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Rapid determination of logarithmic partition coefficients between *n*-octanol and water using micellar electrokinetic capillary chromatography

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Abstract

Micellar electrokinetic capillary chromatography (MECC) was evaluated as a rapid screening tool for the determination of logarithmic partition coefficients between *n*-octanol–water ($\log P_{ow}$). The technique is performed by electrochromatographing a mixture of standards of known $\log P_{ow}$. The logarithmic capacity factor of each standard was plotted against its $\log P_{ow}$ to form a linear calibration curve for a given set of chromatographic conditions. The $\log P_{ow}$ of an unknown is calculated by using its chromatographically determined capacity factor and extracting the $\log P_{ow}$ value from the calibration curve. The method was evaluated with a set of model compounds with known $\log P_{ow}$. The accuracy of the method was examined and found to be within the limits required for screening purposes. The correlation of $\log P_{ow}$ values determined using HPLC and MECC for some novel compounds was examined. This technique allows the screening of $\log P_{ow}$ at a rate of four samples per hour with minimal sample requirements ($< \mu g$) and with extremely small solvent waste generated.

1. Introduction

n-Octanol–water partition coefficients ($\log P_{ow}$) have been widely used to predict lipodal transport of species across cell membranes [1,2]. More recently, $\log P_{ow}$ values have been successfully used in quantitative structure–activity relationships (QSAR) to predict bioactivity [3]. $\log P_{ow}$ values are traditionally measured using the "shake-flask" method combined with UV spectroscopy and can be both tedious and time-consuming. The "shake-flask" method also requires a pure sample and milligram quantities. This technique becomes increasingly difficult with compounds that are poorly soluble in water or that are prone to emulsion formation. High-performance liquid chromatography (HPLC) has been used extensively for the screening of $\log P_{ow}$ with much success [4–6]. However, due to the cost of waste solvent disposal and increased cost in synthesizing new compounds, alternative microscale techniques must be explored.

Since the introduction of micellar electrokinetic capillary chromatography (MECC) by Terabe et al. in 1984 [7], hundreds of papers have been presented displaying the enormous power of the technique [8–11]. MECC not only produces the high separation efficiencies inherent to capillary electrophoresis, but also allows

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the separation of neutral species in an electrical field. Recently, Takeda et al. [12] used literature $\log P_{ow}$ values to predict the migration behavior of phthalate esters in MECC. Muijselaar et al. [13] applied the retention index concept to MECC and found a linear relationship with $\log P_{ow}$. These works suggest that MECC could provide a rapid tool for the screening of $\log P_{ow}$ values.

2. Theory

In *n*-octanol–water systems, the distribution coefficient (K_{oct-aq}) equals:

$$K_{oct-aq} = \left(\frac{c_{oct}}{c_{aq}}\right) \quad (1)$$

where c_{oct} and c_{aq} are the concentrations of the analyte in the *n*-octanol and aqueous phases, respectively. By definition, $\log P_{ow} = \log K_{oct-aq}$ for a neutral species. The capacity factor, k', in MECC is defined as [14]:

$$k' = K_{mic-ac}\frac{V_{mic}}{V_{aq}} = K_{mic-aq}\phi \quad (2)$$

where K_{mic-aq} is the distribution coefficient between the micellar and aqueous phases, V_{mic} and V_{aq} are the volumes of the micellar and aqueous phases, respectively, and their ratio is defined as the volumetric phase ratio, ϕ. The capacity factor, k', in MECC can be calculated experimentally by [7]:

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \quad (3)$$

where t_r is the migration time of the analyte, t_0 is the migration time of the unretained species and t_{mc} is the migration time of micelle. It has been shown that a linear relationship exists between $\log P_{ow}$ and $\log K_{mic-aq}$ for some species using sodium dodecyl sulfate micellar phases (SDS) [12,17]. By taking the logarithm of Eq. (2), it can be expanded to the form of:

$$\log k' = \log \phi + \log K_{mic-aq} \quad (4)$$

If $\log K_{oct-aq}$ is linearly related to $\log K_{mic-aq}$, a plot of $\log k'$ values obtained in an MECC system versus their corresponding $\log P_{ow}$ values should yield a straight line in the form of:

$$log\ k' = b + m(\log P_{ow}) \quad (5)$$

for a given micellar system. Based on this equation, $\log P_{ow}$ can be readily estimated using electrochromatographic data from a given calibrated MECC system. Thus this paper explores the potential of MECC as a tool for the rapid screening of $\log P_{ow}$ values.

3. Experimental

3.1. Instrumentation

This work was performed on a HP^{3D}CE capillary system, Model G1602A, from Hewlett-Packard (Waldronn, Germany) equipped with a real time UV–visible diode array detector (DAD) and accompanying data acquisition and analysis software. The capillary temperature was maintained at 30°C. The detection wavelength was set at 210 nm with an 8-nm bandwidth. The electrical field strength was maintained at 790 V/cm.

Fused-silica capillaries having an inner diameter of 50 μm and an outer diameter of 375 μm were obtained from Polymicro Technology (Phoenix, AZ, USA). The total length of the capillary used was 38.0 cm with an effective length of 30 cm, i.e., from the injection end to the point of detection.

3.2. Reagents

Sodium lauryl sulfate (SDS), ultra grade, and all model analytes used in this study were purchased from Sigma (St. Louis, MO, USA). The series of alkyl phenyl ketones (APK) was obtained from Aldrich (Milwaukee, WI, USA). Optima grade 2-propanol, dimethyl sulfoxide

(DMSO) and all chemicals used for electrolyte preparation were purchased from Fisher Scientific (Pittsburgh, PA, USA).

3.3. Methods

The running electrolyte was prepared by dissolving the proper amount of each reagent in MilliQ water to yield a solution of 5 m*M* boric acid, 5 m*M* dibasic sodium phosphate and 50 m*M* SDS. The pH was adjusted to 7.4 using concentrated phosphoric acid. After pH adjustment, 2-propanol was added on a volume/volume basis to achieve the final buffer composition. Before use, the running electrolyte was filtered with a 0.2 μm syringe filter to avoid capillary plugging followed by briêf degassing in an ultrasonic bath (ca. 3 min).

Stock sample solutions were prepared by diluting pure compound in methanol or acetonitrile to yield a 1000 μg/ml solution. Sample solutions were prepared by adding aliquots of stock solution to 900 μl of running electrolyte. Methanol was added to bring the final volume to 1000 μl, which ensured the percentage of running electrolyte was constant at 90% in the injected sample. Injected sample concentrations ranged from 10 to 25 ppm for each analyte. DMSO was added (0.03% v/v) to measure the migration time of the unretained species, t_0. The micelle migration time, t_{mc}, was determined using the iterative method of a homologous series. All calculations of the electrophoretic mobility of the micelle ($\mu_{ep(mc)}$) and resulting t_{mc}, were performed using a spreadsheet in Microsoft Excel.

Before use, a new capillary is washed with 5 *M* NaOH for 20 min, followed by an additional 5 min wash with 0.5 *M* NaOH, 0.1 *M* NaOH and water, consecutively. Prior to an injection, the capillary was flushed for 3 min with the running electrolyte, which corresponds to roughly 15 capillary volumes. After the completion of a run, the capillary was flushed with 0.5 *M* NaOH for 1 min, 0.1 *M* NaOH for 1 min and water for 1 min, consecutively. This procedure ensured the capillary was always left in water at the completion of a series of runs.

4. Results and discussion

4.1. Calculation of $\mu_{ep(mc)}$

The calculation of an accurate k' value demands that t_0 and t_{mc} be precisely known. Attempts using sudan III, sudan black and anthracene as a t_{mc} marker produced nonlinear plots of $\log k'$ versus the carbon number of a homologous series of APKs when using 10% (v/v) or greater organic modifier in the running electrolyte. The "true" t_{mc} was calculated using the iterative method as described by Bushey and Jorgenson [15] using the migration times of the individual homologues of the APK series from acetophenone to decanophenone. Fig. 1 illustrates an electropherogram of the APK homologous series. The migration time of each homologue was used in the calculation of t_{mc}. At an electrical field strength of 790 V/cm, this running electrolyte produced a current of less than 50 μA. Attempts using 10 m*M* phosphate and 10 m*M* borate in the running electrolyte produced prolonged migration times due to the reduction in electro-osmotic flow at increased ionic strength. Due to the low buffer capacity of the running electrolyte and the high field strengths used, migration times started to change after three runs using the same buffer in the inlet and outlet reservoirs due to changes in pH. This can be easily avoided by using the buffer replenishment system of the Hewlett-Packard CE system to supply fresh running electrolyte at the start of each run. The use of the buffer replenishment system yielded excellent reproducibility, even over two days of consecutive runs.

Using the t_0 and t_{mc} values, the electrophoretic mobility of the micelle, $\mu_{ep(mc)}$, can be calculated for a given set of conditions using [14]:

$$\mu_{ep(mc)} = \frac{lL}{V}\left(\frac{1}{t_0} - \frac{1}{t_{mc}}\right) \quad (6)$$

where l and L are the effective and total capillary lengths, respectively, and V is the applied electrical field strength. Since it is not practical to have APK homologues in every sample, Eq. (6) can be rearranged to provide t_{mc} for a given electropherogram using the measured t_0, once

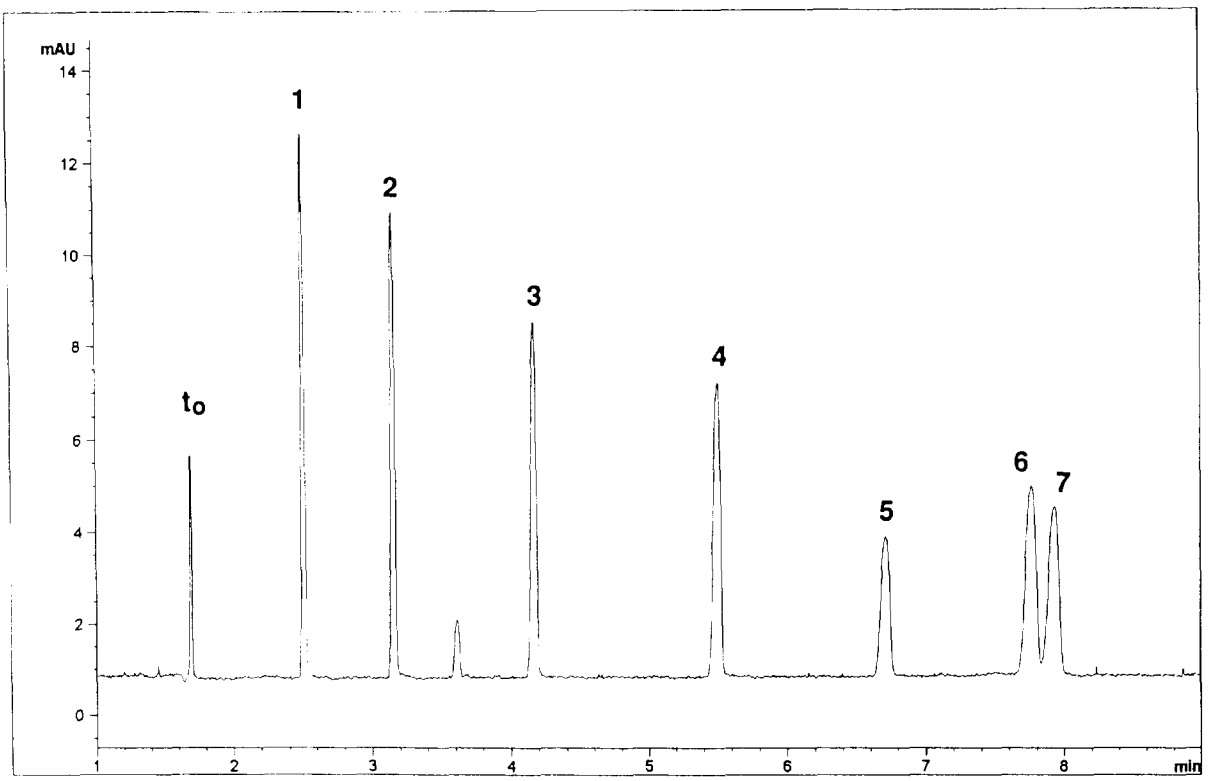

Fig. 1. Electropherogram of APK homologous series. Experiment conditions: running electrolyte 50 m*M* SDS, 5 m*M* borate, 5 m*M* phosphate, pH 7.4 with 10% (v/v) 2-propanol; capillary, untreated fused-silica, 38.0 cm (total length) × 50 μm I.D., 30 cm to detection point; voltage 30 kV; injection 50 mbar for 2 s. Analytes: 1 = acetophenone; 2 = propiophenone; 3 = butyrophenone; 4 = valerophenone; 5 = hexanophenone; 6 = octanophenone; 7 = decanophenone.

$\mu_{ep(mc)}$ has been calculated. This procedure allows the accurate calculation of t_{mc} without the presence of an internal marker t_{mc}.

To test the validity of this procedure, the $\mu_{ep(mc)}$ was measured systematically over the period of two days of continuous operation. Even though the electro-osmotic flow varied slightly due to charges in the capillary surface, $\mu_{ep(mc)}$ varied less than 1% ($n = 20$).

4.2. Calibration curve

Fig. 2 illustrates the electropherogram of eleven compounds of known $\log P_{ow}$ used as standards in the construction of a calibration curve. These compounds are all aromatic in nature, varying in structure as well as $\log P_{ow}$, from 1.1 to 4.8, which ensures calibration over a wide range. All compounds are baseline resolved with exception of the last pair, anthracene and bibenzyl. The k' for each compound was calculated using the "calculated t_{mc}" and plotted versus literature $\log P_{ow}$ values obtained using the shake-flask method [16]. Fig. 3 illustrates a typical calibration curve obtained. Good linearity was observed with a R value of 0.996. It should be mentioned that 10% 2-propanol as an organic modifier was selected only to produce desired resolution at increased hydrophobicity. Increasing the 2-propanol to 20% (v/v) produced baseline resolution of anthracene and bibenzyl, but at the expense of compressing less hydrophobic regions of the electropherogram and extended analysis times; 10% 2-propanol produced a balance between hydrophobic resolution and rapid analysis time. This provided the maxi-

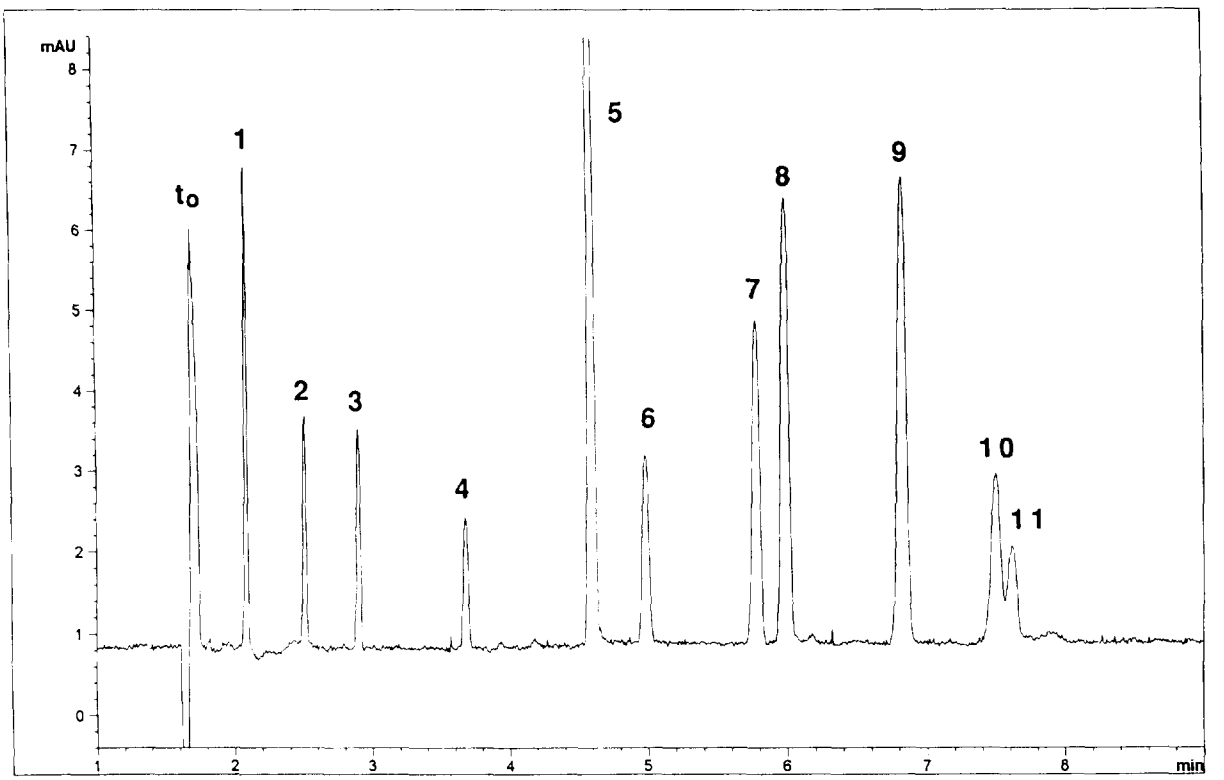

Fig. 2. Electropherogram of log P_{ow} standards used for calibration of the MECC system. Experimental conditions as in Fig. 1. Analytes: 1 = benzyl alcohol; 2 = acetophenone; 3 = anisole; 4 = 4-chlorophenol; 5 = 1-naphthol; 6 = 3-methyl-4-chlorophenol; 7 = naphthalene; 8 = diphenylamine; 9 = diphenyl ether; 10 = anthracene; 11 = bibenzyl.

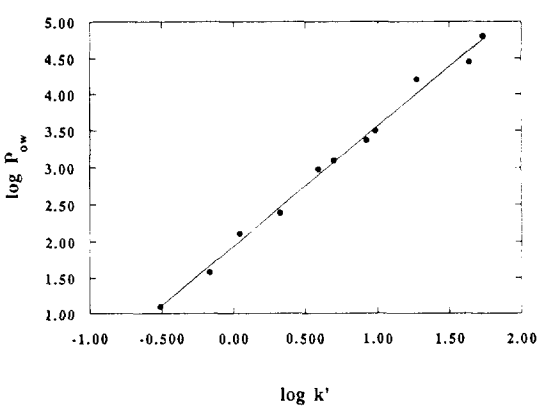

Fig. 3. Calibration curve constructed by plotting log k' for each analyte in Fig. 2 versus their log P_{ow} value; $y = 1.934 + 1.6335x$; $r = 0.99628$.

mum resolution for compounds with log P_{ow} between 2 and 4. Both methanol and acetonitrile were explored at various percentages, and both produced linear calibration curves.

4.3. Measurement of log P_{ow}

Eleven model compounds were selected to evaluate this system. Each compound was injected individually after the determination of $\mu_{ep(mc)}$ and running the standards for the calibration curve. Using Eq. (3), k' was calculated for each compound and its corresponding log P_{ow} was extracted from the calibration curve. Table 1 lists the measured and literature log P_{ow} values for the model compounds. These conditions produced an average error of 0.26 log P_{ow} units for the model compounds. In general, greater

Table 1
Comparison of measured and literature log P_{ow} values for model compounds[a]

Compound	Measured log P_{ow}	Literature log P_{ow}
p-Methoxyphenol	1.13	1.34
Phenol	1.21	1.46
Catechol	1.31	0.95
m-Cresol	1.86	1.96
p-Cresol	1.89	1.94
4-Nitrophenol	2.27	1.91
2,4-Dimethylphenol	2.49	2.36
2,4,5-Trichlorophenol	3.63	3.72
Biphenyl	4.27	3.76
n,*n*-Dibutylphthalate	4.99	5.15
Hexachlorobenzene	5.54	6.18

[a] Conditions as in Fig. 1.

error is associated with compounds of high log P_{ow}. This trend can be attributed to the exponential distribution of k' across the migration time window, approaching infinity as the analyte's migration time approaches t_{mc}. For highly lipophilic compounds, k' can be decreased by increasing the percentage of organic modifier, but at the expense of increased analysis time. For screening purposes, this error is well within acceptable limits.

Fig. 4 illustrates the separation of a mixture of three common drugs, acetaminophen, guaifenesin and phenacetin. This example illustrates the high resolving power of MECC allowing the determination of log P_{ow} for the individual components of a mixture. The average log P_{ow} error for these drugs is less than 0.12 units. The quantity of material required for the analysis was on the order of nanograms.

For further study of the applicability of the technique, the correlation between log P_{ow} measured by HPLC and by MECC for 23 new potential pharmaceutical compounds made by Monsanto was examined. These compounds vary greatly in structure and have molecular masses between 200 and 600. Fig. 5 illustrates the

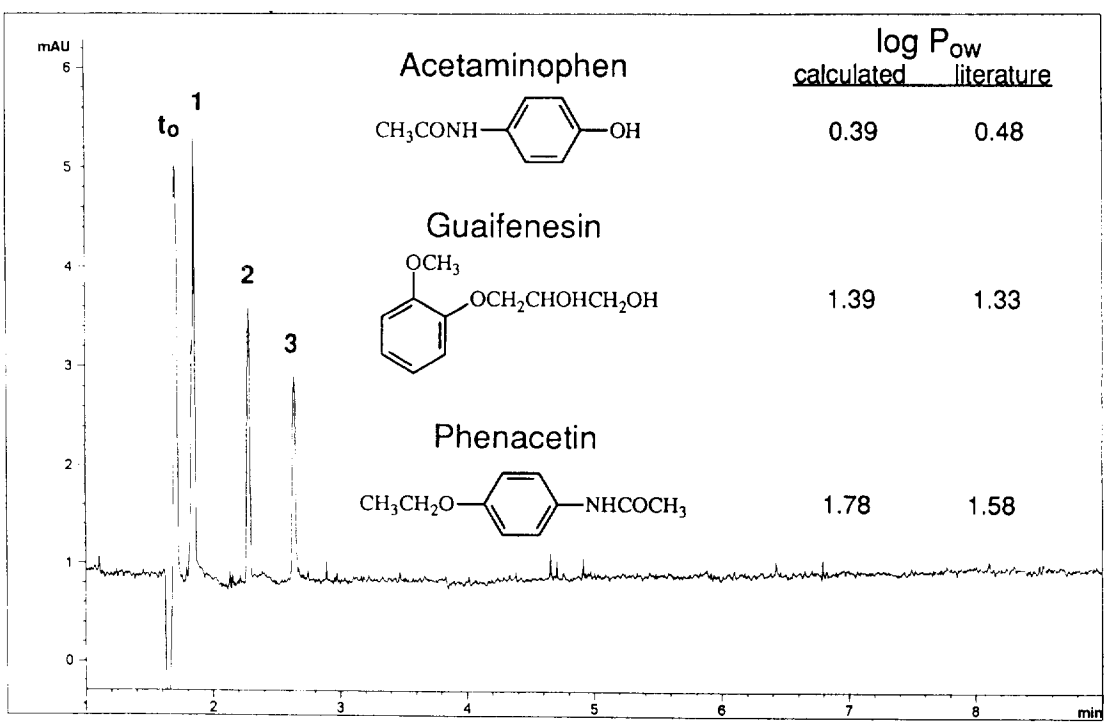

Fig. 4. Electropherogram of a mixture containing three common drugs and their measured and literature log P_{ow} values. Experimental conditions as in Fig. 1. Analytes: 1 = acetaminophen; 2 = guaifenesin; 3 = phenacetin.

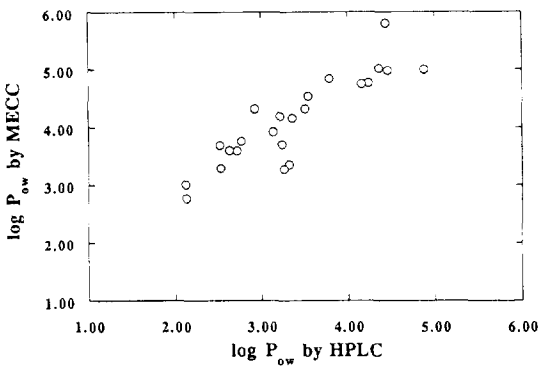

Fig. 5. Correlation between log P_{ow} measured using MECC methods and HPLC method for 24 new potential pharmaceutical compounds. Experimental conditions: MECC, as in Fig. 1; HPLC, Vydac C18, 4 m*M* phosphate at pH 7.4, 10–80% acetonitrile gradient in 20 min. Curve fit: $\log P_{ow(MECC)} = 1.19 + 0.87 \log P_{ow(HPLC)}$, $r = 0.89$.

correlation between the two techniques. Linear regression yielded the line: $\log P_{ow(MECC)} = 1.19 + 0.87 \log P_{ow(HPLC)}$, $r = 0.87$. As can be seen from the slope, the MECC method gave slightly higher $\log P_{ow}$ values than observed in HPLC. Since the "true" $\log P_{ow}$ is not known for any of these compounds it is hard to assess the accuracy of this data.

5. Conclusions

This technique proved to be very effective in the rapid screening of $\log P_{ow}$ values of neutral compounds. The SDS micellar phase is a good mimic of the octanol–water system and yielded a linear relationship between $\log P_{ow}$ and $\log k'$. The average error in $\log P_{ow}$ for eleven model compounds was found to be 0.26 units. Once $\mu_{ep(mc)}$ was determined and the calibration standards were run, samples could be analyzed with a throughput of 15 min per sample, including equilibration and column washes.

The addition of an organic modifier proved necessary to resolve compounds with $\log P_{ow}$ above 4.0. If the samples of interest have very low $\log P_{ow}$ (< 1.0) it would be beneficial to remove the organic modifier and increase the surfactant concentration. The opposite is also true: the organic modifier should be increased to 20 or 25% (v/v) for samples with high $\log P_{ow}$.

This method is designed for use with neutral compounds. Determination of $\log P_{ow}$ for ionic species could prove to be a difficult task. Any electrophoretic mobility of the analyte must be corrected in the k' equation. Also ion-pairing between the micelle and analyte could result in erroneous data.

This method of $\log P_{ow}$ measurement provides a useful alternative to the current techniques used for the screening of $\log P_{ow}$ values for neutral species. Due to the low cost of buffer components used in this system and the comparatively low price of fused-silica capillary, a sample can be ran for pennies of consumable materials.

It should be noted that SDS was chosen as the micellar phase due to its relative availability in our laboratory and is generally considered the "first choice" micellar phase. We have explored other micellar phases for $\log P_{ow}$ determinations, namely nonanoyl-*N*-methylglucamide (MEGA 9) and octyl-β-D-glucoside as borate-sugar complexes, both of which yielded linear relationships between $\log k'$ and $\log P_{ow}$. Since these micellar phases typically require alkali pH, i.e., pH > 9.0, they where not used in these studies since many phenolic compounds have pK_a in this region. This paper simply addresses the ability to perform rapid $\log P_{ow}$ screening with SDS as a micellar phase. Other micellar phases and/or modifiers may yield better correlations between $\log k'$ and $\log P_{ow}$ and provide more accurate $\log P_{ow}$ determinations.

References

[1] A.J. Leo, C. Hansch and D. Elkins, Chem. Rev., 7 (1971) 525.
[2] C. Hansch and T. Fujita, J. Am. Chem. Soc., 86 (1964) 1616.
[3] R. Kaliszan, Quantitative Structure-Chromatographic Retention Relationship, John Wiley and Sons, New York, 1987.
[4] K. Valkó and P. Slégel, J. Chromatogr., 631 (1993) 49.
[5] D.J. Minick, D.A. Brent and J. Frenz, J. Chromatogr., 461 (1989) 177.

[6] T. Braumann, J. Chromatogr., 373 (1986) 191.
[7] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56 (1984) 111.
[8] B.L. Karger, A.S. Cohen and A. Guttman, J. Chromatogr., 492 (1989) 585.
[9] K.D. Altria and S.D. Filbey, J. Liq. Chromatogr., 16 (1993) 2281.
[10] G.M. Janini and H.J. Issaq, J. Liq. Chromatogr., 15 (1992) 927.
[11] W.G. Kuhr and C.A. Monnig, Anal. Chem., 64 (1992) 389.
[12] S. Takeda, S. Wakida, M. Yamane, A. Kawahara and K. Higashi, Anal. Chem., 65 (1993) 2489.
[13] P.G.H.M. Muijselaar, H.A. Claessens and C.A. Cramers, Anal. Chem., 66 (1994) 635.
[14] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 834.
[15] M.M. Bushey and J.W. Jorgenson, J. Microcol. Sep., 1 (1989) 125.
[16] C. Hansch and A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York, 1980.
[17] N. Chen, Y. Zhang, S. Terabe and T. Nakagawa, J. Chromatogr. A, 678 (1994) 327.