DESCRIPTION FOR THE GENERAL PUBLIC

Crystallization of monoclonal antibodies by forced convection evaporation

Recently, a growing number of applications of bioactive proteins in biotechnology and medicine can be observed. Particular interest is put in production of monoclonal antibodies that are used for innovative treatments of cancer and autoimmune diseases in medicine.

Therapeutic proteins are produced in industry using genetic engineering methods. In the first step polypeptides are synthesized by modified bacterial, mammal or plant cells. Next step is Downstream Processing (DSP), in which the target protein is isolated from post-fermentation mixture using specific methods of separation. Generally, for this purpose costly chromatographic methods are used. Industrial application of proteins is limited by high coasts of their production. A large portion of this coast (even up to 80%) is generated by DSP, because the final product must fulfill a high purity demand and demonstrate proper biological activity. This causes that a large amount of research is focused on development new methods of separation and purification of proteins that allow reducing the production costs.

An attractive method that allows meeting that goal is crystallization. In past, crystallization of proteins was almost exclusively used to obtain large crystals for crystallography measurements. However, nowadays protein crystallization becomes more often used in DSP in purification or formulation step. The advantages of this method are not only low cost but also high efficiency and stability of crystalline phase obtained. The latter feature is very important for product storage, because polypeptide in the crystalline form preserves its activity for a long time.

Bulk crystallization is usually performed batchwise in stirred tank crystallizers, where nucleation and growth of protein crystals is induced by salting out in the presence of a crystallization agent, which typically is an aqueous solution of kosmotropic salts. Such an approach can lead to local concentration gradients that may cause protein aggregation or generation of amorphous phase. Therefore, nowadays new innovative techniques for the process realization have been searched.

One of such new techniques is crystallization by forced convection evaporation that has been developed in the frame of my PhD project. In this process, the desired supersaturation level was achieved by controlled water removal from the protein solution under mild temperature conditions. Water evaporation is caused by flow of air in a drying chamber in which the crystallization solution is situated. The protein solution is cooled down during the process as a result of water evaporation. Such an approach allows avoiding local concentration gradients due to slow increase in the concentration of precipitating agent. Moreover, crystallization can be performed for dilute protein solutions that are typical in biopharmaceutical industry.

The goal of the project is implementation of crystallization by forced convection evaporation in purification of the monoclonal antibodies. That concept was only realized for ovalbumin as a model protein. In the frame of this project, crystallization by forced convection evaporation will be used for purification of a post-fermentation industrial mixture containing a monoclonal antibody. The mixture will be supplied by an external company.

The output of the project shall be a new technique for purification of therapeutic monoclonal antibodies based on crystallization by forced convection evaporation. Additionally, the influence of process parameters on the crystallization course will be determined, and process optimization will be conducted. A generic design procedure shall be developed, which can potentially be applied for purification of other therapeutic monoclonal antibodies produced in industry.