How can we better realize the potential of immobilized artificial membrane chromatography in drug discovery and development?

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1. Introduction

Biological membranes define boundaries between and within cells and organelles, which drugs have to cross in order to reach the various compartments where their receptors are located. For years, transport across membranes has been associated with lipophilicity, expressed by octanol–water partition coefficient, logP, or distribution coefficient logD for ionizable drugs [1,2]. Despite its extensive use in prediction models for both ADME properties and receptor affinity, the octanol–water system has received serious criticism as a superficial and oversimplified simulation for membrane partitioning [2,3], although the microscopic structure of wet octanol differs from the structure of the dry solvent, consisting of cylindrical micelles with water cores [4]. On the other hand, the use of liposomes as direct models for biological membranes is restricted due to difficulties in their preparation, standardization, and stability as well as to the limited reproducibility of partitioning experiments [5]. Cell-culture techniques, like the Caco-2 and MDCK (Madin Darby Canine Kidney) cell lines, offer another option for assessing membrane permeability. They need however long culture periods and extensive cost, while results are influenced by inter-laboratory conditions [6].

Advances in column technology enabled the development of Immobilized Artificial Membrane Chromatography (IAM), which exhibits complexity in-between that of octanol–water partitioning and cell line approaches, combining the advantages and characteristics of chromatographic techniques with simulation of the environment in cell membranes [7]. In Figure 1, a schematic comparison of wet n-octanol, liposomes, IAM stationary phase, and membrane bilayer is presented. During three decades, a large number of publications support the use of IAM chromatography for rapid estimation of membrane permeability as well as for drug–membrane interactions in early drug discovery [2,7,10]. However, its advantages are still not completely realized by the medicinal chemistry community and IAM retention is in fact not yet integrated in candidate optimization protocols. Further information on experimental issues, understanding of the retention mechanism, updating of IAM models in respect to different biological processes, and eventually prediction on IAM retention per se would strengthen its position to this direction.

2. IAM column characteristics and measurements

IAM stationary phases consist of monolayers of amphipphilic phospholipids covalently bound to a propylamino-silica skeleton. Commercialized IAM columns, produced by Regis, are based on the prevalent membrane lipid, phosphatidylcholine [11]. The first IAM column (IAM.PC) was developed by Pidgeon [7] and was intended to be used for protein separation, while it offers the basis for cellular membrane affinity chromatography, permitting further immobilization of enzymes and transmembrane proteins [10]. New generation IAM columns are IAM.PC.MG and IAM.PC.DD2, differing in the end-capping procedure (Figure 2). The IAM Fast-Screen Mini Column specially designed for high throughput estimation of drug permeability has a very short analysis time and cost-effectiveness [11]. IAM chromatography is easy to automate, while, under similar conditions, there is sufficient lab-to-lab reproducibility [12]. Retention on IAM is expressed as isocratically determined retention factors corresponding to 100% aqueous mobile phase (logk_w) [10]. Alternatively, the Chromatographic Hydrophobicity Indices (CHI-IAM), obtained by gradient elution, can be used. CHI-IAM corresponds to the fraction of organic modifier (acetoniitrile) which leads to equal concentration between stationary and mobile phase [13]. Usually, there is a good correlation between CHI-IAM and logk_w values.

3. Understanding the retention mechanism

The molecular factors underlying in IAM retention mechanism denote the ambivalent nature of IAM chromatography, as a border case between passive diffusion and binding. In fact, next to the predominant role of hydrophobicity, which is the driving force for solute partitioning into the hydrophobic core, electrostatic interactions contribute significantly to retention. Such interactions are more pronounced between protonated bases and the phosphate anions of the phospholipids, which are located close to the hydrophobic core. More to the point the glycerol moiety may provoke hydrogen bonding [10]. The overall polar component may be expressed by Δlogk_w or Δlogk_w, which is derived from the difference between experimental logk_w and

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the expected value for neutral compounds having the same logP or logD respectively. $\Delta \log k_w$ or $\Delta' \log k_w$ reflects the interaction of solutes with phospholipids and depend on the magnitude of their lipophilicity, being higher for less lipophilic compounds, while high lipophilicity produces negative $\Delta \log k_{w(IAM)}$ or $\Delta' \log k_w$ [14]. $\Delta \log k_w$ or $\Delta' \log k_w$ can be used as parameters per se in modeling permeability of certain biological barriers.

4. Applications of IAM chromatography

The ambivalent nature of IAM chromatography enables multiple applications relevant not only to permeability but also to processes involving drug–membrane interactions and tissue binding. IAM retention has been successfully used to estimate intestinal permeability by establishing robust models, usually in combination with additional molecular descriptors, such as molecular weight, polarity terms, or/and fraction ionized [12]. Evidently, such models concern transport through passive diffusion. Identification of outliers, not fitting to the IAM/pas-

sive diffusion model, provides strong evidence that these compounds follow other routes of permeability. IAM-based models for Blood–Brain Barrier permeability have been reported, usually in combination with calculated pharmacoki-

netic parameters; however they refer to rather limited data sets, while the use of $\Delta \log k_{w(IAM)}$ values leads to a negative linear correlation [14]. Moreover, a classification model for rapid screening of CNS penetration has been proposed, defining upper and lower limits for the expression $k(IAM)/MW^n$, where $n = 4$ [15]. IAM retention is also suitable to model skin partition but not skin permeability, which however is well correlated with $\Delta \log k(IAM)$ [16].

IAM retention has been employed in relationships with non-

receptor specific biological activity, like anti-hemolytic activity or nonspecific bacteriostatic activity [17]. In addition, IAM retention (converted to IAM binding constant) in combination with plasma protein binding, also determined by biomimetic chromatogra-

phy, using a Human Serum Albumin (HSA) column, enables rapid estimation of complex pharmacokinetic properties such as volume of distribution and unbound volume of distribution, which involve binding to tissues [18].

Combination of IAM and HSA chromatography has been suggested for estimation of the in vitro maximum drug efficiency ($D_{eff}$) and thereupon the in vitro Drug Efficiency Index (DEI). DEI is a metric for ranking drug candidates in early lead optimization and is analogous to in silico Ligand Efficiency (LE), bridging the gap between in silico and in vivo assays [19].
Further applications of IAM chromatography concern its potential to provide information on the induction of phospholipidosis [20], while under investigation is its role in cell accumulation or cell retention, issues that are very important for drugs intended to target intracellular receptors or intracellular pathogens [21].

5. Expert opinion

Biological membranes are essential for drug efficacy, triggering research in the field of artificial membrane technology in the aim to face impediments in the early drug discovery phase. The development of IAM chromatography, by immobilizing crucial phospholipids on silica support, has been a key advancement toward this direction, providing an intermediate step between in silico and more complex in vitro assays. Initially, IAM Chromatography was intended to meet the criticism toward the octanol-water system and to provide an alternative to traditional lipophilicity. However, understanding of the molecular factors, involved in IAM retention, which define it as a border case between passive diffusion and binding, highlights many other applications. Thus, one measurement on an IAM column provides information for more issues relevant to multi-objective drug discovery, while it offers the possibility to determine more than one index, allowing the medicinal chemist to choose the most appropriate for his/her purpose. Isocratic retention factors log\(k_w\), their gradient elution alternative CHI, as well as their discrete polar component \(\Delta\log k_w\) have been so far successfully used to model ADME properties, in particular permeability through essential biological barriers, such as human intestinal and blood–brain barrier. The binding component in IAM retention permits rapid estimation of complex pharmacokinetic properties that involve nonspecific binding, while increased IAM retention may be an indication for phospholipidosis [20], extending its use to drug safety. Moreover, IAM retention may be associated with drug intracellular concentration, cell accumulation, or retention [17].

The ambivalent nature of IAM chromatography may however raise some ambiguity on the information encoded in IAM measurements and their correct interpretation. In order to draw sound conclusions from the multiple applications of IAM chromatography, appropriate experience in regard to distinct structural characteristics of drug candidates is required, while better characterization of IAM columns in regard to their potential to simulate particular biological processes, as attempted by Lazaro et al. [22], would contribute to a more focused and consistent use of the technique. More to the point, one should have in mind that IAM retention indices are not stand-alone parameters and they often necessitate the incorporation of additional structural descriptors in the models.

It should be noted that in many cases IAM models show similar performance with models constructed using experimental logP or logD. However, the advantages of IAM chromatography concern its simplicity, speed, and high reproducibility, while minimal amount of solute is required. On the other hand, compared to models based on in silico logP (or logD), IAM retention still has the advantage to be an experimental quantity. Predictions of IAM retention as a more realistic alternative to traditional lipophilicity would be an important step further to support its use in early drug discovery, complementary to logP or logD. To this direction an on-line free access calculator of IAM retention factors on both IAM.PC.MG and IAM.PC.DD2 has recently been developed as a web service, available at https://nova.disfarm.unimi.it/vegaol/logkwiam.htm, which however is restricted to molecules included in the PubChem collection [23]. The position of IAM retention would be further strengthened and become more familiar to the Medicinal Chemist if cut off values or ranges for distinct biological processes are established, as it is already the case for blood–brain barrier permeation and the metric DEI for ranking drug candidates.

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