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Biomimetic chromatography—A novel application of the chromatographic principles

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Abstract

Biomimetic chromatography is the name of the High Performance Liquid Chromatography (HPLC) methods that apply stationary phases containing proteins and phospholipids that can mimic the biological environment where drug molecules distribute. The applied mobile phases are aqueous organic with a pH of 7.4 to imitate physiological conditions that would be encountered in the human body. The calibrated retention of molecules on biomimetic stationary phases reveals a compound's affinity to proteins and phospholipids, which can be used to model the biological and environmental fate of molecules. This technology, when standardised, enables the prediction of in vivo partition and distribution behaviour of compounds and aids the selection of the best compounds for further studies to become a drug molecule. Applying biomimetic chromatographic measurements helps reduce the number of animal experiments during the drug discovery process. New biomimetic stationary phases, such as sphingomyelin and phosphatidylethanolamine, widen the application to the modelling of blood–brain barrier distribution and lung tissue binding. Recently, the measured properties have also been used to predict toxicity, such as phospholipidosis and cardiotoxicity. The aquatic toxicity of drugs and pesticides can be predicted using biomimetic chromatographic data. Biomimetic chromatographic separation methods may also be extended in the future to predict protein and receptor binding kinetics. The development of new biomimetic stationary phases and new prediction models will further accelerate the widespread application of this analytical method.

KEYWORDS
biomimetic stationary phases, lipophilicity, phospholipid binding, protein binding, toxicity

1 | INTRODUCTION

Biomimetic High Performance Liquid Chromatography (HPLC) stationary phases, such as immobilised artificial membrane (IAM, Regis Technologies Inc.), human serum albumin (HSA; ChiralPak-HSA, Chiral Technologies, Daicel Inc.) and α-1-acid glycoprotein (ChiralPak-alpha-1-acid glycoprotein [AGP], Chiral Technologies, Daicel) can mimic the in vivo interactions of drug molecules with lipids and proteins.[1] Figure 1 shows the commercially available biomimetic stationary phases. The methodology can be applied for new modalities in drug discovery, such as peptides[2] that would be difficult to characterise by traditional methods such as equilibrium dialysis to estimate their tissue binding and volume of distribution.

Abbreviations: AGP, α-1-acid glycoprotein; CNS, central nervous system; hERG, human ether-à-go-go-related gene; HSA, human serum albumin; IAM, immobilised artificial membrane; IAM.PC. DD2, immobilised artificial membrane column with phosphatidylcholine, drug discovery; IAM. SPH, immobilised artificial membrane with sphingomyelin; Log P, logarithm of octanol/water partition coefficient; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SPH, sphingomyelin

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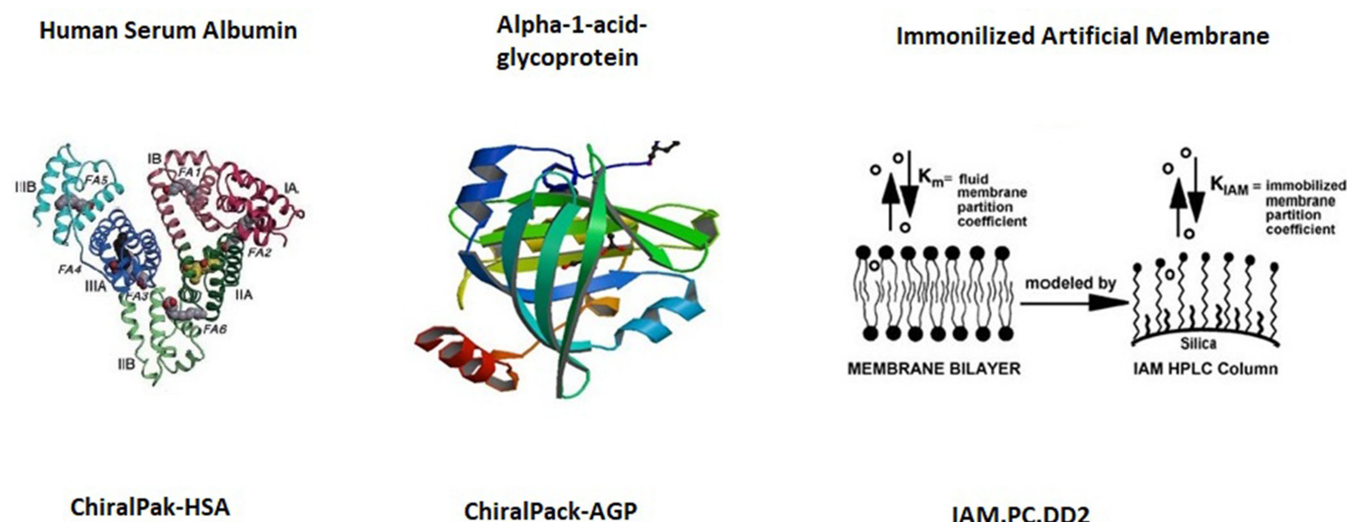

FIGURE 1 Commercially available biomimetic stationary phases using immobilised human serum albumin (HSA) and α-1-acid glycoproteins (AGP) and phosphatidylcholine are ChiralPak-HSA, ChiralPak-AGP and immobilised artificial membrane (IAM) column with phosphatidylcholine, drug discovery

Biomimetic chromatographic methods can be used for lipophilicity, protein binding and phospholipid-binding measurements. The data obtained are suitable to model blood/brain distribution, plasma protein binding, tissue partition, skin penetration, soil absorption, toxicity, aquatic toxicity and the environmental fate of compounds. Chromatographic methods require small quantities of not necessarily pure compounds, as separation occurs during the measurement. Characterising the physicochemical properties of putative drug molecules and new chemical entities is important in the drug design process and evaluating their environmental fate.[3,4] In this respect, the most important properties of the molecules are lipophilicity,[5] protein binding[6] and phospholipid binding.[7] These properties describe quantitatively how the compound prefers the lipid or protein environment relative to a purely aqueous environment. Traditionally, octanol/water partition coefficients are used to model biological partition processes. Table 1 summarises the similarities and the differences between the octanol/water bi-phasic partition system and the biomimetic chromatographic dynamic distribution system. Logarithm of octanol/water partition coefficients (log P) is used to measure the lipophilicity of the compounds. As a large amount of measured log P data has been accumulated in the past 40 to 50 years, several in silico calculation methods have become commercially available, such as in silico calculated logarithm of octanol/water partition coefficients by ACD Labs In, log P from the Pharma Algorithm, Ghose–Crippen–Viswanadhan in silico octanol–water partition coefficient, Moriguchi octanol–water partition coefficient, ChemAxon logP and many more. Octanol/water partition coefficients have been suggested to mimic the biological distribution processes.

The natural distribution processes occur on large surfaces and via a dynamic equilibrium process. In this respect, it is very similar to the chromatographic distribution processes, resulting in a separation of very similar compounds. When protein and phospholipid (such as IAM) stationary phases are used together with aqueous mobile phases

TABLE 1 The similarities and differences between the octanol/water partition and the biomimetic chromatographic dynamic distribution systems

Octanol/water partition	Biomimetic partition/distribution	Biological distribution
Contains water	Contains water	Contains water
Non-aqueous phase contains a polar group	Non-aqueous phase contains a polar group	Non-aqueous phase contains a polar group
Non-aqueous phase is non-polar	Non-aqueous phase is non-polar	Non-aqueous phase is non-polar
Non-aqueous phase does not contain charged groups	Non-aqueous phase contains charged groups	Non-aqueous phase contains charged groups
Bulk partition, the interface is not characterised	Partition on a large interface	Partition on a large interface
It has no shape selectivity	It has shape selectivity	It has shape selectivity

with a physiological pH of 7.4, the chromatographic system mimics the in vivo partition processes, as the proteins and lipids can mimic the majority of the non-specific binding sites in the body. That is why the name ‘biomimetic chromatography’ is used. Broadly speaking, the traditional reversed-phase stationary phases with a long alkyl chain surface can model the inner hydrophobic layer of the cell membrane. Therefore, by using pH 7.4 aqueous mobile phases, the retention may be proportional to the compound’s distribution to the inner core of the cell membrane. In practice, we use organic modifiers to help elute the strongly retained compounds. In isocratic mode, we can extrapolate the logarithmic retention factors to the 100% aqueous mobile phase concentration. When high-throughput compound characterisation

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is required, gradient elution can be used. In this method, the gradient retention times of compounds with known protein binding and phospholipid-binding data are used to convert the gradient retention times of the unknown compounds to binding data.

The retention of neutral compounds on the biomimetic stationary phases is related to lipophilicity, which may correlate with octanol/water partition coefficients.

The reversed-phase chromatographic partition/distribution coefficient can directly relate to the chromatographic retention factor.[1] The biomimetic chromatographic retention on protein (HSA and AGP) and immobilised IAM are considered reversed-phase retention mechanisms, as the mobile phase is more polar than the stationary phase. The retention factors obtained in the biomimetic stationary phases are proportional to the compound distribution between the aqueous mobile phase and the protein or phospholipid in the stationary phase. It was also shown that the gradient chromatographic retention times are proportional to the chromatographic hydrophobicity index obtained by isocratic measurements.[8–10] The chromatographic retention can be converted to HSA binding[10] and phospholipid[11] binding of molecules with appropriate reference molecules and reference binding data. The standardised and calibrated gradient retention time measurements using biomimetic stationary phases significantly accelerated the compound characterisation for hundreds of thousands of compounds.[11] It was shown that the HSA binding and IAM binding data obtained by the high throughput standardised gradient chromatographic methods could be used to predict human and rat volume of distribution,[12] the unbound volume of distribution[13] and drug efficiency.[14] New chromatography-based methods have been discovered to characterise larger molecules' embedded polar surface area to predict cell or intestinal permeability.[15] Recent developments published in the past few years regarding new biomimetic HPLC columns and applications are discussed in this review.

1.1 | Recent developments in biomimetic stationary phases and chromatographic methods

This field has the potential to achieve important milestones with new commercially available biomimetic stationary phases. Immobilising biologically active molecules, such as proteins, that are significant in determining the fate of the compounds in the body or in the environment and stationary phases that can mimic biological membranes could accelerate the development and applications of this approach.

The emerging biomimetic HPLC stationary phases, which apply lipids and proteins directly from biological systems, revealed the weaknesses of the octanol/water lipophilicity, especially when considering charged compounds. More significant discrepancies can be observed for charged compounds, such as in the model volume of distribution.[16,12] The immobilisation and chemical bonding of phospholipids are not trivial, as these amphiphilic molecules form micelles and liposomes. It is difficult to ensure the density and the polar head group arrangements on the stationary phase surface that mimic the biological membranes. The first IAM stationary phase introduced by Pidgeon et al.[17] is still manufactured commercially by Regis Technologies. It contains the phosphatidylcholine (PC) head group. Since then, several efforts have been published for procedures for preparing phospholipid stationary phases.[18,19]

Besides PC, other phospholipids, such as phosphatidylethanolamine (PE), are also abundant in vivo,[20] for example, in lung tissues. A new PE-functionalised biomimetic monolith stationary phases have been synthesised and compared to the commercially available PC stationary phase from Regis Technologies.[21] The authors investigated the retention mechanism and the reproducibility of the new HPLC column. It showed remarkable similarity to the IAM PC. DD2 column. As PC and PE are the most abundant phospholipids, and PE is even more abundant in the lungs, it is important to understand the similarities and differences between the two types of lipid membranes. This biomimetic monolith exhibited good separation selectivity for proteins and small molecules, which is also important for the measurement and differentiation of the properties of the molecules. It would be a significant achievement if these HPLC columns became commercially available and other research groups could use and investigate the properties.

Another new phospholipid stationary phase using sphingomyelin (IAM with sphingomyelin [IAM. SPH]) was first synthesised and published in 2012.[22] Sphingomyelin (SPH) is a type of sphingolipid found in animal nerve tissues; therefore, the SPH stationary phase represents an ideal tool to measure the interactions between active pharmaceuticals and neurons. The IAM. SPH stationary phase showed unique selectivity of acids and bases, similar to the IAM. PC phases. The synthesis and analysis of the new IAM. SPH biomimetic stationary phase for investigating putative drug molecules' blood/brain barrier distribution was published in 2021.[23] A good correlation was found[23] between the new SPH phase named IAM. SPH and the IAM membrane column with phosphatidylcholine, drug discovery (IAM.PC. DD2) phase based on block relevance analysis.[24] It also showed the potential improvements in predicting the compounds' blood/brain barrier permeability.

It was found that the negatively charged intra-lysosomal lipids play an important role in the formation of drug-induced phospholipidosis.[25] A novel IAM column was prepared to mimic this negatively charged lipid surface better. This showed that it is suitable to predict the phospholipidotic potential of putative drug molecules.[26] Figure 2 shows the concept of using the new biomimetic stationary phase to predict drug-induced phospholipidosis. The recent paper in Expert Opinion on Drug discovery discussed how we could better realise the potential of IAM chromatography in drug discovery and development.[27] The development of IAM chromatography by immobilising various phospholipids on a silica stationary phase provided an intermediate step between in silico and more complex in vitro assays. Although the IAM retention selectivity and the derived binding data show similarity to octanol/water partition, they also show significant differences, especially towards charged molecules. Positively charged compounds partition more strongly to the IAM phase than to the octanol phase, which is more similar to compounds partition to biological membranes. Understanding the molecular factors, involved in IAM retention, thus provides information for more issues relevant to multi-objective drug discovery than the octanol/water partition. The models based on

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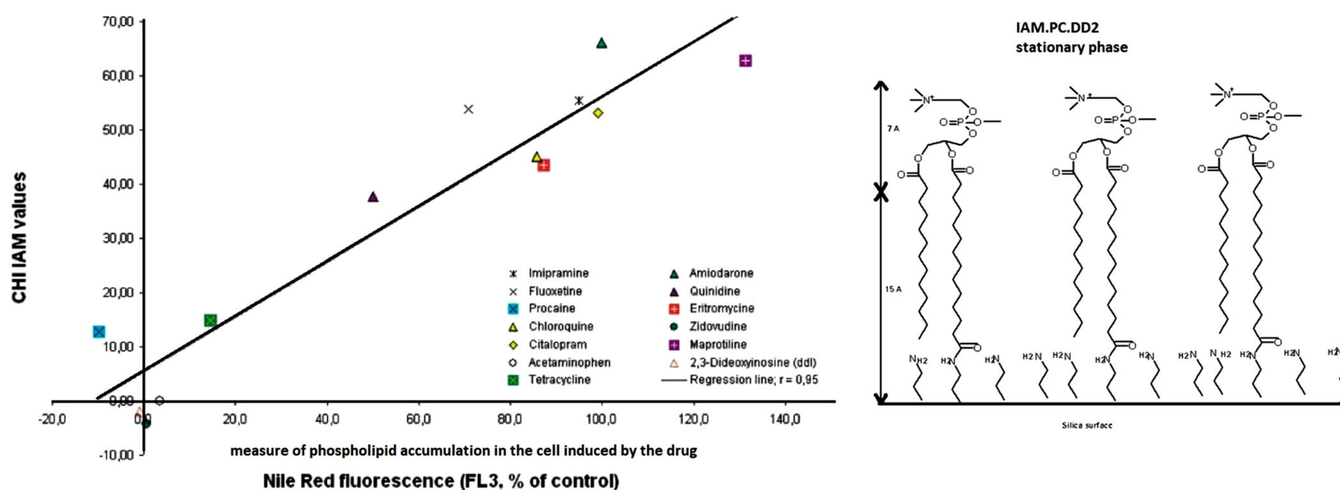

FIGURE 2 The concept of using an IAM stationary phase to predict drug-induced phospholipidosis. Drug-induced phospholipidosis occurs when the drug molecule binds strongly to phospholipids and disturbs its metabolism in the cell, resulting in the accumulation of phospholipid vesicles. This can be detected by Nil-red staining of the cells. It was found that IAM retention (CHI IAM), which measures compound affinity to phospholipids, correlated very well with the staining

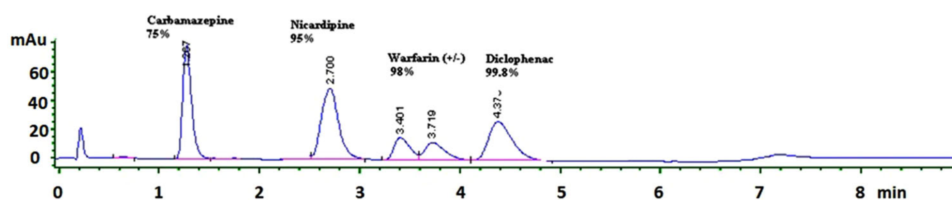

FIGURE 3 The chromatogram of marketed drugs with known plasma protein binding data that are used to calibrate the retention times on an albumin column. Column: ChiralPack-HSA 50 × 3 mm, mobile phases: (A) 50 mM ammonium acetate pH 7.4, (B) 2-propanol. Flow rate: 1.2 ml/min, gradient profile: 0 to 3 min, 0% to 30% 2-propanol, 3 to 9 min 30% 2-propanol, 9 to 10 min, 30% to 0% 2-propanol

biomimetic chromatographic measurements are not intended to model log P that was introduced to model biological partition processes and represent a much better model to the real world as we use the actual phospholipids and proteins that are present in the body.

The protein stationary phases (Chiralpak-HSA and Chiralpak-AGP, containing immobilised HSA and AGP) were developed for chiral separation[28] and have become commercially available. Several authors proved that the retention on the chiral albumin phase was proportional to the albumin binding of the compounds obtained by the equilibrium dialysis method.[29–32] It is important to check that the immobilised albumin phase has retained its native form. The suitability of an HSA column for HSA binding measurements can be checked by injecting racemic warfarin. When albumin is in its native form, it should separate the warfarin enantiomers, showing that the warfarin binding site is intact. Figure 3 shows the chromatogram of known drugs with known albumin binding. Strongly bound compounds (binding greater than 99.8%) can be measured and differentiated using the applied conditions. When chiral separation occurs using mobile phases at physiological pH, it reveals the different binding characteristics of the enantiomers under physiological conditions. The human plasma protein binding of drugs can reveal potential drug–drug interactions and influence the free concentration available at the target. The application of immobilised HSA and the α-1-acid glycoprotein phases is often used to reveal the structure and binding property relationships and aid the computational approach to drug design.[33] There have been efforts to immobilise various proteins on silica surfaces and to use them as stationary phases in what is called bioaffinity chromatography.[34] Several protein-based stationary phases were synthesised for studying drug–protein interactions.[35] It is important that these stationary phases become commercially available so that research institutions can evaluate and use them with the advantages that the chromatographic principle offers.

New chromatography-based methods have been discovered to characterise larger molecules' embedded polar surface area to predict permeability.[15] Controlled supercritical fluid chromatography provided a polarity readout derived from the retention time on a specific column, Chirex 3014. This stationary phase was selected for its balance of lipophilic and polar attributes and its capacity to separate compounds with wide polarity differences. Under normal phase conditions, supercritical chromatography provides a low dielectric constant environment conducive to intramolecular hydrogen bonding formation. In this way, it enabled the characterisation of cyclic peptides embedded on the polar surface area that showed a relationship with the retention of compounds. Other so-called bioaffinity chromatographic methods[36]

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use protein stationary phases for measuring drug–protein interactions that are not necessarily based on HPLC retention time measurements. This area has not been included in the scope of this review. Other types of separation techniques, such as capillary electrophoresis and microemulsion electrokinetic chromatography[37,38,] are also excluded from this review. These methods have the advantages to apply in situ biomimetic components in the mobile phase or in the emulsion to measure drug–protein or drug membrane interactions.

2 | NEW APPLICATIONS AND MODELS

The perspective of using HPLC-based biomimetic properties in early drug discovery will help to reduce later-stage attrition of candidate molecules due to disadvantageous absorption, distribution, metabolism and elimination properties. A compound's binding to lipids and proteins can be measured by biomimetic chromatographic retention and can be used to build distribution models. It was found that the difference between the IAM and HSA binding can be used to estimate the in vivo volume of distribution of the compounds. Scientists from several pharmaceutical companies involved in early-stage drug discovery have published the measurement and application of biomimetic properties for a large number of compounds.[11,39,40] A recent review article discusses the advantages and applications of IAM.PC. DD2 biomimetic stationary phases for their potential in predicting intestinal absorption.[41] Biomimetic retention data obtained on IAM stationary phase have been used to predict the intestinal permeability of compounds. It has to be emphasised that the chromatographic retention is proportional to the dynamic chromatographic partition coefficient of the compounds between the stationary and mobile phases. Therefore, applying these data to model rate descriptors in principle is incorrect. Good correlations can be found because the membrane permeability rate constant is dependent on the membrane partition coefficients. The article also mentions biopartitioning micellar chromatography[42] when the biomimetic micelles are in the mobile phase. Commercially available, reversed-phase stationary phases are used in this chromatography mode. Other stationary phases and surfactants to form micelles are also suggested to mimic better the biological partition processes.[43]

Modelling the blood–brain distribution is important when designing central nervous system (CNS) active drugs. It is also important to assess when the side effects reduce the effectiveness of the new CNS drug molecule. The application of separation methods, especially biomimetic chromatography, has been discussed in a recent publication.[44] The property of the anti-depressant drug candidates was predicted using biomimetic chromatography, genetic algorithms and multiple regression analysis.[45] It was found that lipophilicity, membrane permeability and drug–protein binding measured by biomimetic chromatography successfully characterised the antidepressant drug candidates and helped the drug discovery process by reducing the cost of drug development.

The interaction of antifungal isoxazolo-pyridine derivatives with human serum proteins was investigated using biomimetic chromatography, and meaningful and helpful quantitative structure-retention relationships were established that helped predict the affinity of compounds to HSA.[46] Similar studies and results were published using the methodology for antimicrobial peptide compounds.[47] This demonstrated that the method could be applied for large molecules that are outside the traditional drug property space, where traditional octanol/water partition measurements or equilibrium dialysis methods encounter difficulties.[2]

IAM chromatography proved to be especially useful in predicting various toxicity endpoints of drug molecules. Thus, the phospholipidotic potential of drugs is related to their interactions with the phospholipid membrane, disturbing its metabolism and causing the accumulation of phospholipid vesicles inside the cells.[48] As a prerequisite, the compound should bind strongly to phospholipids that manifest by long retention times on IAM columns. IAM chromatography has been applied to estimate toxicological endpoints regarding drug safety, such as phospholipidosis potential or chemical risk hazards, including the bioconcentration factor and toxicity in aquatic organisms.[49] The speed, reproducibility and low analyte consumption suggest that a broader application of IAM chromatography may save time and money in initial drug candidate selection and contribute to a reduced risk hazard of chemicals. The compounds' binding ability to phospholipids showed a good correlation to their aquatic toxicity. It was studied both for drugs[50] and pesticides.[51]

A study published recently highlighted that the IAM binding and the AGP binding together can be used to model the cardiotoxicity potential of compounds measured by the human ether-à-go-go-related gene (hERG) enzyme inhibition.[52] The mechanistic model is based on the assumption that the compound has to be able to go through the phospholipid membrane where the hERG receptor resides. The IAM chromatography models this assumption. The hERG receptor shows a similar shape to the α-1-acid glycoprotein (AGP) that binds positively charged compounds only if the charged part of the molecules sterically can reach the AGP and the hERG receptor negatively charged area as shown in Figure 4.

It is known that lipophilic compounds are toxic to the environment, as they are bound strongly to non-aqueous, lipophilic layers that natural water cannot wash off. The octanol/water partition coefficients (log P), often the in silico calculated values, are used to assess chemicals' and pesticides' environmental impact. However, the studies showed that the membrane partition measured by IAM chromatography could provide a more reliable assessment, especially when the compounds are charged. The thermodynamic aspects of the binding of new antiepileptic compounds to C-18 and IAM stationary phases have been investigated and compared using differential scanning calorimetry.[53] It was found that heteroatoms such as oxygen and sulphur in the triazole ring were responsible for the selectivity differences in the IAM interactions supported by molecular dynamics calculations. This type of study supports the observation that lipophilic interactions can be very different when different conditions are used for the measurements. It also emphasises the importance of chromatography mimicking the biological environment to obtain better models and predictions of the compounds' behaviour. The measured lipophilicity depends on the nature of the lipid to which the lipophilicity refers.

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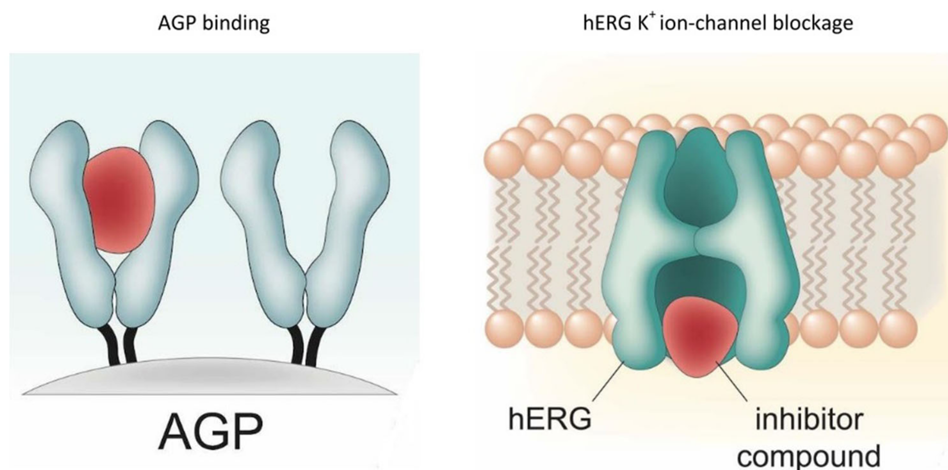

FIGURE 4 The model to predict human ether-à-go-go-related gene (hERG) inhibition by IAM and AGP biomimetic chromatography (from reference[52] with permission). The hERG receptor is located inside the membrane and has similar funnel-like shape (right) as the immobilised AGP on the stationary phase. Both AGP and the hERG channel have negative charges at the narrow end of the funnel shape. The compound that interacts with the hERG channel has to go through the membrane that is modelled by the IAM retention, while the hERG channel binding is modelled by the AGP binding

When the protein-based stationary phases are used to measure plasma or tissue protein binding, it is important to convert the biomimetic chromatographic retention to binding constants or percentages of binding used by absorption, distribution, metabolism and toxicity scientists. It is also important to compare other in vitro methods.[33] It is worth highlighting the differences and similarities between various methods. The chromatographic methods are becoming more precise and differential for strongly bound compounds, unlike the equilibrium dialysis and ultrafiltration methods, which are less accurate at the higher binding range. The chromatographic method using the chiral protein phase also allows the separation of enantiomers and the differences in their protein binding to be determined.

The chromatographic measurements could provide additional information about the kinetic aspects of the binding/partitioning of the compounds to proteins and phospholipids by investigating the peak shape.

Recent studies[54] have pointed out that the kinetics of the drug-receptor binding process could be as important as the affinity in determining the drug efficacy. Therefore, the characterisation and prediction of the rate constants of protein–ligand and lipid–ligand associations and dissociations are required. Slow or fast offset or onset rates of binding alter the drug distribution. Therefore, understanding the molecular features underlying binding and unbinding processes is of central interest to rationalising drug binding kinetics. The chromatographic peak shape can assess the binding kinetics.[55] The chromatographic distribution process is usually fast and results in narrow and symmetrical peaks. However, during protein binding or IAM binding measurements, wide and asymmetrical peaks are often observed for certain compounds due to slower than usual offset or onset rates.[56] The slow rates could be caused by steric hindrance or delocalised charge–charge interactions. Although there are traditional peak broadening effects in chromatography, it was observed that certain compounds gave much wider peaks than the others with similar retention times on the same column on the same system and on the same day. These phenomena could be used to derive binding kinetics information of the compounds using biomimetic chromatography. With a suitable reference method, this potential could be further investigated. The chromatographic separation principle and biomimetic stationary phases using immobilised receptors may provide further powerful applications of these techniques.[57]

3 | SUMMARY AND OUTLOOK

Biomimetic chromatographic data have already been used in several drug discovery organisations. More than half a million compounds have been characterised under standardised conditions.[11] In silico calculation methods have been developed based on the excellent quantitative structure-property relationships. Numerous models and applications have been compiled in a book.[58] The impact on drug discovery has already been observed. Several research groups are working on new models and applications. In this paper, selected achievements published in recent years in biomimetic chromatography have been reviewed. The focus was on efforts to develop new biomimetic stationary phases and published efforts with new applications and models.

Traditional and commercially available biomimetic stationary phases are immobilised IAM containing a PC head group, immobilised HSA and α-1-acid glycoprotein, which have already been proven to be invaluable for characterising early drug discovery compounds. Numerous models have been published in the past 20 years to predict the volume of distribution, drug efficiency, tissue partition and blood/brain barrier distribution. The method has been used for several hundred thousand compounds in several pharmaceutical companies. This was made possible because these columns are manufactured in a reproducible manner and commercially available. The traditional

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C-18 reversed-phase columns are now widely used to determine the lipophilicity of the compounds, which is different from octanol/water partition. It can also be considered a biomimetic phase as it mimics the inner part of the biological membrane bi-layer. Efforts to synthesise new types of phospholipid stationary phases were reviewed that could better model the blood-brain barrier, such as the SPH phase or the second most abundant, the PE phase. These would potentially achieve wider use when they are commercially available.

Still, there is resistance to relying on biomimetic chromatographic data in decision making and lead optimisation in drug discovery. The traditional so-called 'gold standard' methods are often preferred, such as octanol/water partition measurements, equilibrium dialysis measurements or cellular and in vivo animal experiments. These methods are more expensive and time-consuming and often have a greater error in the measurements due to the complexity of the systems. Due to the lack of resolution, these traditional methods often cannot distinguish small structural changes in the molecules. Biomimetic chromatography would gain more popularity when more reproducible stationary phases would become commercially available and standardised procedures were used. The chromatographic conditions are versatile and can affect the measured retention data. Therefore, it is important to measure the retention times of a calibration set of compounds that have fixed binding data. In this way, the retention time and binding relationship can be established whenever the conditions are changed. Every time a change in conditions is applied, method validation procedures must be carried out. Applying a calibration set of compounds with standard binding data would help the interlaboratory comparison and reproducibility.

In recent years, new models have been published that apply the traditional phases to predict the toxicity of drug molecules, such as phospholipidosis and hERG inhibition. Recent publications have discussed the application of biomimetic chromatography and building models to predict a compound's environmental impact and aquatic toxicity. Predicting the environmental fate of drugs and pesticides is gaining increasing importance in the hope of selecting more environmentally friendly drugs and pesticides.

CONFLICT OF INTEREST

The author declared no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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