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An orientation sensitive approach in biomolecule interaction quantitative structure–activity relationship modeling and its application in ion-exchange chromatography

Jörg Kittelmann[a], Katharina M.H. Lang[a], Marcel Ottens[b], Jürgen Hubbuch[a,*]

[a] Section IV: Biomolecular Separation Engineering, Institute of Engineering in Life Sciences, Karlsruhe Institute of Technology (KIT), Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany
[b] Bioprocess Engineering (BPE), Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

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ABSTRACT

Quantitative structure–activity relationship (QSAR) modeling for prediction of biomolecule parameters has become an established technique in chromatographic purification process design. Unfortunately available descriptor sets fail to describe the orientation of biomolecules and the effects of ionic strength in the mobile phase on the interaction with the stationary phase. The literature describes several special descriptors used for chromatographic retention modeling, all of these do not describe the screening of electrostatic potential by the mobile phase in use. In this work we introduce two new approaches of descriptor calculations, namely surface patches and plane projection, which capture an oriented binding to charged surfaces and steric hindrance of the interaction with chromatographic ligands with regard to electrostatic potential screening by mobile phase ions. We present the use of the developed descriptor sets for predictive modeling of Langmuir isotherms for proteins at different pH values between pH 5 and 10 and varying ionic strength in the range of 10–100 mM. The resulting model has a high correlation of calculated descriptors and experimental results, with a coefficient of determination of 0.82 and a predictive coefficient of determination of 0.92 for unknown molecular structures and conditions. The agreement of calculated molecular interaction orientations with both, experimental results as well as molecular dynamic simulations from literature is shown. The developed descriptors provide the means for improved QSAR models of chromatographic processes, as they reflect the complex interactions of biomolecules with chromatographic phases.

1. Introduction

Chromatography is the most widely applied technique in downstream processing of biopharmaceutical products. Due to the great interest in the underlying process mechanistics and the consequential research, the fundamental understanding of chromatographic separation and adsorption mechanisms have widely advanced in the last decades. Today, a variety of modeling techniques can be applied to describe the influence of different parameters on ion-exchange chromatography, including mobile-phase composition, resin types, and protein characteristics. Comprehensive studies on protein retention in different mobile phase conditions have lead to semi-empirical approaches of protein behavior modeling. Rounds and Regnier introduced the stoichiometric displacement model, describing the interaction between proteins and counter ions [1]. The steric mass action (SMA) model by Brooks and Cramer [2] incorporates the shielding of adsorber charges by bound macromolecules. An extension of this model was introduced by Bosma and Wesselingh, who used the charge of proteins to describe protein binding at different pH values [3]. Although, they assumed a constant electrostatic charge, neglecting the pH dependent protonation of charged residues. These models require the determination of protein and adsorbent specific parameters, and therefore cannot be applied to predict the behavior of unknown molecules. Further, they are based on the assumption that the binding mechanistics remain unchanged throughout the conditional space inquired.

That this is not the case in chromatographic interactions, was shown by molecular dynamic (MD) simulations, which aim to describe the interaction of molecules on the atomic level. It has been shown that specific orientations are predominant in binding of biomolecules to charged surfaces, dependent on charge distribution of the molecule, steric factors, charge type, and capacity of

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* Corresponding author.
E-mail address: Juergen.Hubbuch@kit.edu (J. Hubbuch).

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the corresponding surface. The interaction energies and orientation of biomolecules in cation-exchange chromatography were investigated experimentally and *in silico* via MD simulations by Dismer et al. [4–6]. This approach was later extended to complex proteins (i.e. including non-standard residues) and anion-exchange chromatography by Lang et al. [7]. Dismer and Lang determined a pH dependent change in orientation for lysozyme on a SP Sepharose FF adsorbent. The primary binding orientation was determined by the distribution of the amino acids lysine and arginine with a change of the main binding factor from lysine 13 at low pH toward lysine 96 and 97 at high pH values [6,7]. Despite fast computational development and detailed information to be gained, MD simulations remain inapplicable to large datasets and structures, e.g. monoclonal antibodies, due to the computational costs required.

Quantitative structure–activity relationship (QSAR) techniques aim at developing predictive models based on descriptors derived from molecular structural information. A critical part are the descriptors utilized to build a model, as they need to incorporate the structural features which lead to the observed activity. To capture the molecular information corresponding to ion-exchange chromatography, Mazza et al. [8] introduced an approach to map electrostatic potential onto the surface of a molecule to derive pH dependent descriptors. They predicted retention times for a variety of beta-blockers and similar chemical structures published by Law and Weir [9].

Malmquist et al. [10] developed their own set of pH sensitive descriptors, based on the surface mapping of atomic charges and applied them successfully in cation and anion-exchange chromatography modeling of model proteins. Based on this work, Yang et al. [11] developed a set of electrostatic potential descriptors, and applied them in SMA parameter modeling, wherein different pH conditions of the same molecule were presented as separate observations to the model. All approaches have in common that they do not reflect the orientation of the molecule and characteristics of the mobile phase. This is especially the case, if predicted parameters are subsequently applied in aforementioned semi-empirical models.

In this approach a new set of QSAR descriptors is introduced, describing the physical proximity of biomolecules to a surface in 3D space. Thereby describing steric hindrance and interaction as well as interaction shielding by the mobile phase. This allows for orientation sensitive QSAR modeling over different ionic strengths and pH values, reflecting the parameter range in ion-exchange chromatography. The predictive capabilities are demonstrated in Langmuir isotherm parameter modeling for model proteins in cation-exchange chromatography. A detailed comparison of interaction orientations of Lysozyme on SP Sepharose FF to experimental and *in silico* data from MD simulations, shows consistent results without the need of comprehensive and time-consuming simulations.

2. Theory

2.1. Parameter projection

A molecular property on a given point in space is generated as the result of the intermolecular interactions between all fragments of the molecule and the mobile phase at the given point.

$$MP_k = \sum_{i}^{N} f_i fct(d_{ik}) \tag{1}$$

With MP being the molecular property at a given point k in space, i denotes the fragment and N the total number of fragments in the molecule. f_i represents the parameter constant of the fragment and fct the distance function with d_{ik} being the distance between the fragment i and the point in space k.

This applies to calculation of both molecular surface properties as well as intermolecular forces as the projection toward the representation of adsorbent surface structures as described in this work. Three types of projections are used, namely the complete molecular surface, a projection toward an adjunct plane, and patches of the surface.

2.1.1. Molecular surface

To represent the distribution and values of molecule electrostatic potential and hydrophobicity characteristics on the molecule surface, these properties are mapped to the solvent accessible surface area (SASA) of the molecule. Single values for atoms (electrostatic potential) and amino acids (hydrophobicity coefficient) are mapped over distance toward the surface represented by distinct points with a density of 1 point per Å^2. Descriptors based on the molecular surface of the molecule are then calculated for all points on the surface. The attenuation of electrostatic and hydrophobicity characteristics distance are described in Sections 2.2 and 2.3, respectively.

2.1.2. Plane projection

To represent different orientations of a molecule toward the adsorbent surface, a representation of said surface was constructed as a grid of points in three dimensional space, which is depicted in Fig. 1. This representation can be repositioned around the molecule to capture the effects of reorientation of molecules.

To represent all possible orientations of the molecule toward a surface, the molecule is represented by a sphere with the geometric center of the molecule in the sphere center. Based on the sphere 120 equidistant orientations were calculated. This compares to 62 grid based orientations used by Dismer et al. [6], which lead to an over-representation of the sphere poles and 50 equidistantly distributed orientations used by Lang et al. [7].

Orientation vectors were calculated based on the Thomson equilibrium of a sphere [12] with the vector origin placed in the geometric center of the molecule, yielding equidistantly distributed orientations.

For each vector a plane of 120 Å edge length and a grid point density of 5 Å was calculated and positioned with the vector as normal vector in the plane center. The plane was shifted along the normal vector to fit the distance to the molecular surface, which was set to 5.0 Å, in accordance to the set-up of orientation sensitive MD simulations performed by Dismer [6] and Lang [7].

2.1.3. Surface patch

To capture properties on the molecular surface sensitive to the orientation of the molecule toward an interacting surface, surface patches were calculated. Based on the calculated planes, surface patches were defined to include the SASA with a distance of below 20 Å toward the corresponding plane representation.

2.2. Electrostatic potential

It is known that charge and electrostatic potential properties govern the interactions of molecules in ion-exchange chromatography. In fact, the adjustment of counter ion concentration in the mobile phase to alter the long range electrostatic forces in biomolecule-adsorbent interaction is one of the most used parameters to direct protein separation (i.e. salt gradient).

Coulomb interactions between molecules are reduced due to the polarization of the particles in a dielectric medium as water. Particles, depending on their character, form induced and permanent dipoles which will be oriented around a free charge so to terminate some of the field lines originating from the charge. This effect

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