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Enhanced interpretation of adsorption data generated by liquid chromatography and by modern biosensors

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ABSTRACT

In this study we demonstrate the importance of proper data processing in adsorption isotherm estimations. This was done by investigating and reprocessing data from five cases on two closely related platforms: liquid chromatography (LC) and biosensors. The previously acquired adsorption data were reevaluated and reprocessed using a three-step numerical procedure: (i) preprocessing of adsorption data, (ii) adsorption data analysis and (iii) final rival model fit. For each case, we will discuss what we really measure and what additional information can be obtained by numerical processing of the data. These cases clearly demonstrate that numerical processing of LC and biosensor data can be used to gain deeper understanding of molecular interactions with adsorption media. This is important because adsorption data, especially from biosensors, is often processed using old and simplified methods.

1. Introduction

It is of increasing importance to adequately measure molecular and bio-molecular interactions since understanding of these interactions is essential in many classic and new research areas such as pharmacology, pharmacokinetics, pharmacodynamics, drug discovery and proteomics. In pharmacokinetics, binding studies are important because only the unbound concentration of the drug is pharmacologically relevant [1]. The unbound concentration of a drug is typically determined by first separating it from the bound fraction with classic techniques such as ultrafiltration or equilibrium dialysis. These time-consuming techniques produce very uncertain results for drugs with high-affinity binding, because of the low resulting unbound concentrations. In drug discovery, rapid determination of drug–target interactions is crucial in a competitive industrial environment and FDA has recently emphasized the need to distinguish enantioselective interactions [2]. Binding studies are also important for potential drug targets and diagnostic markers, including the process to select and optimize lead compounds during drug discovery.

Nonlinear chromatography theory has been extensively developed over the last 30 years and can be used to perform computer simulations [3–8]. The reason for this development is the need to predict/optimize process chromatography and here knowledge about the thermodynamics and kinetics of the system is crucial for successful simulations. High-pressure liquid chromatography (LC) instruments have become much more robust and precise over the years and LC therefore serves as a perfect platform for detailed and advanced binding studies. We have recently focused on improving data processing for nonlinear LC. E.g., we have developed an adsorption energy distribution (AED) calculation tool and that has been used, in combination with traditional Scatchard plots, to determine the degree of heterogeneity of the solute-surface interactions without prior assumption of a specific model [6]. Here the adsorption data is processed in a three-step procedure,

(i) Scatchard plots are used to roughly reveal the category of the adsorption: i.e., adsorption of type I, II, III, etc. [9].
(ii) AED is calculated to determine the degree of heterogeneity in the interaction: i.e., how many different adsorption sites are present and what is their individual energy of interaction/their abundance (monolayer capacity).
(iii) Model fit: this can be done with only one, or perhaps two, models since the pre-steps have reduced the number of possible models considerably [10–13].

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Using modern LC systems and this three-step procedure we have revealed the complicated thermodynamics for several molecular/biomolecular interactions of importance in the life sciences [6,7,12,14,15].

The principles of modern biosensors are very similar to LC because in both platforms we have a surface containing immobilized molecules which is percolated with a solution containing the analyte to be studied. Thus, biosensors can also be used for adsorption studies and for more detailed investigations of the interactions between drug molecules and proteins. We have recently transferred our AED-calculations tools to process data from modern biosensors, such as Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance (QCM), in order to clarify the molecular interactions between lipoproteins and chondroitin-6-sulfate [14]. From this it is clear that in many cases chromatography models can also be applied to biosensors. The opposite also holds; models developed for biosensors can in many cases be applied to chromatography because similar interactions are studied. In LC we have molecular interactions with an adsorption media and in this sense LC can be regarded as a "sensor"; therefore there are many similarities between the two techniques.

For biosensors based on SPR the studied protein is immobilized on a surface (chip) and then analyte molecules flows over the surface in an analysis cell [10,11]. The binding of analytes to the immobilized molecules leads to changes of the refractive index at the surface; this in turn causes a shift of the so called SPR-angle which is monitored in real time by an optical sensor. The SPR-signal is proportional to the adsorbed mass and quantification and determination of binding constants can be made. When the adsorbed mass is small, because the analyte molecules to be measured have low molecular weight, the signal-to-noise ratio is low. In recent years, much effort has been made to enhance the optical system to improve the signal-to-noise ratio [16] and to invent new immobilization techniques and chips constructions [17].

QCM is another biosensor technique that can be used to study adsorption phenomena. The technique determines small (in the nano-gram range) changes in the mass loaded onto a sensor. Although originally designed for studying adsorption of rigid materials from a gas phase and the formation of very thin and rigid layers from a liquid [18], the technique has evolved. Nowadays the adsorption of soft viscoelastic material suspended, or dissolved, in a viscoelastic medium can also be studied [19]. This has opened the possibility to study the adsorption of polymer films [19] and of biological material [20–25] as well as to characterize interactions of biological importance [26–31]. In QCM one studies the formation of thin and rigid adsorbed films: the relationship between the measured changes in the quartz crystal oscillation frequency (Δf) and the adsorbed mass (Δm) is given by the Sauerbrey equation [18],

$$\Delta m = -\frac{k}{n}\Delta f, \tag{1}$$

where k is a constant depending on the properties of the quartz piece (its fundamental resonance frequency, thickness and density) and n is the overtone number. For a standard quartz crystal with a resonance frequency of 5 MHz, the constant k is 17.7 ng cm^{-2} Hz^{-1}. Eq. (1) holds even if the crystal is oscillating in a liquid environment. However, certain conditions must be fulfilled: (i) the adsorbed mass is smaller than the mass of the crystal, (ii) the deposited film is rigid and (iii) the adsorbed material is evenly distributed on the sensor surface.

It is necessary to consider that the QCM response in a liquid environment considers the solvent included in the adsorbed film and can therefore give biased data if the adsorption of heavily solvated compounds is studied. Furthermore, a major drawback of QCM in liquids is that viscous and elastic contributions of the solvent could affect the frequency shift, particularly if the adsorbed material itself forms a viscoelastic film. This is the case for most polymers and proteins, as well as for intrinsically soft structures such as liposomes and lipodisks (planar lipid bilayer structures stabilized by PolyEthyleneGlycol, PEG, modified lipids). Applying Eq. (1) to these systems gives inaccurate results as the loss of energy due to viscoelastic effects is ignored. For the study of these materials by QCM different approaches are employed. Noteworthy are the use of the electromechanical analogy, e.g. see [32], and the use of *ring down* techniques that involves turning off the AC voltage exciting the crystal and measure the decay of the oscillation amplitude. This latter approach is the basis of the QCM with dissipation monitoring technique (QCM-D), which besides the shifts in frequency reports changes in the "dissipation factor" D, i.e., a measurement of the energy damping due to the deposited film viscoelasticity. Quantitative determination of the adsorbed mass can be done using the model proposed by Voinova et al. [19] for the formation of viscoelastic films when both the frequency and the dissipation shifts are available at several overtones. In the case of very thin adsorbed films in a bulk liquid, an expression relating the adsorbed mass (Δm), the shift in frequency (Δf) and the shift in dissipation (ΔD) is given by [19,33],

$$\frac{\Delta f}{n} = -\frac{\Delta m}{k} + B(n\,\Delta D), \tag{2}$$

where k, n are the same as in Eq. (1) and B is a parameter given by the fundamental oscillation frequency of the crystal and by the viscosity/elastic modulus ratio of the formed film. According to this equation, a plot of $\Delta f n^{-1}$ vs. $n\Delta D$ for different values of n should be a line that intercepts the y-axis at $-\Delta m/k$ and from which the adsorbed mass can easily be calculated [33]. When the thin film assumption does not hold, Eq. (2) cannot be fitted to a line and specialized software such as QTools [34] for QCM-D data analysis is required. QTools can determine both the mass adsorbed and the viscoelastic properties of the film.

For investigations of binding and adsorption behavior in biologically and pharmaceutically important systems there has been a great expansion and refinement of available techniques – such as LC, SPR, QCM, NMR and photo physical techniques [16,17,19,21,22]. Unfortunately, much of the potential information from these improved techniques is lost since the data is often processed and analyzed using simplified methods developed around 50 years ago when the lack of computers made linearization of the data necessary.

The goals of this review are three: (i) to illustrate the transfer of recently developed numerical tools, for processing adsorption data in LC, to a wide variety of different biosensor assays, (ii) to discuss what the signals really tells us and what more detailed information about the interactions can be achieved by data processing and (iii) to compare the two platforms, i.e., nonlinear LC and biosensor technology. We will demonstrate all this be reprocessing and reevaluating data from five previously published studies, two chromatographic cases and three different biosensor assays cases. Case I shows how the evaluation procedure can be used to gain deeper understanding of chromatographic interactions. Case II shows how adsorption data, in this case generated by LC perturbation experiments, can be further processed for deeper understanding of retention and adsorption mechanisms. For this case the same interactions was studied using SPR and the two techniques are compared. In case III, Quartz Crystal Microbalance (QCM) is used to characterize the adsorption behavior of phosphorylated peptides on titanium dioxide using a rigid film approach. In case IV QCM sensors modified with viscoelastic films is used for peptide–lipodisk interaction studies. Finally, in case V the same interactions as in case IV are studied, but by using a fluorimetric approach instead.