Journal of Chromatography A, 1396 (2015) 25–33

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Insight into the retention mechanism on immobilized artificial membrane chromatography using two stationary phases☆

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ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 22 March 2015
Accepted 23 March 2015
Available online 30 March 2015

Keywords:
Immobilized artificial membrane chromatography
Electrostatic interactions
IAM.PC.DD2
IAM.PC.MG
Octanol–water partitioning

ABSTRACT

The retention behavior of sixty structurally diverse drugs on two immobilized artificial membrane (IAM) columns, IAM.PC.MG and IAM.PC.DD2 types, at two pH values, 7.4 and 5.5, was established. Extrapolated to pure aqueous phase retention factors, $\log k_{w(IAM)}$, were determined and the role of acetonitrile as organic modifier was explored, considering the relationships with the slopes, S, of the extrapolation procedure. Good interrelations between retention factors on the two IAM stationary phases were observed, although $\log k_{w(IAM.PC.DD2)}$ values are generally higher than $\log k_{w(IAM.PC.MG)}$. In order to investigate the underlying retention mechanism, relationships between IAM retention factors and lipophilicity, expressed as $\log P$ or $\log D$ at pH 7.4 were established. Electrostatic interactions were considered by introducing the positively and negatively charged molecular fractions as additional parameters in the $\log k_{w(IAM)}/\log D$ relationships. The positive contribution of these fractions supported the involvement of the electrostatic interactions in the retention mechanism. Special attention was given to the retention behavior of zwitterionic compounds and for compounds with special structural characteristics.

1. Introduction

Immobilized artificial membrane (IAM) chromatography is considered as a useful tool in Medicinal Chemistry and Drug Design permitting rapid evaluation of membrane permeation of drug candidates and/or drug-membrane interactions [1,2]. Numerous studies report correlation of IAM retention factors with pharmacokinetic properties, such as unbound volume of distribution and tissue binding [3], blood–brain uptake [4], or human oral absorption [5].

IAM stationary phases are prepared by immobilization of monolayers of phospholipids (e.g. phosphatidylcholine, PC) on propylamine-silica skeletons. Among the commercially available IAM stationary phases, IAM.PC.DD2 and IAM.PC.MG, contain double chain phosphatidylcholine analogues, differing in end-capping of the free propylamino residues. Double chain IAM columns are considered most suitable for simulation of biological processes in comparison with the first generation single chain IAM.PC.DD [6]. Details on the characteristics of IAM columns can be found in refs. [7–9].

Retention on a IAM stationary phase is governed mainly by hydrophobic interactions, while, in case of charged molecules, electrostatic interactions with the opposite charged centers of phospholipids are anticipated [9,10]. The role of electrostatic interactions on IAM.PC.DD2 column has been systematically investigated by some of us for a set of structurally diverse neutral, basic and acidic drugs. Stronger interactions between the positively charged compounds with the phosphate anions than between solute anions and the positively charged choline nitrogen were demonstrated [11,12]. This behavior is consistent with the pH-piston hypothesis formulated for liposome partitioning [13].

Up to now comparison between IAM.PC.DD2 and IAM.PC.MG has been restricted to congeneric compounds [9,14,15], while more recently two publications on limited data sets of structurally non-related drugs appeared in literature [16,17]. No substantial

☆ Selected papers from the 16th edition of ExTech, the International Symposium on Advances in Extraction Technologies, held in Chania, Crete, Greece, 25th - 28th May 2014.

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http://dx.doi.org/10.1016/j.chroma.2015.03.060
0021-9673/

differences in the elution mechanism on both columns were observed in those studies [9,14–17], apart from a report based on a data set of 47 neutral compounds according to which IAM.PC.MG stationary phase is hardly discriminative for polar compounds [14]. However, more systematic investigation is needed in order to gain insight into any possible differentiation in solute interactions with IAM.PC.DD2 and IAM.PC.MG stationary phase and to propose the IAM column type, better simulating biological processes under certain conditions.

In the present work, a comparative study of the retention behavior of sixty (60) structurally diverse drugs on IAM.PC.MG and IAM.PC.DD2 stationary phases was carried out at physiological pH 7.4 and at pH 5.5 corresponding to the pH of the upper part of the small intestine. The interrelation between the IAM retention factors, determined on the two columns was investigated and the role of lipophilicity and electrostatic interactions as the factors governing retention mechanism was explored.

2. Experimental

2.1. Materials

The investigated drugs included neutral, acidic, basic and zwitterionic species and they were donated by local pharmaceutical companies. They are listed in Table 1. All reagents were of analytical grade unless otherwise mentioned. For the preparation of mobile phases KCl (≥99.5%, Riedel-de Haen), KH_2PO_4 (≥99.5%, Merck), Na_2HPO_4 (≥99.0%, Sigma-Aldrich), NaCl (≥99.5%, Sigma-Aldrich), CH_3CN (≥99.9%, LC gradient grade, Merck) and High Purity Water (HPW) were used. The HPW was obtained by means of an EASYpure II (Barnstead International, USA) water purification system. As void volume markers, sodium citrate·2x H_2O (≥99.0%, Sigma-Aldrich), $(COONH_4)_2{\cdot}H_2O$ (≥99.5%, Riedel-de-Haen) and L-Cystine (≥99%, Fluka Biochemika) were used.

2.2. Chromatography

All experiments were carried out under constant temperature (24 ± 1 °C). The liquid chromatographic system was consisted of a Knauer K-1001 HPLC gradient pump with a K-1500 degasser and solvent organizer (Model Wellchrom) and a Knauer K-2501 UV–vis detector (180–800 nm). The flow rate was adjusted at 1–3 ml min^{-1} and an injection loop of 20 μl was selected. The stability of the pressure was evaluated during the chromatographic procedure. Pharmaceutical compounds and the appropriate void volume markers [18] were detected at 254 or 210 nm, depending on structure (acetonitrile UV cut off value 190–200 nm). Chromatographic data were processed with the Eurochrom 2000 Version 2.05 software.

As stationary phases, the following chromatographic columns were used:

A. Rexchrom IAM.PC.MG Drug-Discovery Column (Regis Technologies, Inc., USA, 150 mm × 4.6 mm, 10 μm particle size).
B. Rexchrom IAM.PC.DD2 Drug-Discovery Column (Regis Technologies, Inc., USA, 30 mm × 4.6 mm, 10 μm particle size).

For strongly retained drugs, acetonitrile was added in the mobile phase at concentrations ranging from 5% to 25%, except for amitriptyline, candesartan cilexitil, chlorpromazine, fluoxetine, nortriptyline, promethazine, protriptyline, thioridazine and tioconazole, whose retention behavior was investigated in acetonitrile fraction up to 35%.

Retention times were determined at least in triplicate for each chromatographic condition and they were converted to the logarithm of retention factors, $\log k$ values by means of Eq. (1):

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right) \quad (1)$$

where t_r is the retention time of the solute investigated and t_0 is the column void time being measured by the substance giving the lowest retention time under each chromatographic condition, according to our previous work, e.g. ammonium oxalate and L-cystine at pH 5.5, as well as sodium citrate at pH 7.4 [18].

In many cases, $\log k$ values were determined using 100% aqueous mobile phase, corresponding to actual $\log k_w$ values. For strongly retained drugs, $\log k_w$ values were obtained by linear extrapolation of isocratic $\log k$ values, measured in presence of at least four different acetonitrile fractions (φ) in the mobile phase, according to Eq. (2):

$$\log k = \log k_w - S\phi \quad (2)$$

The extrapolated and actual $\log k_w$ values at pH 7.4 and 5.5 for IAM.PC.MG and IAM.PC.DD2 stationary phases along with the corresponding slopes S are presented in Tables 2 and 3, respectively.

2.3. Physicochemical properties

2.3.1. Octanol–water partition and distribution coefficients

Octanol–water partition coefficients ($\log P$) and distribution coefficients at the pH values of 7.4 and 5.5, $\log D_{7.4}$ and $\log D_{5.5}$, respectively, are presented in Table 1. The $\log P$, $\log D_{7.4}$ and $\log D_{5.5}$ data were taken from refs. [12,19–24] and therein cited references. If no experimental $\log D_{5.5}$ data were available, they were calculated according to their experimental $\log D_{7.4}$ values and their $\log D_{7.4}/\log D_{5.5}$ differences predicted by ADME Boxes v. 3.0 software (PharmaAlgorithms). This software was also used to calculate $\log D_{7.4}$ and $\log D_{5.5}$ values for ampicillin and exp-3174, as experimental data were not available. In the case of cefoxitin and 4-hydroxycoumarin, MedChem Designer software (Simulations Plus Inc.) was used to estimate $\log D_{7.4}$ and $\log D_{5.5}$ values, since ADME Boxes produced unreliably low values in regard to the reported experimental $\log P$.

2.3.2. Fractions of molecular species

The molecular fractions of positively charged (F^+), negatively charged (F^-) and zwitterionic species (F^z) at working pH values 7.4 and 5.5 were calculated according to ADME Boxes v. 3.0 software (PharmaAlgorithms). They are included in Table 1.

2.4. Statistical analysis

Regression analysis of experimental data was carried out using the Statistica-Axa 7.0 software package (StatSoft, Tulsa, Oklahoma, USA).

The following statistical data were given for the evaluation of regression equations: the correlation coefficients R and R^2, standard deviation s and Fisher test F at 95% significance level. For the statistical significance of variables, the student test absolute value $|t|$ was considered, which should be ≥2.

3. Results and discussion

The sixty structurally diverse drugs were investigated on the two IAM columns using PBS at pH 7.4 in order to mimic the physiological environment. Retention factors of acidic and zwitterionic drugs, as well as representative neutral (allopurinol, caffeine, paracetamol, theophylline) and basic (pentazocine) solutes were also determined at the pH value of 5.5 on both columns, since