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Indirect determination of octanol–water partition coefficients by microemulsion electrokinetic chromatography

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Abstract

Microemulsion electrokinetic chromatography (MEEKC) was assessed and developed as a screening tool for the indirect determination of octanol–water partition coefficients. The capacity factors from MEEKC were correlated to the octanol–water partition coefficients. The same microemulsion (50 m*M* SDS, 400 m*M* butanol and 32 m*M* heptane) was used at pH 1.19 and pH 12 allowing most compounds to be run in their neutral state. This procedure was evaluated using a set of 24 structurally diverse solutes and 4 homologs. It was found that MEEKC can determine a range of over 5 orders of magnitude in the log K_{ow} covering from −1 to over 4. MEEKC provides all the advantages of an HPLC system to estimate lipophilicities including automation, small sample size, short run and analysis times and good reproducibility. However, MEEKC has neither the disadvantages of HPLC including pH limitations, column degradation and homologous series limitations nor the disadvantages of shake flask methods including large sample size, no automation and long turnaround.

Keywords: Microemulstion electrokinetic chromatography; Octanol–water partition coefficients; Lipophilicity

1. Introduction

The modern discovery process for new xenobiotic materials and pharmaceuticals is a multi-disciplinary approach using a wide variety of scientific expertise. In the last 25 years, hydrophobic, electronic and steric properties of new bioactive compounds have been recognized as important for bioactive compound design and optimization. Thousands of quantitative structure-activity relationships (QSARs) have been developed relating the log of the octanol–water partition coefficient (log K_{ow}) and perhaps some other physical parameter such as pK_a, to bioactivity [1]. For example, Klier and co-workers have modeled and correlated phloem mobility of a series compounds in several structural classes to pK_a values and membrane permeabilities [2]. They extrapolated the model even further and related the membrane permeability to the octanol–water partition coefficient for efficient screening and modeling purposes. Users of this model will thus plot optimum mobility versus pK_a and log K_{ow} to determine the desired physical properties of a future potential herbicide. Indeed, the octanol–water partition coefficient is the most commonly used index of lipophilicity and has become the de-facto standard to which other methods of lipophilicity are compared.

Direct K_{ow} measurements can be time consuming, expensive and require relatively large amounts of scarce test solutes. As a means of simplifying the measurement of K_{ow}, LC-determined capacity factors have been used commonly as a predictive tool via a Collander relationship [3]. However, any LC procedure suffers from some very limiting constraints.

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For example, most LC procedures are limited to pH values between 2 and 7. Also, the variability of stationary phases over time requires frequent calibrations. In addition, users are subject to the manufacturing constraints of both the silica and column producers which includes "continuous improvement" of the manufacturing process (leading to changes in selectivity), discontinuation of selected phases and the financial health of the producer. Separations are often multimodal, not just relying on the bonded phase. Hence, LC generated indices of lipophilicity are undesirable although they have been useful for many short term studies.

A method based on solution chemistry provides the best technique for generating a lipophilicity index value because it is inherently reproducible relative to a technique that relies on a solution–surface interaction. Solutions can be made reproducibly whereas surfaces are more difficult to make and maintain consistently. With this in mind, other groups have correlated the capacity factors, k, in micellar electrokinetic chromatography (MEKC) or microemulsion electrokinetic chromatography (MEEKC) to K_{ow} [4–10]. The best correlations in these studies were obtained with MEEKC. However, the MEEKC work presented was done over a pH range of 7 to 9.2 thus limiting the utility of this lipophilicity measurement for QSAR. Indeed, K_{ow} values are reported here as the neutral species because many biological transport properties are driven by pH gradients; knowing the pK_a and an index of the lipophilicity of the neutral solute can thus serve to model these solutes' behavior. In this paper, we extend the usefulness of the MEEKC generated capacity factors by using a combination of structural homologs and structurally dissimilar analytes at extreme pH regions where nearly all solutes of interest can be characterized as neutral species.

2. Experimental

2.1. Apparatus

A Beckman model 5010 P/ACE capillary electrophoresis unit with a photodiode array detector scanning from 190 to 290 nm and monitoring at 225 nm was used with 2 modes of operation depending on the pH of the microemulsion. For the pH 1.19 runs, a 27 cm×25 μm uncoated fused-silica column with a 75 mm pathlength window (Hewlett-Packard) was used at an electric field of −5 kV. For the pH 12 runs, a 57 cm×50 μm fused-silica column (Polymicro Technologies, Phoenix, AZ, USA) was used at 24 kV. All runs were at 25°C. Buffer pH was measured on an Orion EA940 ion analyzer (ATI, Boston, MA, USA) using a Ross pH electrode.

2.2. Sample and buffer preparation

The microemulsions were prepared by blending butanol and heptane and then adding a solution of sodium dodecyl sulfate (SDS) and adjusting the solution pH with concentrated solutions of HCl or NaOH. The resulting mixture was diluted with more acid or base to the appropriate concentration volume yielding a stable and clear solution. The microemulsion in the pH 12 experiments consisted of 50 mM SDS, 400 mM butanol and 32 mM heptane in 0.01 M NaOH. The microemulsion in the pH 1.19 experiments consisted of 50 mM SDS, 400 mM butanol and 32 mM heptane in 0.1 M HCl. The samples were prepared at a concentration of 0.2 to 2 mg per ml using the microemulsion made with water (instead of NaOH or HCl solutions) as the solvent. All solutions were filtered with a 0.45 μm membrane filter before use. All water used was ASTM type I quality from a Barnstead Nanopure II system.

2.3. Method calculations

At pH 12, each capacity factor was calculated from the time of the electroosmotic flow marker, t_o, the migration time of the solute, t, and the migration time of a solute fully partitioned into the micelle, t_m [11]

$$k = \frac{t - t_o}{t_o\left(1 - \frac{t}{t_m}\right)} \quad (1)$$

Since the pH 1.19 method was operated at a negative polarity, the solute which fully partitions into the micelle was the first to elute. The electroosmotic flow marker compound was never detected because the flow direction was from the detector to the

Table 1
Microemulsion pH 12 data used to determine and characterize the elution window

Solute	log K_{ow} [13]	Migration time (min)
Urea	−2.11	7.80
Formamide	−1.51	7.80
Dimethylsulfoxide	−1.34	7.80
Dimethylformamide	−1.01	7.91
Benzamide	0.64	9.79
Benzyl alcohol	1.10	10.15
Benzene	2.13	13.93
Toluene	2.73	20.33
Ethylbenzene	3.15	27.33
Propylbenzene	3.72	33.94
Butylbenzene	4.38	37.15
Hexylbenzene	5.52	39.05
Octylbenzene	6.34	39.05
Dodecylbenzene	7.40	39.05

injection end of the column. Several runs were made at pH 1.19 with positive polarity thus aligning the flow from injector to detector and the electroosmotic flow marker eluted at 19.2 min. Negative 19.2 min was taken as the t_o and equation 1 was used to calculate the capacity factor.

3. Results

3.1. Measurements at pH 12

The pH 12 microemulsion was selected to determine the capacity factors of bases and neutrals. The choice of appropriate markers for solutes fully partitioned or fully excluded from the microemulsion was determined in this mixture by running compounds over a wide range of K_{ow} values. The results in Table 1 indicate that hexylbenzene and formamide, urea or dimethylsulfoxide serve as appropriate markers for the elution window of neutral compounds. As the log K_{ow} increased, the elution time became a constant starting with hexylbenzene and thus hexylbenzene serves as the t_m. As the log K_{ow} decreased, the elution time also becomes constant starting with dimethylsulfoxide and thus it may be used for t_o. At times, dimethylformamide was used as the electroosmotic flow marker and a capacity factor of 0.0177, calculated from Table 1, was used to determine the actual time of the electroosmotic flow. Fig. 1 shows a representative pherogram including some of the solutes used in a retention index [12] approach. Modeling the capacity factors via the retention index concept was reported to be independent of the micelle concentration in micellar electrokinetic chromatography. This concept has the possible advantage of leading to a more rugged method for lipophilicity indexing. We have not applied this approach for relating retention index to K_{ow} because of the arbitrary nature of assigning an index to compounds outside of a homologous series such as benzaldehyde, benzamide and benzyl alcohol. The approach is useful for quality control purposes. The log of the capacity factors are presented in Table 2 for a series of test solutes at pH 12.

3.2. Measurements at pH 1.19

The pH 1.19 microemulsion was selected to determine the capacity factors of acids and neutral

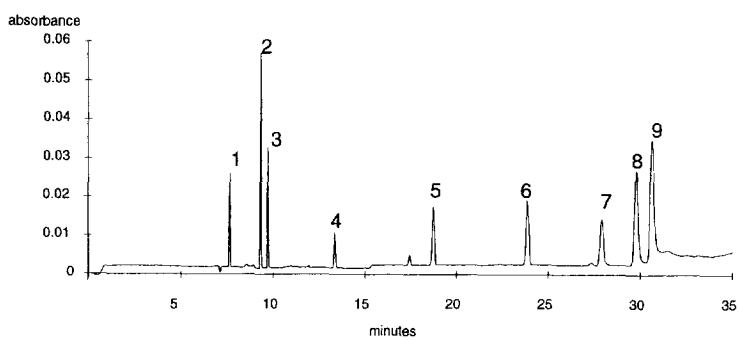

Fig. 1. Separation of migration index markers in pH 12 microemulsion. 1 = dimethylformamide, 2 = benzamide, 3 = benzylalcohol, 4 = benzene, 5 = toluene, 6 = ethylbenzene, 7 = propylbenzene, 8 = butylbenzene, 9 = hexylbenzene.

Table 2
Capacity factor determinations in pH 12 microemulsion

Solute	log k	Log K_{ow} [13–15]	pK_a
Dimethylformamide	−1.75	−1.01	
Benzamide	−0.47	0.64	
Benzyl alcohol	−0.38	1.10	
Benzene	0.13	2.13	
Toluene	0.58	2.73	
Ethylbenzene	0.99	3.15	
Propylbenzene	1.48	3.72	
Butylbenzene	2.02	4.38	
Biphenyl	1.72	4.09	
Spinosad [14]	2.22	5.20	8.36
Isoxaben [15]	−0.75	0.94	1.30
Naphthalene	1.18	3.30	
Quinoline	0.27	2.03	4.81

solutes at a pH low enough for the acids which we encounter in our work to be neutral. The capacity factors determined on this system are in Table 3.

A pherogram of a retention index mixture is in Fig. 2. The peaks are wider in this system than the pH 12 system. The system was operated within the linear portion of a current versus voltage plot and thus, Joule heating was not likely to be the reason for this problem. We believe the more likely reason for the broad peaks lies in the high ionic strength of the pH 1.19 system. The ionic strength of the pH 1.19 system is 10 times that of the pH 12 system. The higher ionic strength aqueous solution results in a greater partitioning into the organic phase. Indeed, capacity factors in the pH 1.19 system were roughly twice that of those in the pH 12 system. The

Table 3
Capacity factor determinations in pH 1.19 microemulsion

Solute	log k	log K_{ow} [13,14]	pK_a
Benzamide	−0.14	0.64	
Benzyl alcohol	−0.07	1.10	
Benzene	0.50	2.13	
Toluene	0.97	2.73	
Ethylbenzene	1.39	3.15	
Propylbenzene	1.79	3.72	
Butylbenzene	2.30	4.38	
Acetylsalicylic acid	0.01	1.19	3.48
Anthranilic acid	0.08	1.21	2.29, 4.59
Benzoic acid	0.23	1.85	4.21
p-Chlorobenzoic acid	0.87	2.55	3.82
Clopyralid [14]	0.02	1.06	2.30
Metosulam [14]	0.58	2.39	5.34
3,6-Dichloro-*o*-anisic acid	0.60	2.21	1.90
2,4-Dichlorophenoxyacetic acid	0.99	2.81	2.60
2,6-Dimethoxybenzoic acid	−0.19	0.66	3.44
Fluroxypyr [14]	0.53	1.74	2.94
Phenol	−0.03	1.42	10.00
3-Phenoxybenzoic acid	1.59	3.91	3.95
Phthalic acid	−0.24	0.73	2.95, 5.41
Salicylic acid	0.36	2.20	3.08
Thymol	1.14	3.33	10.31
Triisopropylbenzoic acid	1.89	3.86	3.41

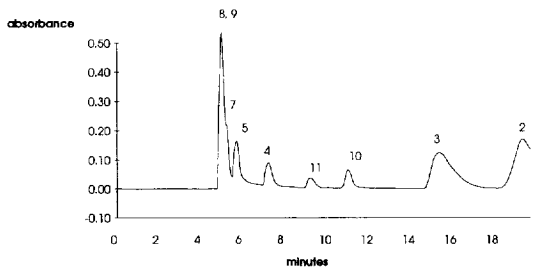

Fig. 2. Separation of migration index markers in pH 1.19 microemulsion. 2 = benzamide, 3 = benzylalcohol, 4 = benzene, 5 = toluene, 6 = ethylbenzene, 7 = propylbenzene, 8 = butylbenzene, 9 = hexylbenzene, 10 = benzaldehyde, 11 = benzoic acid.

enhanced partitioning into the organic phase may also, in part, be due to a larger sized emulsion. These ionic strength related phenomena limit the number of theoretical exchanges between the phases. Hence, ionic strength may be limiting the efficiency. A more complete study of this phenomena such as investigating similar column dimensions and field strengths and techniques to characterize the micelle such as light scattering was outside of the scope of our investigation and thus our explanation should be considered preliminary.

3.3. Prediction of the octanol–water partition coefficient

Figs. 3 and 4 contain graphs of the K_{ow} versus the microemulsion capacity factors from Table 2 and

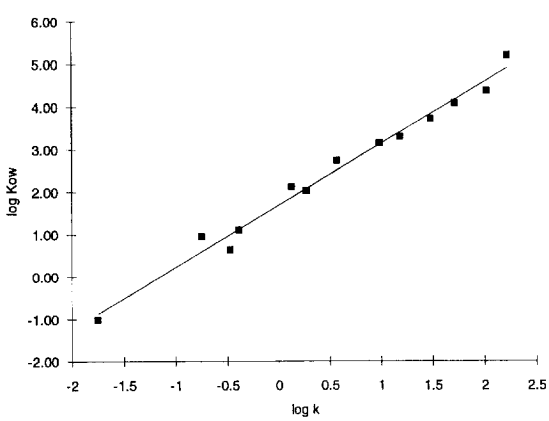

Fig. 3. Octanol–water partition coefficient versus microemulsion capacity factors for pH 12 system.

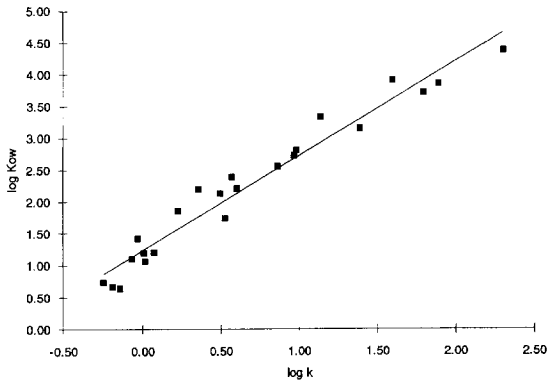

Fig. 4. Octanol–water partition coefficient versus microemulsion capacity factors for pH 1.19 system.

Table 3. Acids, bases and non-ionizable substances were used in this solute set; the capacity factors were determined at a pH where the solutes were not ionized. When used for log K_{ow} prediction, the following equation is determined by linear regression for pH 12:

$$\log (K_{ow}) = 1.46(\pm 0.05) \cdot \log (k) + 1.68(\pm 0.07)$$
$$r^2 = 0.98 \quad n = 13 \qquad (3)$$

For pH 1.19 a similar equation was determined:

$$\log (K_{ow}) = 1.49(\pm 0.07) \cdot \log (k) + 1.23(\pm 0.07)$$
$$r^2 = 0.96 \quad n = 23 \qquad (4)$$

The numbers in parentheses are the standard errors of the coefficients. Inspection of the residuals from both regressions combined (Fig. 5) shows an error

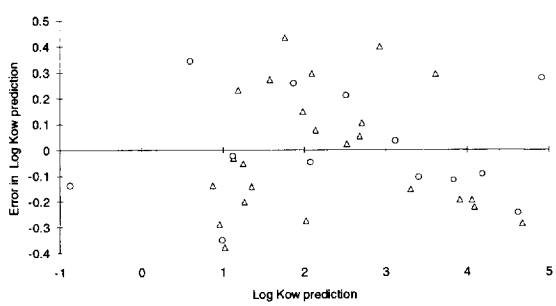

Fig. 5. Residuals plot of error in K_{ow} prediction at pH 1.19 (△) and pH 12 (o) versus K_{ow} as determined by the linear regression of the data plotted in Figs. 3 and 4.

range of 0.40 to −0.38 for pH 1.19 and 0.34 to −0.35 for pH 12 in log K_{ow} units. The overall standard deviation of the error for both datasets combined was 0.22.

4. Conclusions

A solute's K_{ow} may be indirectly determined by MEEKC at pH 1.19 and 12. At these extremes, all but the strongest acids and bases are neutral (unionized) which is important for use in developing a QSAR. The microemulsion investigated which mimics the octanol–water system consisted of 50 m*M* SDS, 400 m*M* butanol and 32 m*M* heptane. The capacity factors determined from a set of structurally diverse solutes with a wide range of K_{ow} values were found to have a first order linear relationship with the K_{ow}. The technique was demonstrated to give accurate predictions over a log K_{ow} range of between 0.6 and 4.4 at pH 1.19 and between −1 and 4.4 at pH 12. The range in the determination error was about ±0.4 log K_{ow} units and the overall standard deviation was 0.22.

References

[1] C. Hansch and A. Leo, Exploring QSAR, American Chemical Society, Washington, DC, Vol. 1, 1995.
[2] B.T. Grayson and D.A. Kleier, Pestic. Sci., 30 (1990) 67.
[3] R. Collander, Acta. Chem. Scand., 5 (1951) 774.
[4] M. Greenaway, G. Okafo, D. Manallack and P. Camilleri, Electrophoresis, 15 (1994) 1284.
[5] A. Schmutz and W. Thormann, Electrophoresis, 15 (1994) 1295.
[6] Y. Ishihama, Y. Oda, K. Uchikawa and N. Asakawa, Chem. Pharm. Bull., 42 (1994) 1525.
[7] Y. Ishihama, Y. Oda, K. Uchikawa and N. Asakawa, Anal. Chem., 67 (1995) 1588.
[8] B.J. Herbert and J.G. Dorsey, Anal. Chem., 67 (1995) 744.
[9] J.T. Smith and D.V. Vinjamoori, J. Chromatogr. B, 669 (1995) 59.
[10] S. Yang and M.G. Khaledi, Anal. Chem., 67 (1995) 499.
[11] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 834.
[12] P.G.H.M. Muijselaar, H.A. Claessens and C.A. Cramers, Anal. Chem., 66 (1994) 635.
[13] C. Hansch and A. Leo, Exploring QSAR, American Chemical Society, Washington, DC, Vol. 2, 1995.
[14] M.H. Benkö, DowElanco, unpublished results.
[15] C. Tomlin (Editor), The Pesticide Manual, British Crop Protection Council and The Royal Society of Chemistry, Cambridge, 1994, p. 614.