

Validation and Long-Term Assessment of an Approach for the High Throughput Determination of Lipophilicity ($\log P_{OW}$) Values Using Multiplexed, Absorbance-Based Capillary Electrophoresis

KIT-SUM WONG, JEREMY KENSETH, ROY STRASBURG

CombiSep, Inc., 2711 South Loop Drive, Suite 4200, Ames, Iowa 50010

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ABSTRACT: A critical evaluation of the use of 96-capillary multiplexed microemulsion electrokinetic chromatography (MMEEKC) for the indirect determination of octanol–water partition coefficients ($\log P_{OW}$ values) for a wide range of structurally different compounds is presented. The various components of the microemulsion solution were evaluated and optimized for use in a multiplexed capillary format. A six-component calibration mixture and 23 different solutes ($n = 4$ each) were analyzed simultaneously, providing a throughput of up to 46 samples/h, which translates to greater than a 20-fold improvement over existing indirect $\log P_{OW}$ methods. Agreement to within $\pm 0.5 \log P$ units of literature values was obtained for 51 of the 54 tested neutral and basic (uncharged) solutes. A linear free energy relationship (LFER) analysis performed on the MMEEKC system supports its use as a viable and effective model of the classical shake-flask method for $\log P_{OW}$ determinations. Moreover, a standard deviation of 0.1 or less $\log P$ units was obtained for 35 of 36 solutes analyzed repeatedly over an 8-month time period, documenting the long-term effectiveness of the analysis format. Critical comparisons between the proposed MMEEKC method and existing separation methods for the indirect determination of $\log P_{OW}$ values are also made. Overall, the results indicate that 96-capillary MMEEKC can serve as a high throughput, cost effective and robust approach and as a valid model for $\log P_{OW}$ determinations. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 93:916–931, 2004

Keywords: octanol–water partition coefficient; capillary electrophoresis; multiplexed; $\log P$; high throughput technologies

INTRODUCTION

The early-stage determination of various physicochemical properties such as the octanol–water partition coefficient ($\log P$), ionization constant (pK_a), solubility, and membrane permeability can minimize extensive and expensive testing of new chemical entities (NCEs) that possess unsatisfac-

tory pharmaceutical properties, thereby reducing overall attrition rates in drug discovery.¹ The evolution of parallel synthetic technologies has enabled the rapid creation of large quantities of unique compounds in a short period of time. This capability has subsequently prompted a quest for effective high throughput analytical methods to determine the pharmaceutical properties of compounds early in the drug discovery pipeline.

Capillary electrophoresis (CE) with UV absorption detection at 214 nm has emerged as a valuable tool for the analysis of pharmaceuticals. This situation reflects the unique attributes of CE, which include rapid analysis time, high separation

Correspondence to: Jeremy Kenseth (Telephone: 515-294-6567; Fax: 515-294-7141);
E-mail: jeremy.kenseth@combisep.com

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efficiency, small sample size, low solvent consumption, and near universal detection. In many cases, CE has been increasingly used as an alternative or complementary technique to high-performance liquid chromatography (HPLC). An additional unique feature of CE is the ability to substantially increase sample throughput by performing concurrent analysis in a parallel 96-capillary array format. Multiplexed CE in a 96-capillary format with UV detection has been successfully employed for a variety of applications.²⁻⁷ Achieving a comparable increase in throughput is, however, far more challenging in HPLC, where pressure requirements, column sizes, packing materials, and detector design have severely limited the development of highly multiplexed systems.

As a subset of CE, electrokinetic chromatography (EKC) methods, such as micellar electrokinetic chromatography (MEKC),⁸⁻¹² have gained in popularity for the indirect estimation of compound $\log P_{OW}$ values (the partition coefficient in the 1-octanol/water system). The $\log P_{OW}$ values of compounds have been traditionally determined directly by the shake-flask technique, and this method is the "gold standard" for characterizing the lipophilicity of a drug compound and for establishing structure-activity relationships.¹³⁻¹⁷ Compared to the shake-flask method, MEKC has the advantages of speed, high sample throughput, wide dynamic range, small sample size, and tolerance of sample impurities.

Other EKC techniques, such as vesicular electrokinetic chromatography (VEKC)^{18,19} and liposome electrokinetic chromatography (LEKC),²⁰ have been applied for $\log P_{OW}$ determinations. However, vesicle and liposome systems are in general more difficult to reproducibly prepare and maintain than micelle systems.

An alternative EKC method that has shown great promise for the indirect determination of $\log P_{OW}$ values is microemulsion electrokinetic chromatography (MEEKC). MEEKC uses a microemulsion as a migrating pseudophase. Microemulsions are immiscible oil droplets of most often heptane or octane in water that are stabilized by surfactants and cosurfactants positioned on the surface of the droplet.²¹ Sodium dodecyl sulfate (SDS) is most commonly used as the surfactant and 1-butanol as a cosurfactant. High pH buffers are generally used for $\log P_{OW}$ analysis because the majority of drugs are weak bases and thus will be present in their neutral form. At high pH, an electroosmotic flow (EOF) is present in the capillary due to the ionized silanol groups of the

capillary wall and is directed from the anode to the cathode. The microemulsion will have an overall negative charge, and thus be attracted to the anode. However, the EOF at high pH is greater than the electrophoretic flow of the microemulsion, so the microemulsion will eventually migrate to the cathode for detection.²¹ With proper selection of composition and preparation, microemulsions are thermodynamically stable and optically transparent at low UV wavelengths.

The separation mechanism in MEEKC is based on the differential partitioning of solutes into the migrating microemulsion pseudophase, and the partitioning of solutes is related to solute hydrophobicity.²² Therefore, the migration time of a solute is directly proportional to its hydrophobicity, i.e., solute capacity factor ($\log k'$) can be directly related to solute $\log P_{OW}$ value. Once a mathematical correlation between $\log k'$ and $\log P_{OW}$ is established on a set of solutes with known $\log P_{OW}$ values, the correlation can be applied to determine the $\log P_{OW}$ of other solutes.

To determine the $\log k'$ it is necessary to accurately measure the migration times of the EOF and microemulsion along with the solute (see eq. 1). Dimethylsulfoxide (DMSO) has no interaction with the microemulsion and is therefore commonly used as an EOF marker. Conversely, a highly hydrophobic solute such as dodecylbenzene that is exhaustively taken up into the microemulsion is used as a microemulsion marker (ME marker). By taking advantage of this two internal marker approach for standardization, MEEKC has been successfully used to determine $\log P_{OW}$ values of structural homologs and structurally diverse solutes at pH 7,²² at extreme pH (pH 1 for acids and pH 12 for bases),^{23,24} and at pH 3 (acids) or pH 10 (bases).^{25,26} In these studies it has been demonstrated that MEEKC can be applied to determine the $\log P_{OW}$ values of solutes ranging from -1.0 to 6.6.

Compared to MEKC, MEEKC has better solubilization properties for working with insoluble compounds²⁴ and is more flexible when manipulating the migration window.²⁷ The migration window is defined as the space between the EOF marker and ME marker. Increasing the migration window will increase the migration time ratio of the microemulsion to the EOF (t_{me}/t_{eof}), which is known to increase the separation resolution of neutral solutes.^{28,29} In addition, the influence of hydrogen bonding during $\log P_{OW}$ analysis in MEEKC was minimal compared to MEKC or RP-HPLC, which may arise from the presence of the

1-butanol cosurfactant. MEEKC has been shown to provide a better correlation with the octanol/water system in partitioning behavior compared to MEKC.²² MEEKC has neither the pH, column degradation, and homologous series limitations of HPLC nor the disadvantages of shake-flask methods (i.e., large sample size, lack of automation, long turnaround, and pure sample requirements).

In a recent study,³⁰ we introduced the use of multiplexed-MEEKC (MMEEKC) with UV detection at 214 nm for indirect $\log P_{OW}$ determinations and demonstrated with a series of solutes covering a wide range of $\log P_{OW}$ values that MMEEKC could serve as a high throughput, rapid, accurate, and reproducible method for $\log P_{OW}$ determinations. The present article extends the development of this technique by critically evaluating the operating parameters suitable for MMEEKC $\log P_{OW}$ analysis and assessing the long term performance of the method. Due to the significant differences in instrument design between single-capillary CE systems and multiplexed-capillary CE, technology transfer from a single capillary to a multiplexed system often involves adjustments to a variety of operating variables. In this article, the limitations and criteria for designing a suitable microemulsion buffer system for MMEEKC are addressed and the individual components used in microemulsion systems are evaluated and optimized for MMEEKC $\log P_{OW}$ application. A linear free energy relationship (LFER) analysis has been performed using solvation parameters to verify that the proposed MMEEKC method encodes similar information to the classical shake-flask technique. In addition, a comprehensive study has been undertaken over an 8-month time period to evaluate long-term system reliability and accuracy, and comparisons of the proposed MMEEKC method to existing indirect $\log P_{OW}$ analysis methods are presented and discussed.

EXPERIMENTAL

Chemicals

Naphthalene was purchased from Acros Organics (Fair Lawn, NJ) and SDS (electrophoresis grade), 1-butanol (HPLC grade) and *n*-heptane (HPLC grade) were from Fisher Scientific (Fair Lawn, NJ). All other chemicals were from Sigma-Aldrich (Milwaukee, WI). Deionized water was obtained from a Synergy Ultrapure Water System (Millipore, Benford, MA).

Microemulsion Solution Preparation

Microemulsions were prepared according to the following procedures unless otherwise noted. Into a 1.00-L volumetric flask, 66.0 g of 1-butanol, 8.0 g of *n*-heptane, and 33.0 g of SDS were added in order. The mixture was swirled gently before and after the addition of 200 mL of 68 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer at pH 10.3. Additional CAPS buffer was then added to bring the solution volume to 900 mL. The solution was sonicated for 30 min and was allowed to stand at room temperature for at least 1 h before diluting to mark with the CAPS buffer. The microemulsion was then passed through a 0.45- μ m nylon filter membrane prior to use.

Sample solutions were prepared by adding solutes at an approximate concentration of 0.5 mg/mL or 0.5 μ L/mL into a microemulsion solution containing 25 μ L/mL DMSO and 0.5 μ L/mL dodecylbenzene. Prepared samples were sonicated for 5 min to 1 h depending on solute stability and solubility.

Pyrazine (6 mg/mL), benzamide (0.5 mg/mL), nicotine (0.5 μ L/mL), quinoline (0.25 μ L/mL), naphthalene (0.25 mg/mL), and imipramine (0.5 mg/mL) were used as standards. Standards were prepared following the same procedures used for the sample solutions.

Capillary Electrophoresis

The MMEEKC experiments were performed on a MCE 2000TM instrument (CombiSep, Ames, IA) utilizing UV detection at 214 nm. A 96-capillary array was composed of fused-silica capillaries with 75 μ m i.d., 150 μ m o.d., and 76 cm length (50 cm effective length). Prior to initial use, the capillary array was conditioned with 0.1 N NaOH for 5 min, water for 10 min and microemulsion buffer for 10 min in sequence. The capillary array was rinsed with microemulsion for 10 min between analyses, washed three times with deionized water for 5 min each at the end of a work day, and rinsed with 0.1 N HCl as needed to restore separation efficiency. Importantly, the same capillary array was used over an 8-month time period without a notable decrease in performance. Samples were arranged in a 96-well plate and injected by applying a vacuum at -0.2 psi for 20–25 s. Separations were performed at 6.5 kV. A VACE (vacuum-assisted CE) level of up to -0.1 psi was applied during the separation. MCE Manager[®] software (CombiSep, Inc., Ames, IA)

was employed for system control, data collection, and data processing.

Calculation of MMEEKC $\log k'$ and $\log P_{OW}$ Values

Solute capacity factors ($\log k'$ values) were calculated according to eq. 1:²⁷

$$k' = \frac{t_s - t_{eof}}{t_{eof}(1 - t_s/t_{me})} \quad (1)$$

where t_{eof} , t_s , and t_{me} are the migration times of the electroosmotic flow (DMSO), solute, and microemulsion (dodecylbenzene), respectively. Calibration curves were constructed by plotting MMEEKC determined $\log k'$ values versus literature $\log P_{OW}$ values of the standard solutes to establish the mathematical correlation between $\log P_{OW}$ and $\log k'$, which is given in eq. 2.

$$\log P_{OW} = A \times \log k' + B \quad (2)$$

In eq. 2, A is the slope and B is the y-intercept. The $\log P_{OW}$ values of sample solutes analyzed in the same CE run were subsequently obtained by applying the MMEEKC determined $\log k'$ value into eq. 2.

MCE $\log P$ CalculatorTM software (CombiSep, Inc., Ames, IA) was developed to facilitate the rapid transformation of CE data into solute $\log P_{OW}$ values. The program automatically calculates solute capacity factors ($\log k'$ values) according to eq. 1; determines the values of A and B in eq. 2 from MMEEKC $\log k'$ values and the entered literature $\log P_{OW}$ values of the standard solutes; and calculates the MMEEKC $\log P_{OW}$ values of the unknown solutes by substituting the MMEEKC $\log k'$ of the solute into eq. 2.

RESULTS AND DISCUSSION

Design of a Microemulsion Suitable for use in Multiplexed CE

As mentioned above, the principal components of a microemulsion buffer are oil droplets, surfactant, cosurfactant, and aqueous buffer. The ideal microemulsion buffer for use in a multiplexed CE system should be thermodynamically stable, generate low current, have low absorption at 214 nm, and provide a wide migration window. This section will discuss how each microemulsion component was evaluated for use in a multiplexed CE format.

The most common oil phase and cosurfactant used in microemulsion buffers are 0.8% (w/v)

n-heptane and 6.6% (w/v) 1-butanol. Although octane has been reported to give more reproducible microemulsions³¹ *n*-heptane is often chosen as the oil phase because it has a lower toxicity than octane.³² In addition, the $\log P_{OW}$ values obtained using microemulsion systems with *n*-heptane as the oil phase provide good correlations to the octanol–water system.^{22,24–26,33}

Studies on microemulsion cosurfactants have suggested that the increase in solubility of hydrophilic and hydrophobic solutes with microemulsions relative to micelles arises from the capability of the alkyl chains of normal alcohols to incorporate into the micellar phase. This incorporation loosens the micelle structure, allowing increased access for the partitioning of analytes into the oil droplet.²⁴ Although normal alcohols with alkyl chains shorter than 1-butanol barely distributed to the micellar phase,³⁴ those with alkyl chains longer than 1-butanol yielded thermodynamically unstable microemulsions.²² Therefore, 0.8% (w/v) *n*-heptane and 6.6% *n*-butanol were kept as basic ingredients in the microemulsion used for our $\log P_{OW}$ analysis via MMEEKC.

Several buffer systems including 3-(cyclohexylamino) propanesulfonic acid (CAPS), Tris (hydroxymethyl) aminomethane (TRIS), 4-(cyclohexylamino)-1-butananesulfonic acid (CABS), and phosphate/borate were evaluated for use in MMEEKC. Phosphate/borate buffers generated relatively high currents, inducing Joule heating over time. Although manageable in various single-capillary CE systems, high-current generation is a major concern in a multiplexed CE system where up to 96 capillaries are aligned in parallel. The multiplexed CE system used herein relies on air as the heat-exchanging medium. High operating currents (>40 μ A per capillary) can lead to baseline instability and irregular solute electrophoretic mobility, both of which can decrease the accurate determination of $\log P_{OW}$ values. TRIS, CABS, and CAPS are zwitterionic buffers and thus generate relatively low currents. However, TRIS buffers have limited buffering capacity at higher pH (pH 10), where most basic compounds are in their neutral form. The CABS buffer ($pK_a = 10.7$) has excellent buffering capacity at high pH values, but was found to induce turbidity in the sample solutions of several tested solutes. We suspect that this turbidity is due to the longer alkyl chain of CABS relative to CAPS.

The CAPS buffer best met all the requirements for MMEEKC. It is a low conductivity zwitterionic buffer, which enables the use of high buffer

concentrations to prevent buffer depletion without increasing Joule heating. It has a high pK_a value (10.4),³⁵ which renders an optimum buffering capacity at higher pH values. As a consequence, CAPS buffer yields a relatively stable baseline at 214 nm and the operating conditions are highly compatible with the multiplexed CE system.

The surfactant is a critical ingredient for generating stable microemulsions and controlling the length of the separation window in MEEKC. Although the most widely utilized microemulsion solution is composed of 1.4% (w/v) SDS,^{22,24–26,36} reports have shown that when the SDS concentration is reduced to 2% (w/v) or below, the microemulsion can become unstable.^{22,37} Therefore, to preserve the stability and shelf life of the microemulsion, the SDS concentration was held at levels above 2% (w/v). However, SDS is an anionic surfactant, and increasing its concentration will increase the migration time of the microemulsion. This situation can be addressed by adding a nonionic surfactant such as Brij 35 to lower the microemulsion charge-to-size ratio.³⁸ Attempts to exploit this possibility revealed that microemulsion solutions containing 2.3% SDS and 0.75% Brij 35 in fact yielded shorter microemulsion migration times. Unfortunately, the separation efficiencies of solutes using this type of microemulsion preparation were much lower than those for microemulsions prepared without Brij 35 (data not shown).

Although higher SDS concentrations will increase the migration window and enhance the stability of the microemulsion, lower concentrations of SDS will reduce the operational current and allow the use of higher field strengths (E = applied voltage/capillary length) to increase the separation efficiency. Two MMEEKC systems have been evaluated for $\log P_{OW}$ analysis: one with higher SDS concentration (3.3% w/v SDS), higher buffer concentration (68 mM CAPS at pH 10.3), and lower field strength (85.5 V/cm); and a second with lower SDS concentration (2.2% w/v SDS), lower buffer concentration (34 mM CAPS at pH 10.3), and higher field strength (111.8 V/cm). Both MMEEKC buffers performed equally well for the determination of $\log P_{OW}$ values (data not shown). A microemulsion buffer with 2.2% SDS (w/v) was employed in our previous study for MMEEKC $\log P_{OW}$ analysis.³⁰ We have found, however, that a microemulsion with a higher buffer and SDS concentration can provide increased buffer capacity and a wider separation window,²² notably favoring the separation of high $\log P_{OW}$ solutes. Consequently, a microemulsion

buffer containing 0.8% (w/v) *n*-heptane, 6.6% (w/v) 1-butanol, 3.3% (w/v) SDS, and 92% (v/v) 68 mM CAPS at pH 10.3 was selected for this study.

Indirect $\log P_{OW}$ Analysis via MMEEKC

As demonstrated in our previous report,³⁰ a unique feature of using multiplexed MEEKC for $\log P_{OW}$ analysis is that standards and samples can be analyzed simultaneously in a single CE run. This attribute serves to minimize run-to-run systematic errors due to variations in running buffer, applied voltage, or temperature. Standards can be analyzed individually or as a mixture to maximize sample throughput.

To develop a generalized approach for determining $\log P_{OW}$ values using MMEEKC, it is vital to establish a universal calibration curve to effectively characterize solutes with diverse structures and lipophilicities. Therefore, calibration standards should not be limited to the same structural analog, must encompass a wide range of $\log P_{OW}$ values, and must be chemically and physically stable and neutral in the selected microemulsion system. Solute such as benzene, toluene, and propylbenzene are not suitable standards, as their volatility limits their shelf life and large batches of standard solutions cannot be prepared for continuous use. As a consequence, standard solutes selected for this study (with literature $\log P_{OW}$ values³⁹) were pyrazine (−0.26), benzamide (0.64), nicotine (1.17), quinoline (2.03), naphthalene (3.30), and imipramine (4.42). These standards were found to be stable for at least 3 months when prepared in the microemulsion buffer and stored at 4°C. In general, the concentration of each standard was approximately 0.5 mg/mL. However, pyrazine was used at a concentration of 6 mg/mL to achieve a response sufficiently large for accurate peak identification and quinoline and naphthalene were lowered to 0.25 mg/mL to avoid obscuring the smaller peaks of the other solutes. The adjustments in solute concentrations were due to differences in the molar absorptivities of the standard compounds.

Figure 1 shows the typical sample arrangement in a 96-well plate used for MMEEKC $\log P_{OW}$ analysis. The standard mixture and 23 different sample solutions were each loaded in four consecutive wells of the tray. For example, the standard mixture was loaded in wells A1, B1, C1, and D1, and ethyl *p*-aminobenzoate sample was loaded in E1, F1, G1, and H1. A portion of a representative 96-capillary electropherogram displaying each

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	standard mixture											
D		pyrimidine	caffeine	2-aminopyridine	aniline	benzylamine	coumarin	phenyl acetate	α -methylbenzylamine	indazole	3,5-lutidine	nitrobenzene
E												
F												
G	ethyl p-aminobenzoate											
H		4-chloroaniline	procaine	3,5-methylaniline	1-aminonaphthalene	licocaine	ethyl benzoate	netopam	pyrilamine	propylbenzene	tetracaine	pyrene

Figure 1. Typical sample arrangement of the standard mixture and 23 different solutes in a 96-well plate used for MMEEKC analysis. Each sample was loaded into four consecutive wells and subjected to four replicate, simultaneous analyses. Solutes utilized in standard mixture were (with literature $\log P_{OW}$ values): pyrazine (-0.26), benzamide (0.64), nicotine (1.17), quinoline (2.03), naphthalene (3.30), and imipramine (4.42).

sample as analyzed by MMEEKC is shown in Figure 2. The first and last peaks of each electropherogram are the EOF marker (DMSO) and ME marker (dodecylbenzene), respectively. It is evident from Figure 2 that the absolute migration times of the EOF and ME markers varied from capillary to capillary. However, these variations are minimized through the normalization of k' via eq. 1.

The MMEEKC $\log k'$ value for each standard and solute sample was calculated according to eq. 1. The average MMEEKC $\log k'$ values from the four replicate standard analyses were used for the $\log P_{OW}$ calibration. The calibration was accomplished by plotting the $\log k'$ against the corresponding literature $\log P_{OW}$ value, yielding a linear calibration curve. The resulting calibration curve is shown in Figure 3, which fit a linear equation of $\log P_{OW} = 1.657 \log k' + 1.088$, with a R^2 of 0.982. The calibration curve was then utilized to calculate the $\log P_{OW}$ values of the other 23 solutes based on their averaged ($n = 4$) MMEEKC $\log k'$ values.

These results are summarized in Table 1, which lists the MMEEKC determined $\log k'$ and $\log P_{OW}$ values of 54 tested solutes along with the differences between the MMEEKC $\log P_{OW}$ values and available literature $\log P_{OW}$ values ($\Delta \log P_{OW}$).

This table shows not only that deviations of the $\log k'$ values for 47 of the 54 tested solutes obtained in the same run were within $\pm 0.03 \log k'$ units, but also that 51 out of 54 MMEEKC determined $\log P_{OW}$ values were in agreement to within $\pm 0.5 \log P_{OW}$ units of their corresponding literature $\log P_{OW}$ values, a $\Delta \log P_{OW}$ generally considered to be satisfactory when considering the variation of reported values in the literature.^{25,39–42}

Of the 54 solutes examined, three were found to give $\Delta \log P_{OW}$ values greater than $\pm 0.5 \log P_{OW}$ units. Both bifonazole (MMEEKC $\log P_{OW} = 4.21 \pm 0.15$; literature $\log P_{OW} = 4.77$) and fluoranthene (MMEEKC $\log P_{OW} = 4.77 \pm 0.06$; literature $\log P_{OW} = 5.16$) possess relatively high $\log P_{OW}$ values and are above the highest calibration standard used (imipramine, literature $\log P_{OW} = 4.42$). The agreement with literature $\log P_{OW}$ values for bifonazole and fluoranthene may be improved by using a calibration mixture that contains a solute with a $\log P_{OW}$ value above 5 to extend the calibration span. In contrast, estradiol ($\Delta \log P_{OW} = -0.86$) has a literature pK_a value of 10.4 due to the presence of a phenolic group. Estradiol therefore exists in both its neutral and anionic forms at pH 10.3. The presence of ionic mobility, combined with the potential for electrostatic repulsion between estradiol and the negatively charged microemulsion, likely account for the discrepancy observed between the MMEEKC and literature $\log P_{OW}$ values. We note that the difficulty with estradiol serves as a good example of one constraint of the current MMEEKC method. That is, the solute of interest should be present in its neutral form. To effectively evaluate the $\log P_{OW}$ value for estradiol, a low pH buffer system should be employed. Although several of the basic solutes in the tested data set may also have been slightly ionized at pH 10.3, no significant deviation greater than $\pm 0.5 \log P_{OW}$ units was observed.

Figure 4 shows a plot of the residuals between the MMEEKC determined $\log P_{OW}$ values and literature $\log P_{OW}$ values as a function of $\log P_{OW}$ value. As noted, several tested solutes with $\log P_{OW}$ values close to 5 are underestimated using the current set of calibration standards. Overall, however, Figure 4 shows that there is no predominant underlying bias for the $\Delta \log P_{OW}$ value across the tested $\log P_{OW}$ range, as the differences are randomly scattered both above and below literature values. Taken together, the results from Figures 1–4 and Table 1 demonstrate that our MMEEKC methodology can serve as a highly

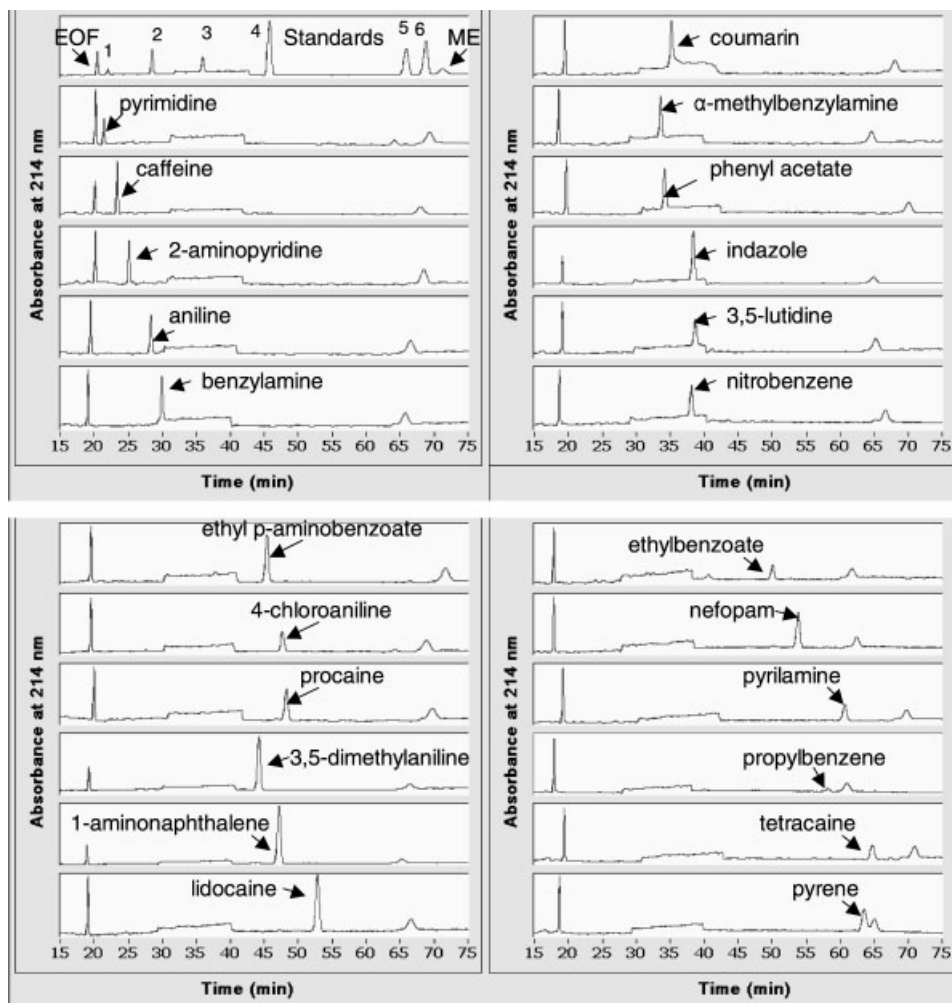


Figure 2. Representative MMEEKC electropherograms obtained from the simultaneous analysis of the standard mixture and 23 solutes arranged in a 96-well plate as shown in Figure 1. The first and last peaks in each electropherogram were DMSO (EOF marker) and dodecylbenzene (ME marker), respectively. The migration order of the standards was as follows: 1. pyrazine; 2. benzamide; 3. nicotine; 4. quinoline; 5. naphthalene; 6. imipramine.

effective strategy for determining accurate $\log P_{OW}$ values of compounds in a highly parallel fashion.

Linear Free Energy Relationship (LFER) Analysis

The shake-flask method is the classical standard for determining the partition coefficient of compounds⁴³ and an extensive database of $\log P_{OW}$ values has been developed.^{16,39,44} To further assess whether the proposed MMEEKC system can serve as a viable alternative to the shake-flask system for $\log P_{OW}$ determinations, a LFER analysis was performed. The LFER is determined by using the solvation parameters described by

Abraham.⁴⁵ The general LFER equation is presented in eq. 3:^{45,46}

$$\log SP = c + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2 + vV_x \quad (3)$$

where SP is a property for a series of solutes in a fixed solvent system, R_2 is the excess molar refraction, π_2^H is the solute dipolarity/polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2$ are the solute overall effective hydrogen-bond acidity and basicity, and V_x is the McGowan characteristic volume.⁴⁷ The constant r is the tendency of the phase to interact through π - and n -electron pairs, s is the phase dipolarity-polarizability, a is a measure of the difference in basicity between water and octanol, b is the phase hydrogen-bond acidity, and v is a measure of the

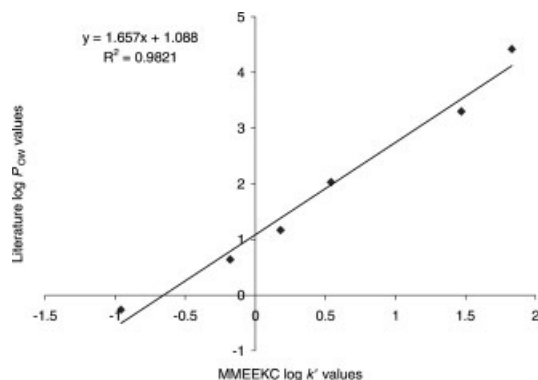


Figure 3. Calibration curve constructed from the averaged ($n = 4$) MMEEKC $\log k'$ values of solutes in the standard mixture and their literature $\log P_{OW}$ values. Data were obtained from the run shown in Figure 2.

difference between the hydrophobicity or lipophilicity of the two phases.⁴⁵ For solutes that have variable hydrogen-bond basicity, Abraham et al.⁴⁶ suggested that $\Sigma\beta_2^O$ be used in the MEEKC system.

The LFER equation based on the shake-flask $\log P_{OW}$ values for 613 solutes has been reported:^{46,48}

$$\log P_{OW} = 0.088 + 0.562R_2 - 1.054\pi_2^H + 0.034\Sigma\alpha_2^H - 3.460\Sigma\beta_2 + 3.481V_x \quad (4)$$

where $N = 613$, $R = 0.997$, $R^2 = 0.995$, $s = 0.116$, and $F = 223162$.

We note that it has been previously stated that it is sufficient that the ratios of the system constants when normalized by division with v are in close agreement to those in the shake-flask method when comparing methods.⁴⁶ Normalization with v was therefore used to authenticate the relationship between the MMEEKC and shake-flask methods.

A valid set of solutes for the LFER study should span a variety of solute types and have no significant crosscorrelation among the solute descriptors, and no clustering of individual descriptor values should be observed.^{45,49} There are 37 solutes listed in Table 1 that have solute descriptors available from the literature,^{26,40,45,46,50–52} and their values are listed in Table 2. The linear regression correlations among the solvent descriptors were calculated and are also listed in Table 2. These solutes therefore possess a wide range of structural properties as evidenced by the diverse solvation parameters. The linear regression values suggest that there is no significant

correlation among the descriptors. Thus the set of 37 solutes are satisfactory for the LFER study.

To further demonstrate that the selected 37 solutes are appropriate for validation of the MMEEKC system, the LFER (eq. 5) based on the shake-flask $\log P_{OW}$ values for the 37 solutes listed in Table 2 was evaluated:

$$\begin{aligned} \log P_{OW} = & 0.133 (\pm 0.057) + 0.523 (\pm 0.049)R_2 \\ & - 1.072 (\pm 0.055)\pi_2^H + 0.051 (\pm 0.106)\Sigma\alpha_2^H \\ & - 3.431 (\pm 0.062)\Sigma\beta_2 + 3.849 (\pm 0.071)V_x \end{aligned} \quad (5)$$

where $N = 37$, $R = 0.997$, $R^2 = 0.995$, $s = 0.126$, and $F = 1142$.

The system constant ratios of eq. 5 are in good agreement with those of eq. 4 (Table 3). Therefore, the 37 solute series can be used to reasonably represent the 613 solute series in eq. 4.

The LFER equation based on the MMEEKC $\log P_{OW}$ values for the 37 solutes is expressed in eq. 6:

$$\begin{aligned} \text{MMEEKC } \log P_{OW} = & 0.345 (\pm 0.121) + 0.474 (\pm 0.104)R_2 \\ & - 1.062 (\pm 0.118)\pi_2^H + 0.014 (\pm 0.225)\Sigma\alpha_2^H \\ & - 3.136 (\pm 0.133)\Sigma\beta_2 + 3.569 (\pm 0.150)V_x \end{aligned} \quad (6)$$

where $N = 37$, $R = 0.986$, $R^2 = 0.972$, $s = 0.269$, and $F = 214$.

The system constant ratios of eq. 6 for the MMEEKC system are very close to those of the shake-flask method (Table 3). The system constant ratios of LFERs for other reported validated systems, RP-HPLC⁴⁰ and single capillary MEEKC,^{26,46} are also listed in Table 3 for comparison. No appreciable differences exist between the various methods and the shake-flask method. These results further argue that MMEEKC using the microemulsion buffer system described herein is a viable alternative to the shake-flask method for $\log P_{OW}$ determinations.

Long-Term Performance Assessment of the MMEEKC Method

Our previous article reported on the short-term reliability and reproducibility of a similar MMEEKC system over a three day period.³⁰ To demonstrate the long-term reliability and reproducibility of the proposed MMEEKC system, the $\log k'$ values and $\log P_{OW}$ values for a group of

Table 1. Data^a Obtained Using MMEEKC

Solute	MMEEKC $\log k^b$	MMEEKC $\log P_{\text{OW}}^c$	Literature $\log P_{\text{OW}}^d$	$\Delta \log P_{\text{OW}}$
Acebutolol	0.40 ± 0.00	1.74 ± 0.01	1.71	0.03
1-Aminonaphthalene	0.72 ± 0.01	2.29 ± 0.01	2.25	0.04
2-Aminopyridine	-0.42 ± 0.01	0.40 ± 0.01	0.49	-0.09
Aniline	-0.10 ± 0.01	0.91 ± 0.02	0.90	0.01
Anthracene ^e	2.14 ± 0.08	4.63 ± 0.14	4.45	0.18
Benzamide	-0.18 ± 0.00	0.78 ± 0.01	0.64	0.14
Benzylamine	0.01 ± 0.01	1.10 ± 0.02	1.09	0.01
Bifonazole	1.89 ± 0.03	4.21 ± 0.15	4.77	-0.56
Butylbenzene	1.98 ± 0.02	4.35 ± 0.11	4.38	-0.03
Caffeine	-0.60 ± 0.01	0.08 ± 0.01	-0.07	0.15
Carbamazepine	0.76 ± 0.01	2.34 ± 0.01	2.45	-0.11
Chloramphenicol	0.19 ± 0.01	1.39 ± 0.02	1.14	0.25
4-Chloroaniline	0.64 ± 0.05	2.16 ± 0.08	1.88	0.28
Chlorpromazine	2.21 ± 0.02	4.74 ± 0.03	5.19	-0.45
Chlorthalidone	0.06 ± 0.01	1.17 ± 0.01	0.85 ^f	0.32
Coumarin	0.21 ± 0.01	1.44 ± 0.02	1.39	0.05
3,5-Dimethylaniline	0.60 ± 0.02	2.09 ± 0.03	2.17 ^g	-0.08
Estradiol	1.25 ± 0.02	3.15 ± 0.04	4.01	-0.85
Ethyl <i>p</i> -aminobenzoate	0.56 ± 0.01	2.02 ± 0.02	1.86	0.16
Ethylbenzene	1.49 ± 0.03	3.54 ± 0.06	3.15	0.39
Ethylbenzoate	0.99 ± 0.01	2.72 ± 0.02	2.64	0.08
Fluoranthene	2.07 ± 0.04	4.47 ± 0.06	5.16	-0.69
Hydrocortisone	0.59 ± 0.01	2.04 ± 0.01	1.61	0.43
Hydrocortisone-21-acetate	0.67 ± 0.04	2.17 ± 0.07	2.19	-0.02
Hydroquinine	1.31 ± 0.07	3.24 ± 0.12	3.43 ^g	-0.19
Imipramine	1.83 ± 0.01	4.13 ± 0.02	4.42 ^h	-0.29
Indazole	0.36 ± 0.02	1.69 ± 0.04	1.77	-0.08
Lidocaine	0.94 ± 0.01	2.65 ± 0.01	2.26	0.39
2,4-Lutidine	0.30 ± 0.01	1.58 ± 0.01	1.90 ^g	-0.32
3,5-Lutidine	0.40 ± 0.01	1.74 ± 0.01	1.78	-0.04
α -Methylbenzylamine	0.21 ± 0.01	1.45 ± 0.01	1.49 ^g	-0.05
2-Methylbenzylamine	0.33 ± 0.01	1.62 ± 0.02	1.62 ^g	0.00
3-Methylbenzylamine	0.45 ± 0.01	1.82 ± 0.02	1.62 ^g	0.20
Metronidazole	-0.68 ± 0.01	-0.05 ± 0.02	-0.02	-0.03
Naphthalene	1.47 ± 0.01	3.52 ± 0.02	3.30	0.22
Nefopam	1.17 ± 0.01	3.02 ± 0.01	3.05 ^g	-0.03
Nicotine	0.18 ± 0.00	1.39 ± 0.00	1.17	0.22
Nifedipine	0.96 ± 0.01	2.66 ± 0.01	3.17 ⁱ	-0.51
Nitrobenzene	0.40 ± 0.02	1.75 ± 0.02	1.85	-0.10
Nitrofurazone	-0.21 ± 0.01	0.74 ± 0.01	0.23	0.51
Pentoxifylline	-0.25 ± 0.01	0.67 ± 0.01	0.29	0.38
Phenanthrene	1.92 ± 0.05	4.37 ± 0.08	4.46	-0.14
Phenyl acetate	0.16 ± 0.01	1.35 ± 0.02	1.49	-0.01
Procaine	0.67 ± 0.01	2.20 ± 0.01	1.92	0.28
Propylbenzene	1.66 ± 0.03	3.85 ± 0.04	3.72	0.13
Pyrazine	-0.96 ± 0.01	-0.50 ± 0.00	-0.26 ^h	-0.24
Pyrene	2.00 ± 0.04	4.40 ± 0.07	4.88	-0.48
Pyrilamine	1.22 ± 0.01	3.12 ± 0.01	3.27	-0.15
Pyrimidine	-1.06 ± 0.00	-0.67 ± 0.01	-0.40	-0.27
Quinidine	1.03 ± 0.03	2.77 ± 0.05	2.88	-0.11
Quinine	1.10 ± 0.02	2.89 ± 0.03	2.64	0.25
Quinoline	0.54 ± 0.01	1.98 ± 0.01	2.03	-0.05

(Continued)

Table 1. (Continued)

Solute	MMEEKC $\log k'$ ^b	MMEEKC $\log P_{OW}$ ^c	Literature $\log P_{OW}$ ^d	$\Delta \log P_{OW}$
Tetracaine	1.43 ± 0.01	3.46 ± 0.01	3.73	-0.27
Toluene	1.02 ± 0.02	2.77 ± 0.04	2.73	0.04

^aAverage of at least four replicates ± standard deviation in a single run.

^bCalculated from equation:

$$k' = \frac{t_s - t_{eof}}{t_{eof}(1 - t_s/t_{me})}$$

where t_{eof} , t_s , and t_{me} are the migration times of the DMSO, solute, and dodecylbenzene, respectively.

^cCalculated from equation: $\log P_{OW} = A \times \log k' + B$, where A and B were slope and y-intercept, respectively, of standard calibration curve constructed from literature $\log P_{OW}$ values versus $\log k'$ values of solutes in standard mixture.

^dFrom ref. 39 unless otherwise noted.

^eAverage of three replicates ± standard deviation in a single run.

^fFrom ref. 32.

^gCalculated value from ref. 42.

^hFrom ref. 25.

ⁱFrom ref. 40.

solutes were analyzed repeatedly using different batches of microemulsion preparations (>10) over a period of 8 months. The results of these analyses are summarized in Table 4. The MMEEKC $\log k'$ values of the majority of solutes (27 of the 36 solutes tested) had standard deviations of 0.05 $\log k'$ units or less and only pyrene exceeded a standard deviation of 0.1 $\log k'$ units. It is noted that the slopes and intercepts of the calibration curves were different from run to run. These differences are most likely due to batch-to-batch variations in microemulsion compositions. This variability, however, does not significantly affect the accuracy of the MMEEKC $\log P_{OW}$ determination as shown by the small standard deviations in Table 4 for which 33 of the 36 solutes tested were 0.1 $\log P$ unit or less. The observed level of reproducibility is attributed in part to the fact that the MMEEKC $\log P_{OW}$ values were calculated from calibration curves constructed during the same

run, thereby minimizing the systematic error. As mentioned previously, extending the calibration span by adding a standard solute with a $\log P_{OW}$ value above 5 should improve the accuracy of the MMEEKC $\log P_{OW}$ determination for high $\log P_{OW}$ compounds.

Comparison of MMEEKC to Indirect Methods for $\log P_{OW}$ Analysis

For absolute accuracy, direct methods for $\log P_{OW}$ analysis such as the shake-flask method or stir-flask method are considered as "gold standard" methods. However, in today's modern pharmaceutical environment, speed is paramount in early discovery screening applications, and often a slight decrease in accuracy while substantially increasing throughput is an acceptable trade-off. In such situations, indirect $\log P_{OW}$ methods such as RP-HPLC, MEKC, MEEKC, and MMEEKC are clearly appropriate. A further advantage of these indirect approaches over the direct flask methods is the ability to separate sample impurities that may potentially interfere with the $\log P_{OW}$ measurement.

The throughput characteristics of various indirect methods are compared in Table 5. It is noted that none of these indirect methods offer a single generic approach for measuring the $\log P_{OW}$ values of acidic, neutral, and basic solutes with good accuracy. In general, anionic solutes have very different chromatographic behavior from cationic and neutral solutes and thus acidic solutes often require the development of a separate subset of analytical conditions as compared to basic and neutral solutes.

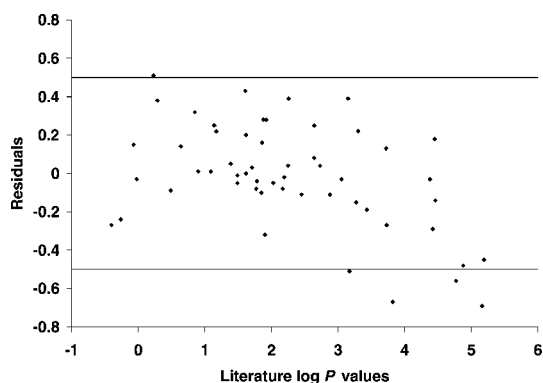


Figure 4. Plot of residuals versus literature $\log P_{OW}$ value.

Table 2. Solvation Descriptors of Solutes

Solute	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^a$	V_x	Ref. ^b
1-Aminonaphthalene	1.670	1.26	0.20	0.57	1.185	52
Aniline	0.955	0.94	0.26	0.50	0.816	52
Anthracene	2.290	1.34	0.00	0.26	1.454	52
Benzamide	0.990	1.50	0.49	0.67	0.973	52
Benzylamine	0.829	0.88	0.10	0.72	0.987 ^c	50
Bifonazole	2.41	2.25	0.00	1.12	2.501	40
Butylbenzene	0.600	0.51	0.00	0.15	1.280	52
Caffeine	1.50	1.60	0.00	1.33	1.363	40
Carbamazepine	2.15	2.07	0.52	1.13	1.811	40
Chloramphenicol	1.85	0.72	0.34	2.09	2.073	40
4-Chloroaniline	1.060	1.13	0.30	0.35	0.939	52
Coumarin	1.06	1.79	0.00	0.46	1.062	26
Estradiol	1.80	1.77	0.86	1.10	2.199	40
Ethyl <i>p</i> -aminobenzoate	1.040	1.52	0.32	0.64	1.313 ^c	45
Ethylbenzene	0.613	0.51	0.00	0.15	0.998	52
Ethylbenzoate	0.689	0.85	0.00	0.46	1.214	52
Fluoranthene	2.377	1.53	0.00	0.20	1.585	52
Hydrocortisone	2.03	3.49	0.71	1.90	2.798	40
Hydrocortisone-21-acetate	1.89	2.88	0.46	2.16	3.095	40
Imipramine	1.480	1.75	0.00	1.19	2.402	26
Indazole	1.180	1.35	0.54	0.30	0.905 ^c	50
3,5-Lutidine	0.659	0.79	0.00	0.44	0.957 ^c	50
Metronidazole	1.05	1.60	0.18	1.03	1.192	40
Naphthalene	1.340	0.92	0.00	0.20	1.085	52
Nicotine	0.865	0.75	0.00	1.14	1.371	26
Nifedipine	1.50	2.45	0.23	1.45	2.495	40
Nitrobenzene	0.871	1.11	0.00	0.28	0.891	52
Nitrofurazone	1.65	1.79	0.40	1.08	1.264	40
Pentoxifylline	1.64	2.28	0.00	1.84	2.083	40
Phenanthrene	2.055	1.29	0.00	0.26	1.454	52
Phenylacetate	0.661	1.13	0.00	0.54	1.072	26
Propylbenzene	0.604	0.50	0.00	0.15	1.139	52
Pyrazine	0.629	0.95	0.00	0.61	0.634	46
Pyrene	2.808	1.71	0.00	0.29	1.585	52
Pyrimidine	0.606	1.00	0.00	0.65	0.634	46
Quinoline	1.268	0.97	0.00	0.51	1.044 ^c	50
Toluene	0.601	0.52	0.00	0.14	0.857	46
Linear regression correlation matrix						
R_2	1					
π_2^H	0.391	1				
$\Sigma\alpha_2^H$	0.068	0.251	1			
$\Sigma\beta_2$	0.127	0.442	0.200	1		
V_x	0.422	0.600	0.150	0.579	1	

^aFor compounds that have variable basicity, $\Sigma\beta_2^0$ were used.^bReferences of solute solvation descriptors.^cCalculated according to ref. 47.

RP-HPLC was initially considered to be an unreliable method for estimating the $\log P_{OW}$ values of chemically unrelated solutes due to the interaction of solutes with the stationary phase. Recently, improvements have been made to over-

come this problem by coating the silica surface with an artificial membrane⁸ or by adding octanol to the mobile phase.^{40,53} The advantage of these improved RP-HPLC methods is that distribution coefficients of ionic solutes ($\log D$) at pH 7.4 can be

Table 3. System Constant Ratios in the LFER Solvation Equation

System	r/v	s/v	a/v	b/v	v	Reference
Octanol–water ($n = 613$)	0.15	-0.28	0.01	-0.91	1	48
Octanol–water ($n = 37$)	0.14	-0.27	0.01	-0.89	1	This work
RP-HPLC	0.12	-0.27	-0.01	-0.89	1	40
MEEKC (pH 7)	0.09	-0.23	-0.02	-0.92	1	46
MEEKC (pH 10)	0.17	-0.24	—	-0.92	1	26
MMEEKC	0.13	-0.30	0.00	-0.88	1	This work

Table 4. Long-Term Performance Characteristics of MMEEKC $\log k'$ and $\log P_{OW}$ Values Collected over a Period of 8 Months

Solute	n^a	MMEEKC $\log k'$		MMEEKC $\log P_{OW}$		Literature $\log P_{OW}^b$	$\Delta \log P_{OW}$
		Avg. \pm Std.	%RSD	Avg. \pm Std.	%RSD		
Acebutolol	42	0.41 \pm 0.03	7.32	1.80 \pm 0.04	2.22	1.71	0.09
1-Aminonaphthalene	37	0.71 \pm 0.03	4.23	2.31 \pm 0.03	1.30	2.25	0.06
2-Aminopyridine	34	-0.41 \pm 0.01	2.44	0.41 \pm 0.01	2.44	0.49	-0.08
Aniline	36	-0.12 \pm 0.02	16.67	0.90 \pm 0.02	2.22	0.90	0.00
Anthracene	6	2.09 \pm 0.10	4.78	4.54 \pm 0.17	3.74	4.45	0.09
Benzamide	50	-0.17 \pm 0.02	11.76	0.81 \pm 0.02	2.47	0.64	0.17
Caffeine	35	-0.59 \pm 0.02	3.39	0.11 \pm 0.07	63.64	-0.07	0.18
4-Chloroaniline	36	0.62 \pm 0.03	4.84	2.16 \pm 0.04	1.85	1.88	0.28
Chlorpromazine	7	2.21 \pm 0.04	1.81	4.74 \pm 0.06	1.27	5.19	-0.61
Chlorthalidone	38	0.07 \pm 0.02	28.57	1.22 \pm 0.05	4.10	0.85	0.37
Coumarin	26	0.22 \pm 0.02	9.09	1.48 \pm 0.05	3.38	1.39	0.09
3,5-Dimethylaniline	15	0.57 \pm 0.03	5.26	2.04 \pm 0.05	2.45	2.17	-0.13
Ethyl <i>p</i> -aminobenzoate	36	0.40 \pm 0.09	22.50	1.78 \pm 0.15	8.43	1.86	-0.08
Ethylbenzene	6	1.49 \pm 0.01	0.67	3.54 \pm 0.01	0.28	3.15	0.39
Ethylbenzoate	38	0.97 \pm 0.04	4.12	2.75 \pm 0.04	1.45	2.64	0.11
Hydroquinine	42	1.26 \pm 0.06	4.76	3.23 \pm 0.10	3.10	3.43	-0.20
Imipramine	52	1.86 \pm 0.08	4.30	4.23 \pm 0.08	1.89	4.42	-0.19
Indazole	46	0.38 \pm 0.03	7.89	1.75 \pm 0.08	4.57	1.77	-0.02
Lidocaine	36	0.89 \pm 0.04	4.49	2.62 \pm 0.03	1.15	2.26	0.36
2,4-Lutidine	12	0.31 \pm 0.01	3.23	1.60 \pm 0.03	1.88	1.9	-0.3
3,5-Lutidine	14	0.42 \pm 0.02	4.76	1.77 \pm 0.03	1.69	1.78	-0.01
α -Methylbenzylamine	8	0.24 \pm 0.03	12.50	1.48 \pm 0.04	2.70	1.49	-0.01
2-Methylbenzylamine	12	0.34 \pm 0.02	5.88	1.65 \pm 0.03	1.82	1.62	0.03
3-Methylbenzylamine	9	0.47 \pm 0.02	4.26	1.86 \pm 0.04	2.15	1.62	0.24
Naphthalene	53	1.36 \pm 0.07	5.15	3.40 \pm 0.09	2.65	3.30	0.10
Nefopam	32	1.14 \pm 0.05	4.39	3.04 \pm 0.04	1.32	3.05	-0.01
Nicotine	53	0.18 \pm 0.02	11.11	1.40 \pm 0.02	1.43	1.17	0.23
Nitrobenzene	35	0.40 \pm 0.02	5.00	1.79 \pm 0.04	2.23	1.85	-0.06
Phenanthrene	13	1.92 \pm 0.06	3.13	4.29 \pm 0.11	2.56	4.46	-0.17
Phenylacetate	36	0.18 \pm 0.02	11.11	1.41 \pm 0.03	2.13	1.49	-0.08
Pyrazine	53	-0.96 \pm 0.01	1.04	-0.51 \pm 0.03	5.88	-0.26	-0.25
Pyrene	8	2.21 \pm 0.23	10.41	4.75 \pm 0.38	8.00	4.88	-0.13
Pyrilamine	35	1.18 \pm 0.06	5.08	3.11 \pm 0.05	1.61	3.27	-0.16
Pyrimidine	36	-1.05 \pm 0.02	1.90	-0.67 \pm 0.03	4.48	-0.4	-0.3
Quinoline	53	0.54 \pm 0.03	5.56	2.00 \pm 0.04	2.00	2.03	-0.03
Tetracaine	38	1.42 \pm 0.07	4.93	3.52 \pm 0.10	2.84	3.73	-0.21

^aNumber of analyses.^bSee Table 1.

Table 5. Comparison of Sample Throughput among Indirect Methods for $\log P_{OW}$ Analysis

Method	Average Analysis Time per Sample (min)	Approximate Throughput (Samples/h)	Ref.
RP-HPLC	20	3	40, 53
MEKC	15	4	10
MEEKC	18–23	2–3	54
	—	100 (per week)	25
	30	2	55
MMEEKC	1.25	46 ^a	This work

^aFour out of 96 capillaries are used for the standard mixture.

measured for basic and neutral solutes with good accuracy.⁵³ However, many reversed-phase columns are not stable at $\text{pH} > 9$, limiting their utility for the determination of the $\log P_{OW}$ values of basic compounds with high pK_a values. In addition, an initial estimation of $\log P_{OW}$ values is utilized to presort solutes into one of three lipophilicity ranges to identify an appropriate group of mobile phases for the solutes.^{40,53} Each group is composed of mobile phases with three different percentages of organic solvent (e.g., methanol), and a three-point extrapolation is constructed to determine the k' at zero percentage of organic solvent.^{40,53} The resulting capacity factors strongly correlated with the $\log P_{OW}$ values. However, the analysis of each sample required 20 min, limiting the throughput to 3 samples/h⁵³ (Table 5).

The micelles and microemulsions employed in electrokinetic chromatography are permanently charged, and therefore are not applicable for the determination of $\log D$ values of charged solutes. However, much success has been achieved in employing these methods for $\log P_{OW}$ determination of neutral solutes. The bare uncoated fused silica capillaries employed for MEKC and MEEKC are more tolerable to extreme pH values compared to reversed phase columns and thus are readily adapted to the experimental conditions required for maintaining acidic or basic solutes in their neutral forms. The two single-capillary CE methods, MEKC¹⁰ and MEEKC,^{25,54,55} have a sample throughput ranging from two to four samples/h, which are similar to that of the RP-HPLC method (Table 5).

In contrast, our multiplexed MEEKC $\log P_{OW}$ method can dramatically increase the throughput by up to 20 \times over the reported single channel approaches while still providing high quality data. Using the 96-well plate sample loading format shown in Figure 2 and assuming a total analysis

time of 2 h per plate (including flushing cycles and data analysis), a throughput of 46 sample analyses/h can be achieved (with four capillaries used for the standard mixture). It is also anticipated the MMEEKC method will be economically favorable, as the modest cost of reagents should translate to a cost per sample of less than one dollar.

CONCLUSION

We have demonstrated that multiplexed, absorbance-based CE can provide a high throughput, cost effective, and accurate method for indirect $\log P_{OW}$ determination when operated in a MEEKC mode. The favorable correlation obtained between MMEEKC $\log k'$ values and literature shake-flask $\log P_{OW}$ values and a LFER analysis supports the use of MMEEKC as a viable and effective model of the classical shake-flask method for $\log P_{OW}$ determinations. Moreover, the high throughput nature of multiplexed CE provides an approach to literally conduct hundreds of analyses per day, and with proper handling and maintenance, capillary arrays can provide reproducible and accurate performance for at least an 8-month time period. We note that it is also possible to evaluate the pK_a values of compounds in a high throughput manner using the same instrument platform, by simply exchanging capillary arrays and buffer solutions.⁵⁶

This study has focused on the use of MMEEKC for the $\log P_{OW}$ analysis of neutral and slightly basic compounds (pK_a values < 9.5). MEEKC is, however, not suitable for determining the partition coefficients of charged compounds due to the difficulties introduced by solute electrophoretic migration and ionic interactions with the charged microemulsions. Therefore, for acidic solutes, a buffer system of low pH should be used to assure the tested solutes are present in their neutral

form. Future work will be focused on the application of MMEEKC at low pH for the high throughput log P_{OW} analysis of acidic solutes, and on expanding the scope of tested solutes to additional compounds of pharmaceutical interest.

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