Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1037 (2004) 3–14
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Determination of liquid–liquid partition coefficients by separation methods

A. Berthod [a,*], S. Carda-Broch [b]

[a] *Laboratoire des Sciences Analytiques, CNRS, Université de Lyon 1, Bat CPE-308, 43 Boulevard du 11 November 1918, 69622 Villeurbanne Cedex, France*
[b] *Area de Química Analítica, Universidad Jaime I, Cra. Borriol s/n, 12080 Castellón de la Plana, Spain*

Abstract

By essence, all kinds of chromatographic methods use the partitioning of solutes between a stationary and a mobile phase to separate them. Not surprisingly, separation methods are useful to determine accurately the liquid–liquid distribution constants, commonly called partition coefficient. After briefly recalling the thermodynamics of the partitioning of solutes between two liquid phases, the review lists the different methods of measurement in which chromatography is involved. The shake-flask method is described. The ease of the HPLC method is pointed out with its drawback: the correlation is very sensitive to congeneric effect. Microemulsion electrokinetic capillary electrophoresis has become a fast and reliable method commonly used in industry. Counter-current chromatography (CCC) is a liquid chromatography method that uses a liquid stationary phase. Since the CCC solute retention volumes are only depending on their partition coefficients, it is the method of choice for partition coefficient determination with any liquid system. It is shown that $K_{o/w}$, the octanol–water partition coefficients, are obtained by CCC within the $-1 < \log K_{o/w} < 4$ range, without any correlation or standardization using octanol as the stationary phase. Examples of applications of the knowledge of liquid–liquid partition coefficient in the vast world of solvent extraction and hydrophobicity estimation are presented.

Keywords: Reviews; Partition coefficients; Octanol–water partition coefficients; Thermodynamic parameters; Distribution constants; Hydrophobicity; Counter-current chromatography; Alkylbenzenes; Quinolines

Contents

* Corresponding author. Tel.: +33-472431434; fax: +33-472431078.
E-mail address: alain.berthod@univ-lyon1.fr (A. Berthod).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.01.001

1. Introduction

If a third substance is added to a system of two immiscible liquids in equilibrium, the added component will distribute itself between the two liquid phases until the ratio of its concentrations in each phase attain a certain value: the distribution constant or partition coefficient.

Studied as soon as the end of the 19th century [1], the distribution of solutes in biphasic liquid systems has become an essential field of study. The octanol–water distribution ratio, $K_{o/w}$, is the accepted physicochemical property measuring the hydrophobicity of chemicals [2]. $K_{o/w}$ is the most widely employed descriptor for quantitative structure–activity relationship (QSAR) studies [3,4].

Besides pharmaceutical and biochemical industry that uses QSAR in drug design and toxicology, the measurement of liquid–liquid partition coefficients is extremely important in: (1) fundamental chemistry for studying inorganic and/or organic complex equilibria; (2) industrial chemistry for optimization of production and waste treatment; and (3) food chemistry for purification and extraction of sugars, fat or caffeine [5]. This field is so important that many universities offer a special course in liquid–liquid partitioning and solvent extraction.

Combinatorial chemistry is able to produce large numbers of new compounds that could be potential drugs [6,7]. A drug has to cross four barriers associated with absorption, distribution, metabolism and excretion referred as ADME interface [8]. One of the core properties required to assess ADME characteristics is hydrophobicity that is $K_{o/w}$. If the $K_{o/w}$ determination is not to be a bottleneck in the combinatorial discovery process, it must be at least as easy as it is to synthesize the compound [9].

Partitioning of solutes between a stationary and a mobile phase is the fundamental principle of all kinds of chromatographic methods. Separation methods are the tools of choice to determine liquid–liquid partition coefficients. This article will describe the different ways offered by separation methods focusing on accuracy, ease and speed of the different methods: classical methods, high-performance liquid chromatography (HPLC), capillary electrophoretic (CE) methods and counter-current chromatography (CCC).

2. Partitioning of solutes between two liquid phases

2.1. Nomenclature

The term liquid–liquid partition coefficient is “not recommended” by IUPAC in its 1993 nomenclature for liquid–liquid distribution [10]. Since this term was widely used, “not recommended” is read as “not forbidden” and it continues to be preferred to the “recommended” terms that are *distribution constant* or *partition ratio* whose symbol should be K_D. To be understood by chromatographers, the term partition coefficient will be used throughout this article with the correct symbol, K_D, but the IUPAC definitions are recalled here. IUPAC points the common confusion made between *distribution ratio* and *distribution constant* (or partition coefficient).

2.1.1. Distribution ratio (D)

The ratio of the *total* analytical concentration of a solute in the liquid phase 1, regardless of its chemical form, to its total analytical concentration in the other phase. As defined, the distribution ratio varies with experimental conditions (chemical reaction, precipitation, ionization). It should

not be confused with the distribution constant (or partition coefficient). The distribution ratio is an experimental parameter and its value does not necessarily imply that distribution equilibrium between phases has been achieved [10].

2.1.2. Distribution constant or partition ratio (K_D)

The ratio of the concentration of a substance in a single definite form, A, in the liquid phase 1 to its concentration in the same form in the other phase (liquid phase 2) at equilibrium:

$$K_D = \frac{[A]_1}{[A]_2} \quad (1)$$

In equations relating to aqueous/organic systems, the organic phase concentration is, by convention, the numerator and the aqueous phase concentration the denominator. This definition clearly states that the substance should have a single definite form. It means that the distribution constant (partition coefficient) is a true constant. If the substance evolves, the *distribution ratio* (D) will change; the *distribution constant* (K_D) will not since it is, by definition, invariant. Of course, if the solutes are not ionizable and do not change due to chemical reaction or complexation, then $D = K_D$.

2.2. Thermodynamics

If we consider two practically immiscible solvents, 1 and 2, they form two liquid phases of one solvent saturated in the other. When a solute A is introduced in such a biphasic liquid system, it distributes between the two phases. Assuming ideal mixtures, in the solvent 1 phase, the Gibbs free energy of A, or chemical potential, μ_{1A}, is expressed by:

$$\mu_{1A} = \mu^0_{1A} + RT \ln x_{1A} \quad (2)$$

where μ^0_{1A} is the standard chemical potential of A at infinite dilution in liquid phase 1. Similarly, in the other phase, the chemical potential, μ_{2A}, is:

$$\mu_{2A} = \mu^0_{2A} + RT \ln x_{2A} \quad (3)$$

If the chemical potential is not identical in the two phases, mass transfer of A occurs, the mole fractions x change so that the chemical potential of A becomes equal in both phases, i.e. the equilibrium is reached. Then:

$$\mu^0_{1A} - \mu^0_{2A} = RT \ln \left(\frac{x_{2A}}{x_{1A}} \right) \quad (4)$$

in which x_{2A}/x_{1A} is the distribution constant, K_D, expressed by:

$$\frac{x_{2A}}{x_{1A}} = K_{D2/1} = \exp \left[\frac{\mu^0_{1A} - \mu^0_{2A}}{RT} \right] \quad (5)$$

In case of non-ideal mixtures, the mole fractions, x, should be replaced by activities, $a = xf$, in which f is the activity coefficient. The distribution constant or partition ratio is constant only if the activity coefficients are constant which is not true in concentrated solutions [2]. Partition coefficients are usually expressed as molarity ratio. Molar solubilities, [A], and mole fractions, x_A, are, in first approximation and diluted solutions, proportional and related as follows:

$$[A]_1 = \frac{x_A}{V_1} \quad (6)$$

where V_1 is the solvent 1 molar volume (M^{-1}).

2.3. Effect of temperature

Eqs. (4) and (5) show that the distribution constant, the partition coefficient, is sensitive to temperature. Eq. (4) expresses the free energy of transfer, $\Delta G_{2/1}$:

$$\Delta G_{2/1} = RT \ln K_{D2/1} \quad (7)$$

Assuming the standard molar enthalpy is constant in a limited temperature range, the plot of $\ln K_{D2/1}$ versus $1/T$ (classical Van 't Hoff plots) should produce a straight line with slope $\Delta G_{2/1}/R$. Unfortunately, this is not that simple. The mutual solubility of the two solvents is also temperature dependent. At the critical solution temperature, the biphasic system becomes monophasic [5]. As a general rule, it is possible to consider that the effect of temperature on the K_D value is not great if the solvents are not very miscible and the temperature change is not dramatic. An average change of $0.009 \log K_D$ unit per degree, either positive or negative, was found for a variety of biphasic systems including the octanol–water system [2,3,11].

2.4. Effect of chemical reactions

As clearly defined in the IUPAC definition, when any chemical reaction occurs, the concentration of a particular species will change, the distribution ratio will change, but the distribution constant (partition ratio or partition coefficient) of this particular species does not change. This implies that the concentration of this species will change in the other phase to maintain the chemical potentials equal in the two phases. The distribution ratio, D, can change dramatically.

The case of a compound that has a carboxylic group will be used as an example. This compound can be represented as AH and can ionize:

$$AH \rightleftarrows A^- + H^+ \quad (8)$$

Defining, respectively, K°_D, K^-_D and D as the distribution constants of the molecular and ionized form of AH, and its distribution ratio, it comes:

$$D = \frac{[AH]_2 + [A^-]_2}{[AH]_1 + [A^-]_1} \quad (9)$$

Using the expression of K_a, the dissociation constant of AH, D can be trivially formulated as:

$$D = \frac{K^\circ_D + K^-_D (K_a/[H^+])}{1 + (K_a/[H^+])} \quad (10)$$

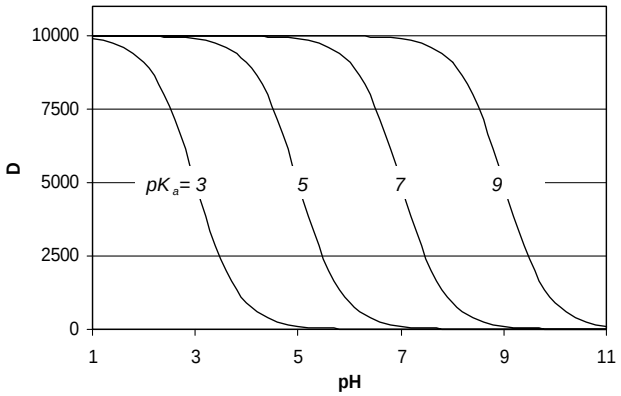

Fig. 1. Change in the distribution ration, D, of ionizable compounds of the AH⇄A$^-$ type with different acidity strength. K_D of all molecular forms is 10 000, K_D of all anionic form is 1. The dissociation constants are indicated as $pK_a = -\log K_a$.

Fig. 1 shows the change in the partitioning of a hydrophobic AH compound in a biphasic liquid system, e.g. the octanol–water system, when the pH changes. At low pH values, AH is essentially located in the organic phase with very high D values. Because the ionized form of AH, A$^-$, is hydrophilic, upon increase of pH, most of the solute is extracted in the aqueous phase in the ionized form. The pH zone corresponding to the change of phases depends on the solute acidity (K_a value, Fig. 1). The distribution ratio is divided by two when pH = pK_a. The case of the partitioning of ionizable solutes was fully studied recently [12]. Applications of the equations developed can also be found in the literature [13].

3. Measurement of partition coefficients

Several review articles describing the various methods used to determine liquid–liquid partition coefficients and especially $K_{o/w}$ appeared recently [2,9,14,15]. The methods are briefly described focusing on accuracy and speed.

3.1. The shake-flask method

K_D values of many solutes are directly determined using the so-called *shake-flask* method. The solute is simply partitioned between the two liquid phases of the proposed solvent system in a test tube. After equilibrium and centrifugation, the relative concentration in each layer is determined using a variety of techniques. These include spectroscopic methods, HPLC, GC, TLC among others.

3.1.1. Small-scale spectroscopic methods

The two phases of the liquid system should be mutually well saturated before use. A suitable quantity, typically 100 μg, of solute is deposited in a test tube. One milliliter of each saturated layer is added to the sample, and the tube is capped and equilibrated for several hours on a wrist-action shaker. When needed, a blank solution is also prepared. The absorbance values of the equilibrated layers and the blanks are read at a suitable wavelength, and the partition coefficient is calculated with the equation:

$$K_D = \frac{[A]_{org} - [A]_{blank}}{[A]_{aq} - [A]_{blank}} \quad (11)$$

The experiment is repeated using a lower quantity, say 50 μg, of solute to increase the method precision and accuracy.

This method is a popular method well suited for compounds that distribute in the liquid system used, i.e. when $0.1 < K_D < 10$. In this case it provides a precision higher than 1% using 1 ml phase volumes and, and can be slightly improved by using 2 ml phase volumes. The method was used with UV spectrometers [16,17] and fluorimeters [18]. Its principal drawback is that pure compounds are needed. If impurities are present, they will distribute also in the liquid phases but with a different K_D value, biasing the measured absorbance in each phase.

3.1.2. Separation methods for phase analysis

The solute purity problem can easily be solved using a method of separation to quantitate the amount of solute present in each phase. It is often not possible to simply substitute a chromatographic method for spectroscopy. Indeed, the technique restrictions apply. It is not possible to use GC if the solute is not volatile or if the liquid phase is aqueous. The organic solvent of the biphasic liquid system may not be compatible with RPLC. However, the major advantage of chromatographic methods is the ability to deal with mixtures and to determine partition coefficients of several solutes in a single assay. This is partly offset by the effort necessary to develop a reliable chromatographic assay for the mixture of interest. HPLC and GC were used in the determination of pesticide partition coefficients [19–21]. TLC was also used [22].

The major point of the shake-flask method is that it is a direct method measuring without approximation the liquid–liquid partition coefficient of the solute in the biphasic liquid system in the flask. The weak point is its limited range that is depending on the method used to analyze the phases. It can be roughly given as $-3 < \log K_D < +3$ with chromatographic phase analysis. Partition coefficients as big as 100 000, $\log K_D = 5$, need to be known accurately. In this case, if 100 μg of the compound is introduced in a test tube containing 2 ml of each phase of a liquid system, only 1 ng will pass in one phase while 99.999 μg will stay in the other phase. The very low concentration, 1 ng in 2 ml or 0.5 μg/l, obtained at equilibrium in one phase may be below the limit of detection of many analytical methods. This level was accurately determined using radiochemical methods working with radioactive isotopes [23]. This method cannot be easily used by most laboratories.

3.1.3. Modern variations on the shake-flask method

Flow injection analysis (FIA) was used to determine $K_{o/w}$ coefficients. The manifold comprised three pumps: one for the aqueous phase, one for the octanol phase and one for the sample that could be dissolved either in the aqueous or in the octanol phase. The octanol and aqueous phases form segments in which the injected compound of interest can partition. A spectrophotometric detector was used to monitor the aqueous phase absorbance. The recorded peak areas obtained at different flow ratios allowed to extrapolate the $K_{o/w}$ solute value [24]. A modified FIA system was designed for rapid $K_{o/w}$ determination adapted for combinatorial chemistry. The result was obtained in less than 4 min consuming less than 1 μl of sample [25]. The FIA method cannot determine accurately $K_{o/w}$ values higher than 2000 ($\log K_{o/w} < 3.3$).

A dialysis tubing was used to contain the aqueous phase and to separate it from the octanol phase. Ultrasonic agitation shortened the equilibration time and HPLC was used to measure the solute concentration in each phase. $K_{o/w}$ values as high as 2.0×10^6 ($\log K_{o/w} = 6.3$, perylene) were accurately determined in less than 6 h [26].

3.2. Liquid chromatography and correlations

3.2.1. A simple and easy method

The use of liquid chromatographic methods using standards and correlations is the most widespread way to measure rapidly liquid–liquid partition coefficients. It is extremely simple: the logarithms of the retention factor of the solutes are linearly correlated with the logarithm of their partition coefficients as first described by Collander [27]:

$$\log K_D = a \log k + b \quad (12)$$

The a and b correlation coefficients are determined measuring the retention factors of a set of solutes on a RPLC column using a mobile phase that differs from the biphasic liquid system in which the K_D values of the test solutes are known. This method is fast and easy (the solute concentration does not need to be known). Several compounds can be measured simultaneously. Unfortunately, the quality of the K_D determination dramatically depends on the test solutes used to determine the a and b correlation coefficients.

By far, this method is used with C_{18} or C_8 columns and methanol–water mobile phases to determine the $K_{o/w}$ partition coefficients in the octanol–water system [28]. Indeed, the hydrophobicity of a compound is often quantitatively expressed by the logarithm of its octanol–water partition coefficient, $\log K_{o/w}$. The prediction of $\log K_{o/w}$ of a compound is essential in various fields such as pharmacology, toxicology, environmental chemistry and food chemistry. With the development of reversed phase HPLC in many laboratories, the readily accessible retention factor of a new compound, compared to the standard but time consuming shake-flask method, made the method extremely popular and widely used.

The method was extensively evaluated in 1988 in an interlaboratory study involving a large set of solutes and different C_{18} columns. The conclusions of the interlaboratory test were [29]:

(1) At least six substances should be used to prepare the correlation line.
(2) The substances should belong to the validated list of reference compounds.
(3) The mobile phase should contain at least 25% (v/v) water.
(4) Extrapolation beyond the calibration range obtained with the selected substances should only be carried out for very hydrophobic substances ($\log K_{o/w} > 6$.
(5) When the conditions are fulfilled, the reliability of the method is, in the range $0 < \log K_{o/w} < 6$, 0.5 log unit or less of deviation from the shake-flask value.

This test was used by the Organization for Economic Cooperation and Development (OECD) to prepare a guideline that should be followed to produce reliable $K_{o/w}$ values using HPLC [30]. The guideline used the five points listed above adding to the second point that "the reference compounds should be structurally related to the test substance". The proposed list of reference compounds are summarized in Table 1. Three main classes of reference compounds are distinguished: neutral, basic and acidic compounds. This list can be adapted for particular cases [31].

3.2.2. Congeneric effect

It is interesting to comment on the five points of the conclusion of the interlaboratory test and the OECD text addition to the second point. The first point proposes that a minimum of six solutes be used to prepare a correlation line. This comes from statistical analysis (and common sense). The second point proposes that the six substances belong to a validated list and the OECD addition says that the substances should be structurally related. This deals with the congeneric effect that is always observed with the HPLC indirect method. The correlation line is very good and the measured $\log K_{o/w}$ values are very accurate when the compounds used to prepare the correlation lines belong all to the same family as well as the unknown compounds. A counter example is: trying to estimate $\log K_{o/w}$ of an amine using a correlation line prepared with aromatic acids gives disastrous results. Alternatively, the correlation line prepared using a set of solutes with widely differing functionalities will have a poor regression coefficient and poor predictive capability.

The third point: "the mobile phase should contain at least 25% (v/v) water", is due to the residual silanols that were always present in 1988 C_{18} columns. Also it makes sense that some water be present in the mobile phase when the partitioning stationary phase octadecyl/mobile phase is correlated to octanol–water. The last point said in clear that if the $\log K_{o/w}$ value of a compound is 2, or $K_{o/w} = 100$, the method can give $\log K_{o/w} = 1.5$, or $K_{o/w} = 32$ as well as $\log K_{o/w} = 2.5$, or $K_{o/w} = 320$. It means that the estimated

Table 1
OECD recommended reference compounds

Reference compound	$\log K_{o/w}$	Class	pK_a
2-Butanone	0.3	n	
4-Acetylpyridine	0.5	B	
Aniline	0.9	B	4.63
Acetanilide	1.0	n	
Benzyl alcohol	1.1	n	
4-Methoxyphenol	1.3	A	10.26
Phenoxyacetic acid	1.4	A	3.12
Phenol	1.5	A	9.92
2,4-Dinitrophenol	1.5	A	3.96
Benzonitrile	1.6	n	
Phenylacetonitrile	1.6	n	
4-Methylbenzyl alcohol	1.6	n	
Acetophenone	1.7	n	
2-Nitrophenol	1.8	A	7.17
3-Nitrobenzoic acid	1.8	A	3.47
4-Chloraniline	1.8	B	4.15
Nitrobenzene	1.9	n	
Cinnamic alcohol	1.9	n	
Benzoic acid	1.9	A	4.19
p-Cresol	1.9	A	10.17
cis-Cinnamic acid	2.1	A	3.89
trans-Cinnamic acid	2.1	A	4.44
Anisole	2.1	n	
Methyl benzoate	2.1	n	
Benzene	2.1	n	
3-Methylbenzoic acid	2.4	A	4.27
4-Chlorophenol	2.4	A	9.10
Trichloroethene	2.4	n	
Atrazine	2.6	B	
Ethyl benzoate	2.6	n	
2,6-Dichlorobenzonitrile	2.6	n	
3-Chlorobenzoic acid	2.7	A	3.82
Toluene	2.7	n	
1-Naphthol	2.7	A	9.34
2,3-Dichloroaniline	2.8	B	2.05
Chlorobenzene	2.8	n	
Allyl phenyl ether	2.9	n	
Bromobenzene	3.0	n	
Ethylbenzene	3.2	n	
Benzophenone	3.2	n	
4-Phenylphenol	3.2	A	9.54
Thymol	3.3	n	
1,4-Dichlorobenzene	3.4	n	
Diphenylamine	3.4	B	0.79
Naphthalene	3.6	n	
Phenyl benzoate	3.6	n	
Isopropylbenzene	3.7	n	
2,4,6-Trichlorophenol	3.7	A	6.0
Biphenyl	4.0	n	
Benzyl benzoate	4.0	n	
2,4-Nitro-6-*sec*-butyl phenol	4.1	n	
1,2,4-Trichlorobenzene	4.2	n	
Dodecanoic acid	4.2	A	4.8
Diphenyl ether	4.2	n	
Phenanthrene	4.5	n	
N-Butylbenzene	4.6	n	
Fluoranthene	4.7	n	
Dibenzyl	4.8	n	
2,6-Diphenylpyridine	4.9	n	
Triphenylamine	5.7	n	
DDT	6.2	n	

From [30]. A: acidic compounds; B: basic compounds; n: neutral compounds.

$K_{o/w}$ value may be three times two big or too low. A recent work with deactivated modern C_{18} columns did not change much the 1988 conclusions. It did show that, with a 50–50 methanol–water mobile phase, the "H-bonding atmosphere of the C_{18} deactivated phase (Capcell Pak C_{18}, a silicone polymer-coated silica gel chemically modified with C_{18}) is very similar to that of octanol" [32].

3.2.3. Variations on the method to improve the correlation quality

Several parameters were added to the $\log K_D$ versus $\log k$ relationship to improve the correlation. For example, Yamagami et al. proposed the equation [33]:

$$\log K_{o/w} = a \log k + b + \rho\sigma_I + sS_{HA} \quad (13)$$

with a, b, ρ and s are the constants for a column and a mobile phase, and σ_I and S_{HA} an inductive electronic constant and the proton acceptor value of the studied solute. Of course, adding parameters does improve the correlation quality, but it decreases the method ease since the σ_I and S_{HA} values of unknown compounds should be also estimated.

It was proposed to use $\log k_w$, the extrapolated solute retention factor in pure water [34,35]. $\log k_w$ is obtained by plotting $\log k$ of the solute versus the organic modifier content in the mobile phase and extrapolating the straight line to 0% modifier (=pure water). Then, $\log k_w$ can be used in Eq. (12) rather than $\log k$. The $\log k_w$ parameter became a widely used chromatographic descriptors of hydrophobicity per se [36–42]. It should be noted that this extrapolated value can yield large errors. This is proved by the fact that the extrapolated $\log k_w$ value, obtained with the same solute and column, may differ significantly depending on the organic modifier used: methanol or acetonitrile.

It was also proposed to use gradient elution to increase the speed of the partition coefficient estimation. It was necessary to use the Abraham linear solvation coefficients to correlate the solute retention factors with $\log K_{o/w}$ [43,44]. Associating methanol gradient elution with a modern stationary phase that can work within the 2–8 pH range showed that $\log K_{o/w}$ values were measured in less than 45 min with a better than 0.3 log unit error in the −0.1 to 4.0 range [45].

The use of octanol- or glycerol-coated stationary phases complicated the method and did not fully suppress the congeneric effect observed with the HPLC methods [46,47]. Micellar liquid chromatography is another HPLC variation that can be used for hydrophobicity measurements [48].

Taking in account the congeneric effect and accepting the 0.5 log unit possible error on the determined $\log K_{o/w}$ values, HPLC is now one of the tools most often used to evaluate quickly the magnitude of the hydrophobicity of a new synthesized compound [49–52].

3.3. Capillary electrophoresis with ordered media

Classical CE uses an electric field to sort charged solutes by differing electrophoretic mobilities. Micellar liquid chro-

matography had shown that the solute micelle binding constants were related to solute hydrophobicity and $K_{o/w}$ coefficients [48]. Micelles were introduced in CE to separate neutral solutes according to their affinity for the micelles [53]. Herbert and Dorsey showed that the retention factors obtained with micellar electrokinetic capillary chromatography (MEKC) allowed to estimate the respective $K_{o/w}$ solute values over 8.5 orders of magnitude ($-2.3 < \log K_{o/w} < 6.2$) with a 0.5 log unit or lower error [54]. However, it was demonstrated that the CE results were also prone to the congeneric effect [55,56].

Ishihama et al. adapted the Herbert and Dorsey's MEKC method proposing to use a microemulsion instead of micelles [57]. Microemulsion electrokinetic capillary chromatography (MEEKC) with a hexane (0.82%, w/w)–1-butanol (6.5%, w/w)–sodium dodecyl sulfate (0.05 M or 1.44%, w/w) microemulsion, the $\log K_{o/w}$ value of compounds could be predicted within the −0.5 to 4.5 range with an error lower than 0.2 log unit. The MEEKC method was used with a cationic microemulsion to work with positively charged solutes [58]. The method was optimized to increase the solute throughput [59].

It was showed that a universal set of standards could be used to calibrate the MEEKC method [60]. Cationic, anionic and surfactant association able to form vesicles were tried to extend the hydrophobicity windows and/or method accuracy [61]. Today, due to its higher throughput and accuracy, the MEEKC method is becoming the dominant indirect method of estimating $\log K_{o/w}$ in industry, supplanting HPLC wherever a CE apparatus is available [8,9].

3.4. The counter-current chromatography method

Counter-current chromatography is a separation technique that uses a liquid mobile phase with a stationary phase that is also liquid. There is no solid support for the liquid stationary phase [62]. Centrifugal fields maintain the two immiscible liquid phase together. The only physicochemical interaction that is responsible for solute retention in a CCC column is liquid–liquid partitioning. The retention equation is:

$$V_R = V_M + DV_S \quad (14)$$

with the subscript R, M and S standing for retention, mobile and stationary phase volumes. Since there are only liquid phases in the CCC column, the column volume, V_C, is:

$$V_C = V_M + V_S \quad (15)$$

The CCC machine (=column) volume is known, and then it is necessary to measure only one volume, either V_M, the mobile phase volume, or V_S, the stationary phase volume.

3.4.1. Direct partition coefficient measurement

3.4.1.1. Method description. In CCC, the retention volume of solute A is directly related, without any correlation, to its distribution ratio, D, in the liquid system used:

$$D = \frac{[A]_{\text{stationary phase}}}{[A]_{\text{mobile phase}}} = \frac{V_R - V_M}{V_S} = \frac{V_R - V_M}{V_C - V_M} \quad (16)$$

Often, there is no chemical reaction, ionization or complexation in the two liquid phases, then $D = K_D$ (see Section 2.1). Otherwise, the distribution ratio is related to the partition coefficient taking in account the chemical changes that occurred in the liquid phases [63,64].

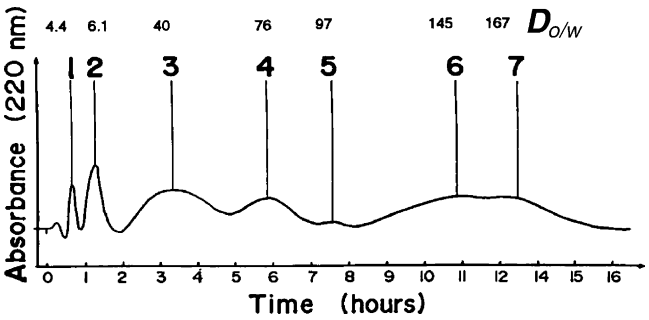

Fig. 2. Direct determination of the $D_{o/w}$ distribution ratio of: (1) benzamide; (2) 2-acetoxy benzoic acid; (3) acetophenone; (4) benzoic acid; (5) 2-chlorobenzoic acid; (6) chlorophenol; (7) 2-chloronitrobenzene. Machine Sanki CPC-LLN, $V_C = 125$ ml, $V_{oct} = V_S = 22.2$ ml, aqueous mobile phase at 5 ml min^{-1} and pH 4. Adapted from [63].

Fig. 2 illustrates the method showing the CCC chromatogram obtained when a mixture of six compounds was injected in a CCC column equilibrated with an octanol stationary phase and eluted with a buffer mobile phase [63]. Without any correlation, the solute retention volumes are related to the $D_{o/w}$ ratio of the solutes by Eq. (16). For example, the retention volume of 2-chlorophenol (compound 6 in Fig. 2) is 3320 ml (retention time of 660 min or 11 h) allowing to calculate its $D_{o/w}$ ratio as 145. Since this compound is not ionized at pH 4, its $D_{o/w}$ ratio is also its $K_{o/w}$ octanol–water partition coefficient. Fig. 2 illustrates perfectly the CCC direct method. Its big advantage is that it gives the D ratio of compounds directly and in any biphasic liquid system. The drawback is the range of the measurable D ratios. Large D values need prohibitive times and mobile phase volumes to be determined. It took 11 h and over 3.2 l of aqueous mobile phase to measure a D ratio of 145 ($\log D = 2.16$). Orders of magnitude higher values should be determined with hydrophobic solutes. The method was used for biological [65], ionizable [12,13,66], or industrial compounds [63,67]. Its use is linked to the availability of CCC chromatographs that are difficult to obtain. This is the main reason why this excellent method for partition coefficient determination is still little known [68].

3.4.1.2. Method range and accuracy. The error on D was established as [64]:

$$\frac{\Delta D}{D} = \frac{\Delta V_R}{V_R - V_M} \quad (17)$$

In the case of 2-chlorophenol already used as an example compound (Fig. 2), the error on reading the retention volume is estimated to be 40 ml because the peak is really broad. Then, $\Delta D/D = 40/(3320 - 103) = 0.0124$ or $\Delta D = 1.8$. This clearly shows the very high accuracy of the CCC method compared to the HPLC method. For 2-chlorophenol, the result is $D = 145 \pm 2$ or $\log D = 2.16 \pm 0.005$ that should be compared to the accuracy of the HPLC method that is commonly 0.05 log unit in this range of hydrophobicity and could be as high as 0.5 log unit with higher hydrophobic compounds and difficult extrapolation (see OECD method, Section 3.2.1). Eq. (17) shows that the accuracy may decrease as the solute retention volume decreases, especially for very low retention volumes. It was practically estimated that the solute retention volume should be at least 5 ml higher than the mobile phase volume (=the "dead" volume) to obtain a maximum acceptable relative error of 10% on D [64]. Then there is a minimum measurable D ratio that is $5/V_S$ (Eqs. (16) and (17)). With Fig. 2, V_S value of 22.2 ml, the minimum measurable D ratio is 0.22. The maximum value depends on detector sensitivity to see the peaks that dramatically broaden with the long retention times (Fig. 2). It cannot be higher than 200. The method range is about $-1 < \log D < 2.3$, that is more than three orders of magnitude with an accuracy better than 0.1 log unit.

3.4.1.3. Method variation. In CCC, the mobile phase can be any one of the two phases of the biphasic liquid system. Fig. 2 shows that 2-chlorophenol is highly retained by the octanol stationary phase. Then, why not use an octanol mobile phase with the aqueous buffer being the stationary phase? Since Eq. (16) still applies, the measured distribution ratio would be $D_{w/o}$ which is trivially $1/D_{o/w}$. Assuming that the CCC column made in these conditions has an aqueous stationary phase volume of 50 ml, the V_M octanol phase would be 75 ml, then Eq. (14) shows that the retention volume of 2-chlorophenol would be $75 + 50(1/145) = 75.3$ ml. The 0.3 ml difference between the "dead" volume and the solute cannot be measured accurately. Clearly, the use of an octanol mobile phase will not help to measure accurately very high $K_{o/w}$ coefficient. It may save time in the case of $K_{o/w}$ coefficient between 10 and 50. These compounds would have measurable and short retention volumes. In the case of octanol, it is recalled that its viscosity is around 7 cP precluding a 5 ml min^{-1} flow rate in an analytical CCC machine [13,63].

3.4.2. Using the liquid nature of the stationary phase

The three orders of magnitude $\log K_D$ range of the CCC direct method is not wide enough. The unique feature of the CCC technique is that the stationary phase is a support-free liquid phase [62]. This allows innovative uses of the technique that cannot be conceived with any other chromatographic technique with a solid or a solid support for the stationary phase.

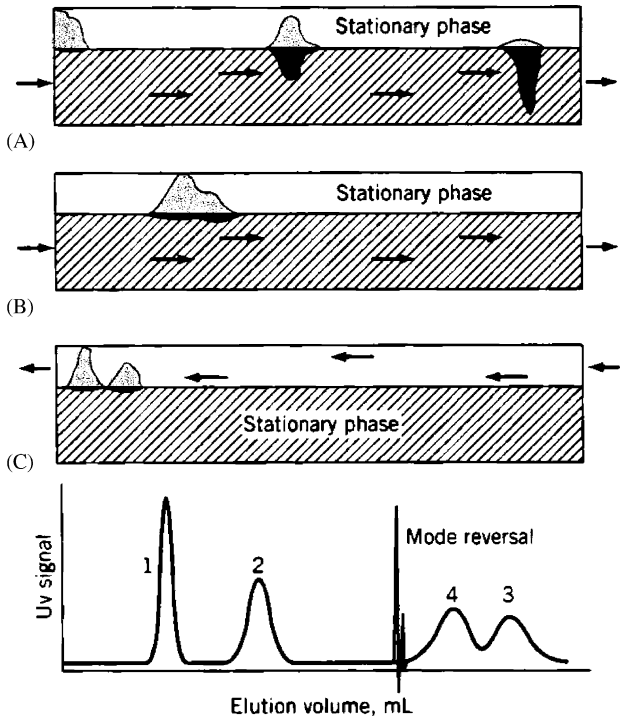

Fig. 3. The dual-mode CCC method. Step A: the mobile phase is the dense hatched liquid phase flowing from left to right and eluting solutes 1 and 2 with D value of 0.2 and 1, respectively. Step B: solutes 3 and 4, with D value of 10 and 20, respectively, move very slowly to the right. Step C: the mobile phase is now the light phase flowing from right to left, solutes 4 and 3 elute rapidly as shown by the chromatogram.

3.4.2.1. Dual-mode CCC. The solutes with very high D values need a very large volume of mobile phase to emerge outside the column because they move very slowly in the column. To force them out of the CCC column, the role of the phases can be reversed after some reasonable flowing time as illustrated by Fig. 3. It was demonstrated that the solute D values was simply expressed by [67,69,70]:

$$D = \frac{V_1}{V_2} \tag{18}$$

with V_1 the volume of phase passed in the normal way (steps 1 and 2, Fig. 3) and V_2, the volume of the other phase passed in the reversed way (step 3, Fig. 3). It cannot be simpler. Neither the machine volume, nor the phase ratio inside the machine or the flow rate matter, they all cancel. There is a detection problem because the liquid phase flowing in the detector during the first phase differs from the one flowing during the reversed mode. Since the V_2 volume is usually much lower than the V_1 volume, it is possible to reduce the flow rate to have more time to re-equilibrate the detector. Using the dual-mode method, $K_{o/w}$ coefficients as high as 20 000 ($\log K_{o/w} = 4.3$) were accurately determined [69,70].

In the case of the $D_{o/w}$ measurements, the distribution ratio is simply V_{aq}/V_{oct}. The error was estimated as:

$$\frac{dD_{o/w}}{D_{o/w}} = \frac{\Delta V_{aq}}{V_{aq}} - \frac{\Delta V_{oct}}{V_{oct}} \tag{19}$$

Since this method is used to measure high $D_{o/w}$ value, the aqueous phase volume is always much higher than the octanol phase volume. The first term of Eq. (19) is always very small compared to the second term. It is critical to minimize the error on the V_{oct} volume using a very low flow rate for the octanol phase. Gluck et al. recommends that the V_{oct} volume be at least 3 ml [69]. If a maximum reasonable V_{aq} volume is 20 l (55 h at 6 ml min^{-1}), then the maximum measurable $D_{o/w}$ is 6700 or $\log D_{o/w} = 3.8$ with a 0.1 log unit error. Accepting the 0.5 log unit error commonly observed with any other methods, $\log D_{o/w}$ as high as 4.5 can be measured ($D_{o/w} \sim 32\,000$) [66,67,69].

3.4.2.2. Cocurrent CCC. This is another method that uses the liquid nature of the stationary phase. The highly retained solutes stay too long inside the liquid stationary phase. Then, pushing slowly the liquid stationary phase in the same direction as the mobile phase will force solutes to elute more rapidly. The full theoretical treatment of the method was given by Berthod [71]. It was shown that V_R, the retention volume of a solute eluted with a F_{aq} aqueous flow rate and a very low F_{oct} octanol flow rate, was given by:

$$V_R = (F_{aq} + F_{oct}) \left(\frac{V_{aq} + D_{o/w} V_{oct}}{F_{aq} + D_{o/w} F_{oct}} \right) \quad (20)$$

Eq. (20) shows that the retention volume of a lipophilic solute will decrease dramatically with F_{oct}. For example, a compound with a $D_{o/w}$ of 1000 has a retention volume of 40 l or a retention time of 4 days and 15 h at 6 ml min^{-1} with a 125 ml machine containing only 40 ml of octanol (Eq. (14)). If a second pump is used to push slowly (0.1 ml min^{-1}) the octanol "stationary" phase, Eq. (20) shows that the retention volume drops to 2.3 l or 6 h and 20 min, a 94% reduction in experiment duration [64].

It was demonstrated that the minimum error (maximum selectivity) was obtained around the $D_{o/w}$ range equal to the flow ratio F_{aq}/F_{oct} [71]. For example, to determine accurately a $D_{o/w}$ value of 1000, an octanol flow rate of 10 μl min^{-1} should be associated with a main aqueous flow rate of 10 ml min^{-1}. The experiment duration will be balanced with the desired accuracy. Low octanol flow rate will produce higher accuracy on high $D_{o/w}$ values at the cost of prohibitive retention times. Solute detection is another serious drawback of the method. As the method was designed, two immiscible liquid phases enter and leave the CCC column. The evaporative light scattering detector can be used to detect solid solutes. Alternately, a mixing agent such as 1-propanol can be added post-column. The UV detector will be usable but the effluent cannot be recycled [64,70]. Table 2 lists the phase volumes, retention times (experiment duration) and $D_{o/w}$ ratio for some compounds comparing the various CCC techniques that can be used. We recently proposed a new method to extend the hydrophobicity window using again the liquid nature of the stationary phase: the elution-extrusion method [72]. However, this method will not allow enhancing the accuracy of partition coefficient measurement by CCC.

4. Application: homologues and partition coefficients

Homologues are families of compounds having the same functionalities and differing by the length of an alkyl chain. With such homologous series of compounds, it was shown that the hydrophobic contribution of the alkyl chain could be dissociated from the contribution of the functional groups [73,74]. Starting from Eq. (7), it can be written:

$$RT \ln K_D = \sum \Delta G_{\text{functional groups}} + n_{CH_2} \Delta G_{CH_2} \quad (21)$$

Table 2
Octanol–water distribution ratio measured using different CCC methods [60–64,69,70]

Solute	V_R (ml)	t_R (min)	V_{oct} (ml)	$D_{o/w}$[a]	$\log D_{o/w}$	Literature value
Direct measurements[b]						
Benzamide	200	40		4.4 ± 0.1	0.64 ± 0.01	0.64 ± 0.02
Benzyl alcohol	383	76.5		12.6 ± 0.1	1.1 ± 0.01	1.1 ± 0.02
Phenol	760	152		29.5 ± 0.2	1.47 ± 0.01	1.5 ± 0.02
2-Chlorophenol	3320	664		145 ± 2	2.16 ± 0.01	2.17 ± 0.03
Dual-mode measurements[c]						
Phenol	157	42	5.15	30.5 ± 0.8	1.48 ± 0.02	1.5 ± 0.02
2-Chlorophenol	487	103	2.8	174 ± 2	2.24 ± 0.05	2.17 ± 0.03
Toluene	2627	536	5.5	478 ± 5	2.68 ± 0.02	2.71 ± 0.06
Naphthalene	4795	962	1.6	3000 ± 40	3.48 ± 0.02	3.23 ± 0.2
Cocurrent measurements[d]						
2-Chlorophenol	2700	337	6.8	147 ± 5	2.17 ± 0.01	2.17 ± 0.03
Toluene	5570	697	14	490 ± 10	2.69 ± 0.01	2.71 ± 0.06
Naphthalene	9280	1162	23.2	5100 ± 100	3.70 ± 0.03	3.23 ± 0.2
Phenanthrene	9810	1229	24.6	20000 ± 1000	4.3 ± 0.1	4.4 ± 0.3

[a] Most compounds cannot ionize, so the $D_{o/w}$ distribution ratio can be read as the $P_{o/w}$ octanol–water partition coefficient.
[b] Machine volume 125 ml, flow rate 5 ml min^{-1}, $V_{oct} = 22.2$ ml.
[c] Machine volume 125 ml, flow rate aqueous phase 5 ml min^{-1}, octanol phase 0.5 ml min^{-1} in the reversed direction after switching mode.
[d] Machine volume 49 ml, flow rate $F_{aq} = 8$ ml min^{-1}, $F_{oct} = 0.02$ ml min^{-1} in the same direction and simultaneously with F_{aq}.

where n_{CH_2} is the number of methylene groups in the alkyl chain.

4.1. Alkylbenzene partition coefficient in the heptane–methanol–water system

The alkylbenzene partition coefficients were studied by CCC in biphasic liquid systems made with heptane, methanol and 20% (v/v) or less water [75]. In these biphasic liquid systems, it was found that $\log K_D$ of the alkylbenzenes were always linearly related to their carbon number. However, the regression lines were excellent ($r^2 \geq 0.999$) if the water content was expressed in mole fraction, χ, rather than the volume percentage. It was experimentally established that, in this biphasic liquid system containing 20% (v/v) or less water, for alkylbenzenes up to dodecylbenzene and at 295 K (22 °C), the heptane–hydrated methanol partition coefficients of the alkylbenzene homologues could be expressed by:

$$\log K_D = (0.342 n_{CH_2} + 1.72)\chi + 0.080 n_{CH_2} + 0.034 \quad (n_{CH_2} < 13,\ \chi < 0.361 \text{ and } T = 295\,\text{K}) \tag{22}$$

For example, the heptane–hydrated methanol partition coefficient of hexylbenzene in the biphasic liquid system heptane–(methanol–water (85–15, v/v)) is 38.5, $\log K_D = (0.342 \times 6 + 1.72) \times 0.284 + 0.08 \times 6 + 0.034 = 1.585$. The CCC measured value was 40 or $\log K_D = 1.60$ [75]. Trivially, the hydrated methanol–water partition coefficient is $1/38.5 = 0.026$ or -1.585 for the log value.

The partitioning of the alkylbenzenes in the heptane–(methanol–water) biphasic liquid system could be related to the similar partitioning occurring between the liquid-like octadecylsilyl-bonded layer in RPLC with methanol rich mobile phases. The correlation of the alkylbenzene retention factors with the water content in the mobile phase is a common selectivity test in RPLC [73,74,76,77].

4.2. Petroleum components and waterless biphasic liquid systems

In another study, the partition coefficients of alkylbenzenes and polyaromatic hydrocarbons (PAH) were studied in waterless biphasic liquid systems such as heptane–dimethyl sulfoxide (DMSO), heptane–2-furancarboxaldehyde (furfural), heptane–dimethylformamide (DMF), heptane–*N*-methylpyrrolidone (NMP), all four solvents of the dipolar and aprotic family, and heptane–methanol, a polar and protic solvent [78]. The linear relationship of Eq. (22) was verified in aprotic solvents. It was also found that a similar linear relationship could be established between the $\log K_D$ values of the PAHs and the number of sp^2 hybridized carbons in their molecules.

The very interesting point was that, for all four aprotic solvents studied, the solute affinity for the heptane phase increased with the length of the alkyl chain and decreased with the number of sp^2 hybridized carbons. From a thermodynamic point of view, the addition to a hydrocarbon molecule of a methylene group produced a positive ΔG_{CH_2} contribution when the addition of a sp^2 hybridized carbon produced a negative ΔG_{sp^2} contribution. Table 3 lists the ΔG_{CH_2} and ΔG_{sp^2} contribution for the four solvents studied. It also gives the ratio of the two contributions. This ratio shows how many sp^2 hybridized carbon should be added to the molecule of a compound to compensate for one more methylene group so that the new compound partition coefficient does not change. For example, the heptane–DMSO $\Delta G_{CH_2}/\Delta G_{sp^2}$ ratio is 3.93, almost 4. In this system, the partition coefficient of ethylbenzene is 2.12, 33% lower than the 3.16 value for propylbenzene [78]. However, the heptane–DMSO partition coefficient of propylnapthalene is likely very close to 2.12 (not measured in [78]). Propylnaphthalene has four more sp^2 hybridized carbons compared to propylbenzene. These four sp^2 hybridized carbons in propylnaphthalene compensate for the methylene group and ethylbenzene and propylnaphthalene should have both similar partition coefficients in the heptane–DMSO system.

Table 3
Transfer energy for alkylated (CH_2) and arylated (sp^2) hydrocarbon in waterless heptane/polar solvent systems

Parameter	Solvent			
	NMP	DMF	Furfural	DMSO
ΔG_{CH_2} (kJ mol^{-1})	0.33	0.42	0.56	0.94
ΔG_{sp^2} (kJ mol^{-1})	−0.56	−0.39	−0.40	−0.24
Ratio $-\Delta G_{CH_2}/\Delta G_{sp^2}$	0.59	1.07	1.42	3.93

Data from [78]. Temperature 298 K or 25 °C; NMP: *N*-methylpyrrolidone; DMF: dimethylformamide; furfural: 2-furancarboxaldehyde; DMSO: dimethylsulfoxide.

These results clearly show the interest of knowing the partition coefficients of compounds in liquid–liquid biphasic systems. Like known for decades, the study shows that DMSO is a solvent that will selectively extract the PAHs with little alkyl substitution from a crude oil or petroleum fraction [5]. However, it allowed a clear view of the properties of several similar solvents. For example, NMP with its low $\Delta G_{CH_2}/\Delta G_{sp^2}$ ratio (Table 3) will extract aromatic compounds and PAHs including the alkyl-substituted ones, leaving a raffinate rich in alkanes and saturated hydrocarbons.

4.3. Quinoline homologues in heptane–acetonitrile–methanol systems

It was found that 2-alkylquinolines could be very active against leishmaniasis, a tropical disease due to a protozoan of the genus *Leishmania*. A French team synthesized 2-alkylquinolines with 1–16 carbon atoms in the alkyl chain and separated them using CCC and the heptane–methanol–acetonitrile biphasic liquid system [79]. In this waterless

system they found relationship similar to Eq. (21) and listed the slopes and intercepts of the lines in four different biphasic compositions. The $K_{\text{heptane/polar phase}}$ partition coefficients were increasing exponentially with the carbon number in the alkyl chain and also with the methanol content in the polar non-aqueous phase.

Using the published data [79], it is possible to derive the general equation relating, $K_{\text{heptane/polar phase}}$, the partition coefficient of the 2-alkylquinoline:

$$\ln K_{\text{heptane/polar phase}} = -1.3 + 0.3n_C - 0.235\%_{\text{MeOH}} - 0.145n_C \times \%_{\text{MeOH}} \quad (23)$$

where n_C carbon number in the alkyl chain and in the heptane/polar phase, with the polar phase made of $\%_{\text{MeOH}}$ (v/v) methanol percentage. For example, the $K_{\text{heptane/polar phase}}$ of 2-hexylquinoline and 2-octylquinoline in the heptane–acetonitrile–methanol (5:1:4, v/v/v) liquid system are calculated as 0.681 and 0.984 with $n_C = 6$ and 8, respectively, and $\%_{\text{MeOH}} = 0.8$ or 80% of methanol in the polar phase. The experimental values were measured as 0.68 and 0.99 (accuracy not given) in [79]. The exact knowledge of these partition coefficients will be very useful in QSAR studies on the activity of these quinoline homologues on the protozoan parasite. They will be used also to separate and purify a particular member of the family [79].

5. Conclusion

Separation methods can be used to determine liquid–liquid partition coefficient of solutes. The most needed liquid–liquid partition coefficient is the octanol–water partition coefficient. $K_{o/w}$ is accepted as a good reference parameter for solute hydrophobicity. This parameter may not be the best one for this purpose and this could be extensively discussed. However, it is convenient to have it and it is striking that any new method able to estimate solute hydrophobicity systematically refers to $K_{o/w}$ or $\log K_{o/w}$ to show its efficacy. Indeed, $K_{o/w}$ can be rapidly estimated using capillary electrophoresis with a micellar or microemulsion solution and/or RPLC. Both methods give rapidly the $K_{o/w}$ order of magnitude. Next, if needed, a more accurate value can be obtained again using RPLC with a careful standardization taking in account the congeneric effect. CCC working with the octanol–water biphasic system can produce very accurate $K_{o/w}$ value without any approximation but within a limited range of hydrophobicity and with an intensive hardware. It was shown that the distribution ratio, D, of ionizable compounds could also be measured by CCC. Almost any biphasic liquid system can be used in CCC allowing to measure partition coefficients in a wide variety of solvent environments. We are considering the new class of solvents: the room temperature ionic liquids [80,81].

Acknowledgements

The authors gratefully acknowledge the financial support from the European Community through the Marie Curie Fellowship HPMF-CT-2000-00440 and the INTAS grant No. 00-00782. A.B. thanks the Centre National de la Recherche Scientifique FRE2194-UMR5180 for continuous support.

References

[1] M. Berthelot, E. Jungfleish, Ann. Chim. Phys. 26 (1872) 396.
[2] J. Sangster, Octanol–Water Partition Coefficients, Fundamentals and Physical Chemistry, Wiley, Chichester, 1997.
[3] C. Hansch, A. Leo, D. Hoelkman, Exploring QSAR: Fundamentals and Applications in Chemistry and Biology, American Chemical Society, Washington, DC, 1995.
[4] P.C. Taylor, in: J.C. Deardon (Ed.), Comprehensive Medicinal Chemistry: The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds, Pergamon Press, New York, 1990, pp. 241–294.
[5] J. Rydberg, C. Musikas, G.R. Choppin, Principle and Practices of Solvent Extraction, Marcel Dekker, New York, 1992.
[6] A.W. Czarnik, Anal. Chem. 70 (1998) 378A.
[7] S. Borman, Chem. Eng. News November (2002) 43.
[8] S.K. Poole, S. Patel, K. Dehring, H. Workman, J. Dong, J. Chromatogr. B 793 (2003) 265.
[9] S.K. Poole, C.F. Poole, J. Chromatogr. B 797 (2003) 3.
[10] N.M. Rice, H.N.M.H. Irving, M.A. Leonard, Pure Appl. Chem. 65 (11) (1993) 2373.
[11] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525.
[12] A. Berthod, S. Carda-Broch, M.C. Garcia-Alvarez-Coque, Anal. Chem. 71 (1999) 879.
[13] S. Carda-Broch, A. Berthod, J. Chromatogr. A 995 (2003) 55.
[14] L.G. Danielsson, Y.H. Zhang, Trends Anal. Chem. 15 (1996) 188.
[15] K. Valko (Ed.), Separation Methods in Drug Synthesis and Purification, Elsevier, Amsterdam, 2000.
[16] W. Szczepaniak, A. Scymanski, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1217.
[17] N. Gulyaeva, A. Zaslavsky, P. Lechner, M. Chlenov, A. Chait, B. Zaslavsky, Eur. J. Pharm. Sci. 17 (2002) 81.
[18] B. de Castro, P. Gameiro, C. Guimaraes, J. Lima, S. Reis, Biophys. Chem. 90 (2001) 31.
[19] M.C. Bowman, M. Beroza, Anal. Chem. 38 (1966) 1544.
[20] D. Stopher, S. McClean, J. Pharm. Pharmacol. 42 (1990) 144.
[21] J. Thomas, O. Adetchessi, F. Pehourcq, P. Dallet, C. Jarry, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 671.
[22] D.G. Martin, S.A. Mizsak, W.C. Krueger, J. Antibiot. 38 (1985) 746.
[23] J. Tools, D.T.H. Sum, Environ. Toxicol. Chem. 14 (1995) 1675.
[24] L.G. Danielsson, Y.H. Zhang, J. Pharm. Biomed. Anal. 12 (1994) 1475.
[25] K. Carlsson, B. Karlberg, Anal. Chim. Acta 423 (2000) 137.
[26] J.T. Andersson, W. Schräder, Anal. Chem. 71 (1999) 3610.
[27] R. Collander, Acta Chem. Scand. 5 (1951) 774.
[28] S.H. Hunger, P.S. Cheung, G.H. Chiang, J.R. Cook, in: W.J. Dunn, J.H. Block, Pearlman, R.S. (Eds.), Partition Coefficient Determination and Estimation, Pergamon Press, New York, 1986, pp. 69–82.
[29] W. Klein, H. Kordel, M. Weiss, H.J. Poremski, Chemosphere 17 (1988) 361.
[30] OECD, Guideline for Testing of Chemicals, vol. 117, 1989, http://www.oecd.org.
[31] M. Cichna, P. Markl, J.F.K. Huber, J. Pharm. Biomed. Anal. 13 (1995) 339.
[32] C. Yamagami, K. Kawase, K. Iwaki, Chem. Pharm. Bull. 50 (2002) 1578.

[33] C. Yamagami, K. Kawase, T. Fujita, Quant. Struct.–Act. Relat. 18 (1999) 26.
[34] T. Braumann, J. Chromatogr. 373 (1986) 191.
[35] C.H. Lochmüller, M. Hui, J. Chromatogr. Sci. 36 (1998) 11.
[36] R.J.E. Growls, E.W. Ackerman, H.H.M. Korsten, L.J. Hellebrekers, D.D. Breimer, J. Chromatogr. B 694 (1997) 421.
[37] C.M. Du, K. Valko, C. Bevan, D. Reynolds, M.H. Abraham, Anal. Chem. 70 (1998) 4228.
[38] A. Kaune, R. Brüggemann, A. Kettrup, J. Chromatogr. A 805 (1998) 119.
[39] S. Griffin, S.G. Willye, J. Marckham, J. Chromatogr. A 864 (1999) 221.
[40] L. Novotny, M. Abdel-Hamid, H. Hamza, J. Pharm. Biomed. Anal. 24 (2000) 125.
[41] Q.C. Meng, H. Zou, J.S. Johansson, R.G. Eckenhoff, Anal. Biochem. 292 (2001) 102.
[42] T.L. Djakovic-Sekulic, S.M. Petrovic, N.U. Perisic-Janjic, S.D. Petrovic, Chromatographia 54 (2001) 60.
[43] K. Valko, C.M. Du, C. Bevan, D.P. Reynolds, M.H. Abraham, Curr. Med. Chem. 8 (2001) 1137.
[44] M.C. Du, K. Valko, C. Bevan, D.P. Reynolds, M.H. Abraham, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 635.
[45] N.C. Dias, M.I. Nawas, C.F. Poole, Analyst 128 (2003) 427.
[46] A. Kaune, R. Bruggemann, A. Kettrup, J. Chromatogr. A 805 (1998) 119.
[47] K. Miyake, M. Nobayasu, H. Terada, J. Chromatogr. 439 (1988) 227.
[48] A. Berthod, C. Garcia-Alvarez-Coque, Micellar Liquid Chromatography, Chromatographic Science Series, vol. 83, Marcel Dekker, New York, 2000.
[49] W.J. Lambert, J. Chromatogr. A 656 (1993) 469.
[50] S. Griffin, S.G. Wyllie, J. Markham, J. Chromatogr. A 864 (1999) 221.
[51] M. Nakamura, T. Suzuki, K. Amano, S. Yamada, Anal. Chim. Acta 428 (2001) 219.
[52] S.K. Sahu, G.G. Pandit, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 26.
[53] S. Terabe, K. Otsuka, K. Ichikawa, T. Ando, Anal. Chem. 56 (1984) 111.
[54] B.J. Herbert, J.G. Dorsey, Anal. Chem. 67 (1995) 744.
[55] M.D. Trone, M.S. Leonard, M.G. Khaledi, Anal. Chem. 72 (2000) 1228.
[56] K.A. Kelly, T.S. Burns, M.G. Khaledi, Anal. Chem. 73 (2001) 6057.
[57] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, Anal. Chem. 67 (1995) 1588.
[58] Y. Ishihama, Y. Oda, N. Asakawa, Anal. Chem. 68 (1996) 4281.
[59] S.J. Gluck, M.H. Benko, R.K. Hallberg, K.P. Steele, J. Chromatogr. A 744 (1996) 141.
[60] W.L. Klotz, M.R. Schure, J.P. Foley, J. Chromatogr. A 930 (2001) 145.
[61] W.L. Klotz, M.R. Schure, J.P. Foley, J. Chromatogr. A 962 (2002) 207.
[62] A. Berthod, CCC, The Support-Free Liquid Stationary Phase, Comprehensive Analytical Chemistry, vol. 38, Elsevier, Amsterdam/New York, 2002.
[63] A. Berthod, Y.I. Han, D.W. Armstrong, J. Liq. Chromatogr. 11 (1988) 1441.
[64] A. Berthod, in: A. Foucault (Ed.), Centrifugal Partition Chromatography, Chromatographic Science Series, vol. 68, Marcel Dekker, New York, 1995, Chapter 7, pp. 167–197.
[65] P. Vallat, N. El Tayar, B. Testa, I. Slacanin, A. Martson, K. Hostettmann, J. Chromatogr. 504 (1990) 411.
[66] A. Berthod, V. Dalaine, Analusis 20 (1992) 325.
[67] S.J. Gluck, E.J. Martin, J. Liq. Chromatogr. 13 (1990) 3559.
[68] A. Finizio, M. Vighi, D. Sandroni, Chemosphere 34 (1997) 131.
[69] S.J. Gluck, E. Martin, M.H. Benko, in: A. Foucault (Ed.), Centrifugal Partition Chromatography, Chromatographic Science Series, vol. 68, Marcel Dekker, New York, 1995, Chapter 8, pp. 199–218.
[70] A. Berthod, R.A. Menges, D.W. Armstrong, J. Liq. Chromatogr. 15 (1992) 2769.
[71] A. Berthod, Analusis 18 (1990) 352.
[72] A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, Anal. Chem. 75 (2003) 5886.
[73] W.R. Melander, Cs. Horváth, Chromatographia 15 (1982) 86.
[74] G. Guiochon, in: Cs. Horvath (Ed.), HPLC: Advances and Perspectives, vol. 2, Academic Press, New York, 1980.
[75] A. Berthod, M. Bully, Anal. Chem. 63 (1991) 2508.
[76] H. Colin, G. Guiochon, J.C. Diez-Masa, Anal. Chem. 53 (1981) 146.
[77] M. Czok, H. Engelhardt, Chromatographia 27 (1989) 5.
[78] A. Berthod, A.I. Mallet, M. Bully, Anal. Chem. 68 (1996) 431.
[79] P. Duret, M.A. Fakhfakh, C. Herrenknecht, A. Fournet, X. Franck, B. Figadere, R. Hocquemiller, J. Chromatogr. A 1011 (2003) 55.
[80] A. Berthod, S. Carda-Broch, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 1493.
[81] A. Berthod, S. Carda-Broch, D.W. Armstrong, Anal. Bioanal. Chem. 375 (2003) 191.