Journal of Chromatography A, 1286 (2013) 127–136

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

The use of biopartitioning micellar chromatography and immobilized artificial membrane column for *in silico* and *in vitro* determination of blood–brain barrier penetration of phenols☆

Katarzyna E. Stępnik*, Irena Malinowska

Faculty of Chemistry, Chair of Physical Chemistry, Department of Planar Chromatography, Maria Curie – Skłodowska University, M. Curie – Skłodowska Sq. 3, 20-031 Lublin, Poland

ARTICLE INFO

Article history:
Received 25 October 2012
Received in revised form 14 January 2013
Accepted 20 February 2013
Available online 27 February 2013

Keywords:
Biopartitioning Micellar Chromatography
Immobilized Artificial Membrane
Blood–brain barrier
Linear Solvation Energy Relationship

ABSTRACT

Biopartitioning Micellar Chromatography (BMC) is a mode of micellar liquid chromatography that uses C18 stationary phases and micellar mobile phases of Brij35 under adequate experimental conditions and can be useful to mimic human drug absorption, blood–brain barrier distribution or partitioning processes in biological systems. BMC system can be useful in constructing good predictive models because the characteristics of the BMC system are similar to biological barriers and extracellular fluids. Immobilized Artificial Membrane (IAM) chromatography uses stationary phase which consists of a monolayer of phosphatidylcholine covalently immobilized on an inert silica support. IAM columns are thought to mimic very closely a membrane bilayer and are used in a HPLC system with a physiological buffer as eluent. In this paper the usefulness of BMC and IAM system for *in silico* and *in vitro* determination of blood–brain barrier (BBB) penetration of phenols has been demonstrated. The most important pharmacokinetic parameters of brain have been obtained for the determination of BBB penetration, *i.e.* BBB permeability – surface area product (PS), usually given as a log PS, brain/plasma equilibration rate ($\log(PS \times f_{u,brain})$) and fraction unbound in plasma (F_u). Moreover, the relationships between retention of eighteen phenols and different parameters of molecular size, lipophilicity and BBB penetration were studied. Extrapolated to pure water values of the logarithms of retention factors ($\log k_w$) have been compared with the corresponding octanol–water partition coefficient ($\log P_{o-w}$) values of the solutes. In addition, different physicochemical parameters from Foley's equation for BMC system have been collated with the chromatographic data. The Linear Solvation Energy Relationship (LSER) using Abraham model for the describing of phenols penetration across BBB has been used. Four equations were developed as a multiple linear regression using retention data from IAM and BMC system (QRAR models) and molecular volume parameter (V_m) and Abraham descriptors to correlate the log BB values. Moreover, in order to establish the relationships between different variables, the principal components analysis (PCA) has been done. The results of PCA were obtained using chromatographic data from IAM and BMC systems as well as from the structures of tested phenols. The four parameters: $\log k_{wIAM(exp)}$, $\log k_{wBMC(exp)}$, analyte-micelle association constant (K_{ma}) and $\log P_{o-w}$ have been checked.

1. Introduction

Biopartitioning Micellar Chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of polyoxyethylene (23) lauryl ether, Brij35 and C18 reversed stationary phase, under adequate experimental conditions. This technique can be useful in describing the biological behavior of different kinds of organic compounds and can mimic many biological processes such as blood–brain barrier penetration, skin permeability, intestinal absorption and drug partitioning process in biological systems [1–7]. The usefulness of BMC in constructing good biological models could be attributed to the fact that the characteristics of the BMC system are similar to biological barriers and extracellular fluids which are basically composed of water, salts, glucose, amino acids, cholesterol, phospholipids, fatty acids and proteins [8]. As many articles confirm [9–11], BMC is actually treated like a useful model for describing many important biological processes in a living organism. The main disadvantage of BMC system is that the analysis rate of the strong hydrophobic solutes may be slow

☆ Presented at the 29th International Symposium on Chromatography, Torun, Poland, 9–12 September 2012.

* Corresponding author. Tel.: +48 81 537 55 64.
E-mail addresses: katarzyna.stepnik@poczta.umcs.lublin.pl (K.E. Stępnik), irena.malinowska@poczta.umcs.lublin.pl (I. Malinowska).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.02.071

and usually a small amount of organic solvents needs to be added [12].

The retention of compounds in BMC as well as in classical micellar chromatography depends on the types of interactions between modified reversed stationary phase and micelles present in the mobile phase. These interactions are governed by hydrophobic, electronic and steric properties of compounds [6].

Octadecyl bonded silica stationary phases, used in BMC, are also often utilized to determine hydrophobicity [13,14]. However, a simple hydrocarbon chain cannot truly mimic biological membranes. Since the alkyl chains of reversed phase do not resemble the real penetration behavior of a cell membrane, the introduction of phosphatidylcholine phases should result in a more realistic model [15]. Biomimetic chromatographic partition systems have been successfully introduced with Immobilized Artificial Membranes (IAMs) as chromatographic packing materials [16]. These columns, containing a monolayer of phosphatidylcholine immobilized on a silica support, are used in HPLC systems with a physiological buffer as eluent [17]. The first work about IAM columns for HPLC determination of lipophilicity of drug was published by Kaliszan et al. [18].

Although a monolayer of phosphatidylcholine covalently bounded to a propylamino-silica core (IAM) is an approximation of a biomembrane phospholipid bilayer, it is a better model than n-octanol–water partitioning to mimic drug/biomembrane interactions [19]. It does not only support charged moieties but it is also an anisotropic phase, having polar and apolar moieties ordered in the three-dimensional space, so offering different interaction capability depending on the side they are approached by the analyte [20].

The IAM columns increase the means of characterization of various aspects of hydrophobicity. Their advantages over the slow equilibrium methods of hydrophobicity determination are the simplicity of operation and the suitability of the generated retention measures for predicting specific bioactivity parameters [21] such as blood–brain barrier penetration. The blood–brain barrier (BBB) is a highly regulated and efficient barrier that provides a sanctuary to the brain. It is designed to regulate brain homeostasis and to permit selective transport of molecules that are essential for brain function. Unfortunately, drug transport to the brain is hampered by this almost impermeable, highly selective and well coordinated barrier. The BBB is a dynamic barrier protecting the brain against invading organisms and unwanted substances. It is also the most important barrier impeding drug transport into the brain *via* the blood circulation [22].

The BBB is a selective barrier with the endothelium forming a much tighter interface than peripheral endothelia, because the gaps between capillary endothelial cells in most part of the brain are sealed by tight junctions and thus have a severely limited permeability [23]. The passive passage of a drug across a biological barrier can occur through paracellular or transcellular pathway. Paracellular pathway can only occur for small, usually hydrophilic, solutes. These molecules simply diffuse through the tight junction but not to any great extent [24]. Small lipid soluble substances like alcohol and steroid hormones penetrate transcellularly by dissolving in their lipid plasma membrane. For almost all other substances, including essential materials such as glucose and amino acids, transport proteins (carriers), specific receptor-mediated or vesicular mechanisms (adsorptive transcytosis) are required to pass the BBB. In the case of transport proteins or known as carrier-mediated transport, there is binding of a solute such as glucose or amino acids to a protein transporter on one side of the membrane that triggers a conformational change in the protein, resulting in the transport of the substance to the other side of membrane, from high to low concentration [22].

In this work IAM and BMC chromatographic retention factors were measured for a group of eighteen phenols which are interesting from a toxicological point of view. They are widely used in many branches of industry and can elicit a number of toxicities to different species [25]. Phenols have been used to stabilize organic materials, including foods, petroleum products and plastics against degradation by autoxidation [26].

The usefulness of BMC and IAM chromatographic systems for *in silico* and *in vitro* determination of blood–brain barrier (BBB) penetration of phenols was demonstrated. The logarithms of retention factors extrapolated to pure water ($\log k_w$) of investigated substances using these two techniques were determined. These values were compared with the corresponding obtained octanol–water partition coefficient ($\log P_{o-w}$) values of the solutes. The logarithms of retention factors extrapolated to pure water ($\log k_w$) with the analyte-micelle association constant (K_{ma}) were correlated.

Blood–brain distribution (BB) is the measure which is defined through Eq. (1) [27]:

$$\mathrm{BB} = \frac{\text{conc. in brain}}{\text{conc. in blood}} \tag{1}$$

The BBB penetration in the presented work is shown as the logarithm of the ratio between brain and blood concentration of tested substances:

$$\log \mathrm{BB} = log \frac{\text{conc. in brain}}{\text{conc. in blood}} \tag{2}$$

In this investigation the relationships between log BB values and various partition indexes were checked to compare their possible effectiveness in describing BBB passage.

The Quantitative Structure–Activity Relationship (QSAR) studies play an important role in contemporary drug design, toxicology and environmental monitoring. In QSAR, the biological activity is viewed as a summation of the different interactions that a compound undergoes both during the transport through biological membranes and in the reaction with the sites of action (receptor). These interactions are assumed to be governed by the chemical structure of the compound. A change in structure can result in a change in biological response [28].

Alternatively to QSAR models, the Quantitative Retention–Activity Relationship (QRAR) models represent other kind of modeling techniques, in which chromatographic retention parameters are used as descriptor and/or predictor variables of a given biological response of chemicals [29]. QRAR models based on retention factors ($\log k$) obtained using BMC and IAM chromatographic systems have been compared.

In this study several aims have been taken. First of all, it was the most important to investigate how commonly existing empirical models based on the retention data, lipophilicity parameters, molecular size parameters and Abraham descriptors, can estimate the possibility of BBB penetration of phenols. Therefore, QRAR as well as QSAR models of phenols penetration across BBB were established. The log BB values obtained through QRAR and QSAR models have been correlated. For this purpose, the Linear Solvation Energy Relationship (LSER) of Abraham model [30–34] was applied to characterize IAM and BMC systems, and utilized to compare the systems with the other physicochemical and biological parameters.

Moreover, the comparison between retention data from different chromatographic techniques (BMC and IAM) was achieved. It was necessary because of many differences between these two techniques in the stationary phase structures as well as in the components of the mobile phases. Owing to this comparison it can be easier to predict which of these two techniques is more useful for *in silico* and *in vitro* determination of BBB penetration of phenols.

However, BMC and IAM techniques are miscellaneous. In the case of BMC system, significant physicochemical parameters based on Foley's equation, have been received. These parameters can describe possible interactions in the micellar system.