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Estimation of octanol–water partition coefficients for neutral and weakly acidic compounds by microemulsion electrokinetic chromatography using dynamically coated capillary columns

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Abstract

Microemulsion electrokinetic chromatography (MEEKC) using dynamically coated capillary columns is shown to be suitable for estimating the octanol–water partition coefficient (log *P*) for neutral and weakly acidic compounds at pH 3. The solvation parameter model is used to demonstrate that the retention properties of sodium dodecyl sulfate (1.4% w/v), *n*-butanol (8% v/v) and *n*-heptane (1.2% v/v) microemulsion are strongly correlated with the octanol–water partition system. For compounds of varied structure and log *P* values from 0.3 to 5.15, the correlation model is able to estimate log *P* to better than 0.25 log units. The dynamically coated columns consisting of a bilayer of poly(vinylsulfonate) adsorbed on top of polybrene provide a suitable electroosmotic flow at pH 3 without interfering in the retention properties of the microemulsion. For automated measurements the microemulsion run buffer should be replenished after 10 runs to maintain a stable cycle time and the coated columns replaced after 40–70 runs, depending on sample properties.

Keywords: Partition coefficients; Microemulsion electrokinetic chromatography; Dynamically coated capillary columns

1. Introduction

A potential drug has to cross several barriers until it binds with its target site. These barriers are associated with absorption, distribution, metabolism and excretion or the ''ADME'' interface. The early prediction of ADME properties serves as an indication of the likely development success of compounds produced by combinatorial or traditional synthetic methods. The core properties required to assess ADME characteristics are solubility, lipophilicity, stability and acid–base character [1–3]. Oil–water partition coefficients, and the octanol–water partition coefficient in particular, are widely used as a measure of lipophilicity [3–6]. The octanol–water partition coefficient is one of the most commonly reported physicochemical properties of drugs and industrial chemicals and the most widely employed descriptor for quantitative structure–activity relationships (QSARs) for all kinds of biological, pharmaceutical and environmental property estimates [3–8].

Traditional shake flask methods for the determination of octanol–water partition coefficients are time consuming, tedious, require relatively large

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amounts of pure compounds and are incompatible with the needs of high throughput methods in the pharmaceutical industry [2,5,9]. Indirect approaches using separation methods are commonly used in industry, and are reviewed elsewhere [2–6,8,10–12]. With a few exceptions, approaches based on reversed-phase liquid chromatography employing chemically bonded phases are limited to estimating log *P* for compounds of similar structure with a narrow range of log *P* values. Fortunately, a large number of microemulsion and micellar electrokinetic chromatographic systems possess the required properties for the construction of suitable correlation models for estimating log *P* for compounds of diverse structure [12]. In addition, capillary electromigration separation methods are more compatible with the needs of high throughput laboratories. Microemulsion electrokinetic chromatography (MEEKC) using the microemulsion prepared from sodium dodecyl sulfate, *n*-butanol, *n*-heptane and buffer was first used for estimating log *P* by Ishihama and co-workers [13]. This MEEKC method was placed on a firm chemical basis by Abraham and co-workers [14]. Based on the solvation parameter model, these authors showed that the ratio of the system constants for retention in the MEEKC system were virtually identical with those of the octanol–water partition system. Poole and co-workers subsequently confirmed the finding of Abraham and introduced chemically bonded sulfonic acid capillary columns for the determination of log *P* for weak acids [15]. With minor modifications, other groups have used the same general method for estimating log *P* [16–18].

There are few problems in estimating log *P* for neutral and weakly basic compounds where operation at a pH between 7 and 12 ensures adequate electroosmotic flow. At low pH, ionization of the surface silanol groups on the fused-silica capillary column is suppressed and the electroosmotic flow reduced to values too low for practical retention factor measurements in MEEKC. For this reason, sulfonic acid coated capillary columns with a pH 3 buffer were introduced for estimating log *P* for weakly acidic compounds together with overpressure at the inlet vial to reduce the separation time [15]. The high cost of commercially available sulfonic acid coated capillary columns and the variation in batch-to-batch quality made it desirable to find an alternative approach for estimating log *P* of weak acids. Elimination of the pH-dependency of the electroosmotic flow requires a chemical modification of the capillary surface accomplished by either covalent modification or dynamic coating procedures. The later approach is easier to implement and, given our disappointment with the variation in the quality of commercially produced covalently modified capillary columns, we chose to explore the dynamic coating method.

The dynamic coating of fused-silica capillary columns with neutral and ionic polymers [19,20] and small-molecule additives [20,21] was recently reviewed. While many methods have been reported to produce neutral and positively charged capillary surfaces with pH-independent electroosmotic flow, relatively few methods were reported for the preparation of negatively charged capillary surfaces. Katayama and co-workers [22] prepared capillary columns with stable electroosmotic flow over the pH range 2–11 by successive adsorption of layers of polybrene and dextran sulfate. The intra- and intercapillary variations in electroosmotic flow were shown to be better than 0.5% and 3% RSD, respectively. Graul and Schlenoff [23] reported a similar coating procedure consisting of adsorbed multilayers of poly(diallydimethylammonium) salt and poly(styrenesulfonate) which afforded a stable electroosmotic flow over the pH range 4–8 for capillary electrophoresis. Bendahl and co-workers [24] described a dynamic coating method for the formation of bilayers consisting of the anionic polymer, poly(vinylsulfonate), adsorbed onto a capillary surface previously coated with the cationic polymer, polybrene. With poly(vinylsulfonate) added to the run buffer the intra- and intercapillary variation in electroosmotic flow for capillary electrophoresis was less than 1% and 2% of RSD, respectively. The coated capillaries were stated to be suitable for separations employing MEKC over the pH range 2–9. Boone and co-workers [25] reported the use of a proprietary reagent, CElixir®, for the dynamic bilayer coating of capillaries for the separation of basic drugs at pH 2.5 by capillary electrophoresis.

As far as we are aware the utilization of dynamic coated capillary columns for estimating log *P* of neutral and weakly acidic compounds by MEEKC

has not been described previously. In this work: (1) we report the adaptation of the method proposed by Bendahl and co-workers [24] for use in MEEKC; and (2) demonstrate the suitability of MEEKC with dynamically coated capillary columns for estimating log *P* of neutral and weakly acidic compounds based on comparison of the system constant ratios from the solvation parameter model and correlation of retention factors with literature log *P* values.

The solvation parameter model in a form suitable for evaluating retention in MEEKC and partition in the octanol–water system is set out below [12,14,15,20,26–28]

$$\log k \text{ or } \log P = c + eE + sS + aA + bB + vV \quad (1)$$

where *k* is the retention factor. The model consists of product terms representing solute properties (descriptors), indicated by capital letters, and the complementary system properties, indicated by the lower case letters in italics. Each product term describes the relative contribution of defined intermolecular interactions to the correlated solute property, in this case, either log *k* or log *P*. The contribution from electron lone pair interactions is defined by *eE*, interactions of a dipole type by *sS*, hydrogen-bond interactions by *aA* and *bB*, and differences in cavity formation and dispersion interactions for transfer of the solute from one phase to the other by *vV*. The solute descriptors are formally defined as the excess molar refraction, *E*, dipolarity/polarizability, *S*, effective hydrogen-bond acidity, *A*, effective hydrogen-bond basicity, *B*, and McGowan's characteristic volume, *V*. Solute descriptors are available for about 4000 compounds with others available using estimation methods [6,26–30].

The system constants reflect the difference in solvation properties in the two phases. The system constants are defined as the difference in contributions from electron lone pair interactions, *e*, dipole-type interactions, *s*, hydrogen-bond basicity, *a*, hydrogen-bond acidity, *b*, and cohesion and dispersion interactions, *v*, for the two phases. The system constants are calculated by multiple linear regression analysis for a varied group of solutes selected to satisfy the statistical and chemical requirements of the model [27–29,31–33]. The system constants for the octanol–water partition system are: $c = 0.09$ (± 0.02); $e = 0.56$ (± 0.01); $s = -1.05$ (± 0.02); $a = 0.03$ (± 0.02); $b = -3.46$ (± 0.03); and $v = 3.81$ (± 0.01) [6,34,35]. System constants with a positive sign favor transfer from the aqueous phase to wet octanol. Thus wet octanol is more hydrophobic and polarizable than water but less dipolar and hydrogen-bond acidic. The hydrogen-bond basicity of water and wet octanol is about the same.

A separation system will emulate the octanol–water partition system if the system constants for both processes are (nearly) identical. It will correlate with the partition system through a relationship, such as Eq. (2), if the ratios of the system constants (e.g. e/v, s/v, a/v, b/v) for both models are (nearly) identical [8,27,36–38].

$$\log P = p \log k + q \quad (2)$$

where *p* and *q* are regression constants.

2. Experimental

2.1. Materials

Sodium dodecyl sulfate, polybrene (hexadimethrine bromide, technical grade, 90% pure), poly(vinylsulfonate) sodium salt (molecular mass 4000–6000 as a 25% aqueous solution), dimethyl sulfoxide, dodecanophenone and all compounds used in the evaluation and validation data set were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium borate, sodium phosphate, *n*-butanol, *n*-heptane and methanol were obtained from Mallinkrodt (Phillipsburg, NJ, USA). Fused-silica capillary columns, 50 cm long (effective length 39.8 cm) and 50 μm I.D were purchased from PolyMicro Technologies (Phoenix, AZ, USA). Covalently bonded sulfonic acid fused-silica capillary columns 50 cm long (effective length 39.8 cm) and 50 μm I.D. were purchased from Phenomenex (Torrance, CA, USA), cat No. 04650-HF. Windows for on-column detection were prepared using a frit burner from Innovatech (Stevenage, UK).

2.2. Dynamic coating of capillary columns

Prior to coating the fused-silica capillary column was rinsed with sodium hydroxide (1.0 *M*) for 30

min at 20 p.s.i and then flushed with deionized water for 15 min at 50 p.s.i. The capillary was then dynamically coated with a 2% (w/v) aqueous solution of polybrene for 15 min at 10 p.s.i. followed by a 2% (v/v) aqueous solution of poly(vinylsulfonate) for 15 min at 10 p.s.i. After coating the capillary column was flushed with deionized water for 5 min at 50 p.s.i. The column was then conditioned using the standard operating conditions for 45 min with the microemulsion solution as the run buffer.

2.3. Preparation of the microemulsion

The acidic buffer at pH 3 was prepared by adding phosphoric acid (85%) drop wise to a stirred solution of 50 m*M* sodium phosphate until the required pH measured by a potentiometer fitted with a pH electrode was obtained. The microemulsion was prepared by adding sodium dodecyl sulfate (1.44 g) to 90 ml of buffer followed by *n*-butanol (8 ml) and heptane (1.2 ml). The final solution was made up to 100 ml with buffer and stirred at room temperature with a mini stirrer, model 200, from VWR Scientific (Batavia, IL, USA) until the solution became clear. The solution can be used immediately or stored in a closed container for several weeks.

2.4. Instrumentation

Retention factor measurements were made on a Beckman P/ACE system MDQ (Fullerton, CA, USA) equipped with a photodiode array detector operated at 222 nm. For the dynamically coated capillary columns the applied voltage was 15 kV producing a current between 35 and 40 μA. The run time was about 15 min without pressure applied to the inlet vial. For the sulfonic acid coated capillary column the voltage was 20 kV producing a current of 50–55 μA. A pressure of 0.2 p.s.i. was applied to the inlet vial to maintain the run time at about 30 min. All retention measurements were made at 30 °C.

Samples were introduced into the capillary by applying a pressure of 0.5 p.s.i. for 6 s. The retention factor, *k*, was calculated using Eq. (3)

$$k = (t_R - t_{eo})/(1 - t_R/t_{mc})t_{eo} \quad (3)$$

where t_{eo} is the migration time of the electroosmotic flow marker (methanol), t_{mc} the migration time of the microemulsion marker (dodecanophenone), and t_R the solute migration time.

2.5. Standard solutions

Standard solutions were prepared in dimethyl sulfoxide to match the solvent used for drug samples at a concentration of 1–2 mg/ml; 25 μl of this solution was mixed with 25 μl of dodecanophenone solution (15 mg/ml in methanol) and then diluted by addition of 400 μl of the microemulsion solution.

2.6. Data analysis

The solute descriptors for the solvation parameter model [14,15,26,30] and log *P* [3,5] for the evaluation data set were taken from several sources and are summarized in Table 1 together with the experimental retention factors for the reader's convenience. Multiple linear regression analysis and statistical tests were performed on an IBM Vista PC computer (Atlanta, GA, USA) using the program SPSS/PC V. 11.0 (Chicago, IL, USA).

3. Results and discussion

Our goal was to develop a stable separation system for estimating log *P* values for neutral and weakly acidic compounds at pH 3 using MEEKC. The choice of microemulsion was based on our previous experience, and that of others, which indicate that the microemulsion system described in the experimental section provides a suitable MEEKC system for estimating log *P* [13–18]. The difficulty was to establish a separation system based on MEEKC that provided sufficient electroosmotic flow at the desired pH to enable practical measurements. Based on literature precedent, dynamically coated capillary columns with an adsorbed bilayer structure were selected for this purpose [22–24]. Our main concerns were the effect that the presence of the column coating materials would have on the retention properties of the oil droplets (stationary phase of the microemulsion), the statistical validity of the correlation between retention in MEEKC and log *P*, and the repeatability and durability of the coated columns when used for MEEKC. These factors are

Table 1
Solute descriptors, log P and retention factors (log k) used in the solvation parameter and correlation models

Compound	Solute descriptor					Log P	Log k
	V	E	S	A	B		
Acetophenone	1.014	0.818	1.01	0.00	0.48	1.58	0.064
Acetylsalicylic acid	1.288	0.781	0.80	0.49	1.00	1.19	−0.123
Anisole	0.916	0.708	0.75	0.00	0.29	2.11	0.315
Anthracene	1.454	2.290	1.34	0.00	0.26	4.45	2.060
Acetaminophen	1.172	1.060	1.78	1.09	0.81	0.51	−0.829
Benzamide	0.973	0.990	1.50	0.49	0.67	0.64	−0.508
Benzaldehyde	0.873	0.820	1.00	0.00	0.39	1.48	−0.113
Benzoic acid	0.932	0.730	0.90	0.59	0.40	1.87	0.205
Benzophenone	1.481	1.447	1.50	0.00	0.50	3.18	0.963
Benzyl alcohol	0.916	0.803	0.87	0.39	0.56	1.01	−0.396
Bromobenzene	0.950	1.060	1.15	0.70	0.16	2.63	0.620
Butyrophenone	1.296	0.800	0.95	0.00	0.51	2.76	0.772
4-Chlorobenzoic acid	1.054	0.840	0.97	0.63	0.27	2.65	0.714
4-Chlorophenol	0.898	0.920	1.08	0.67	0.20	2.39	0.486
Cortisone	2.755	1.960	3.50	0.36	1.87	1.42	0.236
Coumarin	1.062	1.060	1.79	0.00	0.46	1.60	−0.075
1,4-Dimethylbenzene	0.998	0.613	0.52	0.00	0.16	3.15	0.978
3,5-Dimethylphenol	1.057	0.820	0.84	0.57	0.36	2.35	0.412
2,4-Dinitrophenol	1.124	1.200	1.50	0.10	0.55	2.36	0.093
1,3-Dichlorobenzene	0.961	0.847	0.73	0.00	0.02	3.53	1.329
Ethylbenzene	0.988	0.613	0.51	0.00	0.15	2.64	1.046
Estradiol	2.199	1.800	3.30	0.88	0.95	2.69	1.149
Estrone	2.156	1.730	3.10	0.56	0.91	2.76	1.051
Eugenol	1.354	0.946	0.99	0.22	0.51	2.99	0.655
Fluoranthene	1.585	2.377	1.53	0.00	0.20	4.50	2.483
4-Hydroxybenzoic acid	0.990	0.930	0.92	0.71	1.90	1.28	−0.122
Hydrocortisone	2.798	2.030	3.49	0.71	1.87	1.55	0.444
Ibuprofen	1.777	0.700	0.92	0.60	0.60	3.50	1.526
Iodobenzene	0.974	1.188	0.82	0.00	0.12	3.25	1.105
Methylbenzoate	1.073	0.733	0.85	0.00	0.48	2.12	0.389
1-Methylnaphthalene	1.226	1.344	0.90	0.00	0.20	3.87	1.575
4-Methylphenol	0.916	0.820	0.87	0.57	0.31	1.94	0.166
Naphthalene	1.085	1.340	0.92	0.00	0.20	3.37	1.188
1-Naphtol	1.144	1.520	1.05	0.61	0.37	2.84	0.719
4-Nitrobenzamide	1.147	1.250	2.17	0.75	0.60	1.93	−0.326
1-Nitronaphthalene	1.260	1.600	1.51	0.00	0.29	3.19	0.988
4-Nitrophenol	0.949	1.070	1.72	0.82	0.26	1.91	0.067
4-Nitrotoluene	1.032	0.870	1.11	0.00	0.28	2.37	0.411
Phenanthrene	1.454	2.055	1.29	0.00	0.26	4.46	2.138
Phenol	0.775	0.805	0.89	0.60	0.31	1.46	−0.224
Phenylbenzoate	1.540	1.330	1.42	0.00	0.47	3.59	1.234
Prednisolone	2.755	2.210	3.10	0.71	1.92	1.62	0.382
Pregnenolone	2.665	1.360	3.29	0.32	1.18	3.13	1.704
Progesterone	2.622	1.450	3.29	0.00	1.14	3.26	1.339
Resorcinol	0.834	0.980	1.00	1.10	0.58	0.80	−0.619
Toluene	0.857	0.601	0.52	0.00	0.14	2.69	0.688
Valerophenone	1.437	0.800	0.95	0.00	0.50	3.11	1.069

of course interdependent, and will be treated separately in the following sections.

In initial screening experiments, to arrive at the standard conditions reported in the experimental section, several variations on the method described by Bendahl and co-workers were explored [24]. During the coating process a 15-min rest period between the initial coating with polybrene and subsequent coating with the poly(vinylsulfonate) solution was evaluated against no waiting time. The coated capillaries were rinsed with the poly(vinylsulfonate) solution between runs or no rinse step was used. Different concentrations of poly(vinylsulfonate) solution [0, 0.001, 0.01, 0.05, 0.1 and 0.2% (v/v)] were added to the microemulsion solutions used for the separation. Five standard compounds (acetylsalicylic acid, phenylacetic acid, salicylic acid, ketoprofen and 4-bromo-3-nitrobiphenyl) with a range of log *P* values from 1.19 to 4.66 were used for the evaluation. The relative standard deviations (RSD) were calculated for the retention factor of each compound for all combinations of the experimental conditions. The retention factors were mainly affected by the above changes in experimental conditions with an average relative standard deviation for all compounds of less than 5%. No systematic change or variation in solute retention between the experimental condition variants indicated that each set of experimental conditions performed comparably. Since neither reproducibility nor durability could be related to the use of a poly(vinylsulfonate) rinse solution or as an additive to the microemulsion system, these procedures were not incorporated into the standard method adopted for estimating log *P*. The standard method described in the Experimental section was developed to allow all steps of the dynamic coating and measurement procedures to be sequenced automatically by the Beckman P/ACE software.

3.1. Suitability of the MEEKC system as a model for log P

The solvation parameter model was employed to establish that the fundamental intermolecular interactions that contribute to retention in MEEKC for the standard operating conditions were correlated to those responsible for partition in the octanol–water system. An evaluation set of 48 neutral and weakly acidic compounds, Table 1, was selected for this purpose. This is the same evaluation set used previously for evaluation of covalently bonded sulfonic acid capillary columns for estimating log *P* by MEEKC [15].

The experimental retention factors for the evaluation data set were fit to the solvation parameter model resulting in the following system constants: $c = -1.09$ (± 0.06); $e = 0.41$ (± 0.05); $s = -0.53$ (± 0.05); $a = -0.08$ (± 0.06); $b = -2.28$ (± 0.09); and $v = 2.44$ (± 0.09). The model statistics are $r^2 = 0.978$, SE = 0.119, $F = 384$ and $n = 48$, where r^2 is the overall coefficient of determination, SE the standard error in the estimate, F the Fischer statistic, and n the number of compounds. The descriptive statistics indicate that the model is sound and provides a suitable representation of the contribution of the various intermolecular interactions to retention for the MEEKC system. The system constants are similar, but not identical, to those reported for the microemulsion system using covalently bonded sulfonic acid capillary columns [15]. There are small but statistically significant differences in the v and b system constants, likely reflecting the presence of small amounts of soluble poly(vinylsulfonate) and polybrene in the separation buffer. The mobile phase is slightly less cohesive ($v = 2.44 \pm 0.09$ compared with 2.16 ± 0.10) and more hydrogen-bond acidic ($b = -2.28 \pm 0.09$ compared with -2.10 ± 0.09) for the dynamically coated column compared with the covalently bonded column.

For retention in the MEEKC system to provide a suitable correlation model to estimate log *P* it is necessary that the system constant ratios for the MEEKC retention model and log *P* are (nearly) identical. The system constant ratios for the octanol–water partition system are: $e/v = 0.15$; $s/v = -0.28$; $a/v = 0.01$; and $b/v = -0.91$ [6,12,14]. The system constant ratios for the MEEKC retention model are: $e/v = 0.17$; $s/v = -0.22$; $a/v = -0.03$; and $b/v = -0.93$. The agreement between the two sets of system constant ratios is excellent. Retention in the MEEKC system should be strongly correlated with log *P*. Structural diversity should have little effect on the predictive ability of the model, since the same range of intermolecular interactions that contribute to one system contribute proportionally to the other.

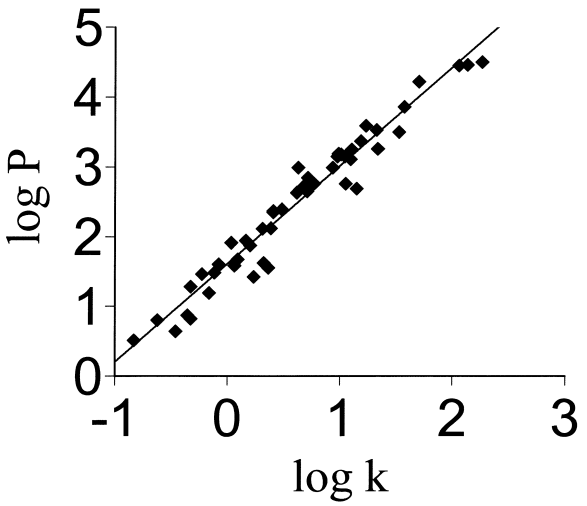

Fig. 1. Plot of log *P* against the retention factor (log *k*) from MEEKC on a dynamically coated capillary column for compounds of varied structure (see Table 1).

3.2. Correlation of retention in the MEEKC system with log P

Fig. 1 shows a plot of literature log *P* values against the retention factor (log *k*) in the MEEKC system for the 48 neutral and weakly acidic compounds of the evaluation set. The correlation model and statistics for the fit are

$$\log P = 1.41(\pm 0.05) \log k + 1.59(\pm 0.04) \quad (4)$$

$$n = 48, \quad r^2 = 0.972, \quad \mathrm{SE} = 0.234, \quad F = 911$$

The model is suitable for estimating log *P* to better than 0.25 log units, at least for the range of log *P* values (0.5–4.5) represented in the evaluation data set. The uncertainty of the estimated log *P* values is acceptable for a wide range of property estimates in the pharmaceutical industry. The results are comparable to those obtained previously using covalently bonded sulfonic acid capillary columns, which allowed log *P* to be estimated to about 0.28 log units [15].

3.3. Validation of the correlation model for log P

A second set of 31 varied compounds, not included in the evaluation set, was used to validate the correlation model for log *P* (Table 2). The compounds were selected to cover a wide range of log *P* values (0.34–5.15). The average relative standard deviation in estimated log *P* values is 6.2% ($n = 6$). The average difference between the estimated log *P* and literature log *P* values is ±0.16 log units. The plot of log *P* against estimated log *P* values from the microemulsion system is shown in Fig. 2. The slope of the plot is 0.997 (±0.03) and the intercept 0.123 (±0.07). The slope is indistinguishable from 1.00, indicating that the chemical factors affecting the two measurements are the same. The small intercept indicates a systematic difference between the two measurements, but is not of a magnitude to cause concern. The results are satisfactory and confirm that the MEEKC system using dynamically coated capillary columns is suitable for estimating log *P*.

3.4. Reproducibility and repeatability of dynamically coated capillary columns for estimating log P

The repeatability of the dynamically coated capillary columns for MEEKC was evaluated by variation of the retention factors for a standard mixture using the recommended conditions for estimating log *P* described in the Experimental section. The mean retention factors and their standard and relative standard deviations for eight consecutive runs are summarized in Table 3. The relative standard deviations of 1.04–2.13% indicate excellent short-term precision. These results compared favorably with the results for the covalently bonded sulfonic acid capillary column, which gave relative standard deviations of 2.02–3.65% for the same compounds. To evaluate the reproducibility of the method the same test compounds were separated using eight newly prepared microemulsion solutions, on eight newly coated capillary columns, installed in eight different capillary electrophoresis instruments. The results are summarized in Table 3. There is no significant difference in the mean values for the retention factors compared with the repeatability test and the relative standard deviations for the retention factors at 4.2–6.5% are acceptable.

It was observed that after the first run the migration window for the separation system expands slowly with each run. This expansion, however, has

Table 2
Estimated and literature log *P* values for the validation data set

Compounds	Log *P* estimated ($n=6$)			Log *P*	Difference (Δ)
	Mean	SD	RSD (%)		
4-Androstene-3,17-dione	2.69	0.110	4.09	2.75	0.06
4-Bromo-3-nitrobipheyl	4.82	0.339	7.06	4.66	−0.16
Benzonitrile	1.43	0.089	6.25	1.56	0.13
4-Chloroanisole	2.90	0.136	4.70	2.82	−0.08
4-Chlorobenzyl alcohol	1.99	0.104	5.20	1.96	−0.03
4-Chlorotoluene	3.33	0.183	5.49	3.33	0.00
2-Chloro-5-nitrobenzoic acid	2.13	0.132	6.17	2.03	−0.10
2-Chloro-4-nitrobenzoic acid	2.19	0.142	6.47	2.03	−0.16
4-Cyanophenol	1.28	0.086	6.74	1.63	0.32
Diethylacetamide	0.37	0.067	18.11	0.34	−0.03
2,4-Dihydroxybenzoic acid	1.46	0.069	4.76	1.63	0.17
N,*N*-Dimethyl benzamide	0.72	0.010	1.39	0.62	−0.10
1,3-Dimethylbenzene	2.95	0.247	8.36	3.20	0.25
Ethyl benzoate	2.55	0.147	5.75	2.64	0.09
Flufenamic acid	4.81	0.305	6.33	5.15	0.34
4-Iodophenol	2.71	0.160	5.89	2.91	0.20
Ketoprofen	2.81	0.148	5.29	3.12	0.31
4-Methylbenzyl alcohol	1.53	0.095	6.16	1.59	0.06
3-Methylphenol	1.67	0.111	6.66	1.96	0.29
2-Methylphenol	1.63	0.099	6.09	1.95	0.32
2-Naphthol	2.57	0.108	4.18	2.84	0.27
Nitrobenzene	1.65	0.078	4.79	1.85	0.20
2-Nitrotoluene	2.17	0.044	2.03	2.30	0.13
2-Phenoxybenzoic acid	2.63	0.138	5.24	3.11	0.48
Phenyl acetate	1.43	0.111	7.76	1.41	−0.02
Phenylacetic acid	1.29	0.091	6.99	1.43	0.14
Propiophenone	1.99	0.049	2.45	2.19	0.20
Pyrene	4.99	0.462	9.25	4.88	−0.11
Salicylic acid	2.08	0.099	4.75	2.26	0.18
4-Toluic acid	2.17	0.102	4.70	2.27	0.10
1,2,4-Trichlorobenzene	4.02	0.247	6.14	3.98	−0.04

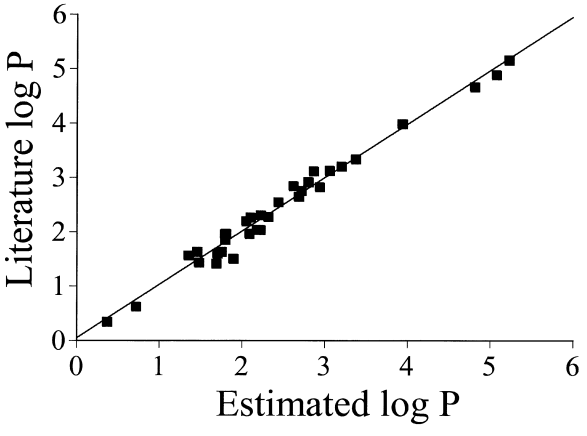

Fig. 2. Plot of log *P* against log *P* estimated by the MEEKC correlation model for compounds of varied structure (see Table 2).

little effect on the calculation of retention factors. The migration window can be restored to its original value by exchanging the microemulsion solution (run buffer) after 10 runs. For example, the migration window was monitored for 50 consecutive runs and the microemulsion solution replenished for runs 11 and 21. For run 2 the migration window was 8.55 min with retention factor values (log *k*) for the five test compounds of −0.11, 0.22, 0.41, 0.89 and 1.89, respectively. For run 11 (after replenishment of the microemulsion solution) the migration window was 9.06 min with log *k* values for the test compounds of −0.10, 0.22, 0.43, 0.91 and 2.03. For run 21 the migration window was 8.88 min with log *k* values for the test compounds of −0.098, 0.25, 0.48, 0.99 and 2.07. The microemulsion solution was not

Table 3
Repeatability and reproducibility (log k) for the MEEKC system ($n=8$)

Statistic	Acetylsalicyclic acid	Phenylacetic acid	Salicyclic acid	Ketoprofen	4-Bromo-3-nitrobiphenyl
(i) Repeatability (dynamically coated column)					
Mean	−0.111	0.223	0.418	0.891	1.852
SD	0.002	0.004	0.009	0.009	0.022
RSD	2.13	1.72	2.23	1.04	1.21
(ii) Repeatability (covalently bonded sulfonic acid column) [15]					
Mean	−0.123	0.215	0.402	0.869	1.802
SD	0.008	0.006	0.015	0.019	0.036
RSD	3.38	2.73	3.65	2.14	2.02
(iii) Reproducibility (dynamically coated column)					
Mean	−0.119	0.210	0.411	0.903	1.934
SD	0.008	0.013	0.023	0.038	0.085
RSD	6.48	6.39	5.67	4.16	4.39

replenished between run 21 and 50. The window expanded to 22.16 min at run 50 with log k values of −0.12, 0.23, 0.39, 0.89, and 1.84. Thus, to maintain a constant cycle time and minimize the separation time for automated measurements, it is important to replenishing the microemulsion solution at periodic intervals. Since the Beckman P/ACE instrument does not have an automated buffer replenishment feature, the sequence is set to either increment the separation time for each sequence of 10 runs, or to increase the applied voltage and/or pressure applied to the inlet run buffer vial for each sequence of 10 runs. Attempts to stabilize the migration window by rinsing the capillary with poly(vinylsulfonate) solution between runs, by adding different concentrations of poly(vinylsulfonate) to the microemulsion solution during the runs were not effective. The useful lifetime of each column depends on the compounds used. For standards, the average column lifetime is about 70 runs and for reaction mixtures about 40 runs.

4. Conclusions

Dynamically coated capillary columns prepared with a bilayer of poly(vinylsulfonate) adsorbed on top of the cationic polymer, polybrene, are suitable for estimating log P values of neutral and weakly acidic compounds at pH 3 by MEEKC. These columns are an acceptable alternative to commercially available covalently bonded sulfonic acid capillary columns. The agreement in system constant ratios for the solvation parameter models demonstrates that the retention properties of the sodium dodecyl sulfate, n-butanol and n-heptane microemulsion are strongly correlated with the octanol–water partition system. The correlation model between the retention factor (log k) in the microemulsion separation system and log P allows log P to be estimated to about 0.25 log units for compounds with log P values in the range 0.3–5.2. To stabilize the migration window the microemulsion solution should be replaced after each 10 runs. Alternatively, the run sequence can be programmed to either increase the run time or the applied voltage and/or the pressure applied to the inlet vial after each 10 runs.

References

[1] E.H. Kerns, J. Pharm. Sci. 90 (2001) 1838.
[2] K. Valko (Ed.), Separation Methods in Drug Synthesis and Purification, Elsevier, Amsterdam, 2000.
[3] C. Hansch, A. Leo, Exploring QSAR; Fundamentals and Applications in Chemistry and Biology, American Chemical Society, Washington, DC, 1995.
[4] W.J. Lambert, J. Chromatogr. A 656 (1993) 469.
[5] J. Sangster, Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry, Wiley, New York, 1997.
[6] V. Pliska, B. Testa, H. van de Waterbeemed (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1995.

[7] R.S. Boethling, D. Mackay, Handbook of Property Estimation Methods for Chemicals, Environmental and Health Sciences, Lewis Publishers, Boca Raton, FL, 2000.
[8] C.F. Poole, A.D. Gunatilleka, S.K. Poole, Adv. Chromatogr. 40 (2000) 159.
[9] L.-G. Danielsson, Y.-H. Zhang, Trends Anal. Chem. 15 (1996) 188.
[10] R. Kaliszan, J. Chromatogr. B 717 (1998) 125.
[11] S.F. Donovan, M.C. Pescatore, J. Chromatogr. A 952 (2002) 47.
[12] S.K. Poole, C.F. Poole, J. Chromatogr. B, submitted.
[13] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, Anal. Chem. 67 (1995) 1588.
[14] M.H. Abraham, C. Treiner, M. Roses, C. Rafols, Y. Ishihama, J. Chromatogr. A 752 (1996) 243.
[15] S.K. Poole, D. Durham, C. Kibbey, J. Chromatogr. B 745 (2000) 117.
[16] S.J. Gluck, M.H. Benko, R.K. Hallberg, K.P. Steele, J. Chromatogr. A 744 (1996) 141.
[17] Y. Mrestani, R.H.H. Neubert, A. Krause, Pharm. Res. 15 (1998) 799.
[18] W.L. Klotz, M.R. Schure, J.P. Foley, J. Chromatogr. A 930 (2001) 145.
[19] J. Horvath, V. Dolnik, Electrophoresis 22 (2001) 644.
[20] C.F. Poole, The Essence of Chromatography, Elsevier, Amsterdam, 2003.
[21] P.G. Righetti, C. Gelfi, B. Verzola, L. Castelletti, Electrophoresis 22 (2001) 603.
[22] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[23] T.W. Graul, J.B. Schlenoff, Anal. Chem. 71 (1999) 4007.
[24] L. Bendahl, S.H. Hasen, B. Gammelgaard, Electrophoresis 22 (2001) 2565.
[25] C.M. Boone, E.Z. Jonkers, J.P. Franke, R.A. de Zeeuw, K. Ensing, J. Chromatogr. A 927 (2001) 203.
[26] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[27] C.F. Poole, S.K. Poole, J. Chromatogr. A 965 (2002) 263.
[28] C.F. Poole, S.K. Poole, M.H. Abraham, J. Chromatogr. A 798 (1998) 207.
[29] M.H. Abraham, C.F. Poole, S.K. Poole, J. Chromatogr. A 842 (1999) 79.
[30] M.H. Abraham, J.A. Platts, J. Org. Chem. 66 (2001) 3484.
[31] A.M. Zissimos, M.H. Abraham, M.C. Barker, K.J. Box, K.Y. Tam, J. Chem. Soc. Perkin Trans. 2 (2002) 470.
[32] M.D. Trone, M.G. Khaledi, J. Chromatogr. A 886 (2000) 245.
[33] E. Fuguet, C. Rafols, E. Bosch, M.H. Abraham, M. Roses, J. Chromatogr. A 942 (2002) 237.
[34] M.H. Abraham, J. Phys. Org. Chem. 6 (1993) 660.
[35] M.H. Abraham, H.S. Chadha, J.P. Dixon, A.J. Leo, J. Phys. Org. Chem. 7 (1994) 712.
[36] J.H. Zhao, P.W. Carr, Anal. Chem. 71 (1999) 2623.
[37] M.H. Abraham, M. Roses, C.F. Poole, S.K. Poole, J. Phys. Org. Chem. 10 (1997) 358.
[38] D.M. Cimpean, C.F. Poole, Analyst 127 (2002) 724.