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Review

Effect of separation conditions on chromatographic determination of hydrophobicity of acidic xenobiotics

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Abstract

Problems encountered in the chromatographic determination of hydrophobicity of acidic xenobiotics are discussed. First, the definition and meaning of hydrophobicity is briefly presented. Next, the methods of determination of the hydrophobicity parameter by reversed-phase high-performance liquid chromatography are described. The methods of determination of the dead volume are analyzed with regard to calculation of the thermodynamically valid retention parameters. Relationships between retention factors and pH of mobile phase which have been reported in the literature are presented. The effects of ionic strength and buffer composition on the apparent retention parameters are discussed. The reversed-phase stationary phase materials presently employed for hydrophobicity determinations are reviewed. Application of micellar electrokinetic chromatography in the determination of hydrophobicity of ionizable analytes is presented. The ability of chromatography to provide the measures of hydrophobicity of xenobiotics best modelling their biological activity is underlined.

Keywords: Reviews; Hydrophobicity; Xenobiotics, acidic

Contents

1. Introduction

Hydrophobicity is usually understood as a measure of the relative tendency of an analyte to prefer a nonaqueous to an aqueous environment. It is also defined as a measure of the tendency of an analyte molecules to aggregate in aqueous solutions [1]. Both measures express some kind of phobia of the analytes towards the aqueous medium. Chromatographers treat the term ‘hydrophobicity’ as synonym-

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ous to 'lipophilicity'. Medicinal chemists insist on distinguishing the two phenomena. For example, Pliška et al. [2] provide the following definitions: 'Hydrophobicity is the association of nonpolar groups or molecules in an aqueous environment which arises from the tendency of water to exclude nonpolar molecules. Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behavior in a biphasic system, either liquid–liquid (e.g., partition coefficient in 1-octanol–water) or solid–liquid (e.g., retention in RP-HPLC or TLC) systems'.

The importance of the hydrophobic (lipophilic) properties of xenobiotics for their pharmacological and toxicological potency has been recognized for a century. There was a wide acceptance of the hydrophobicity parameter introduced by Hansch and Fujita [3] and defined as the logarithm of the ratio of the concentrations of an analyte in a saturated two phase system formed by 1-octanol and water, log *P*. The tediousness of the determinations of log *P* and limited inter-laboratory reproducibility on one hand, and Martin's observation [4] of linear relationships between chromatographic retention and slow-equilibrium partition parameters, on the other hand, gave rise to substituting the reference log *P* by conveniently determined chromatographic hydrophobicity parameters.

The general methodology of chromatographic determination of hydrophobicity has been the subject of detailed [5–7] and comprehensive [8–10] recent reviews. The reader interested in basic methodology is referred to the indicated literature and the works cited therein. Here the emphasis will be put on specific problems encountered in the chromatographic evaluation of hydrophobicity of acidic organic analytes.

2. Chromatographic parameter of hydrophobicity

The logarithm of retention factor corresponding to pure water (buffer) mobile phase, log k_w, is the most commonly employed chromatographic hydrophobicity parameter determined by reversed-phase HPLC. Analogous is the R_M^0 parameter from TLC. The values of log k_w (R_M^0) are obtained based on a series of isocratic measurements at various compositions of binary organic–water (buffer) eluents and extrapolation of the relationship between log k and volume fraction of organic solvent, φ, to 100% water (buffer). Normally, linear log k vs. φ relationships are assumed after Soczewiński and coworkers [11,12]. Occasionally, a quadratic dependence of ln k on φ is employed after Schoenmakers et al. [13].

The performance of these and several other less popular models describing dependence of HPLC retention on the composition of eluent was studied by Sadlej-Sosnowska and Śledziñska [14]. No evident advantages of the more sophisticated models were found.

The extrapolated from polycratic measurements hydrophobicity parameters, log k_w, are not necessarily identical with the values actually measured using pure water (buffer) as the mobile phase. Nonetheless, these values are considered to be more reliable structural parameters of analytes than the individual isocratic log k, especially as predictors of log P [15,16]. However, the determination of log k_w is quite tedious. In such a situation the recently proposed chromatographic hydrophobicity parameters determined by gradient elution HPLC [17,18] are worthy of further testing.

3. Problems of dead volume determination in reversed-phase HPLC

To calculate retention factors, k, in HPLC one needs the values of the dead volume or the dead time of the column used. The determination of these quantities presents both theoretical and practical problems.

From the thermodynamic point of view, dead volume denotes the total volume of all eluent components within the column bed, i.e., the eluent in the inter-particle space plus the eluent within the pores of the particles of column packing [19]. However, bonded stationary phases preferentially adsorb certain components from eluents. Should one consider these adsorbed eluent components as the part of the mobile or the stationary phase?

In the case of HPLC on porous packing materials an unambiguous definition of what constitutes mobile phase and what constitutes stationary phase is impossible. On the other hand, to make thermo-

dynamically valid measurements of k one must provide a clear-cut definition of dead volume so that the data can be compared for various analytes. The value used for dead volume should ideally be the same for a single column for all eluents and should be readily determined with adequate precision. Unfortunately, the popular recipes to determine dead volume do often not provide correct data [19].

The following methods of determination of void volume, V_M, in reversed-phase HPLC may be found in the literature [6,20]. Sometimes V_M is the elution volume of a solvent disturbance or system peak obtained by injecting an eluent component. As the V_M one often takes the elution volume of a nonionized analyte which gives the lowest retention and is small enough not to be sterically excluded. Some researchers recommend for V_M the elution volume of an isotopically labelled component of eluent, e.g., ^2H_2O. Often V_M is considered the elution volume of a salt or ion, usually a UV-absorbing ion. According to another recipe, V_M is the volume of liquid which the column contains (obtained by weighing the column full of liquid and then empty) less the volume of any absorbed eluent components. A more complex definition describes V_M as the volume which, when subtracted from elution volumes, V_{Rn}, of a series of homologues, provides a linear dependence of log $(V_{Rn}-V_M)$ against n, the number of carbon atoms in the homologues.

Probably the determination of V_M by flushing the column with a single component eluent and measuring the elution volume of the isotopically labelled eluent sample gives most reliable and reproducible data. Deuterated eluent can easily be detected with a UV detector due to its different refraction coefficient. The method is not ideal, however, because deuterium substitution may have an isotope effect on the distribution constants [21,22].

In spite of all the above mentioned objections most of the reversed-phase HPLC capacity factors used as measures of hydrophobicity of analytes have been calculated based on elution volumes of inorganic salts.

4. Retention as a function of pH

Dependence of the capacity factor, k, from reversed-phase HPLC on pH has been the subject of numerous papers (e.g., [23–27]). For a weak monoprotic acid the observed capacity factor, k, is:

$$k = (k_0 + k_i K_a/[H^+])/(1 + K_a/[H^+]) \quad (1)$$

where k_0 is the capacity factor for neutral (protonated) form of the acid and k_i refers to the retention of an anion; K_a is the dissociation constant of the acid and $[H^+]$ is the actual concentration of hydrogen ions in the eluent.

Eq. (1) results if one assumes the observed capacity factor, k, to be a weighted average of the capacity factors of individual species, k_0 and k_i [24]:

$$k = k_0[HA]([HA] + [A^-])^{-1} + k_i[A^-]([HA] + [A^-])^{-1} \quad (2)$$

Lewis et al. [26] proposed the following notation equivalent to Eqs. (1) and (2):

$$k = k_0(1 - F_i) + k_i F_i \quad (3)$$

where F_i is the fraction of analyte molecules which are ionized. This fraction for an acidic compound is:

$$F_i = (1 + [H^+]/K_a)^{-1} \quad (4)$$

The authors of [26] stress that significant deviations from Eq. (3) sometimes occur, especially for pH values which are either much higher or much lower than pK_a.

Another transformation of Eq. (1) can be:

$$k_0 = k(1 + 10^{pH - pK_a}) \quad (5)$$

In case of TLC data Lepri et al. [28] employed the following equation accounting for the dependence of R_F values of a series of phenols on pH of the eluent:

$$1/R_F - 1 = (1/R_F^0 - 1)[H^+](K_a + [H^+])^{-1} + (1/R_F^i - 1)K_a(K_a + [H^+])^{-1} \quad (6)$$

where R_F^0 refers to the protonated form of the phenol and R_F^i refers to its deprotonated form.

For monoprotic bases the equations corresponding to Eqs. (1)–(6) have the positions of $[H^+]$ and K_a or pH and pK_a exchanged.

Appropriate equations for bifunctional acids and bases can be found [29] although their experimental verification is much more difficult.

Empirical modifications of Eq. (1) were proposed. The equation given by Hanai [30] has the form:

$$k = 0.5(k_0 - k_i)\tanh(pK_a - pH) + 0.5(k_0 + k_i) \quad (7)$$

where tanh means tangent hyperbolic function. Recently Hanai et al. [31] reported an approach to calculate pK_a values of phenolic and nitrogen-containing analytes based on reversed-phase HPLC retention data and Hammett's constants of substituents.

The equation for acids by van der Waterbeemd et al. [9] is as follows:

$$\log k_0 = \log k + [1 - \tanh(pK_a - pH + 1)] \quad (8)$$

All the equations given above assume the pH of the mobile phase to be the same as the pH of its aqueous fraction. This assumption is certainly disputable, especially if higher proportions of organic modifiers are used to prepare individual eluents.

Some authors propose measuring of pH after mixing the organic and aqueous components [32,33]. However, the potentiometric systems are normally calibrated with aqueous standards. Hence, the apparent pH of the mixture may be different from the actual one.

Having the above in mind Bosch and coworkers [34–36] proposed procedures to calculate the true pH value and ionic strength of the methanol–buffer mobile phases. The approach was tested for weak acid analytes and the buffers most often used in HPLC.

Bosch and coworkers [34–36] argue that dissociation of acids in methanol–water mixed solvents is governed by electrostatic interactions and specific analyte–solvent interactions (solvation effects). In the dissociation of acids charges are formed. The process of dissociation is disturbed as the dielectric constant of the medium decreases with increasing content of methanol. In effect, the pK_a of a neutral or anionic acid increases if methanol concentration increases. For a cationic acid the decrease of pK_a by solvation by methanol–water is not balanced by the change of dielectric constant and the pK_a decreases with the formation of the methanol–water complex. Exemplary data collected by Bosch et al. [35] from the literature are given in Table 1. Bosch et al. [36] derived a rather complex equation predicting pK_a values of the acids most often used to prepare buffers for reversed-phase HPLC at different compositions of the methanol–water mobile phases. From the pK_a values and the buffer composition, the pH values were calculated for the buffer at given mobile phase composition. The dependence of retention time, t_R, for an acid HA on the true pH values in the mobile phase derived by Rosés et al. [36] is:

$$t_R = [(t'_{R(HA)} + t_{O(HA)})y_{A^-}10^{pK_a - pH} + (t'_{R(A^-)} + t'_{O(A^-)})] \cdot [y_{A^-}10^{pK_a - pH} + 1] \quad (9)$$

where $t'_{R(HA)}$ and $t'_{R(A^-)}$ are adjusted retention times of a neutral of the acid and its anion, respectively; $t'_{O(HA)}$ and $t'_{O(A^-)}$ are the corresponding holdup times; y_{A^-} is the activity coefficient of the anion and pK_a is actual acidity of the analyte in a given methanol–water mobile phase. Employing Eq. (9) one can calculate pK_a values of acid analytes. These values are increasing with increasing methanol content in mobile phase.

The corrections of the pH values of the organic modifier–buffer mobile phase with regards to the pH of the buffer alone should be kept in mind when using eluents of a high content of the organic modifiers. This concerns, for example, the chromato-

Table 1
pK_a values of selected neutral, anionic and cationic acids in pure water and in 50% (v/v) methanol–water mixture [35].

Acid	pK_a	
	Water	50% Methanol
Phosphoric		
pK_{a1}	2.11	3.21
pK_{a2}	7.19	8.24
Citric		
pK_{a1}	3.13	3.98
pK_{a2}	4.78	5.70
pK_{a3}	6.39	7.59
Succinic		
pK_{a1}	4.20	5.00
pK_{a2}	5.60	6.71
Trichloroacetic	0.65	1.61
Formic	3.73	4.35
Acetic	4.77	5.54
Benzoic	4.19	5.23
Anilinium	4.60	4.23
Ammonium	9.24	8.76

graphically determined pK_a values of analytes. If there would be a reference chromatographic hydrophobicity scale of analytes at fixed pH (e.g., physiological pH 7.2–7.4) appropriate corrections would also make sense. There is no such a scale and the slow-equilibrium partition coefficient, *P*, from octanol–water systems provides the accepted reference scale causing no pH problems.

Normally, one needs chromatographic measures of hydrophobicity for a series of compounds of more or less diverse properties of interest (e.g., bioactivity). To study the effect of differences in hydrophobicity among the analytes on their other properties, during the chromatographic determinations of hydrophobicity the pH of the buffer is usually measured before mixing it with the organic modifier. However, in the case of methanol–water mixtures, the thermodynamic pH (pH^T) can be calculated from the operational pH (pH^0) and the tabulated correction term ϕ [37]:

$$pH^T = pH^0 + \phi \quad (10)$$

where pH^0 is the value measured with a glass electrode calibrated with standard aqueous buffers.

5. Causes of deviations of the observed from the theoretically predicted retention

Lewis et al. [26] identified six main reasons for errors in predicted retention as a function of pH: (i) interactions of analytes with exposed silanols or metal contaminants of stationary phase, whose pK_a or complexing constants can also depend on pH; (ii) effect of ionic strength on K_a; (iii) solvophobic effect of ionic strength on retention; (iv) ion-pair interaction of sample ions with ionized buffer components; (v) change in the microscopic nature and sorption properties of the hydrocarbonaceous silica as a result of changing ionization of silanols; (vi) changes in buffer type, when more than one buffer is used to attain requested pH range.

Other authors mention additional complications, e.g., those caused by the presence of neutral, polybasic and/or amphoteric sample components [32,38]. One has to remember that the retention of neutral forms of ionizable analytes also depends in some instances on pH, although this effect is much weaker than for ionizable species.

A pronounced effect of ionic strength of eluent on retention factors of anionic solutes chromatographed on a silica-based hydrocarbonaceous stationary phase is illustrated in Fig. 1 after Knox et al. [39]. It appears that ionized organic acids can be excluded from the pores of stationary phase available to the eluent. The anions are thus eluted earlier than the molecules of the mobile phase simultaneously introduced on the column. It means negative values of the retention factor, *k*. It can be noted from Fig. 1 that the process of exclusion of acids becomes saturated at a higher ionic strength of the eluent (0.1–1 *M*).

Exclusion of anionic analytes from a reversed-phase material depends in a very complex manner on the composition of eluent. In Fig. 2 a U-shaped plots of *k* versus water content in water–ethanol eluents is given [39] for three organic acids. Maximal exclusion occurs at a composition of about 50% water in mobile phase. With more than 80% water, benzoic acid and salicylic acid are retained, but sulfanilic acid is still excluded.

The plots like Fig. 2 are attributed to the strong

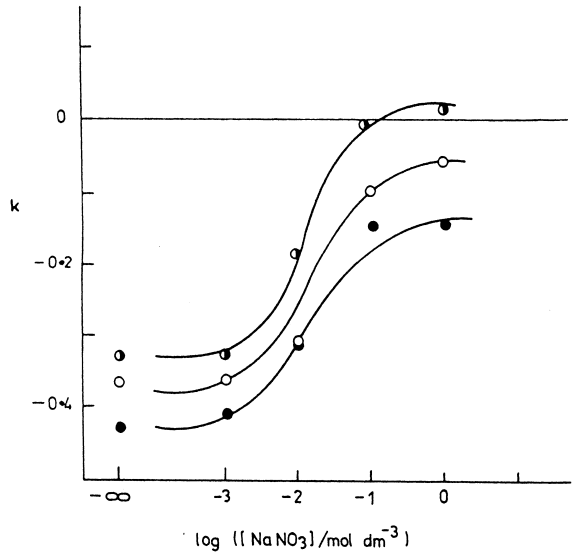

Fig. 1. Dependence of retention factors, *k*, on eluent ionic strength for benzoic acid (◑), salicylic acid (○) and sulfanilic acid (●) eluted from 5 μm ODS Hypersil (Shandon Southern Products, UK). Eluent: water–ethanol (70:30, v/v with added amounts of sodium nitrate). Data taken from Knox et al. [39].

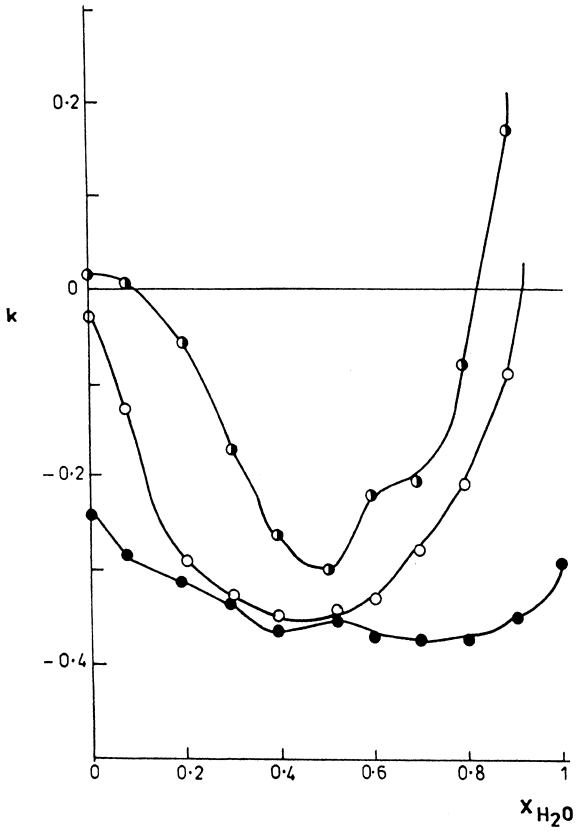

Fig. 2. Dependence on eluent composition of capacity factors, *k*, for benzoic acid (◑), salicylic acid (○) and sulfanilic acid (●) eluted from ODS Hypersil (Shandon Southern Products, UK) by water–ethanol mixtures (eluents contained 10^{-3} *M* $NaNO_3$). Data taken from Knox et al. [39].

interactions between the analytes and the residual silica hydroxyls of stationary phase [40,41]. The increase in retention as the concentration of the organic modifier increases beyond the value corresponding to the minimum is thought to be due to the column taking on some normal-phase character.

The composition of a buffer and/or the type of substance used for adjustment of ionic strength may affect retention of ionizable solutes. According to Wang and Lien [42], for acidic and neutral solutes, phosphate buffer appears to give partition coefficients closer to the values obtained from the octanol–water system than acetate and bicarbonate buffers. Adding a cation to the mobile phase decreases exclusion of acids [39] and decreases the retention of basic analytes [43]. This effect also depends on the kind of the cation applied.

To reduce the effects of free silanols on reversed-phase HPLC retention various practical methods have been proposed. Stadalius et al. [44] suggest using a pH between 2.5 and 3.5 with higher buffer concentrations, potassium salts instead of sodium, and the addition of amine modifiers such as triethylamine or dimethyloctylamine. Minick et al. [45] propose adding 1-octanol and 1-decylamine to the eluent composed of methanol and 4-morpholinopropanesulfonic acid buffer. Unfortunately, neither of these approaches appear to be fully effective.

6. Stationary phases for determination of hydrophobicity of acidic analytes

The access of the analyte to the free silanols causes the main problems in the chromatographic determination of hydrophobicity of ionizable compounds on classical alkyl-bonded silica stationary phases. Even with maximized surface coverage and 'end-capping' techniques approximately 50% of the surface hydroxyls (or 3.5–5 $\mu mol/m^2$) remain unreacted on such phases [46–48]. Besides, the phases are unstable at a pH range wide enough to cover dissociation range of some analytes.

In recent years great progress has been achieved in the technology of silica-based reversed-phase materials. Due to high octadecyl bonding densities, significant protection of octadecylsilica (ODS) phases against hydrolysis at extremes of pH was attained [49]. New commercially available phases exhibit high level of silanol deactivation [50]. The coating of a chromatographic support material with a layer of a polymer was applied to modify the silica-based reversed-phase materials [51,52].

Another approach to preparing stable reversed-phase materials with the surface silanols shielded is the encapsulation of silica with a polymeric layer such as polymethylsiloxanes substituted with long-chain alkyl ligands, polybutadiene [53] and copolymerized vinyl-modified silica with acrylic acid derivatives [54].

When alumina-based reversed-phase materials appeared there was interest in them from the view point of hydrophobicity parametrization. Alumina is stable

over a wide pH range and possesses no interferring silanol groups. The polybutadiene-coated alumina (PBCA) stationary phase was introduced by Bien-Vogelsang et al. [55]. Due to chemical stability of the PBCA stationary phase, the nonionized forms of acids, bases and neutrals can be analyzed in the same HPLC system operated at an appropriately adjusted pH. Thus, a continuous hydrophobicity scale was obtained in an easier, faster and more reproducible manner than is the case with the octanol–water partition system [56].

A monomeric octadecyl-bonded alumina (ODA) was introduced by Haky et al. [57]. The ODA phase has pH stability similar to that of PBCA and exhibits higher chromatographic efficiency. A characteristic feature of the phase is a higher degree of hydrogen-bonding analyte–stationary phase interactions than on octadecyl-bonded silica.

In recent years a number of reversed-phase materials were introduced devoid of the known shortages of silica-based phases. Several such materials based on zirconia, titania and ceria supports were described by Carr and coworkers [58,59] and by Forgács and coworkers [60,61].

Organic polymers have for some time been tested as stationary phases for hydrophobicity determination. Poly(styrene–divinylbenzene) copolymers (PS–DVB) are stable over a pH range of 1 to 14. They are reported to provide rather moderate correlations with log *P* [62,63]. Instead, they were found to mimic water–alkane partition [64]. The problems with PS–DVB columns are that they are characterized by low efficiency and the material suffers from excessive shrinkage and swelling [65,66].

Several polymeric phases having a chemically bonded octadecyl moiety have been tested in hydrophobicity determinations. Such phases like octadecylpolivinyl copolymer or rigid macroporous polyacrylamide with bonded octadecyls do not undergo swelling nor shrinkage and offer the possibility of having reasonable flow-rate without undesirable pressure increase at the column inlet [62,67]. Depending on the specific phase used the reported correlations of retention parameters with log *P* are low [45] or at best as good as obtained with the octadecylsilica phase [62,68–70]. There is evidence, however, that individual polymeric phases provide specific input to retention. For example, the octadecylpolivinyl copolymer was reported to be less hydrophobic than alkylsilica but strongly retains some aromatic compounds [71].

A hydrophobicity scale of anionic analytes from microemulsion electrokinetic chromatography has recently been proposed by Ishihama et al. [72].

The main reason to use the chromatographic system to determine hydrophobicity is to conveniently model processes in the biophase. Hence, the components of the chromatographic and the biological systems should be comparable. An RP-HPLC system which is used to model transport of a drug through biological membranes should be composed of an aqueous phase and an organized phospholipid layer. A new stationary phase material, the immobilized artificial membrane (IAM) [73,74] models natural membranes which are composed of lipids with a polar headgroup and nonpolar chains. In the classical IAM phase a phospholipid ligand, lecithin, is covalently bound to propylamine silica forming confluent monolayer of immobilized membrane lipids. Only one of the alkyl chains is linked to the propylaminosilica surface, and the immobilized lipid headgroups protrude away from the stationary phase surface. These charged moieties are the first contact site between analytes and IAM.

Correlations between retention parameters determined on IAM columns and the standard measure of hydrophobicity, log *P*, were not high [75–79]. However, the hydrophobicity parameter determined on the IAM columns appeared to be a better predictor of bioactivity than log *P* for several classes of drugs. Good modelling of human skin permeation by steroids and phenols was reported [78]. A successful correlation with IAM parameter was obtained of IC_{50} values on cyclooxygenase 2 in intact cells for acidic nonsteroidal antiinflammatory drugs [79]. Also, bile salt–membrane interactions were better predicted by means of retention factors from the IAM columns than from the classical octadecylsilica columns [80].

7. Micellar electrokinetic chromatography in determination of hydrophobicity of ionizable compounds

In standard capillary zone electrophoresis (CZE) mobility of equally charged molecules often correlates well to their molecular mass raised to the power

of −2/3 [81]. CZE separates electrically charged molecules whereas hydrophobicity is referred to uncharged, neutral form of an analyte. Micellar electrokinetic capillary chromatography (MEKC or MECC), introduced by Terabe et al. [82] is a mode of capillary electrophoresis for separation of uncharged compounds. The principle of the separation in MEKC is based on the distribution of the analyte between an aqueous phase and a micellar phase. Micellar liquid chromatography (MLC) is based on a similar separation principle [83,84].

MEKC and MLC attract the special interest of medicinal chemists as convenient and biorelevant methods of determination of hydrophobicity of xenobiotics. This is because micelles are amphiphilic aggregates with anisotropic microenvironments which provide both hydrophobic and electrostatic sites of interactions. In this respect they are more structurally similar to biomembranes than octanol or RP-HPLC materials [85,86].

A number of papers report good correlations between standard reference parameter of hydrophobicity, log *P*, and retention parameters from MLC [84–88] and MEKC [89–95]. As observed by Herbert and Dorsey [93] in a large series of structurally diverse analytes subjected to MEKC with sodium dodecyl sulfate micelles, the correlation between retention parameter and log *P* was good for 59 neutral analytes ($R=0.979$). However, there were difficulties in dealing with analytes which were ionized in the aqueous phase that was buffered at pH 7.0.

To overcome the problems with both acidic and basic analytes microemulsion electrokinetic chromatography (MEEKC) was employed [96,97]. Recently Gluck et al. [98] correlated retention factors from MEEKC for ionizable compounds to log *P*. The same microemulsion was used at pH 1.19 and pH 12. At these extremes, all but the strongest acids and bases are neutral (unionized). The microemeulsion investigated, which mimics the octanol–water system, consisted of 50 m*M* sodium dodecylsulfate, 400 m*M* butanol and 32 m*M* heptane. As stressed by the authors [98], MEEKC provides the advantages of an RP-HPLC system to estimate hydrophobicities including automation, small sample size, short run and analysis times and good reproducibility. However, MEEKC has not the disadvantages of RP-HPLC including pH limitations (this dose not concern the method reported in [56]) and column degradation.

8. Conclusions

Determination of a thermodynamically valid chromatographic parameter of hydrophobicity still remains a challenge in the case of ionizable analytes. Striving for a comparable measure of hydrophobicity, which would be precisely related to the chemical structure of analytes, one has to be cognizant of the difficult-to-control factors affecting retention. The problems start with the definition of the linear free-energy related capacity factors depending on the actually assumed void volume of the column. Then, the ambiguities arise regarding the definition and the measurements of pH of the environment in which the separation takes place. The effect of the ionic strength and the composition of the buffer as well as the effects of concentration of organic modifier in the eluent are also difficult to be theoretically predicted. The nature and specific properties of the stationary phases used for chromatographic determination of hydrophobicity have a prevailing effect on the data obtained. All these factors explain the real situation that the hydrophobicity parameters from individual reversed-phase HPLC systems are scarcely comparable (although often significantly intercorrelated).

MEKC methods of hydrophobicity determination appear convenient and reliable, especially as far as mimicking of the octanol–water partition is considered.

One has to assume that each chromatographic system provides a more or less specific measure of hydrophobicity for the analytes tested. Such a measure does not need to be identical with that provided by the standard reference hydrophobicity measuring system, i.e., octanol–water partition system. On the other hand, there is no reason to assume that hydrophobicity measures provided by individual chromatographic systems should not account for differences in bioactivity within a series of xenobiotics as well as the arbitrary reference hydrophobicity measure, log *P*. The flexibility of HPLC offers a good chance to identify the separation systems modelling biological partition systems.

9. Abbreviations

CZE	Capillary zone electrophoresis
IAM	Immobilized artificial membrane
MEEKC	Microemulsion electrokinetic chromatography
MEKC	Micellar electrokinetic chromatography
MLC	Micellar liquid chromatography
ODA	Octadecyl bonded alumina
ODS	Octadecylsilica
PBCA	Polybutadiene coated alumina
PS DVB	Poly(styrene divinylbenzene)
RP-HPLC	Reversed-phase high-performance liquid chromatography
TLC	Thin-layer chromatography

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