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Characterization of tetradecyltrimethylammonium bromide (TTAB) microemulsion by microemulsion electrokinetic chromatography.

Caracterización de una microemulsión de bromuro de tetradeciltrimetilamonio (TTAB) por cromatografía electrocinética de microemulsiones.

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REPORT

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1. SUMMARY

The aim of this work is to characterize a tetradecytrimethylammonium bromide (surfactant), heptane (oil) and butan-1-ol (co-surfactant) microemulsion by microemulsion electrokinetic chromatography (MEEKC) through the solvation parameter model. This model is especially useful to describe the distribution of neutral solutes between two phases (the aqueous phase and the microemulsion). To do that, 69 neutral compounds with representative enough properties were analysed.

The solvation parameter model is based on the linear free energy relationships (LFERs), which can be written as:

$$\log k = c + eE + sS + aA + bB + vV \quad (3)$$

where k is the MEEKC retention factor and E, S, A, B and V are the Abraham solute descriptors. The coefficients of the system (e, s, a, b and v) can be obtained by multiple linear regression and provide the properties of the studied microemulsion system.

Once the coefficients of the system are determined, the studied system can be compared with other systems with known coefficients. In this work, the TTAB MEEKC system was correlated with octanol-water partition and SDS MEEKC systems. The coefficients are similar enough, thus the TTAB MEEKC system can be used to emulate the octanol-water partition.

The octanol-water partition emulation is especially useful as it is a measure of the lipophilicity of compounds, which plays an important role in drug discovery and development.

Keywords: TTAB, microemulsion, MEEKC, model comparison, solvation parameter model, lipophilicity, capillary electrophoresis.

2. RESUMEN

El objetivo de este trabajo es caracterizar una microemulsión compuesta por bromuro de tetradeciltrimetilamonio (surfactante), heptano (aceite) y 1-butanol (co-surfactante) por cromatografía electrocinética de microemulsiones (MEEKC) a través del modelo de parámetros de solvatación. Este modelo es muy usado para describir la distribución de especies neutras entre dos fases (fase acuosa y microemulsión). Para realizar la caracterización, 69 compuestos neutros con propiedades representativas fueron analizados.

El modelo de parámetros de solvatación se basa en las relaciones lineales de energía libre (*LFERs*), representado como:

$$\log k = c + eE + sS + aA + bB + vV \quad (3)$$

donde k es el factor de retención en MEEKC y E, S, A, B y V son los descriptores de Abraham de los compuestos analizados. Los coeficientes del sistema (e, s, a, b y v) se obtienen por regresión multilineal, proporcionando las propiedades del sistema estudiado.

Una vez se conozcan los coeficientes, el sistema de microemulsión estudiado puede ser comparado con otros sistemas cuyos coeficientes sean conocidos. En este trabajo, el sistema estudiado TTAB MEEKC ha sido correlacionado con los sistemas partición octanol-agua y SDS MEEKC. Los coeficientes son suficientemente similares, por lo tanto el sistema TTAB MEEKC puede usarse para emular el sistema de partición octanol-agua.

La emulación del sistema partición octanol-agua es especialmente útil puesto que es una medida de la lipofilicidad, propiedad de gran importancia en el descubrimiento y desarrollo de medicamentos.

Palabras clave: TTAB, microemulsión, MEEKC, comparación de modelos, modelo de parámetros de solvatación, lipofilicidad, electroforesis capilar.

3. INTRODUCTION

3.1. DRUG DISCOVERY AND DEVELOPMENT

Drug discovery is the process by which drugs are discovered and designed.

It starts when researchers study a disease and how it affects the organism. They look for the altered gen or protein, known as target, and how it interacts with cells and tissues. Once the researchers have tested and proved that the gen or protein is related to the disease of interest, it starts a research to find the molecule or compound which can alter the target in order to affect or stop the disease progress. [1]

High-throughput methods are automated techniques which have been developed to capture up to millions of data points simultaneously [2]. These methods ease the compound selection process, by helping to determinate the properties of interest in a shorter time. The studied MEEKC system is a high-throughput method.

A set of molecules and compounds are proposed based on their properties; then, initial clinical trials are done to abridge the number of candidates. After several tests, a molecule is chosen and its formulation and industrial production process are designed and optimized. [1]

3.2. PARTITION COEFFICIENT

Lipophilicity is a physicochemical parameter which plays an important role in drug discovery, as it reflects the capacity of a compound to transfer from an aqueous phase to cell membranes. Lipophilicity is experimentally determined with the octanol-water partition coefficient. [3]

The partition coefficient (log P) is a property which determines the ratio of concentrations of a compound in a mixture of two immiscible phases in equilibrium. This coefficient is therefore a measure of the differential solubility of a compound between two solvents. When these two solvents are water and 1-octanol, the partition coefficient is known as the 1-octanol-water partition coefficient (log $P_{o/w}$), which provides a prediction of the membrane solubility in several biological systems. [3–5]

The classical method for measuring log$P_{o/w}$ is the shake-flask method. In this method, the tested compound is mixed with a 1-octanol/water mixture and shaken for a given period during which equilibrium between both phases must be achieved. Then, both phases are separated, and the concentration of the compound in the aqueous and the octanol phases is determined.

This method is not suitable for compounds with $logP_{o/w}$ values greater than 4-5 as large volumes of the aqueous phase are required. Several new methods have been developed to overcome these issues, such as reversed-phase high performance liquid chromatography (RP-HPLC) and high performance electrokinetic chromatography (HPCE) [6]. Biopartioning chromatography (BPC) was developed as an extension of conventional HPLC, representing a novel platform for the rapid evaluation of a large number of compounds.

Among the BPC systems, the use of microemulsion electrokinetic chromatography (MEEKC) provides several advantages in terms of predicting the drug membrane permeability properties, such as the ability to control experiment conditions, unique separation selectivity, good stability and enhanced detection sensitivity [7]. This work will focus on MEEKC.

3.3. MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY (MEEKC)

3.3.1. Capillary electrophoresis

Microemulsion electrokinetic chromatography (MEEKC) is based on capillary electrophoresis (CE), a separation technique driven by differences in solute velocity (rate of migration) in an electric field.

An electrophoretic separation is performed by the injection of the sample into an aqueous buffer solution contained in a silica capillary. Voltage is applied on the capillary between two electrodes placed at the entrance and exit of the capillary, creating a field which causes the ions to migrate from one electrode to the other.

The migration rate (v, $cm \cdot s^{-1}$) of a compound in an electric field is a product of the field strength (E, $V \cdot cm^{-1}$) and the electrophoretic mobility (μ_e, $cm^2 \cdot V^{-1} \cdot s^{-1}$) of the compound.

$$v = \mu_e E \quad (1)$$

Electrophoretic mobility is a physical constant determined at the point of full solute charge. It is highly dependent on pH and composition of the running buffer. [8]

3.3.2. Electroosmotic flow

Electroosmotic flow (EOF) is the bulk flow of liquid in the capillary. It appears as a consequence of the surface charge on the capillary wall when applying the electric field on the solution.

At pH values above 4, the silica wall of the capillary is negatively charged due to the ionization of the silanol groups (-Si-O$^-$). The buffer cations accumulate in the electrical double layer adjacent to the negative surface. Then, the cations on the diffuse outer layer are attracted towards the cathode (negative pole), dragging the solvent along with them, as seen in Figure 1.

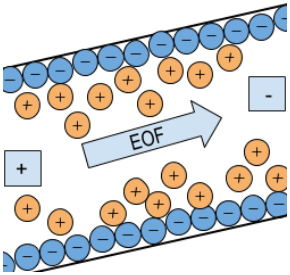

Figure 1. Charge distribution and electroosmotic flow upon application of electric field. [8]

The apparent mobility of the compounds is the vector sum of the electrophoretic mobility and EOF mobility. The magnitude of the EOF can be more than one order greater than the electrophoretic mobilities. Therefore, anions migrate slowest since they are attracted to the cathode but are still flushed by the EOF towards the cathode. Otherwise, cations will migrate fastest since the cathode attraction and the EOF are in the same direction and neutrals will flush at the EOF velocity, as seen in Figure 2. Hence, the EOF causes the movement of nearly all the species, regardless of the charge, on the same direction. [8]

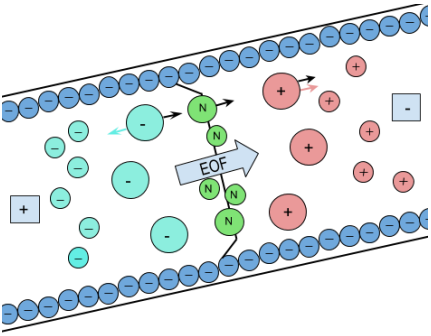

Figure 2. Solute migrations based on their charge and electrophoretic mobility. [8]

3.3.3. Microemulsion composition

MEEKC uses a microemulsion (ME) as a semi-stationary phase and a BIS-TRIS buffer solution as a background electrolyte (BGE), which adjusts ionic strength and maintains pH

constant. A microemulsion is a stable system containing an oil and water, stabilized by a surfactant and a co-surfactant. The most used type of ME is oil-in-water (O/W), where water makes up the bulk phase.

ME formation occurs when mixing in defined ratios the oil (heptane), water, surfactant (TTAB) and co-surfactant (1-butane) as seen in figure 3. In an O/W system, the oil is stabilized by the surfactant lowering the surface tension and the co-surfactant reducing the electrostatic repulsion among the surfactant molecules. [9]

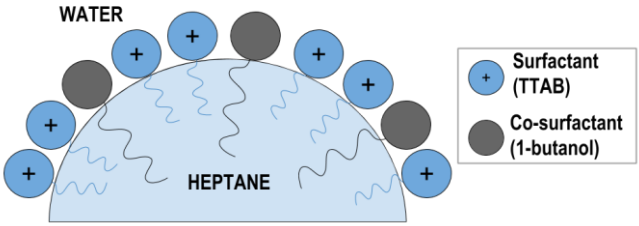

Figure 3. Microemulsion composition.

The ME droplets act as a pseudo-stationary phase, enabling the separation of neutral and charged compounds.

The ME studied in this work is charged positively due to the ionized TTAB molecules, consequently, a negative voltage needs to be applied in order to flush the bulk solution towards the cathode.

3.3.4. Separation principles: log k_{MEEKC} and log $P_{o/w}$

The characteristic parameter of the partition of analytes between the mobile and pseudo-stationary oil phase is the logarithm of the mass distribution ratio (log k_{MEEKC}) defined as:

$$\log k_{MEEKC} = \log \frac{t_R - t_{eof}}{t_{eof}\left(1 - \frac{t_R}{t_{ME}}\right)} \quad (2)$$

where t_R, t_{eof} and t_{ME} are the analyte, the EOF marker and the ME marker migration times.

All neutral solutes have the same mobility as EOF and differ on their lipid affinity towards the ME heptane droplets. Most hydrophilic compounds are expected not to interact with the ME droplets and to have a short elution time while most hydrophobic compounds are expected to do the opposite, but all of the compounds will be flushed between the t_{eof} and the t_{ME}, marking the migration window. [10]

3.3.5. Solvation parameter model

The solvation parameter model has been demonstrated to be very useful in the characterization of many biological and physicochemical processes. It is a very suitable tool to appreciate the similarity of the system with log $P_{o/w}$ partition.

This model relates a solvation property (log $P_{o/w}$ or log k_{MEEKC}) with the sum of specific interaction terms, based on the linear free energy relationships (LFERs), which can be written as:

$$\log k = c + eE + sS + aA + bB + vV \quad (3)$$

where k is the MEEKC retention factor; E, S, A, B and V are the Abraham solute descriptors and c, e, s, a, b and v are the system constants.

E is the excess molar refraction, S the solute dipolarity/polarizability, A and B are the solute hydrogen-bond acidity and basicity descriptors, respectively, and V is McGowan's characteristic volume of the molecule. The values of the coefficients e, s, a, b and v reflect the complementary effect of the solute descriptors on the solvent phases. [10,11]

The system constants can be obtained by multiple linear regression between the experimental log k_{MEEKC} values acquired for a group of analytes and their solute descriptors, which must be sufficiently varied to be representative [11]. In addition, analytes must be neutral at the working pH, as the Abraham model does not contemplate ion interactions. [12]

3.4. MODEL COMPARISON

3.4.1. The *d* parameter

The Abraham model allows the evaluation of the similitude between two systems by comparison of their solute descriptors, allowing the comparison between the studied system and the octanol-water partition system.

Positively charged compounds could not be properly analysed with the previously used microemulsion, as SDS is negatively charged and tends to form aggregates with compounds with positive charge. Consequently, the TTAB MEEKC system was also compared with the previously used SDS MEEKC system in order to see if it could be a good replacement.

In a further study, Abraham and Martins [13] considered the equation descriptors as a point in a five dimension space and proposed a distance *D'* between two points as a measure of the chemical similarity between two systems.

Later, Lázaro, *et al.* [14] proposed to use the distance between two normalized vectors of two systems ($\overrightarrow{w_{ui}}$ and $\overrightarrow{w_{uj}}$) as a measure of their similarity.

The normalized vector of a system ($\overrightarrow{w_u}$) has the following normalized descriptors:

$$e_u=\frac{e}{l};\ \ s_u=\frac{s}{l};\ \ a_u=\frac{a}{l}\ ;\ \ b_u=\frac{b}{l};\ \ v_u=\frac{v}{l} \tag{4}$$

$$l=\sqrt{e^2+s^2+a^2+b^2+s^2} \tag{5}$$

where l is the length (module) of the coefficients vector.

Considering two systems, the *d* distance between their normalized vectors ($\overrightarrow{w_{ui}}$ and $\overrightarrow{w_{uj}}$) provides a measure of the mathematical similarity between them.

The *d* distance can be calculated according to the following equation:

$$d=\sqrt{(e_{ui}-e_{uj})^2+(s_{ui}-s_{uj})^2+(a_{ui}-a_{uj})^2+(b_{ui}-b_{uj})^2+(v_{ui}-v_{uj})^2} \tag{6}$$

The smaller the *d* distance is, the more similar the two compared systems are. In fact, two systems are considered similar enough if d<0.25. [14,15]

Once that two systems are proved to be similar enough, further model comparison is done by analysing their chromatographic precisions.

3.4.2. Chromatographic precision

In order to know if a chromatographic system represents well enough a biological system, the corresponding chromatographic and biological data can be correlated by the following equation:

$$\log SP_{bio}=q+p\log SP_{chrom} \tag{7}$$

where SP_{bio} and SP_{chrom} are the correlated biological data and the correlated chromatographic data. In this work, the correlated biological data is log $P_{o/w}$ and the correlated chromatographic data is log k_{MEEKC}.

The main sources of variance in a system correlation are the biological variance, the chromatographic variance and the error coming from the dissimilarity between the two correlated systems. The precision of the correlation can be expressed in terms of variance as:

$$\sigma^2_{corr} = \sigma^2_{bio} + \sigma^2_{chr} + \sigma^2_{d} \quad (8)$$

where σ^2_{corr} is the overall variance and σ^2_{bio}, σ^2_{chr}, σ^2_{d} are the biological system data precision, the chromatographic system data precision and the error coming from the dissimilarity of the two systems, respectively.

The contribution of the biological system variance is especially important because of the complexity of biological systems. Although, for well characterized biological systems, the standard deviation of the characterization (SD_{bio}) can be taken as an estimation of the precision of the original biological data.

Consequently, σ^2_{bio} can be estimated directly as:

$$\sigma^2_{bio,} = SD_{bio}{}^2 \quad (9)$$

Considering a chromatographic system characterized by the Abraham model, the precision of the chromatographic data can be estimated by means of the standard deviation of its characterization (SD_{chr}). The contribution of this source of error to the overall system precision is affected by the slope; if the slope is larger, it will have a greater contribution.

Consequently, σ^2_{chr} can be estimated as:

$$\sigma^2_{chr,} = (p\ SD_{chr})^2 \quad (10)$$

The dissimilarity error can be estimated by means of the correlation between the two chromatographic systems. When the two compared systems have a small *d* distance (eq 6), the correlation of their data is less influenced by σ^2_{d}. The variance can be estimated by means of a correlation between calculated values for both systems obtained by multiplying the LFER equation (eq 3) of each system by the descriptors of each considered solute. When values are calculated this way, the overall variance is not affected by the precision of the correlated data:

$$SD^2_{corr\ cal} = \cancel{SD^2_{bio}}^{\,0} + \cancel{SD^2_{chr}}^{\,0} + SD^2_{d} \quad (11)$$

Therefore, the contribution of the dissimilarity between two systems can be estimated by means of the correlated values variance:

$$\sigma^2_{d} = SD^2_{d} = SD^2_{corr\ cal} \quad (12)$$

Hence, the overall variance of the chromatographic systems correlation can be expressed as the following equation: [15]

$$SD^2_{corr\ cal} = SD_{bio}{}^2 + (p\ SD_{chr})^2 + SD^2_d \quad (13)$$

The study of the *d* parameter and the chromatographic precision allows to evaluate the interest of several chromatographic systems to estimate biological properties, leading to the selection of the most adequate chromatographic partition systems for its estimation.

4. OBJECTIVES

Lipophilicity is a very informative physicochemical property in the drug discovery and development process. In a previous work [11], Microemulsion electrokinetic chromatography (MEEKC) was proposed as a technique for determining lipophilicity, using Sodium dodecyl sulfate (SDS) as surfactant. The aim of this work is to characterize a microemulsion of tetradecytrimethylammonium bromide (TTAB) through the solvation parameter model and proving that it provides a similar ability to emulate log $P_{o/w}$ as to the SDS microemulsion.

The work plan is:

1) Choosing a representative group of neutral analytes with varied enough log $P_{o/w}$ values.
2) Developing a procedure for the chromatographic separations of the neutral compounds.
3) Characterizing the TTAB microemulsion system by means of its solvation model coefficients.
4) Implementing a model comparison between TTAB MEEKC and SDS MEEKC systems by analysing the *d* distance.
5) Implementing a model comparison between SDS MEEKC and Octanol-water partition by analysing the *d* distance and chromatographic precision.
6) Analysing the log $P_{o/w}$ vs log k_{MEEKC} correlation for the system.

5. EXPERIMENTAL SECTION

5.1. COMPOUNDS

The compounds used, their CAS number, commercial brand and purity are listed in Table 1.

Table 1. CAS number, purity and commercial brand of the analysed compounds.

Compound	CAS number	Purity	Commercial brand
Tetradecyltrimethylammonium bromide (TTAB)	1119-97-7	>98%	Fluka
BIS-TRIS	6976-37-0	>99 %	Fluka
Heptane	142-82-5	99%	Sigma-Aldrich
Methanol	67-56-1	high	Carlo Erba
Propan-1-ol	71-23-8	high	Prolabo
Propan-2-ol	67-63-0	99%	Merck
Butan-1-ol	71-36-3	>99.7%	Sigma-Aldrich
Pentan-1-ol	71-41-0	>99.5%	Carlo Erba
Pentan-3-ol	584-02-1	>98%	Fluka
Propan-1,3-diol	504-63-2	98%	Aldrich
Butan-1,4-diol	110-63-4	99%	Fluka
Pentan-1,5-diol	111-29-5	97%	Aldrich
Thiourea	62-56-6	>99%	Baker
Benzene	71-43-2	99,7%	Merck
Toluene	108-88-3	99,9%	Sigma-Aldrich
Ethylbenzene	100-41-4	>99%	Merck
Propylbenzene	103-61-1	99%	Fluka
Butylbenzene	104-51-8	>99%	Aldrich
***p*-Xylene**	106-42-3	>99%	Carlo Erba
Naphthalene	91-20-3	-	Baker
Chlorobenzene	108-90-7	>99%	Baker
Bromobenzene	108-86-1	>99.5%	Carlo Erba
Anisole	100-66-3	>99%	Carlo Erba
Benzaldehyde	100-52-7	>99%	Fluka
Acetophenone	98-86-2	99%	Sigma
Propiophenone	93-55-0	99%	Aldrich
Butyrophenone	495-40-9	-	Sigma
Valerophenone	1009-14-9	99%	Aldrich
Heptanophenone	1671-75-6	98%	Aldrich
Dodecanophenone	1674-38-0	98%	Aldrich
Benzophenone	119-61-9	99%	Scharlau
Methyl benzoate	93-58-3	>98%	Fluka
Benzyl benzoate	120-51-4		Aldrich
Benzonitrile	100-47-0	99,9%	Sigma
Aniline	62-53-3	99%	Baker
***o*-Toluidine**	95-53-4	>99%	Carlo Erba
3-Chloroaniline	108-42-9	>98%	Merck

Compound	CAS number	Purity	Commercial brand
4-Chloroaniline	106-47-8	>99%	Merck
2-Nitroaniline	88-74-4	>98.5%	Carlo Erba
3-Nitroaniline	99-09-2	>98.5%	Carlo Erba
4-Nitroaniline	100-01-6	>98 %	Merck
Nitrobenzene	98-95-3	>99.5%	Carlo Erba
2-Nitroanisole	91-23-6	>99%	Aldrich
Benzamide	55-21-0	98%	Merck
4-Aminobenzamide	2835-68-9	98%	Merck
Acetanilide	103-84-4	pure	Merck
4-Chloroacetanilide	539-03-7	>98%	Fluka
Phenol	108-95-2	>99.5%	Carlo Erba
3-Methylphenol	108-39-4	pure	Scharlau
2,3-Dimethylphenol	526-75-0	-	Fluka
2,4-Dimethylphenol	105-67-9	90%	Merck
Thymol	89-83-8	>99%	Riedel-de Haën
4-Chlorophenol	106-48-9	>99%	Carlo Erba
Catechol	120-80-9	>99%	Fluka
Resorcinol	108-46-3	99%	Sigma-Aldrich
Hydroquinone	123-31-9	>99%	Carlo Erba
2-Naphthol	135-19-3	-	Sigma
1,2,3-Trihydroxybenzene	87-66-1	>98%	Fluka
Furan	110-00-9	>99%	Sigma-Aldrich
2,3-Benzofuran	271-89-6	>99%	Fluka
Quinoline	91-22-5	>98.5%	Baker
Pyrrole	109-97-7	>98%	Carlo Erba
Pyrimidine	289-95-2	>98%	Fluka
Antipyrine	60-80-0	>99%	Fluka
Caffeine	58-08-2	-	-
Corticosterone	50-22-6	98,5%	Sigma
Cortisone	53-06-5	98%	Aldrich
Hydrocortisone	50-23-7	98%	Aldrich
Estradiol	50-28-2	98%	Sigma
Estratriol	50-27-1	98%	Aldrich
Monuron	150-68-5	99%	Aldrich
Myrcene	123-35-3	-	Aldrich
α-Pinene	7785-26-4	> 99%	Sigma
Geraniol	106-24-1	98%	Aldrich

5.2. PREPARATION OF THE MICROEMULSION SOLUTION

5.2.1. Preparation of the BIS-TRIS buffer

A pH=7 BIS-TRIS 0.4M buffer solution was prepared.

First of all, 4.2g of BIS-TRIS were weighted. Then, 20mL of HCl 1M where added using a burette and under constant agitation, in order to fully deprotonate BIS-TRIS. The solution was levelled to 250 mL with MilliQ water in a volumetric flask.

Afterwards, 50mL of the prepared solution and 20mL of NaOH 0.5M were measured with a burette and added to a beaker under constant agitation. The resulting solution was put at ultrasounds for 30 min in order to assure a proper mixing. Then, it was levelled to 250mL with MilliQ water in a volumetric flask. The pH was determined and adjusted to pH=7 with HCl 0.5M.

5.2.2. Preparation of the TTAB microemulsion solution

A 200mL microemulsion solution was prepared (50mM TTAB, 8.15%v/v 1-butanol, 1.15%v/v heptane).

First of all, 3.4g of TTAB were weighted and solved in the previously prepared BIS-TRIS buffer solution under constant agitation. The solution pH was measured (pH=7).

Then, 16.0mL of 1-butanol and 2.3mL of heptane were measured with a burette and added to the TTAB solution under constant agitation. The microemulsion solution was levelled to 200mL with MilliQ water.

The microemulsion solution was stored at 25ºC.

5.3. PREPARATION OF THE STOCK SOLUTIONS AND ANALYSIS SOLUTIONS

5.3.1. Preparation of the analyte stock solutions

Approximately 2000mg/L analyte stock solutions were prepared, using methanol as solvent. All analyte stock solutions were prepared in 2mL Eppendorf flasks properly labelled. The 69 analytes can be separated in 3 groups: solids, liquids and alcohols.

For solid analytes, 2mg were weighted and solved in 1mL of methanol.

For liquid analytes, 0.4mL were measured and solved in 1mL of methanol.

For alcohols, 1mL of the compound was added to the flask.

All the analyte stock solutions were stored at 4ºC (at the fridge).

5.3.2. Preparation of the dodecanophenone stock solution

A 4000mg/L solution of dodecanophenone was prepared to be the microemulsion marker.

80mg of dodecanophenone were weighted and solved in 20mL of methanol.

Dodecanophenone solution was stored at 4ºC (fridge).

5.3.3. Preparation of the analysis solutions

The analysis solutions were prepared the same day of the separation in 2mL electrophoresis vials.

For solid and liquid analytes, the analysis solutions contained 75µL of analyte stock solution and 75µL of dodecanophenone stock solution and were filled with the microemulsion solution up to 2 mL, with a final concentration of 75mg/L of analyte and 150mg/L of dodecanophenone.

For alcohols, 100µL of methanol were also added to the analysis solutions, with a final concentration of 75mg/L of the analysed alcohol and 150mg/L of dodecanophenone.

5.4. APPARATUS AND ANALYSIS CONDITIONS

All separations were performed with a CE Agilent instrument with a UV diode array detector. The fused-silica separation capillaries were 38.5cm total length, 30cm effective length (with a 0.6cm window) and 50µm diameter.

Retention measurements were made at -12.5kV to avoid alterations caused by the Joule effect and at 25ºC. A pressure of 5mbar was applied to obtain a shorter separation time.

Methanol was used as the electroosmotic flow marker as it is a very polar compound and is not retained by the microemulsion. The methanol peak enables the identification of the electroosmotic retention time.

Dodecanophenone was used as the micellar marker as it is a very non-polar compound and is strongly retained by the microemulsion. The dodecanophenone peak enables the identification of the microemulsion retention time.

Each time the capillary was changed, it was conditioned in the following sequence: 5 min of water, 10 min of NaOH 1M, 5 min of water, 10 min of NaOH 0.1M and 5 min of microemulsion solution.

Prior to each separation, the capillary was flushed in the following sequence: 0.5 min of water, 2 min of NaOH 0.1M, 0.5 min of water and 2 min of microemulsion solution.

All measurements were taken in triplicate.

Water was Milli-Q plus (Millipore) with a resistivity of 18.2 MΩ cm.

6. METHOD DESIGN AND OPTIMIZATION

6.1. VOLTAGE AND PRESSURE

To be able to identify each analyte injected by its migration time, the voltage applied should be as large as possible to obtain a good resolution, but avoiding the Joule effect. This effect consists in the loss of a part of the electrical energy applied in form of dissipated heat through the capillary, which results in wider bands.

According to Ohm's law, when the Joule effect is null, intensity is proportional to the applied voltage. In order to determine the optimum voltage, a plot was made by applying several voltages from 0 to -25 kV and measuring the generated current.

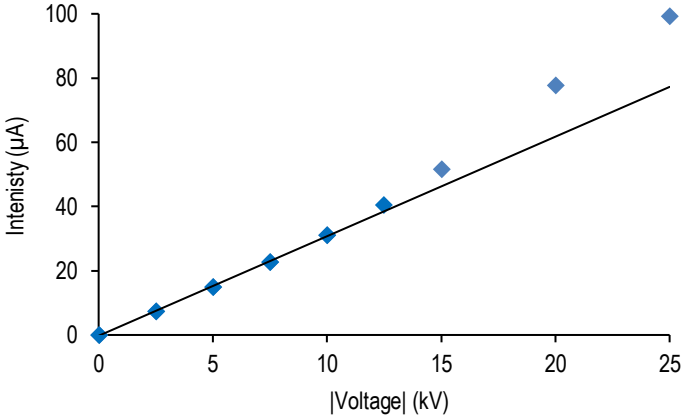

Figure 4. Determination of the Joule effect

As can be seen in Figure 4, Joule effect starts to be relevant at -15kV as current clearly deviates from a linear behaviour. Given that result, a voltage of -12.5 kV was applied to all the separations, which corresponds to an intensity of 40.4 µA.

In order to obtain suitable separation times, some tests were performed applying pressure. First, 0 mbar where applied but separation time was too long. Then, 5mbar where applied, resulting in approximately 15 min separation times.

Finally, 5 mbar were chosen as the analysis pressure.

6.2. ANALYTE SELECTION

In order to obtain accurate solvation coefficients of the model studied in this work, representative analytes were chosen.

In 2001, Fuguet, *et al*. [11] did a study of the solute-solvent interactions in micellar electrokinetic chromatography (MEKC) where 71 compounds were chosen as analytes from a 2975 solute data base, according to their solute-solvent interactions. Discarding methanol and dodecanophenone for being the EOF and microemulsion markers, the other 69 compounds where chosen as analytes for the TTAB MEEKC system characterization. These 69 compounds had varied enough solute descriptor values to provide a representative analysis.

The Abraham solute descriptors from the analytes were extracted from Fuguet, *et al*. [11], which are listed at Table 2. The log k_{MEEKC} values listed at Table 2 were obtained experimentally. The log $P_{o/w}$ values for the 69 analytes are listed and referenced in Table 2.

Table 2. Abraham solute descriptors, log k_{MEEKC} and log $P_{o/w}$ values of the chosen analytes.

Analyte	E	S	A	B	V	log k_{MEEKC}	log $P_{o/w}$
Propan-1-ol	0,236	0,42	0,37	0,48	0,5900	-0,71	0,30 [16]
Propan-2-ol	0,212	0,36	0,33	0,56	0,5900	-0,79	0,05 [16]
Butan-1-ol	0,224	0,42	0,37	0,48	0,7309	0,08	0,85 [17]
Pentan-1-ol	0,219	0,42	0,37	0,48	0,8718	-	1,48 [18]
Pentan-3-ol	0,219	0,36	0,33	0,56	0,8718	-	1,09 [19]
Propan-1,3-diol	0,397	0,91	0,77	0,85	0,6487	-	-1,04 (u)
Butan-1,4-diol	0,395	0,93	0,72	0,90	0,7860	-	-0,83 (u)
Pentan-1,5-diol	0,388	0,95	0,72	0,91	0,9305	-	-0,43 [17]
Thiourea	0,840	0,82	0,77	0,87	0,5696	-1,06	-1,02 (p)
Benzene	0,610	0,52	0,00	0,14	0,7164	0,36	2,13 [20]
Toluene	0,601	0,52	0,00	0,14	0,8573	0,77	2,69 [20]
Ethylbenzene	0,613	0,51	0,00	0,15	0,9982	1,10	3,15 [21]
Propylbenzene	0,604	0,50	0,00	0,15	1,1391	1,53	3,72 [22]
Butylbenzene	0,600	0,51	0,00	0,15	1,2800	-	4,38 [6]
***p*-Xylene**	0,613	0,52	0,00	0,16	0,9982	1,12	3,18 [23]
Naphthalene	1,340	0,92	0,00	0,20	1,0854	1,21	3,37 [24]
Chlorobenzene	0,718	0,65	0,00	0,07	0,8388	0,85	2,90 [6]
Bromobenzene	0,882	0,73	0,00	0,09	0,8914	0,99	2,99 [20]
Anisole	0,708	0,75	0,00	0,29	0,9160	0,28	2,11 [20]
Benzaldehyde	0,820	1,00	0,00	0,39	0,8730	-0,10	1,49 [25]
Acetophenone	0,818	1,01	0,00	0,48	1,0139	-0,03	1,58 [24]
Propiophenone	0,804	0,95	0,00	0,51	1,1548	0,35	2,24 [26]
Butyrophenone	0,797	0,95	0,00	0,51	1,2957	0,68	2,65 [26]
Valerophenone	0,795	0,95	0,00	0,50	1,4366	1,05	3,4 [26]
Heptanophenone	0,720	0,95	0,00	0,50	1,7184	1,88	4,41 [26]
Benzophenone	1,447	1,50	0,00	0,50	1,4808	1,02	3,32 [27]

Analyte	E	S	A	B	V	log k_{MEEKC}	log $P_{o/w}$
Methyl benzoate	0,733	0,85	0,00	0,46	1,0726	0,35	2,12 [20]
Benzyl benzoate	1,264	1,42	0,00	0,51	1,6804	1,49	3,97 (u)
Benzonitrile	0,742	1,11	0,00	0,33	0,8711	-0,06	1,56 [20]
Aniline	0,955	0,96	0,26	0,50	0,8162	-0,28	0,90 [20]
o-Toluidine	0,970	0,90	0,23	0,59	0,9751	-0,04	1,35 [28]
3-Chloroaniline	1,050	1,10	0,30	0,36	0,9390	0,47	1,88 [20]
4-Chloroaniline	1,060	1,10	0,30	0,35	0,9390	0,41	1,84 [26]
2-Nitroaniline	1,180	1,37	0,30	0,36	0,9904	0,25	1,83 [29]
3-Nitroaniline	1,200	1,71	0,40	0,35	0,9904	0,07	1,32 [26]
4-Nitroaniline	1,220	1,91	0,42	0,38	0,9904	0,11	1,39 [20]
Nitrobenzene	0,871	1,11	0,00	0,28	0,8906	0,16	1,85 [20]
2-Nitroanisole	0,965	1,34	0,00	0,38	1,0902	0,17	1,78 [28]
Benzamide	0,990	1,50	0,49	0,67	0,9728	-0,39	0,64 [20]
4-Aminobenzamide	1,340	1,94	0,80	0,94	1,0726	-0,86	-0,44 [30]
Acetanilide	0,870	1,36	0,46	0,69	1,1137	-0,17	1,19 [26]
4-Chloroacetanilide	0,980	1,50	0,64	0,51	1,2357	0,51	2,12 [31]
Phenol	0,805	0,89	0,60	0,30	0,7751	0,05	1,48 [24]
3-Methylphenol	0,822	0,88	0,57	0,34	0,9160	0,38	1,96 [20]
2,3-Dimethylphenol	0,850	0,90	0,52	0,36	1,0569	0,64	2,48 [32]
2,4-Dimethylphenol	0,840	0,90	0,53	0,39	1,0569	0,71	2,42 [5]
Thymol	0,822	0,79	0,52	0,44	1,3387	1,20	3,34 [26]
4-Chlorophenol	0,915	1,08	0,67	0,20	0,8975	0,79	2,39 [20]
Catechol	0,970	1,10	0,88	0,47	0,8338	-0,09	0,86 (u)
Resorcinol	0,980	1,00	1,10	0,58	0,8338	-0,18	0,80 [20]
Hydroquinone	1,000	1,00	1,16	0,60	0,8338	-0,43	0,59 [33]
2-Naphthol	1,520	1,08	0,61	0,40	1,1441	1,04	2,84 [29]
1,2,3-Trihydroxybenzene	1,165	1,35	1,35	0,62	0,8925	-0,29	0,68 [28]
Furan	0,369	0,53	0,00	0,13	0,5363	-0,31	1,31 [25]
2,3-Benzofuran	0,888	0,83	0,00	0,15	0,9053	0,71	2,69 [34]
Quinoline	1,268	0,97	0,00	0,51	1,0443	0,20	2,15 [24]
Pyrrole	0,613	0,73	0,41	0,29	0,5774	-0,44	0,75 [29]
Pyrimidine	0,606	1,00	0,00	0,65	0,6342	-1,03	-0,34 [25]
Antipyrine	1,320	1,50	0,00	1,48	1,5502	-0,67	0,56 [24]
Caffeine	1,500	1,60	0,00	1,33	1,3632	-0,77	-0,01 [24]
Corticosterone	1,860	3,43	0,40	1,63	2,7389	0,65	1,9 [24]
Cortisone	1,960	3,50	0,36	1,87	2,7546	0,23	1,50 [35]
Hydrocortisone	2,030	3,49	0,71	1,90	2,7975	0,39	1,53 [24]
Estradiol	1,800	3,30	0,88	0,95	2,1988	1,35	4,01 [24]
Estratriol	2,000	3,36	1,40	1,22	2,2575	0,67	2,54 [36]
Monuron	1,140	1,50	0,47	0,78	1,4768	0,32	2,01 [37]
Myrcene	0,483	0,29	0,00	0,21	1,3886	-	4,17 (p)
α-Pinene	0,446	0,14	0,00	0,12	1,2574	-	4,83 [38]
Geraniol	0,513	0,63	0,39	0,66	1,4903	1,07	3,47 [39]

(u) unpublished work / (p) private communication – log $P_{o/w}$ found at BioLum.

7. SYSTEM CHARACTERIZATION

7.1. LOG K_{MEEKC} DETERMINATION

The aim of this work is to characterize a tetradecytrimethylammonium bromide (TTAB) microemulsion by microemulsion electrokinetic chromatography (MEEKC). To achieve the objective, retention constants (k_{MEEKC}) of the analytes where determined.

First, all analytes were injected individually to obtain an indicative value of their retention constant.

According to the results obtained, some compounds had to be discarded from the system characterization:

- Butylbenzene and α-Pinene were too much lipophilic and were eluted with the ME marker, making them undistinguishable in the registered electropherograms.

- Propan-1,3-diol, butan-1,4-diol and pentan-1,5-diol were too little lipophilic and were eluted with the EOF marker, making them undistinguishable in the registered electropherograms.

- Pentan-1-ol and pentan-3-ol generated too much distortion on the baseline and their peaks could not be properly identified.

- Myrcene was not soluble enough in methanol to be detected.

Subsequently, the 61 remaining analytes where distributed in groups and analyzed. Separations were repeated until all the analytes had 3 retention time values with a relative deviation smaller than 5 units.

Log k_{MEEKC} was determined for each analyte using the equation 2.

7.2. CORRELATION BETWEEN SOLUTE DESCRIPTORS AND LOG K_{MEEKC}

A correlation was made between the log k_{MEEKC} of each analyte and their Abraham solute descriptors in order to obtain the system coefficients.

A study of the residuals was made to verify the linearity of the model. Analytes with a value of standard residue higher than 2.5 were considered as outliers and were discarded from the model characterization. In this correlation, butan-1-ol, thiourea and estradiol were considered as outliers.

The parameters of the system characterization are shown in Table 3.

Table 3. Abraham solute descriptors of the studied MEEKC system.

	e	s	a	b	v	n	R^2	SD
TTAB MEEKC system	0.470 (±0.08)	-0.692 (±0.06)	0.191 (±0.04)	-2.068 (±0.07)	2.350 (±0.06)	58	0.972	0.109

Therefore, the obtained system equation is the following:

$$\log k_{MEEKC} = -0.95 + 0.47\,E - 0.69\,S + 0.19\,A - 2.07\,B + 2.35\,V \quad (14)$$

Positive coefficients point out a greater affinity for the microemulsion of the determined parameter, while negative coefficients mean that there is a bigger affinity for the aqueous phase. The bigger the absolute value of the parameter, the more influence it has on the retention times.

The large value of the coefficients b and v show that the solute volume and the hydrogen-bond basicity are the most influential parameters. The v coefficient is positive, which means that solutes with a bigger volume are more retained by the microemulsion phase. A negative b coefficient means that highly basic hydrogen bond compounds are less retained by the microemulsion phase.

The e coefficient is positive, so the microemulsion has more polarizability than the aqueous phase. The negative value of the s coefficient indicates that the microemulsion system is less dipolar than the aqueous phase. The coefficient a is close to zero, having a small effect on the system.

8. MODEL COMPARISON

With the aim of comparing the TTAB MEEKC system assayed with the literature log $P_{o/w}$ values, two parameters were determined: the *d* parameter and the chromatographic precision.

8.1. MODEL COMPARISON BY THE *d* PARAMETER

The *d* parameter allows the comparison of two systems by means of their solvation model coefficients.

The TTAB MEEKC, the Octanol-water partition and the SDS MEEKC system coefficients were normalized using the equations 4 and 5. Then, applying the equation 6, the *d* distance was determined between TTAB MEEKC and Octanol-water partition and between TTAB MEEKC and SDS MEEKC, which can be seen in Table 4.

Table 4. Non-normalized and normalized coefficients and *d* distances of the compared systems.

	e	s	a	b	v	n	SD	R^2
TTAB MEEKC	0.470	-0.692	0.191	-2.068	2.350	58	0.109	0.972
Octanol-water	0.562	-1.054	0.034	-3.460	3.814	613	0.116	0.994
SDS MEEKC	0.279	-0.692	0.060	-2.805	3.048	53	0.090	0.988
	e_u	s_u	a_u	b_u	v_u	d	Ref	
TTAB MEEKC	0.145	-0.213	0.059	-0.637	0.724	-	-	
Octanol-water	0.106	-0.199	0.006	-0.654	0.721	0.069	[12]	
SDS MEEKC	0.066	-0.164	0.014	-0.666	0.724	0.107	[12]	

For the TTAB MEEKC and Octanol-water partition comparison, the normalised coefficients of the two systems are very similar. In both systems, the most influential coefficients are b and v. Highly basic hydrogen bound compounds are slightly less retained by octanol in comparison with the TTAB microemulsion, since octanol-water has a more negative b coefficient. Solutes with a bigger volume are also slightly less retained by octanol than the microemulsion, but the difference in the v coefficient is practically insignificant.

The *d* parameter between TTAB MEEKC and Octanol-water partition much smaller than 0.25 [10], which means that TTAB MEEKC is a good approximation of the Octanol-water partition system, and consequently, lipophilicity. Because of the obtained results, further model comparison will be implemented.

For the TTAB MEEKC and SDS MEEKC comparison, the normalised coefficients of both systems are also very similar. Both systems have the same v coefficient value, but according to

the b coefficient values, highly basic hydrogen bound compounds are slightly less retained by the SDS microemulsion.

Although the *d* parameter between TTAB MEEKC and SDS MEEKC is bigger than the distance between TTAB MEEKC and Octanol-water partition, it is still a very small parameter and considered as similar enough [11], concluding that the TTAB microemulsion could be a good replacement for the SDS microemulsion in this technique.

8.2. MODEL COMPARISON BY CHROMATOGRAPHIC PRECISION

In order to know if a chromatographic system represents well enough a biological system, the corresponding chromatographic and biological data can be correlated.

As was explained previously, the main sources of variance in a system correlation are the biological variance, the chromatographic variance and the error coming from the dissimilarity between the two correlated systems. The overall variance of the chromatographic systems correlation can be expressed through the equation 13.

In 1994, Abraham, *et al.* [40] developed an analysis of the Octanol-water partitioning by means of the solvation parameter equation (equation 3) due to its importance is drug research. In that work, 613 compounds were analyzed to determine the system coefficients. The given equation was:

$$\log P_{o/w} = 0.088 + 0.562\,E - 1.054\,S + 0.034\,A - 3.460\,B + 3.814\,V \qquad (15)$$

The standard deviation of this determination can be taken as the biological standard deviation (SD_{bio}), having a value of 0.116.

The variance of the chromatographic system corresponds to the TTAB MEEKC determined variance, which was calculated with equation 10.

Otherwise, for the 613 compounds, $\log P_{o/w}$ was calculated by the substitution of each compound solute descriptors in the Octanol-water regression equation given by Abraham, *et al.* [40] (equation 15) and $\log k_{MEEKC}$ were calculated by doing the same substitution on the TTAB MEEKC equation obtained in this work (equation 14). A correlation between the two calculated parameters was done in order to estimate the dissimilarity between the two correlated systems. The obtained regression values were R^2=0.977, SD_d=0.109 and p=1.603±0.004.

For the TTAB MEEKC and Octanol-water system correlation, the standard deviation and variance values are listed in Table 5. The squared variance values to estimate the variance where obtained using the equations 9, 10, 12 and 13.

Table 5. Standard deviation and their squared values for the log $P_{o/w}$ and log k_{MEEKC} correlation.

	SD_{bio}	SD_{bio}^2	SD_{chr}	$(pSD_{chr})^2$	SD_d	SD_d^2	SD_{corr}^2
TTAB MEEKC and O/W correlation	0.116	0.013	0.109	0.030	0.109	0.012	0.056

As can be seen in Table 5, the largest contribution to the overall correlation standard deviation comes from the chromatographic data, which is reasonable because it is affected by the slope of the correlation, while biological variance and dissimilarity are not.

The squared standard deviation of the dissimilarity between the two systems is lower than squared standard deviation of the biological data ($SD_d^2<SD_{bio}^2$), which means that there is not much error introduced in the correlation regarding to the error which contains the original data. Besides, the correlation calculated squared standard deviation (SD_{corr}^2) is very close to 0.

For this reasons, the correlation between TTAB MEEKC and Octanol-water systems is considered good enough, which means that the TTAB MEEKC system is a good system to estimate the lipophilicity of compounds.

9. CORRELATION BETWEEN LOG $P_{O/W}$ AND LOG K_{MEEKC}

Once that the TTAB MEEKC and Octanol-water systems had been correlated by the *d* parameter and the chromatographic precision and the obtained results showed that the two systems were similar enough, a correlation between the literature values of log $P_{o/w}$ and the estimated log k_{MEEKC} of the 61 analytes was done to observe the similarity of the two systems.

A study of the residuals was made to verify the linearity of the model. In this correlation, butan-1-ol had a standard residue slightly greater than 2.5, but discarding it had no big influence on the result, so it was decided not to consider it as outlier.

The correlation between log $P_{o/w}$ and log k_{MEEKC} is plotted at Figure 6.

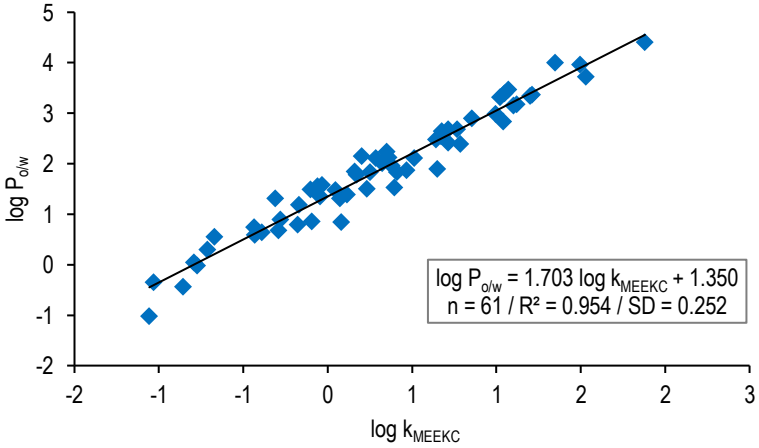

Figure 5. Linear correlation between log $P_{o/w}$ and log k_{MEEKC} for the TTAB MEEKC system.

As can be seen in Figure 5, the value of R^2 is very close to 1, showing that there is a good correlation between the log $P_{o/w}$ and the log k_{MEEKC}. The SD value of the linear regression is small, which indicates that the obtained values of log k_{MEEKC} for the analysed compounds do not have a big error.

Whereby, through the model comparison techniques and the log $P_{o/w}$ and log k_{MEEKC} correlation, the TTAB MEEKC studied system has been proved to provide a good estimation of the log $P_{o/w}$ of compounds.

10. CONCLUSIONS

The aim of this work was to characterize a tetradecytrimethylammonium bromide (surfactant), heptane (oil) and butan-1-ol (co-surfactant) microemulsion by microemulsion electrokinetic chromatography (MEEKC) through the solvation parameter model and comparing its similarity with the Octanol-water partition system.

Several conclusions can be drawn from the obtained results:

1. The solvation model coefficients have been successfully determined for the TTAB MEEKC system, allowing its characterization by the interpretation of the magnitude and sign of each coefficient. The obtained equation was the following:

$$\log k_{MEEKC} = - 0.95 + 0.47\,E - 0.69\,S + 0.19\,A - 2.07\,B + 2.35\,V \qquad (14)$$

   The two predominant coefficients have been demonstrated to be the b coefficient (hydrogen bond basicity) and the v coefficient (solute volume).

2. By the calculation of the *d* parameter, the TTAB MEEKC system has been shown to be a good replacement for the currently used SDS MEEKC system.

3. By two model comparison techniques and the log $P_{o/w}$ vs log k_{MEEKC} correlation, the TTAB MEEKC has been proved to be a good emulation of the Octanol-water partition, which lead to the conclusion that the studied system is suitable to estimate the lipophilicity of compounds. The model comparison also proved that TTAB MEEKC and SDS MEEKC have a very similar ability to emulate log $P_{o/w}$.

4. The log $P_{o/w}$ vs log k_{MEEKC} correlation for the TTAB MEEKC system proved that the chromatographic partition values were a good approximation of the partition coefficient values of a compound, by obtaining a value of R^2 very close to 1 and a low SD value.

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12. ACRONYMS

BGE	Background Electrolyte
BIS-TRIS	Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane
BPC	Biopartioning Chromatography
CAS	Chemical Abstracts Service
CE	Capillary Electrophoresis
EOF	Electroosmotic Flow
HPCE	High Performance Electrokinetic Chromatography
HPLC	High Performance Liquid Chromatography
LFER	Linear Free Energy Relationship
ME	Microemulsion
MEEKC	Microemulsion Electrokinetic Chromatography
O/W	Oil-in-water
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SP	Property of a solute in a partition system
TTAB	Tetradecytrimethylammonium Bromide
UV	Ultraviolet