204 T m^{-1} A^{-1}) was used for the vesicle experiments and a Bruker 5-mm actively shielded gradient probe (coil constant, 0.0534 T m^{-1} A^{-1}) was used for the investigation of ion-pairing. Information about the instrumentation and experimental details of the measurements have been reported previously (22,23).

All FIDs were transferred to a Silicon Graphics Indy workstation, apodized by multiplication with a function equivalent to 1.0 Hz line broadening, and processed using FELIX 97.0 (Biosym) software. Chemical shifts were referenced relative to the HOD resonance (4.78 ppm). A serial file of processed data was created and user-defined spectral regions were analyzed with CONTIN to produce a 50-point diffusion coefficient distribution, G(D). For the ion-pairing experiments, FIDs were zero-filled to 8192 points, Fourier transformed, and regression analysis was performed with the program SCIENTIST (MicroMath) to calculate diffusion coefficients from the integral attenuation as a function of gradient amplitude.

Electron Microscopy

Electron microscopy (EM) was performed with a JEOL EM 100 electron microscope. A 5-μL sample of the vesicle solution was placed on a 400 mesh EM grid. A solution of 15.0 nm colloidal gold and 60 nm latex spheres was added and allowed to dry. The sample was fixed in 2% paraformaldehyde and 0.05% glutaraldehyde for 1 h. The fixed sample was examined with the electron microscope.

THEORY AND CALCULATIONS

Determination of Log k′

Khaledi and co-workers have described the migration behavior of charged analytes in MEKC in detail (14,15). It is

expected that the behavior of analytes in VEKC is very similar. Briefly, the observed mobility of an analyte is a weighted average of the mobility of the analyte in the vesicular phase (μ_{ves}) and the mobility in the aqueous phase (μ_0). It can be described by

$$\mu = [k'/(k' + 1)]/ \mu_{ves} + [1/(k' + 1)] \mu_0 \quad (1)$$

where μ is the observed mobility of the analyte and k' is the retention factor. Rearranging equation 1 gives

$$k' = (\mu - \mu_0)/(\mu_{ves} - \mu) \quad (2)$$

The electrophoretic mobility is related to retention time by

$$\mu = (1/t - 1/t_{eof})(L_t L_d/V) \quad (3)$$

where t is the retention time of the analyte, t_{eof} is the retention time of the electroosmotic flow (EOF) marker, L_t is the total length of the capillary, L_d is the length of capillary to the detector, and V is the applied separation voltage.

To calculate k', the electrophoretic mobility of each analyte was determined in the buffer solution with vesicles (μ) and without vesicles (μ_0). The average values of μ and μ_0 for a series of runs were used in the calculation of k'. Acetonitrile was used in each run to measure the EOF. The mobility of the vesicles (μ_{ves}) was determined with alkylphenone homologues (C_8–C_{12}) using an iterative computational method (24). For all experiments, the value of -4.5×10^{-4} cm^2/V · sec was used for the mobility of vesicles.

Determination of Diffusion Coefficients

The theory and application of PFG-NMR has been described in detail elsewhere (25). Diffusion coefficients can be determined by data inversion using the program CONTIN (26) for polydisperse samples such as phospholipid vesicles (27) and humic substances (23). CONTIN analysis produces a distribution function of diffusion coefficients, G(D), which can be used to determine the most probable diffusion coefficient, D_P, from the maximum of the distribution (23). By analogy to number- and weight-average molecular weights, number- (D_N) and weight-average (D_W) diffusion coefficients can also be calculated. The ratio of D_W/D_N can provide an indication of the polydispersity of the sample, however, it should be noted that although the terminology and equations are analogous to those used for the calculation of molecular weights, D_N and D_W are calculated from the same experimental distribution and cannot be rigorously interpreted as number- and weight-averaged diffusion coefficients. Rather, the NMR signal originates from the number of protons, which is related to both the number and weight of the molecules.

The radius of the vesicles is related to the diffusion coefficient by the Stokes-Einstein equation for spherical species

$$R = (298 \cdot k_b)/(6\pi\eta D) \quad (4)$$

where R is the radius of the species, k_b is Boltzmann's constant, η is the viscosity of D_2O at 298 K, and D is the diffusion coefficient (m^2/s) in D_2O at 298 K.

RESULTS AND DISCUSSION

Characterization of CTAB/SOS Vesicles

Electron microscopy images of vesicles aged for 24 h (not shown) indicated that the diameter of the vesicles was approximately 60 nm and that the vesicles were spherical in shape, as expected. Light scattering experiments were performed on two different batches of 30:70 CTAB/SOS vesicles in 10 mM HEPES, pH 7.3, to determine the change in size and polydispersity over a 2-week period. The apparent diameter of the vesicles steadily increased over time (Table 1). Both batches of vesicles were moderately polydisperse (~0.2) and the polydispersity increased somewhat with age. Although there were small differences between the sizes of the two vesicle preparations, the overall trend in the changes of size and polydispersity was the same.

Because of the variable size and the polydispersity of the vesicles, the interday reproducibility of the apparent mobility of several analytes in the VEKC system was determined. Anisole, antipyrine, atenolol, bromobenzene, ibuprofen, and *p*-nitrotoluene were used in this study because they represent a wide range of log P values and include both charged and neutral species. Two different preparations of 30:70 CTAB/SOS vesicles in 10 mM HEPES, pH 7.3 were used to determine the apparent mobility of the analyte each day for 14 days. With both vesicle preparations, only random variation in analyte mobility between days was observed (data not shown). In addition, the average mobility of each analyte using the first vesicle preparation was not statistically different from the mobility obtained using the second vesicle preparation. Therefore, analyte mobility is neither dependent upon daily variations in vesicle size nor small differences that might occur during vesicle preparation.

Figure 1 shows a 2D DOSY spectrum of the 30:70 CTAB/SOS vesicles in 10 mM HEPES, pD 7.3, with the 1D ^{1}H-NMR spectrum displayed above the 2D plot. The signal at 4.78 ppm is the HOD resonance, the fastest diffusing species. The region from 3.77 to 4.12 ppm consists of overlap between SOS and HEPES, but is dominated by HEPES. Two components are observed, one diffusing rapidly and one diffusing slowly, indicating that a fraction of the HEPES molecules was in the vesicles and that this fraction diffused at the rate of the vesicles. For the regions of SOS and CTAB only (1.11–1.59 and 0.71–1.10 ppm, respectively), there are also two components, signifying that there was a small percentage of surfactant monomers that was not incorporated into the vesicle structures. The presence of two discrete diffusion coefficient distributions suggests that the monomers were in slow ex-

Table 1. Light Scattering Results for 30:70 CTAB/SOS Vesicles in 10 mM HEPES, pH 7.3

	Sample A		Sample B	
Day[a]	Diameter (nm)	Polydispersity	Diameter (nm)	Polydispersity
1	40.1	0.15	48.9	0.17
2	53.3	0.21	56.2	0.18
3	53.7	0.18	63.4	0.18
4	52.6	0.16	61.5	0.16
5	56.1	0.21	61.3	0.18
9	59.4	0.20	63.9	0.18
10	59.3	0.20	67.1	0.20
11	63.0	0.22	67.3	0.19
12	71.7	0.25	72.4	0.23
15	81.7	0.25	74.9	0.23

[a] Vesicles were prepared on day 1, 2 h prior to analysis.

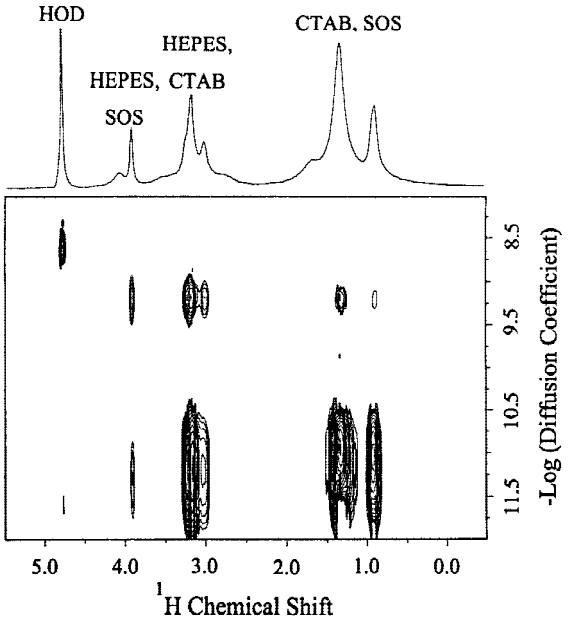

Fig. 1. 2D DOSY plot of CTAB/SOS vesicles. Proton chemical shift region 1 (0.71–1.10 ppm) and region 2 (1.11–1.59 ppm) contained resonances for CTAB and SOS. Region 3 (2.69–3.58 ppm) arose from CTAB protons, as well as protons from the buffer. Region 4 (3.77–4.12 ppm) was mainly from buffer protons, but also contained resonances from SOS.

change with the vesicles on the NMR diffusion time scale. For the slowly diffusing species, D_N, D_W, and D_P were calculated and are listed in Table 2 with the polydispersity ratio D_W/D_N. The trend of $D_W > D_N > D_P$ was expected as a result of the calculations used to determine each D value. For the slowly diffusing vesicle species the ratio D_W/D_N is 1.5–1.6, indicating that the vesicles were fairly polydisperse. The diameter of the vesicles calculated from the average D_N ($8.7 \pm 0.5 \times 10^{-12}$ m^2 s^{-1}) was 42 ± 2 nm. This is in good agreement with the light scattering results for vesicles of the same age (Table 1).

It has been suggested that ion-pair formation between cations and free anionic surfactant monomers in run buffer is possible in MEKC (15). Ion-pairing changes the mobility of the cation in the aqueous phase (μ_0) and therefore has an effect on the observed k′. A second series of PFG-NMR experiments was performed to determine if ion-pairing was a factor in this VEKC system. Alprenolol and lidocaine were chosen as test compounds because they are charged, relatively hydrophobic, and represent two classes of compounds.

Diffusion coefficients for solutions of SOS, alprenolol, lidocaine, 1:1 SOS/alprenolol and 1:1 SOS/lidocaine were determined. The diffusion coefficient for alprenolol alone was $6.06 \pm 0.06 \times 10^{-10}$ m^2s^{-1} and the diffusion coefficient of alprenolol in the presence of SOS was $6.12 \pm 0.09 \times 10^{-10}$ m^2 s^{-1}. For lidocaine, the diffusion coefficients in the absence and presence of SOS were $6.17 \pm 0.05 \times 10^{-10}$ m^2 s^{-1} and $6.2 \pm 0.1 \times 10^{-10}$ m^2 s^{-1}, respectively. The diffusion coefficient of SOS alone was $7.6 \pm 0.1 \times 10^{-10}$ m^2 s^{-1} and in the presence of lidocaine or alprenolol was $7.5 \pm 0.1 \times 10^{-10}$ m^2 s^{-1}. There was no statistical difference between the diffusion of lidocaine, or alprenolol, alone or in the analyte/SOS mixtures, indicating no or insignificant ion-pair formation.

The solution conditions for this second series of PFG-NMR experiments were chosen to favor ion-pair formation, i.e., high concentrations and 1:1 ratio of SOS to analyte. In the CE system, the vesicles provide added competition for the free SOS and analyte, making ion-pair formation less favorable. Because ion-pair formation was not observed by PFG-NMR, under conditions designed to favor such interactions, ion-pair formation should not be a significant complication of the VEKC determination of k′.

Determination of Capacity Factors (Log k′)

Neutral and charged species were included in this study. The analytes studied have a wide range of hydrophobicities and represent several classes of compounds. Table 3 lists the pK_a and log P values for the compounds.

Experimental log P values were available for most analytes, however, in cases where experimental values were not available, published calculated log P values were used (28).

Log k′ values were initially determined at pH 7.3, which is close to physiological pH. At this pH the compounds studied included cations, neutrals, and anions. The cations consisted of amine-containing compounds that are protonated at pH 7.3. The anions were deprotonated carboxylate-containing compounds. The experimental electrophoretic mobilities in the absence and presence of vesicles along with the calculated log k′ for all analytes using VEKC at pH 7.3 are listed in Table 3. The log k′ values for acidic analytes are not included in Table 3 because the mobilities of the anionic compounds in the presence of vesicles were not statistically different from their mobilities in the absence of vesicles. The 30:70 CTAB/SOS vesicles are anionic, thus little interaction with anionic analytes was expected due to electrostatic repulsion. In addition, the vesicles and the anionic analytes electromigrate in the same direction. Therefore, even if the anionic analytes were to partition into the vesicles, only minimal changes in their migration would be observed.

The log k′ values calculated at pH 7.3 were compared to the log P values for the neutral and basic analytes. A correlation between log k′ and log P (slope = 0.55 ± 0.04, intercept = −1.53 ± 0.11, R^2 = 0.879, n = 24) was observed for both neutral and positively charged compounds (Fig. 2). This result

Table 2. Vesicle[a] Diffusion Coefficients from CONTIN-DOSY Analysis of PFG-NMR Data (Average ± Range)

	Region 1 (0.71–1.10 ppm)	Region 2 (1.11–1.59 ppm)	Region 3 (2.69–3.58 ppm)	Region 4 (3.77–4.12 ppm)
D_P ($10^{-12}m^2s^{-1}$)	6.6 ± 0.5	8.4 ± 0.2	7.1 ± 0.2	6.6 ± 0.5
D_N ($10^{-12}m^2s^{-1}$)	8.1 ± 0.7	9.1 ± 0.1	9.0 ± 0.4	8.4 ± 0.6
D_W ($10^{-12}m^2s^{-1}$)	13 ± 1	13.5 ± 0.7	14.8 ± 0.9	13 ± 1
D_W/D_N	1.6 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.2

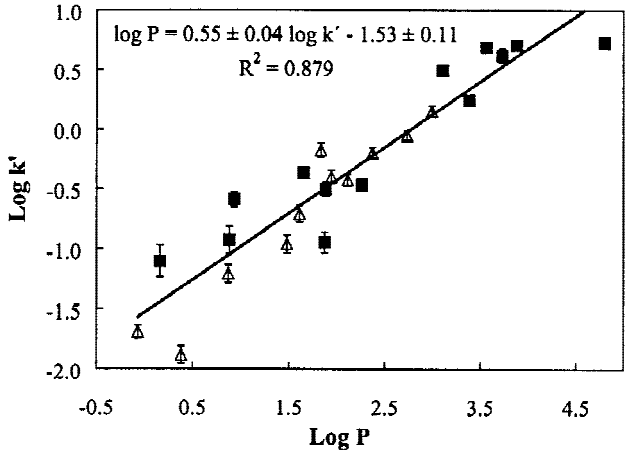

Fig. 2. Correlation between retention factors (log k′) in VEKC with 30:70 CTAB/SOS vesicles at pH 7.3 and octanol-water partition coefficients (log P) for neutral (△) and basic (■) compounds.

was somewhat surprising. It was expected that electrostatic attraction between the positively charged analytes and the negatively charged vesicle would occur in addition to the hydrophobic interaction (30). This electrostatic attraction would increase the interaction of the analyte with the vesicle, and thus increase the capacity factor for positively charged analytes. This interaction would not be a factor in the octanol-water partitioning system. However, as Fig. 2 shows, the log k′-log P correlation is equivalent for both the neutral and basic species.

To determine the relative importance of electrostatic attraction in the overall interaction between vesicles and basic analytes, experiments were performed in which the pH of the run buffer was varied. Log k′ values were calculated for the neutral and basic compounds at various pH values (Table 4).

Figure 3 shows the correlation of log ḱ versus log P for both neutral and basic analytes at pH 4.3 (slope = 0.44 ± 0.05, intercept = –1.28 ± 0.12, R^2 = 0.809, n = 18), pH 6.0 (slope = 0.53 ± 0.04, intercept = –1.48 ± 0.10, R^2 = 0.895, n = 23), and pH 10.2 (slope = 0.56 ± 0.05, intercept = –1.73 ± 0.12, R^2 = 0.869, n = 22). At pH 6.0 and above, all of the neutral and basic analytes are scattered randomly around the regression line. At pH 4.3, the more hydrophobic compounds are randomly scattered about this line (Fig. 3A).

However, for the less hydrophobic compounds (log P less than 1.7) there is a slight positive deviation from the log k′-log P correlation. The log k′ values for the hydrophilic basic analytes were higher than expected, indicating that the interac-

Table 3. Log P, pK_a, and Mobility Data at pH 7.3

Analyte	pK_a[a]	log P[a]	μ[a]	μ_0[a]	log k′
Neutral analytes					
Anisole	–	2.11	–1.2 ± 0.1	0.00	–0.43 ± 0.05
Benzaldehyde	–	1.48	–0.45 ± 0.08	0.00	–0.96 ± 0.07
Benzylalcohol	–	0.87	–0.27 ± 0.04	0.00	–1.20 ± 0.07
Bromobenzene	–	2.99	–2.6 ± 0.2	0.00	0.15 ± 0.04
p-Nitrotoluene	–	2.37	–1.7 ± 0.2	0.00	–0.20 ± 0.05
Toluene	–	2.73	–2.1 ± 0.1	0.00	–0.06 ± 0.04
Antipryine	1.45	0.38	–0.06 ± 0.01	0.00	–1.88 ± 0.07
Caffeine	0.60	–0.07	–0.09 ± 0.01	0.00	–1.69 ± 0.06
Corticosterone	–	1.94	–1.3 ± 0.2	0.00	–0.40 ± 0.06
Dexamethasone	–	1.83	–1.8 ± 0.2	0.00	–0.17 ± 0.06
Hydrocortisone	–	1.61	–0.7 ± 0.1	0.00	–0.71 ± 0.07
Basic analytes					
Alprenolol	9.65	3.10	–2.8 ± 0.2	2.4 ± 0.2	0.49 ± 0.04
Atenolol	9.60	0.16	1.63 ± 0.06	2.1 ± 0.1	–1.1 ± 0.1
Bupivacaine	8.10	3.38[c]	–2.1 ± 0.2	2.0 ± 0.1	0.24 ± 0.03
Doxepin	9.00	3.88[c]	–3.43 ± 0.05	2.0 ± 0.2	0.71 ± 0.03
Ephedrine	9.60	0.93	1.3 ± 0.1	2.8 ± 0.2	–0.58 ± 0.06
Imipramine	9.50	4.80	–3.47 ± 0.09	2.1 ± 0.3	0.73 ± 0.04
Lidocaine	7.90	2.26	0.00	1.5 ± 0.1	–0.47 ± 0.03
Metoprolol	9.70	1.88	0.8 ± 0.2	2.4 ± 0.1	–0.50 ± 0.06
Prilocaine	7.90[b]	1.65[c]	0.00	1.9 ± 0.1	–0.36 ± 0.02
Procainamide	9.26	0.88	1.80 ± 0.04	2.6 ± 0.2	–0.9 ± 0.1
Procaine	9.00[b]	1.87	1.72 ± 0.05	2.4 ± 0.1	–0.94 ± 0.09
Propranolol	9.45	3.56	–3.3 ± 0.1	2.5 ± 0.1	0.69 ± 0.04
Tetracaine	8.39	3.73	–3.3 ± 0.2	1.79 ± 0.07	0.62 ± 0.06
Acidic analytes					
Acetylsalicylic acid	3.50	1.19	–2.24 ± 0.03	–2.48 ± 0.09	–
Ibuprofen	4.40	3.50	–2.05 ± 0.04	–2.09 ± 0.04	–
Indomethacin	4.50	4.27	–2.19 ± 0.04	–2.03 ± 0.01	–
Salicylic acid	2.97	2.26	–2.91 ± 0.03	–3.12 ± 0.02	–
Warfarin	5.10	2.52	–1.79 ± 0.03	–2.03 ± 0.01	–

[a] Value from reference 28.
[b] Value from reference 29.
[c] Calculated log P.

Table 4. Log k′ Values for Neutral and Basic Compounds

Analyte	log k′ pH 4.3	pH 6.0	pH 10.2
Neutral analytes			
Anisole	−0.49 ± 0.01	−0.42 ± 0.05	–
Benzaldehyde	−1.02 ± 0.02	−0.98 ± 0.05	−0.95 ± 0.05
Benzylalcohol	−1.3 ± 0.1	−1.2 ± 0.1	−1.22 ± 0.04
Bromobenzene	0.12 ± 0.02	0.23 ± 0.05	0.25 ± 0.08
p-Nitrotoluene	−0.25 ± 0.01	−0.21 ± 0.06	−0.15 ± 0.03
Toluene	−0.16 ± 0.01	–	−0.03 ± 0.03
Antipryine	–	−1.69 ± 0.03	−1.8 ± 0.2
Caffeine	−1.5 ± 0.1	−1.5 ± 0.2	−1.57 ± 0.07
Corticosterone	−0.57 ± 0.04	−0.43 ± 0.06	−0.39 ± 0.05
Dexamethasone	−0.36 ± 0.03	−0.21 ± 0.06	−0.16 ± 0.05
Hydrocortisone	–	−0.75 ± 0.07	−0.71 ± 0.05
Basic analytes			
Alprenolol		0.49 ± 0.03	0.10 ± 0.09
Atenolol	−0.65 ± 0.02	−1.1 ± 0.1	−1.9 ± 0.7
Bupivacaine	–	0.16 ± 0.05	−0.01 ± 0.03
Doxepin	0.51 ± 0.02	0.71 ± 0.06	0.48 ± 0.07
Ephedrine	–	−0.63 ± 0.07	−1.4 ± 0.5
Imipramine	–	0.77 ± 0.09	0.62 ± 0.06
Lidocaine	−0.32 ± 0.02	−0.46 ± 0.06	−0.71 ± 0.08
Metoprolol	−0.44 ± 0.02	−0.6 ± 0.1	−1.4 ± 0.3
Prilocaine	−0.20 ± 0.01	−0.41 ± 0.07	−0.87 ± 0.05
Procainamide	−0.61 ± 0.03	−1.0 ± 0.2	–
Procaine	−0.58 ± 0.02	−0.9 ± 0.1	−0.5 ± 0.1
Propranolol	0.43 ± 0.01	0.62 ± 0.03	0.5 ± 0.1
Tetracaine	0.36 ± 0.01	0.52 ± 0.03	0.20 ± 0.06

tion between a cationic analyte and vesicle was stronger than the interaction between a neutral analyte and vesicle.

Residual plots for the data at pH 4.3 and 7.3 are shown in Fig. 4. At each pH, the equation of the line was calculated as the linear regression through the neutral data set. At pH 4.3, the residual values are large and positive for the least hydrophobic compounds. This shows that electrostatic interaction made a significant contribution to the interaction between these analytes and the vesicles.

For more hydrophobic compounds, the residual values are small and randomly scattered about zero. At pH 7.3, a similar trend is observed, however, the residual values for the least hydrophobic compounds are smaller at this pH than at pH 4.3. This analysis indicates that electrostatic interactions still made a contribution at pH 7.3 that was not readily obvious from the regression plot (Fig. 2). However, at this pH, the effect was small even for the least hydrophobic compounds.

The observed trend at both pH 4.3 and 7.3 is a reflection of the fact that log P is a hydrophobic parameter, but log k′ calculated in VEKC is due to both electrostatic and hydrophobic interactions. For the least hydrophobic basic analytes, electrostatic interaction between the vesicle and cation was significant at low pH values, resulting in larger log k′ values than predicted from log P. As the pH increased, the basic analytes became less protonated and the electrostatic interaction was reduced.

For more hydrophobic basic analytes, the contribution of the electrostatic interaction was negligible at all pH values, as indicated by the small residual values. Therefore, log k′ for more hydrophobic basic analytes was due mainly to the hydrophobic interaction between the vesicle and cation.

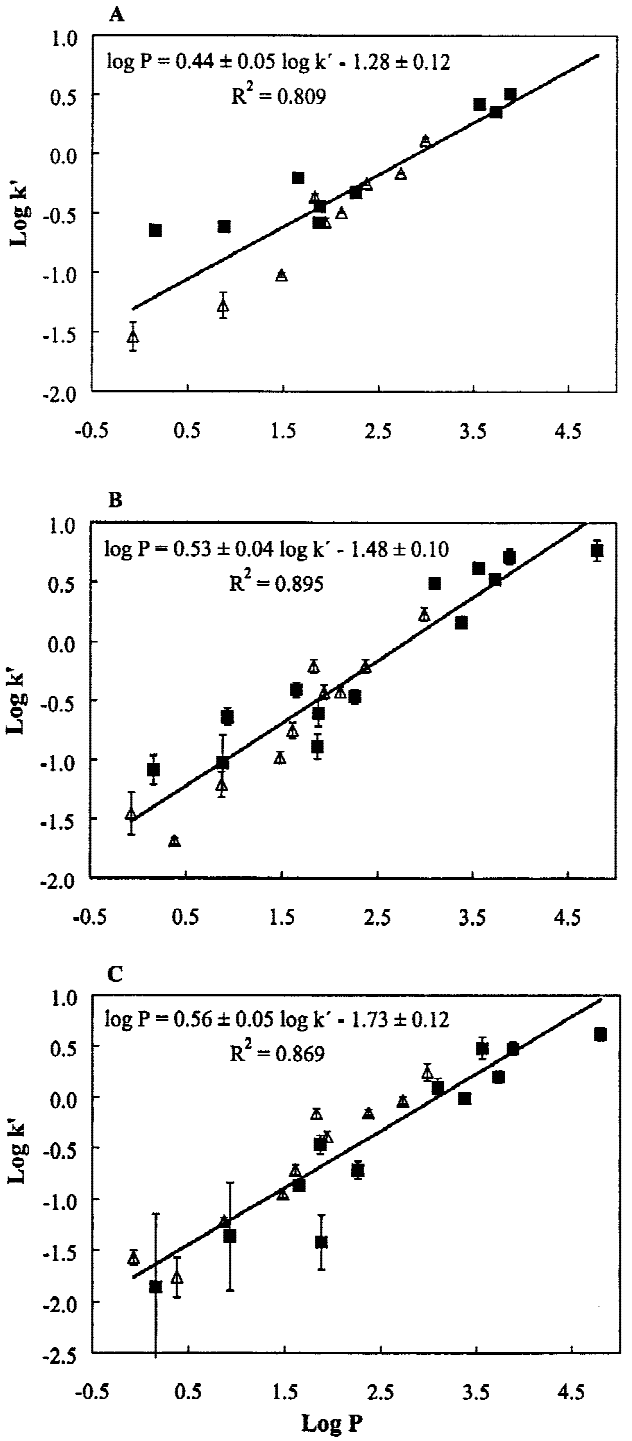

Fig. 3. Correlation between retention factors (log k′) in VEKC and octanol-water partition coefficients (log P) at (A) pH 4.3, (B) pH 6.0, and (C) pH 10.2 for neutral (△) and basic (■) compounds.

CONCLUSIONS

Spontaneous vesicles formed from CTAB and SOS surfactants are suitable for use as pseudostationary phases in capillary EKC. Though the size and polydispersity of these vesicles change over time, the electrophoretic behavior of the vesicles was constant over a period of at least 2 weeks. In addition, ion-pair formation between free SOS monomer and cationic analyte was insignificant.

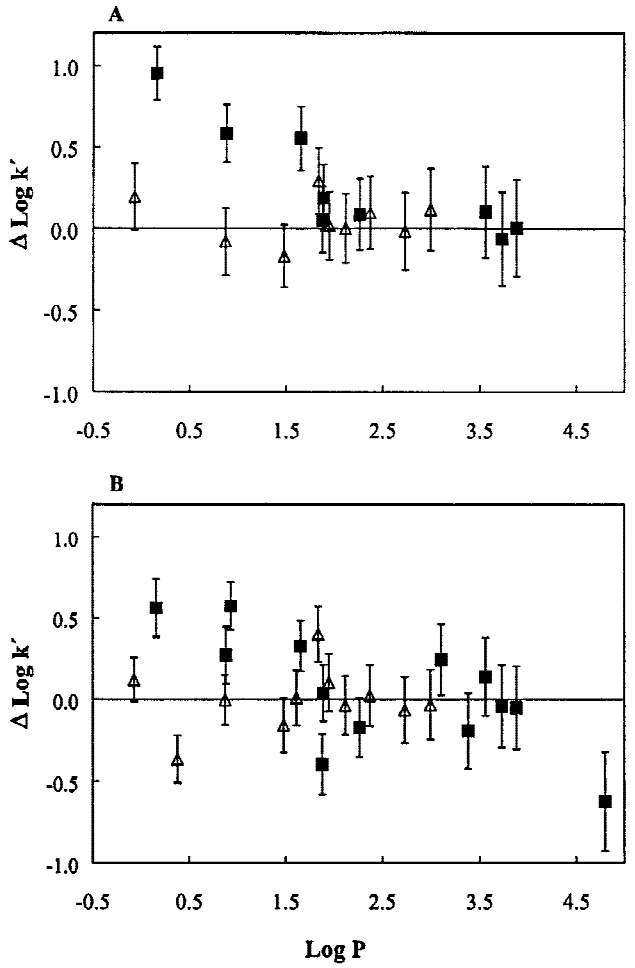

Fig. 4. Residual plots at pH 4.3 (A) and pH 7.3 (B) showing Δlog k′ (log k'_{exp} – log k'_{calc}) versus log P for neutral (△) and basic (■) compounds. Linear regression through the neutral data set was used in determination of the equation of the line. Log k′ values given in Tables 3 and 4 were used in calculation of Δlog k′.

For neutral analytes there was good correlation between log k′ and log P calculated in a VEKC system at all pH values. However, the correlation between log k′ and log P can be dependent upon pH when ionizable species are analyzed. For hydrophilic basic species, the interaction between the analyte and the vesicle was determined by both electrostatic and hydrophobic interactions at low pH. The contribution of the electrostatic interactions between these analytes and vesicles was smaller at higher pH values (6.0, 7.3, and 10.2).

For more hydrophobic basic species, electrostatic interactions seemed to play only a minor role in the interaction between the compound and the vesicles at all pH values.

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