DESCRIPTION FOR THE GENERAL PUBLIC

Mathematical modeling of chromatographic process dynamics for proteins with unstable structures

Efficient purification of substances that have medical application is one of the most important and actual goals of chemical engineering. In the biopharmaceutical industry only the products of a very high purity can be accepted because it ensures biochemical stability and activity of the molecule and minimizes its undesired influence on the organism. This holds true particularly for monoclonal antibodies (mAbs), which, due to their very high selectivity, belong to the most desired proteins for therapeutic, diagnostic and analytical application. mAbs are used as drugs and diagnostic markers in many diseases including different types of cancer (e.g. rheumatoid arthritis, breast cancer).

The efficient purification of proteins in general, and mAbs in particular, is very important and still challenging. This is due to complex adsorption behavior of macromolecules which often involves conformational changes of their structure. The structure stability problems arising from conformational changes of proteins, often results in unexpected reduction in the separation selectivity and yield losses in preparative and industrial protein chromatography. This is caused by formation of inactive forms of proteins due to their unfolding. Although considerable research has been devoted to this issue, the mechanism of protein unfolding and aggregation is still not well understood. It involves complex kinetic and thermodynamic effects which depend on various process variables, and therefore are difficult to describe and predict. Also, due to difficulties in the measurement of proteins stability at solid interface as well as in interpreting the results of such measurements, the unfolding phenomenon cannot usually be controlled during protein processing by chromatography.

Therefore, this research attempts to improve the understanding of protein unfolding on chromatographic media and quantify this phenomenon in the form of a mathematical model, which can be used to determine the operating window for efficient separation of unstable proteins by chromatography. Several orthogonal methods will be used to elucidate the unfolding process and support the model formulation, including methods for analyzing the protein stability in liquid and adsorbed phases, such as: hydrogen exchange with mass spectrometry (HX-MS) and differential scanning fluorimetry (DSF). The former is a robust technique with well-established procedure for the interpretation of measurements, however, it involves a sophisticated and time consuming experimental procedure. The latter one is simple, fast and allows performing a large number of measurements with very low material consumption. Yet, the quantitative description of data generated by DSF is still lacking. Hence, we intend to put efforts to correlate HX-MS and DSF measurements to develop a platform for the interpretation DSF data. A procedure for the formulation of mathematical models for description of protein unfolding on chromatographic surfaces will be developed. This will enable exploiting the potential of chromatographic separations while avoiding undesirable effects arising from yield losses accompanying chromatography of unstable proteins, and a detailed optimization of the whole mAbs purification process, in which chromatography plays the most significant role.

The research will be performed for a few model proteins with the focus on monoclonal antibodies, which belong to the most desired proteins in pharmaceutical and biotechnology industries. Various chromatographic media will be considered, which are commonly used for downstream processing of mAbs, including: ion exchange, hydrophobic interaction and affinity chromatography media.

A part of the research will be performed in cooperation with the research group of prof. Giorgio Carta from University of Virginia, whose laboratory is equipped with suitable apparatus for HX-MS measurements and direct determination of kinetics of proteins adsorption onto surfaces of adsorbent particles.