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Review

Quantitative structure–retention relationships in affinity high-performance liquid chromatography

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Abstract

In this report the affinity high-performance liquid chromatography data, which were determined on silica-based human serum albumin, α_1-acid glycoprotein, keratin, collagen, melanin, amylose tris(3,5-dimethylphenylcarbamate), and basic fatty acid binding protein columns, are discussed. Using a quantitative structure–retention relationship (QSRR) approach the affinity data were interpreted in terms of structural requirements of specific binding sites on biomacromolecules. The unique chromatographic properties of immobilized artificial membrane and cholesterol stationary phases were also analyzed from the point of view of mimicking biological processes. It has been demonstrated that chemometric processing of appropriately designed sets of chromatographic data derived in systems comprising biomolecules provides information of relevance for molecular pharmacology and rational drug design.

Keywords: Reviews; Quantitative structure–retention relationship

Contents

1. Introduction

The fundamental processes of pharmacokinetics and pharmacodynamics: absorption, distribution, ex-

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cretion and receptor activation are similar to the processes governing chromatographic separations. The same basic intermolecular interactions determine the behavior of chemical compounds in both the biological and chromatographic environments [1].

High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) techniques allow for inclusion of biomolecules as active components of separation systems. The extreme complexity of biological systems limits rational design of an individual separation system that would directly mimic a given biological system. On the other hand, HPLC and CE are unique techniques that can readily yield a great amount of diversified but precise and reproducible data. It can be presumed that chemometric processing of appropriately designed and selected sets of chromatographic or electrophoretic data can reveal systematic information regarding both the analytes and the affinity stationary phases studied [2,3].

The first reports on the use of protein stationary phases (PSPs) for HPLC appeared as early as the 1980s [4–6]. For the purpose of chiral separation of drugs, the stationary phases containing serum proteins, such as α_1-acid glycoprotein (AGP) [7], bovine serum albumin (BSA) [8] and human serum albumin (HSA) [9] were introduced. Also, for enantiospecific separations of drugs other protein-bound phases such as ovomucoid [10], avidin [11], flavoprotein [12] as well as enzyme-bound phases such as cellulase [13], trypsin [14], α-chymotrypsin [15], lysozyme [16] and pepsin [17] were developed. The low molecular mass proteins such as riboflavin binding proteins (RfBPs) [18] or basic fatty acid-binding protein (bFABPs) [19] were also used as chiral selectors. A wide range of compounds were separated on the protein-bonded columns, however there is still a need to search for new PSPs which will be able to separate specific important analytes.

Although not actually containing biomacromolecules, interesting from the view point of bioanalysis are the immobilized artificial membrane (IAM) stationary phases introduced by Pidgeon and coworkers [20,21]. These phases resemble natural membranes that are composed of lipids with a polar headgroup and two nonpolar chains. Chromatographic retention parameters determined on the IAM columns appeared suitable for modeling pharmacokinetic properties of drugs [22–24] as well for predicting human skin permeation of various organic substances [25–27].

The HPLC and CE separation techniques have been found to be a convenient tool to quantify drug–protein binding. In recent years Gao et al. [28], Hage and Tweed [29], Heegaard et al. [30] and Shibukawa et al. [31] published reviews concerning studies on drug–protein interactions by HPLC and CE.

Combination of biochromatography and chemometrics was demonstrated to provide information of relevance to molecular pharmacology and rational drug design. That research strategy was introduced and developed in the 1990s [3,32]. The first review concerning discussion of data from affinity HPLC in terms of chemometrics was published in 1998 [33]. In the present report emphasis will be put on the achievements in this field during the last 3 years but some important earlier papers will be mentioned briefly.

2. Human serum albumin column

Human serum albumin (HSA) is a serum protein of ca. 65,000 Da molecular mass. HSA shows affinity mainly to acidic and neutral drugs. It plays an enormous role in drug action because only the free, unbound fraction of a drug in blood undergoes distribution. Therefore, affinity HPLC on immobilized human serum albumin protein stationary phases (HSA–PSPs) can serve as a convenient tool for studying drug–protein interactions [34,35].

In 1992 the first QSRR studies of HPLC retention on the HSA phase were reported [36,37]. Firstly, a set of 22 benzodiazepine derivatives consisting of achiral and chiral compounds was chromatographically analyzed. Next, the structures of compounds were characterized by means of quantum chemical and molecular modeling calculations. Based on multiple linear regression (MLR) analysis, retention parameters of the first- and the second-eluting enantiomers were described by structural descriptors obtained from molecular modeling. According to the derived QSRR, the structural requirements for two postulated modes of benzodiazepine binding to HSA were suggested (Fig. 1) [36]. Binding of the first-

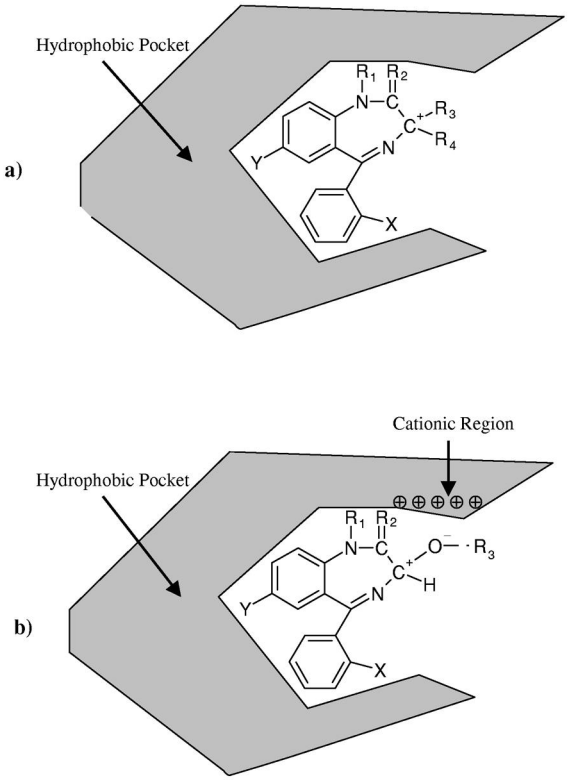

Fig. 1. Two postulated modes of benzodiazepine binding to human serum albumin [36].

eluting enantiomers of benzodiazepines takes place within a hydrophobic cavity in HSA. Substituents at N_1, C_2 and C_5 of the benzodiazepine system provide spatial orientation of the analyte molecules within this cavity. The binding mode of the second-eluting enantiomer involves hydrophobic and electrostatic interactions. Thus, it has been postulated that in addition to the hydrophobic cavity, there must be a cationic region in close proximity. The structure of the HSA–benzodiazepine binding site that has emerged from QSRR analysis appears to be consistent with the structure derived from X-ray crystallographic studies.

Andrisano et al. [38] presented quantitative structure–enantioselective retention relationships (QSERRs) for a series of 12 structurally related chiral arylcarboxylic acids chromatographed on HSA. The effects of solute structure on observed chromatographic retentions and enantioselectivity were investigated. By means of linear regression analysis the QSERR equations with a good predictive ability were constructed. The authors [38] correlated the chromatographic retention factors k' of first and second eluted enantiomers with hydrophobicity (log P) and molecular volume of the analytes. Statistically significant correlations were observed in both series of enantiomers and in each one retention increased with increasing hydrophobicity. Replacing log P by molecular volume gave very similar correlation in case of first eluted enantiomers. Correlation between the k' of second eluted enantiomers and their molecular volumes surpassed that achieved with log P.

The observations derived from the relationships between molecular volume and enantioselectivity led to the conclusion that the binding site at which enantioselective binding occurs is a chiral cavity with defined steric features. For the series of arylcarboxylic acids maximum enantioselectivity was observed when the molecular volume was 136 $Å^3$, suggesting that the optimum chiral selector/selectand fit occurs with solutes near this value.

Chromatographic retention data of the 12 arylcarboxylic acids were also correlated with structural descriptors derived from computational chemistry [38]. Based on multiple regression analysis the ''best'' relationships between retention data of the first and the second eluting enantiomers, k_1' and k_2', respectively, and structural descriptors: MLP (molecular lipophilicity potential), E_{tot} (total energy), E-State sum (electronic state sum) and MEP (molecular electrostatic potential) were developed:

$$\ln k_1' = 4.782\,\mathrm{MLP}_{int} - 3.223E_{tot} + 6.612$$
$$n = 12;\ R = 0.954;\ F = 45.29;\ s = 2.323 \quad (1)$$

$$\ln k_2' = 497.3\,\mathrm{MLP}_{mean} + 2.910\ \text{E-State sum} + 2.194\,\mathrm{MEP}_{min} - 31.24$$
$$n = 12;\ R = 0.973;\ F = 46.57;\ s = 2.623 \quad (2)$$

where n is the number of analytes considered in deriving the regression equation, R is the multiple correlation coefficient, F is the value of the F-test of significance and s is the standard error of estimate.

QSERR studies led Andrisano et al. [38] to the conclusion that enantioselective retention of the solutes takes place at the indole–benzodiazepine site (site II) on the HSA molecule. The predominant

hydrophobic interactions limited by steric volumes of the solutes affect the chiral recognition mechanism. Additionally the electrostatic interactions that take place between the carboxylate moiety of the solute and the cationic groups located in site II stabilize the binding complex.

In 2000 Andrisano et al. [39] published another study on binding characteristics of a series of substituted hydroxypropionic acids to HSA stationary phase. QSRR analysis was performed to obtain insight into the chiral recognition mechanism. Using multiple regression analysis, the chromatographic retention data of the compounds were described in terms of various molecular descriptors derived from molecular modeling. As the obtained log P values did not differentiate lipophilicity for the erythro and threo stereoisomers, the lipophilicity parameters log k'_w were determined by reversed-phase HPLC. The parameter log k'_w expressed the chromatographic retention factor extrapolated to the pure water in mobile phase. According to the chromatographic behavior displayed on the HSA column, all the threo diastereoisomers showed higher log k'_w values than the corresponding erythro diastereoisomers.

The quantitative relationships between retention observed on HSA chromatographic column and physicochemical descriptors of analytes were studied by means of regression analysis and the partial least square (PLS) method. The ''best'' obtained equations describing retention of both the first and the second eluting enantiomers on HSA comprise of a lipophilicity parameter measured by HPLC:

$$\log k'_1 = 0.574(\pm 0.084) \log k'_w - 1.731(\pm 0.446)$$
$$n = 10;\ r^2 = 0.969;\ \text{S.D.} = 0.095;\ q^2 = 0.944;$$
$$F_{1,8} = 249.31 \quad (3)$$

$$\log k'_2 = 0.545(\pm 0.054) \log k'_w - 1.463(\pm 0.285)$$
$$n = 10;\ r^2 = 0.986;\ \text{S.D.} = 0.061;\ q^2 = 0.976;$$
$$F_{1,8} = 547.53 \quad (4)$$

Based on the above equations and having in mind that no other descriptor was found to significantly improve the correlation, the authors [39] concluded that the lipophilicity expressed by log k'_w is the most important physicochemical parameter influencing the affinity for the HSA stationary phase for all the enantiomers.

Beaudry et al. [40] presented a paper describing determination of binding between various drugs and human serum albumin using a HSA column and a QSRR approach. For a series of 40 structurally unrelated pharmaceuticals with binding affinity ranging from 0 to 99%, chromatographic data including retention time, peak width and k', were used to evaluate protein binding. A good correlation coefficient ($r^2 = 0.799$) was observed for the relationship between chromatographic retention data and the percentage of binding determined in standard slow-equilibrium experiments.

3. α_1-Acid glycoprotein column

α_1-Acid glycoprotein (AGP) is one of the main serum proteins, which is characterized by the preferential binding of basic drugs [41]. The prevalent opinion is that AGP has only one binding site which binds drugs through hydrophobic and electrostatic interactions [42,43]. However, neither log P [42,44] nor pK_a [45] could account for binding differences within small sets of tested drugs.

Nasal et al. [46] and Kaliszan et al. [24,47] determined retention factors, log k_{AGP}, for 52 basic drugs of diverse chemical structures and pharmacological activities on an AGP stationary phase. Additionally, for the same set of compounds the retention factors were determined on an immobilized artificial membrane (IAM) column, log k_{IAM}. Molecular modeling calculations were used to determine structural parameters of the compounds analyzed. The following parameters were found significant in describing retention on the AGP column: N_{ch}, electron excess charge on an aliphatic nitrogen atom and S_T, surface area of a triangle having one vertex on the aliphatic nitrogen and the two remaining vertices on the furthest positioned atoms in the drug molecule (Fig. 2) [47]. The QSRR equation relating retention on chemically immobilized AGP to the hydrophobicity measure, log k_{IAM}, electron excess charge on aliphatic nitrogen, N_{ch}, and a size parameter of drugs, S_T, has the form:

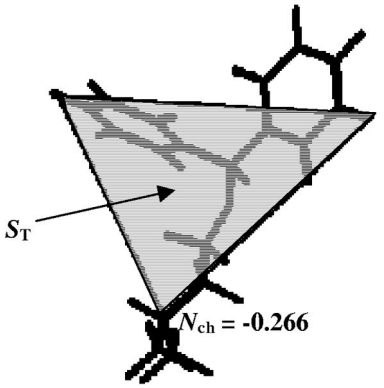

Fig. 2. Graphical presentation of structural descriptors (N_{ch}, S_T) of drugs chromatographed on an α_1-acid glycoprotein (AGP) column used in QSRR studies [47].

$$\log k_{AGP} = 0.6577(\pm 0.0402) \log k_{IAM} + 3.342(\pm 0.841) N_{ch} - 0.0081(\pm 0.0030) S_T + 1.688(\pm 0.245)$$

$$n = 49; R = 0.929; s = 0.163; F = 92; P \leq 10^{-5} \quad (5)$$

The above equation could be used as a first approximation of relative binding of an agent to AGP without the need to perform biochemical experiments.

The reported QSRR equations [24,46,47] as well as independently provided qualitative characteristics of the mode of binding of xenobiotics to AGP [41–45] allow for an indirect identification of structural features of the binding site for basic drugs on the protein (Fig. 3). An open conical pocket can be used as a model of the binding site. The internal surface of the pocket contains lipophilic regions at the base of the cone. There is an anionic region close to the spike of the cone. The probable mechanism of binding is as follows: protonated aliphatic nitrogen guides drug molecules towards the anionic region. Hydrophobic hydrocarbon fragments of the interacting drugs fix the molecules in the lipophilic regions of the binding site. Steric restriction inhibits the ability of the molecules to enter into the binding site. Asymmetric charge distribution accounts for the observed enantioselectivity of binding to AGP.

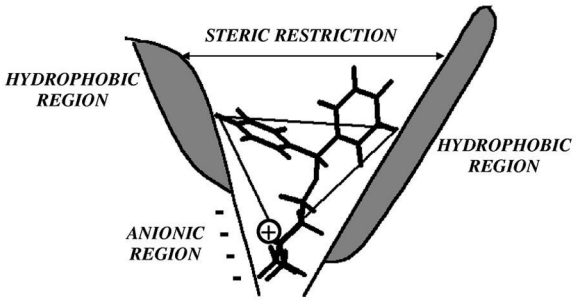

Fig. 3. Hypothetical mode of binding of basic drugs by AGP [33].

Karlsson and Aspergen [48] used statistical experimental design [49,50] to compare two commercially available protein chiral stationary phases: α_1-acid glycoprotein (Chiral-AGP) and cellulase (Chiral-CBH) immobilized to silica particles. Using 12 structurally closely related amino alcohols as the test solutes, enantioselective retention on the two protein-based supports was studied. For each column three important mobile phase descriptors which improved the chiral recognition were chosen as independent variables and retention and separation factors were used as responses. Variables were mobile phase buffer pH and column temperature for both chiral supports and additionally ionic strength for the Chiral-AGP and concentration of 2-propanol for the Chiral-CBH column. The correlations between system descriptors and chromatographic responses were calculated using a partial least-squares method. The effects of changes in mobile phase buffer pH and column temperature on enantioselective retention were shown for both chiral supports as was the effect of ionic strength for the Chiral-AGP column and the effect of 2-propanol concentration when using the Chiral-CBH column. It was also demonstrated that minor changes in the solute structure, e.g. type of alkyl group attached to the nitrogen atom, position of substituent in the aromatic ring and the distance between the stereogenic center and the nitrogen atom had a large impact on enantioselectivity.

4. Keratin column

Keratin immobilized on silica was proposed [51,52] as a new stationary phase for chromatographic modeling of skin permeation by drugs. Keratin is a main constituent of the outermost layer of epidermis. Therefore, when modeling skin permeability one should consider not only the lipo-

philicity of drugs but also their possible interactions with keratin. For a series of test solutes the retention factors, log k_{KER}, were determined on an immobilized keratin column. Independently, a hydrophobicity parameter on an IAM column, log k_{IAM}, was also determined for the drugs studied [27]. The logarithms of human skin permeation coefficient, log K_p, were taken from the literature [53–56]. Equations describing log K_p in terms of the hydrophobicity parameter, log k_{IAM}, proves the importance of drug hydrophobicity for penetration of the skin:

$$\log K_p = 1.458(\pm 0.138) \log k_{IAM} - 6.420(\pm 0.139)$$
$$n = 17; R = 0.899; s = 0.47; P \leq 10^{-4} \quad (6)$$

Significant improvement of the predictiveness of Eq. (6) was achieved by adding the log k_{KER} term:

$$\log K_p = 1.920(\pm 0.242) \log k_{IAM} - 1.039(\pm 0.413) \log k_{KER} - 6.558(\pm 0.130)$$
$$n = 17; R = 0.932; s = 0.40; P \leq 10^{-4} \quad (7)$$

Fig. 4 presents the plot of the observed human skin permeation data, log K_p, against those calculated by Eq. (7).

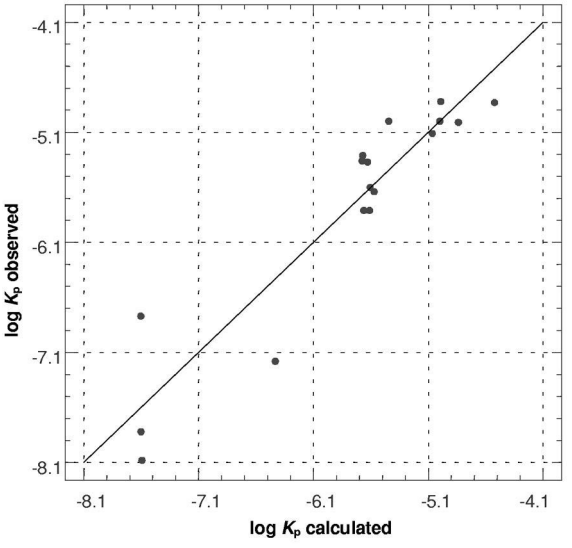

Fig. 4. Plot of logarithms of human skin permeability observed experimentally against the corresponding data calculated theoretically using Eq. (7).

Eq. (7) has interpretable physical sense. The skin permeability increases with the lipophilicity of the agents but at the same time, it decreases with the affinity of agents to keratin. The QSRR expressed in Eq. (7) suggest that keratolytic properties of phenols and other acidic test compounds are in opposition to their lipophilic properties.

5. Collagen columns

Collagen covalently bound to aminopropylsilica and diolsilica was also used to evaluate a skin permeation model by means of affinity chromatographic data [57]. The collagen-based stationary phase was shown to exhibit a different mechanism of retention from that observed on either keratin or IAM columns. Polar and hydrophilic properties of collagen manifested themselves clearly in QSRR analysis.

A statistically significant and physically meaningful QSRR equation was obtained for a series of 13 test solutes chromatographed on a collagen bound to silica stationary phase. The equation incorporates: chromatographic retention, log k_{COLL} described in terms of total dipole moment, μ, and the largest difference (in electrons) between the maximum and the minimum atomic excess charges in the molecule, MaxMin:

$$\log k_{COLL} = -0.043(\pm 0.009)\mu + 0.629(\pm 0.166)\,\text{MaxMin} - 0.982(\pm 0.101)$$
$$n = 13; R = 0.860; s = 0.155; P \leq 0.002 \quad (8)$$

The MaxMin parameter in Eq. (8) probably reflects the local dipole whereas μ is the measure of total dipole moment of the analyte molecule. Both the parameters undoubtedly represent differences in polar properties within the sets of test analytes. None of the numerous molecular size-related structural descriptors tested appeared significant in QSRR analysis. Therefore, one can presume that the normal-phase and not reversed-phase retention mechanism prevails on the collagen phase.

So far, the collagen phase has not been shown to be useful in the modeling of human skin permeation.

6. Melanin column

Natural melanins (rheomelanins containing sulphur and eumelanins that do not contain sulphur) are present in external and internal tissues (skin, hair, ear, eye and brain). Thus, the binding of substances to melanin is of biological and pharmacological interest. High affinity to melanin correlates with ocular toxicity, ototoxicity, pigment disturbances of the skin and hair, carcinogenicity and extrapyramidal disorders caused by drugs [58–60]. Consequently, silica-based stationary phases for HPLC were prepared with physically [61] and chemically [62,63] immobilized synthetic melanin.

For a series of psychotropic drugs the retention parameters, log k_{MEL}, were determined on a chemically immobilized melanin–silica stationary phase [64]. For seven drugs of the series, the binding to synthetic melanin was determined by an ultrafiltration method. The drug–melanin interaction parameters from affinity HPLC and those determined by a standard ultrafiltration method showed significant correlation ($P<0.05$). The chromatographically determined retention factor is highly reproducible and more reliable as a melanin-binding parameter than that obtained by the slow-equilibrium ultrafiltration method. This conclusion supports the studies by Knörle et al. [63] on binding of several drugs to melanin using affinity chromatography.

A QSRR equation derived by Radwańska et al. [64] describes retention factors on a melanin column, log k_{MEL}, in terms of structural parameters obtained either empirically (log k_{IAM}, drug hydrophobicity parameter determined on an IAM column) or from molecular modeling (E_{LUMO}, energy of lowest unoccupied molecular orbital):

$$\log k_{MEL} = -0.225(\pm 0.073)\log k_{IAM} - 0.326(\pm 0.076)E_{LUMO} + 0.696(\pm 0.010)$$
$$n = 13;\ R = 0.933;\ s = 0.056;\ P \leq 0.0001 \quad (9)$$

Eq. (9) provides quantitative proof of the involvement of charge-transfer intermolecular interactions (accounted for by E_{LUMO}) along with hydrophobic interactions (reflected by log k_{IAM}), in the formation of complexes between melanin and the drugs tested. Involvement of charge-transfer interactions in the formation of drug–melanin complexes was previously postulated by other authors [65,66] but have not been proved.

7. Amylose tris(3,5-dimethylphenylcarbamate) column

Booth and Wainer [67,68] applied QSERR analysis to evaluate chiral recognition mechanisms on an amylose tris(3,5-dimethylphenylcarbamate) (AD) stationary phase. For a series of 28 chiral α-alkyl arylcarboxylic acids the retention factors were determined and correlated to hydrogen bonding ability and aromaticity of analytes. The multiple regression equations for the first and for the second eluting enantiomers are as follows:

$$\ln k_1 = -2.499 + 1.369(\pm 0.177)X + 0.791(\pm 0.121)Y + 0.415(\pm 0.094)Z$$
$$n = 26;\ R = 0.947;\ P \leq 0.0001 \quad (10)$$

$$\ln k_2 = -2.659 + 1.498(\pm 0.152)X + 0.896(\pm 0.103)Y + 0.439(\pm 0.080)Z$$
$$n = 26;\ R = 0.967;\ P \leq 0.0001 \quad (11)$$

In Eqs. (10) and (11), X and Y are the number of hydrogen bond donors and acceptors, respectively, and Z is the degree of aromaticity in the molecules. The authors [67] performed molecular modeling studies and identified a site within the helical ravine of AD at which enantioselective discrimination of analytes may occur. They concluded that instead of the standard ''three-point interaction'' model of chiral recognition, enantioselectivity on AD is due to a ''conformationally driven'' chiral recognition process.

In further studies on the molecular mechanism of enantioselective separation on the AD chiral stationary phase, Booth and Wainer [68] analyzed a drug, mexiletine, and a series of 11 structurally related compounds. QSERR equations derived describe re-

tention factors of the first- and the second-eluting enantiomers in terms of fragmental hydrophobicities of selected substituents, π_{R1} and π_{R3}, and polarity parameters of analyte molecules: the total aromatic excess electronic charge, A_C, and substructure dipole, S_d:

$$\ln k_1 = 3.179 - 1.338(\pm 0.555)\ \pi_{R1} - 1.645(\pm 0.416)\ \pi_{R3} + 4.937(\pm 3.231)\ A_C$$
$$n = 12;\ R = 0.958;\ F_{3,8} = 29.74;\ P \leq 0.0001 \qquad (12)$$

$$\ln k_2 = 0.913 - 1.455(\pm 0.521)\ \pi_{R1} - 1.734(\pm 0.370)\ \pi_{R3} - 0.283(\pm 0.172)\ S_d$$
$$n = 12;\ R = 0.970;\ F_{3,8} = 42.71;\ P \leq 0.00009 \qquad (13)$$

As a result of QSERR and enthalpy–entropy compensation analysis, two distinctive retention mechanisms for mexiletine-related drugs on the AD stationary phase were identified. These mechanisms are based on either the presence or absence of secondary hydrogen-bonding groups.

Booth et al. [69] investigated enantioselective separation of amides on three amylose-based chiral stationary phases: amylose tris(3,5-dimethylphenylcarbamate) (AD), amylose tris(*S*-phenylethylcarbamate) (AS), and amylose tris(*R*-phenylethylcarbamate) (AR). The relative retentions and enantioselectives of the analytes on these three amylose-based stationary phases were compared. QSERR equations were derived to describe the chromatographic retention mechanisms on the analyzed stationary phases. The results indicate that for the solutes tested, the observed elution order was a function of the chirality of the amylose backbone and that the magnitude of the enantioselective separations was affected by the chirality of the carbamate side chain.

In the following paper Booth et al. [70] applied multiple regression analysis together with neural network analysis to predict the chiral chromatographic separation of a series of 29 aromatic acids and amides on three amylose-based stationary phases. In the QSERR analysis the most significant structural parameters derived from molecular modeling were: the average molecular electrostatic potential (MEP), average molecular lipophilic potential (MLP), total dipole moment (DIP) and energy of the lowest unoccupied molecular orbital (LUMO). The LUMO and MLP parameters affected retention most strongly. The LUMO parameter suggested that charge transfer interactions occur between the analytes and the amylose-based stationary phases studied. The MLP parameter incorporates a combination of lipophilicity with steric and geometric factors [71].

Multiple regression analysis was able to provide information regarding the fundamental mechanistic interactions determining retention on AD phases. However, with regards to retention prediction based on structural descriptors of analytes, neural networks have been shown to be of a much higher predictive power than multiple regression analysis [70].

8. Basic fatty acid binding proteins column

The fatty acid binding proteins (FABPs) are a class of low-molecular-weight proteins that bind fatty acids and are thought to be involved in their intracellular transport [19]. Basic fatty acid binding protein (bFABP) is present at high concentration in chicken liver and has been isolated and immobilized on silica. Using this stationary phase, successful separation of aryl- and aryloxypropionic acid enantiomers has been achieved. QSRR studies using regression analysis were undertaken to describe the relationships between the chemical structures of the analytes and the observed chromatographic results and to provide information on the structure of the protein binding site. Two structural descriptors of test analytes, the total lipole (TL) accounting for lipophilicity of a whole molecule and the energy of the highest occupied molecular orbital (HOMO) for electrostatic interactions were considered. The retention parameters of 13 aryloxypropionic acid enantiomers were determined and the obtained QSRR for the first (k_1) and for the second (k_2) eluting enantiomers were as follows:

$$\log k_1 = 0.13\ \mathrm{HOMO} + 0.025\ \mathrm{TL} + 2.04$$
$$n = 13;\ R = 0.91;\ F = 24.46;\ s = 0.068 \qquad (14)$$

$$\log k_2 = 0.14\ \mathrm{HOMO} + 0.032\ \mathrm{TL} + 2.2$$
$$n = 13;\ R = 0.92;\ F = 27.79;\ s = 0.07 \qquad (15)$$

Based on these equations, the authors [19] concluded that hydrophobic interactions are predominant in the retention mechanism, and this is in agreement with the hydrophobic character of the protein binding

site [72]. Binding of aryloxypropionic acids to bFABP immobilized on silica increases with the hydrophobicity of analytes. However, electrostatic interactions accounted for by the HOMO energy play an important role in the stabilization of the analyte–protein complexes. These results support the hypothesis of the presence of a characteristic binding site in the bFABP structure which is a chiral cavity with a defined steric structure.

9. Immobilized artificial membrane column

Immobilized artificial membrane (IAM) columns [20,21] are prepared by covalently binding monolayers of cell membrane phospholipids to silica particles. IAM phases were designed to mimic the lipid environment of a cell membrane. Pidgeon et al. [73] claim that the retention factors determined on the IAM column always give better prediction of drug transport through any biological barrier than chromatographic retention parameters determined on typical octadecylsilica columns and better than those provided by the logarithms of *n*-octanol–water partition coefficients, log *P*.

Salminen et al. [74] reported studies on the relationships between IAM chromatographic retention and brain penetration by structurally diverse drugs. Retention factors were determined for a set of 26 acidic, basic and neutral drugs for which the brain/blood concentration ratios were available. The logarithms of brain/blood concentration ratios (log BB) correlated only weakly with the IAM retention parameters (log k_{IAM}) and similarly with the *n*-octanol–water partition coefficient (log K_{oct}). After addition of an indicator parameter accounting for the effect of ionization (I) and the molecular volume (V_m) the regression models improved:

$$\log BB = 0.58 \log k_{IAM} + 0.89 I_2 - 0.01 V_m + 1.28$$
$$n = 21; R = 0.921; s = 0.27; F = 31.5 \quad (16)$$

$$\log BB = 0.35 \log K_{oct} + 0.99 I_3 - 0.01 V_m + 1.25$$
$$n = 23; R = 0.921; s = 0.32; F = 35.2 \quad (17)$$

The two obtained equations (Eqs. (16) and (17)) are of a comparable quality. However, the authors argue that the IAM chromatography is a superior method for predicting solute distribution within biomembranes. This has also been supported by the studies of Kępczyńska et al. [75]. For a series of 30 barbituric acids the retention parameter log k_{IAM} was related to log *P* and various physicochemical and biological parameters. Especially interesting appears to be the relationship between the inhibition of rat brain oxygen consumption (log $1/c$) and log k_{IAM} data of eight barbiturates:

$$\log 1/c = 2.011(\pm 0.087) + 1.548(\pm 0.159) \log k_{IAM}$$
$$n = 8; R = 0.970; s = 0.163; F = 95 \quad (18)$$

This observation suggests the suitability of log k_{IAM} for predicting bioactivities which are dependent on a drug's ability to permeate the blood–brain barrier. Although log k_{IAM} is intercorrelated to some extent with log *P* it may better account for some specific aspects of drug lipophilicity which are of relevance for individual types of bioactivity.

Caldwell et al. [76] employed retention factors, log k_{IAM}, of eight β-adrenolytic drugs obtained with IAM.PC.DD and ^{ester}IAM.PC.MG columns for predicting drug–membrane interactions. Chemical structures of two types of IAM columns are presented in Fig. 5. Using linear regression analysis, the log k_{IAM} values from both types of IAM column were correlated with log *P*, retention parameters determined on regular C_{18} stationary phases and with the liposome partition coefficient. Additionally, various pharmacokinetic parameters were considered. Best correlations were obtained with the ^{ester}IAM.PC.MG column. The authors concluded that their results suggest a deeper partitioning of β-adrenolytic drugs into the ^{ester}IAM.PC.MG stationary phase as compared to the IAM.PC.DD phase. Since partitioning determines β-adrenolytic drug transport across biomembranes, the higher affinity of the ^{ester}IAM.PC.MG stationary phase may account for its superior modeling of physicochemical and pharmacokinetic data.

10. Cholesterol column

Cholesterol is an important component of biological membranes. Because of expected specific

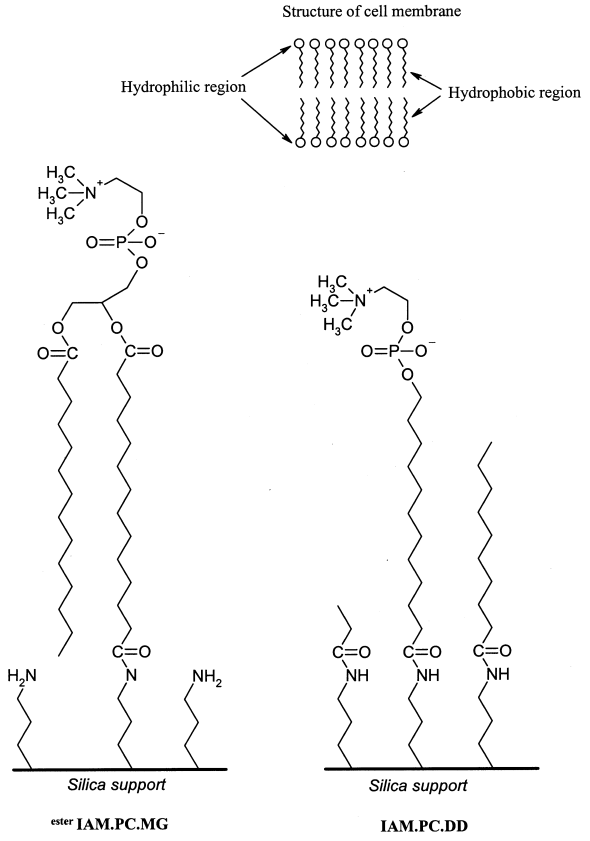

Fig. 5. Chemical structures of ligands of two types of immobilized artificial membrane (IAM) stationary phase and a schematic model of biological membrane.

separation properties of the presumed liquid crystal structure of the immobilized cholesterol layer, there was interest in preparation of such a stationary phase for RP HPLC [77–80]. Recently, Buszewski et al. [81] reported a synthesis and studies on retention and separation performance of a cholesterol–silica stationary phase for HPLC. Al-Haj et al. [82] studied the mechanism of separation on the cholesterol–silica column by QSRR analysis. QSRR were derived by multiple regression analysis using different sets of structural descriptors of analytes and the log k_w data of 24 test analytes. To describe the retention mechanism two sets of structural descriptors were used: linear solvation energy relationship (LSER) parameters (Eq. (19)) and molecular modeling parameters (Eq. (20)):

$$\log k_w = 0.455(\pm 0.404) - 0.748(\pm 0.294)\alpha_2^H - 3.114(\pm 0.310)\beta_2^H + 3.312(\pm 0.331)V_x$$
$$n = 24;\ R = 0.9575;\ s = 0.452;\ F = 73;\ P \leq 10^{-4} \quad (19)$$

$$\log k_w = -0.986(\pm 0.682) + 5.328(\pm 1.410)\delta_{min} - 0.108(\pm 0.026)\mu^2 + 0.018(\pm 0.002)\mathrm{SAS}$$
$$n = 24;\ R = 0.9477;\ s = 0.500;\ F = 59;\ P \leq 10^{-4} \quad (20)$$

The LSER-based structural descriptors in Eq. (19) are: α_2^H and β_2^H, effective hydrogen-bond acidity and basicity, respectively; V_x, the McGowans characteristic volume. Molecular modeling structural descriptors of analytes reported in Eq. (20) are: δ_{min}, highest atomic excess charge in the molecule; μ^2, square of total dipole moment; SAS, solvent accessible molecular surface area. According to QSRR results in relation to two reference stationary phases (IAM and a standard octadecylsilica), the new cholesterol–silica stationary phase possesses distinctive, unique retention properties. These properties may be of analytical value but the application of the proposed column for modeling of the penetration of xenobiotics through biological membranes appears rather unlikely.

11. Conclusions

Affinity high-performance liquid chromatography appears a reliable tool in determination of molecular interactions between drugs (xenobiotics) and biomacromolecules. Quantitative structure–retention and retention–pharmacological activity relationships employing affinity HPLC data and structural parameters of drug analytes may provide information on molecular mechanism of chromatographic separations and biological interactions. To derive reliable relationships, it is necessary to use sufficiently large sets of numerically expressed biorelevant data such as retention data determined by means of HPLC systems containing biomacromolecules or other biologically important agents. Of course, the parameters produced by affinity HPLC for individual drugs are

not identical with the drug–biomacromolecule interaction data obtained in vivo or in slow-equilibrium in vitro experiments. However, assuming linear free-energy relationships (LFERs), one can chromatographically compare the ability of individual analytes to form complexes with a given biomacromolecule, which is an active component of a defined separation system.

The chromatographic parameters measured on immobilized artificial membrane stationary phases can be regarded as overall drug lipophilic affinity to the biomolecules forming the biological membranes. Lipophilicity (hydrophobicity) is a multidimensional physicochemical property and retention data measured on IAM columns are one of a set of diverse features of this biologically important phenomenon.

Based on QSERR, a search for a stationary phase of specific enantioselective properties can be facilitated and made more effective. This type of analysis provides insight into the enantioseparation mechanisms operating at the molecular or even submolecular level.

The combination of affinity chromatography and chemometrics appears to be a promising strategy in biochromatography. It offers increased speed and efficiency in establishing quantitative relationships between the chemical structure of analytes and their ability to participate in intermolecular interactions with biological components introduced into the HPLC system. This may help to reduce the total cost of research for new drugs and at the same time decrease the need to employ experiments using material specially acquired from living organisms.

References

[1] R. Kaliszan, Quantitative Structure–Chromatographic Retention Relationships, Wiley, New York, 1987.
[2] R. Kaliszan, Chemometr. Intell. Lab. Sys. 24 (1994) 89.
[3] R. Kaliszan, Structure and Retention in Chromatography. A Chemometric Approach, Harwood Academic, Amsterdam, 1997.
[4] N.D. Danielson, R.W. Siergiej, Biotechnol. Bioeng. 23 (1981) 1913.
[5] S. Kamada, M. Maeda, A. Tsui, Y. Umezawa, T. Kurahashi, J. Chromatogr. 239 (1982) 773.
[6] S.C. Crowley, R.R. Walters, J. Chromatogr. 266 (1983) 157.
[7] J. Hermansson, J. Chromatogr. 269 (1983) 71.
[8] S. Allenmark, B. Bomgren, H. Borén, J. Chromatogr. 264 (1983) 63.
[9] E. Domenici, C. Bertucci, P. Salvadori, G. Félix, I. Cahagne, S. Motellier, I.W. Wainer, Chromatographia 29 (1990) 170.
[10] T. Miwa, M. Ichikawa, M. Tsuno, T. Hattori, T. Miyakawa, M. Kayano, Y. Miyake, Chem. Pharm. Bull. 35 (1987) 682.
[11] T. Miwa, T. Miyakawa, Y. Miyake, J. Chromatogr. 457 (1988) 227.
[12] N. Mano, Y. Oda, N. Asakawa, Y. Yoshida, T. Sato, T. Miwa, J. Chromatogr. 623 (1992) 221.
[13] P. Erlandsson, I. Marle, L. Hansson, R. Isaksson, C. Pettersson, G. Pettersson, J. Am. Chem. Soc. 112 (1990) 4573.
[14] S. Thelohan, P. Jadaud, I.W. Wainer, Chromatographia 28 (1989) 551.
[15] I.W. Wainer, P. Jadaud, G.R. Schombaum, S.V. Kadodkar, M.P. Henry, Chromatographia 25 (1988) 903.
[16] J. Haginaka, T. Murashima, C. Seyama, J. Chromatogr. A 666 (1994) 203.
[17] J. Haginaka, Y. Miyano, Y. Saizen, C. Seyama, T. Murashima, J. Chromatogr. A 708 (1995) 161.
[18] G. Massolini, E. De Lorenzi, M.C. Ponci, C. Gandini, G. Caccialanza, H.L. Monaco, J. Chromatogr. A 704 (1995) 55.
[19] G. Massolini, E. De Lorenzi, E. Calleri, C. Bertucci, H.L. Monaco, M. Perduca, G. Caccialanza, I.W. Wainer, J. Chromatogr. B 751 (2001) 117.
[20] C. Pidgeon, U.V. Venkatarum, Anal. Biochem. 176 (1989) 36.
[21] S. Ong, C. Pidgeon, Anal. Chem. 67 (1995) 2119.
[22] R. Kaliszan, A. Kaliszan, I.W. Wainer, J. Pharm. Biomed. Anal. 11 (1993) 505.
[23] R. Kaliszan, A. Nasal, A. Buciński, Eur. J. Med. Chem. 29 (1994) 163.
[24] R. Kaliszan, A. Nasal, M. Turowski, J. Chromatogr. A 722 (1996) 25.
[25] H. Thurnhofer, J. Schnabel, M. Betz, G. Lipka, C. Pidgeon, H. Hauser, Biochim. Biophys. Acta 1064 (1991) 275.
[26] C. Pidgeon, C. Marcus, F. Alvarez, in: T.O. Baldwin, J.W. Kelly (Eds.), Applications of Enzyme Biotechnology, Plenum Press, New York, 1992, p. 201.
[27] A. Nasal, M. Sznitowska, A. Buciński, R. Kaliszan, J. Chromatogr. A 692 (1995) 83.
[28] J. Gao, M. Mammen, G.W. Whitesides, Science 272 (1996) 535.
[29] D.S. Hage, S.A. Tweed, J. Chromatogr. B 699 (1997) 499.
[30] N.H. Heegaard, S. Nilsson, N.A. Guzman, J. Chromatogr. B 715 (1998) 29.
[31] A. Shibukawa, Y. Kuroda, T. Nakagawa, J. Pharm. Biomed. Anal. 18 (1999) 1047.
[32] R. Kaliszan, I.W. Wainer, in: K. Jinno (Ed.), Chromatographic Separations Based on Molecular Recognition, Wiley–VCH, New York, 1997, p. 273.
[33] R. Kaliszan, J. Chromatogr. B 715 (1998) 229.
[34] I.W. Wainer, J. Chromatogr. A 666 (1994) 221.
[35] A.-F. Aubry, A. McGann, LC–GC Int. 7 (1994) 389.
[36] R. Kaliszan, T.A.G. Noctor, I.W. Wainer, Mol. Pharmacol. 42 (1992) 512.
[37] T.A.G. Noctor, C.D. Pham, R. Kaliszan, I.W. Wainer, Mol. Pharmacol. 42 (1992) 506.

[38] V. Andrisano, T.D. Booth, V. Cavrini, I.W. Wainer, Chirality 9 (1997) 178.
[39] V. Andrisano, C. Bertucci, V. Cavrini, M. Recanatini, A. Cavalli, L. Varoli, G. Felix, I.W. Wainer, J. Chromatogr. A 876 (2000) 75.
[40] F. Beaudry, M. Coutu, N.K. Brown, Biomed. Chromatogr. 13 (1999) 401.
[41] J.M.H. Kremer, J. Wilting, L.H.M. Janssen, Pharmacol. Rev. 40 (1988) 1.
[42] J. Schley, J. Pharm. Pharmacol. 39 (1987) 132.
[43] L. Soltes, B. Sebille, P. Szalay, J. Pharm. Biomed. Anal. 12 (1994) 1295.
[44] H. Glaser, J. Kriegelstein, Naunyn-Schmiedeberg's Arch. Pharmacol. 265 (1970) 321.
[45] D.L. Goolkasian, R.L. Slaughter, D.J. Edwards, D. Lalka, Eur. J. Clin. Pharmacol. 25 (1983) 413.
[46] A. Nasal, A. Radwańska, K. Ośmialowski, A. Buciński, R. Kaliszan, G.E. Barker, P. Sun, R.A. Hartwick, Biomed. Chromatogr. 8 (1994) 125.
[47] R. Kaliszan, A. Nasal, M. Turowski, Biomed. Chromatogr. 9 (1995) 211.
[48] A. Karlsson, A. Aspergen, J. Chromatogr. A 866 (2000) 15.
[49] A.-M. Andersson, A. Karlsson, M. Josefsson, Chromatographia 38 (1994) 715.
[50] R.C. Williams, J.H. Miyawa, R.J. Boucher, R.W. Brockson, J. Chromatogr. A 844 (1999) 171.
[51] M. Turowski, R. Kaliszan, J. Pharm. Biomed. Anal. 15 (1997) 1325.
[52] R. Kaliszan, M. Turowski, Polish Patent No. P313926.
[53] M.S. Roberts, R.A. Anderson, J. Swabrick, J. Pharm. Pharmacol. 29 (1997) 677.
[54] T. Kurihara-Bergstrom, K. Knutson, L.J. De Noble, C.Y. Goates, Pharm. Res. 7 (1990) 677.
[55] A. Ruland, J. Kreuter, Int. J. Pharm. 72 (1991) 149.
[56] G.C. Mazzenga, B. Berner, F. Jordan, J. Control. Release 20 (1992) 163.
[57] M. Turowski, R. Kaliszan, Biomed. Chromatogr. 12 (1998) 187.
[58] A.M. Potts, Invest. Ophthalmol. 3 (1964) 405.
[59] B. Larsson, H. Tjälve, Biochem. Pharmacol. 28 (1979) 1181.
[60] B.S. Larsson, Pig. Cell Res. 6 (1993) 127.
[61] R. Kaliszan, A. Kaliszan, I.W. Wainer, J. Chromatogr. 615 (1993) 281.
[62] H. Ibrahim, A.-F. Aubry, Anal. Biochem. 229 (1995) 272.
[63] R. Knörle, E. Schniz, T.J. Feuerstein, J. Chromatogr. B 714 (1998) 171.
[64] A. Radwańska, T. Frąckowiak, H. Ibrahim, A.-F. Aubry, R. Kaliszan, Biomed. Chromatogr. 9 (1995) 233.
[65] H. Tjälve, M. Nilsson, B. Larsson, Biochem. Pharmacol. 30 (1981) 1845.
[66] K.B. Stępień, T. Wilczok, Biochem. Pharmacol. 31 (1982) 3359.
[67] T.D. Booth, I.W. Wainer, J. Chromatogr. A 737 (1996) 157.
[68] T.D. Booth, I.W. Wainer, J. Chromatogr. A 741 (1996) 205.
[69] T.D. Booth, W.J. Lough, M. Saeed, T.A.G. Noctor, I.W. Wainer, Chirality 9 (1997) 173.
[70] T.D. Booth, K. Azzaoui, I.W. Wainer, Anal. Chem. 69 (1997) 3879.
[71] K. Azzaoui, M. Lafosse, S. Lazar, V. Thiery, L. Morin-Allory, J. Liquid Chromatogr. 18 (1995) 3021.
[72] E. Schievano, D. Quarzago, P. Spadon, H.L. Monaco, G. Zanotti, E. Peggion, Biopolymers 34 (1994) 879.
[73] C. Pidgeon, S. Ong, H. Liu, X. Qiu, M. Pidgeon, A.H. Dantzig, J. Munroe, W.J. Hornback, J.S. Kasher, L. Glunz, T. Szczerba, J. Med. Chem. 38 (1995) 590.
[74] T. Salminen, A. Pulli, J. Taskinen, J. Pharm. Biomed. Anal. 15 (1997) 469.
[75] E. Kępczyńska, J. Bojarski, P. Haber, R. Kaliszan, Biomed. Chromatogr. 14 (2000) 256.
[76] G.W. Caldwell, J.A. Masucci, M. Evangelisto, R. White, J. Chromatogr. A 800 (1998) 161.
[77] Z. Witkiewicz, J. Mazur, LC–GC Int. 8 (1990) 224.
[78] J.J. Pesek, M.T. Matyska, Interface Sci. 5 (1997) 103.
[79] C. Delaurent, V. Tomao, A.M. Siouffi, Chromatographia 45 (1997) 355.
[80] B. Buszewski, M. Jezierska, M. Welniak, D. Berek, J. High Resolut. Chromatogr. 21 (1998) 267.
[81] B. Buszewski, M. Jezierska, M. Welniak, R. Kaliszan, J. Chromatogr. A 845 (1999) 433.
[82] M.A. Al-Haj, P. Haber, R. Kaliszan, B. Buszewski, M. Jezierska, Z. Chilmonczyk, J. Pharm. Biomed. Anal. 18 (1998) 721.