**The Rational Purification of Proteins: Proteomics, Expert Systems, Modelling, Process Conditions and Optimization**

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**Highlights**

* Selection of optimal chromatographic sequence based on proteomic data of product and contaminants.
* Mathematical modelling of chromatography for accurate simulation including affinity.
* Optimization of chromatography process conditions including “peak cutting”.

**1. Introduction**

Over the past years we have developed a Computer Based Expert System based on Proteomic Data of Proteins for the rational selection of optimal protein purification sequences [1], as well as Mathematical Models that simulate and allow optimization of chromatographic protein purification processes. These systems consider more “generic” purification procedures such as ion-exchange, hydrophobic interaction (HIC) and gel filtration chromatography, but also affinity chromatography both using salt and pH gradients in the selection of chromatographic processes and in the mathematical modelling and simulation of chromatographic performance.

**2. Methodology**

The chosen methodology was implemented in a computer- based Expert System. Two algorithms were developed, the first algorithm was used to select the most efficient purification method to separate a protein from its contaminants based on the physicochemical properties of the protein product and the protein contaminants and the second algorithm was used to predict the number and concentration of contaminants after each separation as well as protein product purity. Once

the type of chromatography is chosen, optimization of the operating conditions is essential. For this two different mathematical models have been used [2]. These were the Plate Model and the more fundamentally based Rate Model. Operational conditions and the peak fraction collection is selected using a cost function of the production process which considers yield, purity, concentration, and process time that are obtained from simulations. This analysis allows optimization of process conditions [3].

**3. Results and discussion**

The theory developed for ion-exchange, HIC chromatography and affinity separations will be analyzed in this presentation. This is a unique contribution that allows investigation of chromatographic protein purification in a holistic approach that includes ion-exchange, HIC, gel filtration and affinity separations [4, 5, 6]. The methodology and experimental data required to carry out such optimization will be analyzed and shown. This analysis that considers a first principles approach to rational protein purification will be presented and discussed.

The first step consists of the selection of the optimal chromatographic sequence of operations (2-3, usually) based on the proteomic data of the protein product and the contaminants. The second step consists of detailed and accurate mathematical modelling and rigorous simulation using experimental data of each chromatographic step including parameter estimation, gradient elution, pH and flow rate. Procedures have been carried out at low and high protein concentrations such as those used in process oriented situations [3].

A mathematical function was built that includes parameters to optimize protein production as well as the effects of chromatography performance such as yield, purity, concentration and the time needed to accomplish the separation. Operational conditions in all Chromatographic processes can be selected using this model [3, 7]. This mathematical function was successfully used for the selection of the operational conditions as well as the fraction of the product to be collected (peak cutting).

Finally, the present interest in carrying out protein separation and purification in a continuous process will be analysed and discussed.

**4. Conclusions**

We have developed a method to select the optimal chromatographic sequence of operations for protein purification based on detailed proteomic data of the protein product and its contaminants. We have also developed accurate mathematical models for the simulation of the chromatographic steps. A detailed procedure was developed that includes all chromatographic parameters to optimize protein production performance including purity, yield, concentration and operational time. This is a holistic approach for rational protein purification [7].

**References**

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