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Short communication

Screening of octanol–water partition coefficients for pharmaceuticals by pressure-assisted microemulsion electrokinetic chromatography

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Abstract

A rapid screening assay for the determination of octanol–water partition coefficients (log P_{ow}) of pharmaceuticals was developed by using pressure-assisted microemulsion electrokinetic chromatography (MEEKC). The microemulsion system contains 50 m*M* sodium dodecyl sulfate, 0.87 *M* 1-butanol, 82 m*M* heptane, and 50 m*M* borate–phosphate (2:3) at pH 10. Ten standard compounds with known log P_{ow} values from −0.26 to 4.88 were used for constructing the calibration curve of log P_{ow} against the MEEKC retention factor, log *k*. The log P_{ow} values of the compounds were calculated based on the log *k* values measured by MEEKC and the slope and intercept of the calibration curve. For 13 literature and 32 Roche compounds, about 90% of the log P_{ow} values measured by MEEKC are within 0.5 log units of the values from the literature and potentiometric titration. The throughput is about 2 samples/h using +20 kV voltage plus 5 mbar air pressure for separation. This MEEKC method is applicable for log P_{ow} screening of weakly basic, weakly acidic, and neutral pharmaceuticals with log P_{ow}=0–5 and pK_a≤10.

Keywords: Octanol–water partition coefficients; Microemulsion electrokinetic chromatography; Electrokinetic chromatography; Pharmaceutical analysis

1. Introduction

Hydrophobic interaction plays a significant role in partitioning into lipid bilayers of biomembranes, bioavailability, and pharmacokinetics of drugs. Solute hydrophobicity is usually expressed by the thermodynamic 1-octanol–water partition coefficient (P_{ow}). Extensive data collection of log P_{ow} values can be found in the literature [1–3].

A number of direct and indirect methods have been applied for log P_{ow} measurement [4]. Direct measurement of log P_{ow} using the conventional shake-flask method was historically considered to be the gold standard assay. An indirect high-throughput method is reversed-phase liquid chromatography (RPLC) utilizing the linear relationship between log P_{ow} and retention factor, log *k* [5,6]. However, the log P_{ow}–log *k* correlation in RPLC may vary due to the changes in solute–stationary phase interactions and solute–solvent interactions [7,8].

Electrokinetic chromatography (EKC) using micelle (MEKC) and microemulsion (MEEKC) has

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been recently used for log P_{ow} assessment. Solute separation is based on the differential partitioning of the compounds into the micelles as in MEKC or into the oil droplets as in MEEKC. Herbert and Dorsey [9] have measured log k values for over 100 compounds by MEKC that correlate well with log P_{ow} (R^2=0.835) for nine orders of magnitude in log P_{ow}. However, congeneric behavior was observed for different groups of compounds using MEKC [10]. It was reported that MEEKC using the sodium dodecyl sulfate (SDS)–butanol–heptane microemulsion system provided better estimation of log P_{ow} than MEKC using the SDS micellar system [11]. Literature results showed that log k values measured by MEEKC in the SDS–butanol–heptane microemulsion system were highly correlated with log P_{ow} (R^2>0.96) over 5–8 orders of magnitude [11–14]. However, only few applications of MEEKC were reported for log P_{ow} determination of pharmaceuticals [15,16].

In this paper, pressured-assisted MEEKC using the SDS–butanol–heptane microemulsion system will be examined for log P_{ow} determination of pharmaceuticals. The MEEKC measured log P_{ow} values are compared with the results from potentiometric titration and commercial prediction program.

2. Experimental

2.1. Chemicals and reagents

The water for solution preparation was deionized and purified through a NANOPure system. Heptane, SDS, 1-butanol, dodecanophenone, and the compounds in Tables 1 and 2 were obtained from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA) with high purity (>98%). The Roche compounds were obtained from Compound Collection Inventory at Roche Palo Alto LLC (Palo Alto, CA, USA).

The microemulsion containing 50 m*M* SDS, 0.87 *M* 1-butanol, 82 m*M* heptane, and 50 m*M* borate–phosphate (2:3) at pH 10 was prepared following the literature procedure [13]. In a 50-ml volumetric flask, 0.72 g of SDS was dissolved into 40 ml of borate–phosphate buffer (pH 10). Then 4 ml of 1-butanol and 0.6 ml of heptane were added sequentially with mixing. The flask was filled to the mark with the pH 10 buffer and sonicated for 30 min to transparent.

Table 1
MEEKC calibration standards [16]

Standard compound	Log k (MEEKC)	Log P_{ow} (literature)	pK_a (CE)
Pyrazine	−1.25	−0.26	<2
Benzamide	−0.49	0.64	None
Nicotine	−0.12	1.17	3.2; 8.75
Indazole	0.13	1.77	~1.6
Benzocaine	0.22	1.86	2.63
4-Chloroaniline	0.29	1.88	3.98
Lidocaine	0.61	2.26	7.92
Pyrilamine	0.96	3.27	3.99; 9.18
Impramine	1.71	4.42	9.21
Pyrene	2.17	4.88	None

A marker solution was prepared in the microemulsion system with 0.2% (v/v) of dimethyl sulfoxide (DMSO) as the electroosmotic flow (EOF) marker and 0.5 mg/ml of dodecanophenone as the micelle marker. The sample solutions (0.2–1.0 mg/ml) were prepared in the marker solution.

2.2. Apparatus and methods

2.2.1. Log P_{ow} by MEEKC

The MEEKC experiments were performed on an Agilent capillary electrophoresis system with a diode array UV–Vis detector. The uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 50 cm total length (L_t) and 40 cm to the detector (L_d) (50 μm I.D.×360 μm O.D.) was used and thermostated at 25.0±0.1 °C. Injections were made at 25 mbar for 3 s. A voltage of +20 kV and external air pressure of 5 mbar were applied for the CE separation. Prior to the first run, the capillary was flushed with 0.1 *M* NaOH for 20 min, water for 10 min, and microemulsion system for 10 min. There was no rinsing with the microemulsion between sample injections.

The retention factor, k, was calculated from the MEEKC migration time according to Eq. (1), where t_R, t_{EOF}, and t_{MC} are the migration times of the solute, the EOF marker, and the micelle marker, respectively [17]. Linear relationship of log P_{ow} versus log k was obtained for the 10 standard compounds and used as calibration curve. The log P_{ow} values for the compounds of interest were

Table 2
Log P_{ow} values measured by MEEKC vs. literature values for the literature compounds [1,5,18,19]

Compound	Log k (MEEKC)	Log P_{ow} (MEEKC)	Log P_{ow} (literature)	ΔLog P_{ow}	pK_a (CE)
Atenolol	−0.67	0.49	0.15	0.34	9.58
Pilocarpine	−0.57	0.66	0.20	0.46	7.08
Aniline	−0.43	0.88	0.94	−0.07	4.61
N-Methylaniline	−0.07	1.46	1.65	−0.20	4.86
Acebutolol	0.14	1.79	1.75	0.04	9.41
Procaine	0.29	2.02	2.03	−0.01	9.04
Quinoline	0.22	1.91	2.15	−0.24	4.97
Quinidine	0.71	2.71	2.64	0.07	4.5; 8.57
Buspirone	0.71	2.70	2.78	−0.08	7.6
Papaverine	0.61	2.54	2.91	−0.37	6.38
3-Bromoquinoline	0.82	2.88	2.91	−0.03	2.74
Propranolol	1.06	3.25	3.35	−0.10	9.53
Chlorpromazine	1.88	4.56	5.34	−0.78	9.24

calculated based on the measured log k and the calibration curve.

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

2.2.2. Log P_{ow} by potentiometric titration

The log P_{ow} values of the compounds were measured by potentiometric titration on a GLpKa or PCA101 titrator (Sirius Analytical Instruments, Forest Row, UK) in 0.15 *M* KCl aqueous solution under an argon atmosphere at 25.0±0.5 °C. The log P_{ow} values were calculated from the difference between aqueous pK_a and apparent p$_o K_a$ (in 1-octanol–water phase) and the volume ratio of 1-octanol–water. Detailed experimental procedures and discussions were described in the literature [18–20]. In general, the potentiometric titration method can measure log P_{ow} values of −2 to 6 with accuracy of less than 0.2 log units depending on the solubility and pK_a of the compounds.

2.2.3. pK_a by CE

For log P_{ow} determination in MEEKC, it is very important that the compounds are neutral at pH 10 and only the partitioning of the neutral form is involved. The pK_a values of the compounds were measured by CE to check if the microemulsion at pH 10 is suitable for log P_{ow} measurement of the selected compounds. The CE experiments were performed on the Agilent CE system [21]. The uncoated fused-silica capillary (L_t = 32 cm, L_d = 24 cm, 50 μm I.D.) was used and thermostated at 25.0 °C. Voltage of +15 kV and external air pressure of 25 mbar was applied for the CE separation. Samples with 0.5% (v/v) DMSO as the neutral marker were injected at 25 mbar for 3 s. The pK_a values were obtained from the non-linear regression of electrophoretic mobility as a function of pH using SigmaPlot (v 4.0) and corrected to zero ionic strength. In the MEEKC system (ionic strength ~0.1–0.2 *M*), the pK_a values for the bases could be 0.1–0.2 log units less than that obtained at zero ionic strength [21].

3. Results and discussion

3.1. Separation conditions

The migration time for dodecanophenone was more than 40 min when +20 kV was applied. In order to reduce the migration time without generating temperature gradient, external air pressure was applied. The migration time for dodecanophenone was reduced to less than 25 min by applying +20 kV plus 5 mbar that will give a throughput of 2 samples/h. Experimental results showed that external air pressure up to 10 mbar has no effect on the measurement of log k. However, further increases in air pressure will narrow the separation window,

which can cause separation problems for compounds with low and high log P_{ow} values and limit the range of log P_{ow} determination. Using the separation conditions of +20 kV plus 5 mbar, the relative standard deviation (RSD) for migration time measurement was less than 3% ($n=6$).

3.2. Calibration curve

Duplicate or triplicate runs for the mixture of 10 standard compounds (Table 1) were performed before and after the sequential run for the sample compounds to eliminate any outliers in calibration. The electrophoregram is shown in Fig. 1 for the separation of the 10 standard compounds. The average correlation coefficient of log P_{ow} versus log k for 12 injections is 0.99 with less than 3% RSD for the slope and intercept as shown in Eq. (2). In MEEKC separations, the migration behavior of a solute is very sensitive to the surface condition of the capillary. Variation of the slope and intercept is within 10% from day-to-day measurements. Therefore, calibration standards should be run in the same sequence with the samples to minimize errors. Based on the MEEKC separation in Fig. 1, pyrazine (log $P_{ow}=-0.26$) and pyrene (log $P_{ow}=4.88$) could set up the lower and upper limits for the determination of log P_{ow}. The range of log P_{ow} measurement by MEEKC could be increased by increasing the separation window when using longer capillary, lower voltage, and lower air pressure, while the throughput will be sacrificed.

$$\log P_{ow} = 1.57(\pm 0.04)\log k + 1.51(\pm 0.03) \quad (R^2 = 0.99, n = 12) \qquad (2)$$

3.3. Log P_{ow} determination for literature compounds

It was estimated that more than 75% of pharmaceutical drugs are weak bases or neutrals [22]. Therefore, log P_{ow} determination for bases has received more attention. Few applications of MEEKC were reported for log P_{ow} determination of weak acids [13,23]. In this work, only the basic compounds were selected for log P_{ow} determination. The MEEKC method was applied to the determination of log P_{ow} for 13 literature compounds with known log P_{ow} values from 0.15 to 5.34 [1,5,18,19]. More than 90% of the log P_{ow} values measured by MEEKC are within 0.5 log units of the literature values as shown in Table 2. Only chlorpromazine, a surface active compound [24], has much lower log P_{ow} value by MEEKC (4.56) than the literature log P_{ow} value (5.34).

3.4. Log P_{ow} determination for Roche compounds

The log P_{ow} values of 32 Roche compounds with diverse chemical structures have been measured both by MEEKC and potentiometric titration. Approximately 90% of the log P_{ow} values measured by MEEKC are within 0.5 log units of the values measured by potentiometric titration. Correlation of the log P_{ow} values measured by MEEKC with the log P_{ow} value from the literature and potentiometric titration is shown in Eq. (3). Good correlation ($R^2=0.846$, $n=45$) of log P_{ow} with log k was also observed for all literature and Roche compounds. These results show that MEEKC can give good estimation of log P_{ow} in the range of 0 to 5 log units.

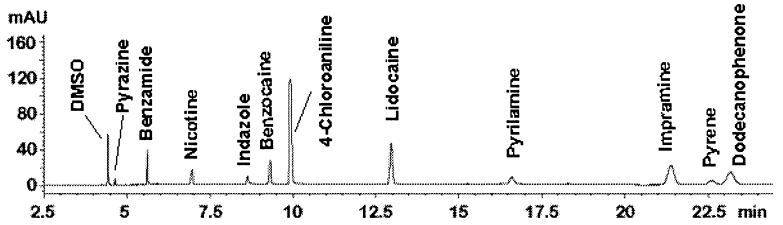

Fig. 1. Electropherogram for the mixture of 10 standard compounds. Capillary, $L_d=40$ cm, $L_t=50$ cm, I.D.=50 μm; separation, +20 kV, 5 mbar, 25.0 °C; injection, 25 mbar, 3 s; detection, 200 nm; microemulsion, 50 mM SDS, 0.87 M 1-butanol, 82 mM heptane, 50 mM borate–phosphate (2:3), pH 10; EOF marker, 0.2% (v/v) DMSO; micelle marker, 0.5 mg/mL dodecanophenone.

$$\log P_{ow}\,(\text{MEEKC}) = 0.86(\pm 0.05)\log P_{ow}\,(\text{reference}) + 0.5(\pm 0.1) \quad (R^2 = 0.873, n = 45) \tag{3}$$

Large discrepancies between MEEKC and potentiometric titration were observed for some compounds with log $P_{ow} \geq 5$ or p$K_a \geq 10$ (not included in Eq. (3)). For compounds with log $P_{ow} \geq 5$, the MEEKC separation from the micelle marker is hardly resolved, which results errors in log P_{ow} measurement. For basic compounds with p$K_a \geq 10$, the large discrepancy (0.5–1.0 log units) was caused by mixed partitioning mechanism [25]. In the microemulsion at pH 10, these basic compounds are partially positive charged. In addition to partitioning into the oil droplets, the positively charged solutes will have ion-pair interactions with the negatively charged oil droplets and surfactants to increase the migration time and also have electrophoretic mobility to reduce the migration time. The higher measured log P_{ow} values indicate that the ion-pair interaction is more significant than the electrophoretic mobility for the drug molecules. Correction of the charged fraction based on electrophoretic mobility measurement cannot be applied when ion-pair interaction is very strong [25].

3.5. Comparison with calculated log P_{ow}

There are about 30 to 50 commercially available programs for log P_{ow} calculation [26]. These calculation programs often generate different log P_{ow} values due to different data sets used for the calibration. In this work, Kow_clogP (calculated values of octanol–water partition coefficient) values were calculated for the literature and Roche compounds using KowWin (v1.57, Syracuse Research, North Syracuse, NY, USA). The KowWin program is based on the atom/fragment contribution method that is able to predict log P_{ow} within 0.8 log units [27]. About 65% of Kow_clogP values are within 0.5 log units of the log P_{ow} values of the literature and potentiometric titration. In general, Kow_clogP tends to over predict the log P_{ow} values. The calculated data are more scattered and the population of errors is less symmetric than the log P_{ow} values by MEEKC. Only 40% of the Kow_clogP values are within 0.5 log units of the measured log P_{ow} values by MEEKC. The correlation of the log P_{ow} values by MEEKC with the calculated Kow_clogP values is described in Eq. (4):

$$\log P_{ow}\,(\text{MEEKC}) = 0.56(\pm 0.04)\text{Kow\_clogP} + 1.0(\pm 0.1) \quad (R^2 = 0.813, n = 56) \tag{4}$$

In drug discovery support, experimental measurements are needed to verify the calculation results and screen for low log P_{ow} compounds. One of the disadvantages of potentiometric titration for log P_{ow} measurement is also inherited from its principle that requires the measurement of pK_a shift from aqueous phase to 1-octanol–aqueous phase. A group of 20 Roche compounds with Kow_clogP values of 5.2–8.5 were selected for testing the applicability of MEEKC. Measurement of the log P_{ow} values for these compounds by potentiometric titration will be difficult and time consuming due to their poor solubility, low pK_a (2–6), and high lipophilicity. However, these compounds are soluble in the microemulsion system. MEEKC was able to rapidly measure and estimate the log P_{ow} values for these compounds without accurately measuring pK_a. For compounds with Kow_clogP of 5.2–7.2, the MEEKC measured log P_{ow} values are 1.2–3.0 log units less. For compounds with Kow_clogP $>$ 7.2, the log P_{ow} values can not be measured by MEEKC and are expected to be greater than 5.

4. Conclusion

It was demonstrated that pressure-assisted MEEKC is applicable for log P_{ow} screening of weakly basic, weakly acidic, and neutral pharmaceuticals. The current MEEKC method is simple, rapid, and reproducible. It has some advantages over the potentiometric titration method, such as small sample size ($<$0.1 mg), low purity and aqueous solubility requirement for the compounds, and applicability for compounds with low or without pK_a.

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