FLOW-THROUGH CHROMATOGRAPHY AS A CONTINUOUS AND INTEGRATED PURIFICATION METHOD

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Continuous manufacturing is expected to improve the efficiency and economics of protein and other bio-product production processes. However, it is not easy to design and operate the continuous process especially for downstream processing as many unit operations (chromatography and membrane filtration) are involved.
An operation method known as flow-through chromatography (FTC) is considered to be an efficient purification method as the flow is continuous. In FTC, a target bio-product is eluted from the chromatography column without adsorption whereas contaminants are strongly bound. Usually two different modes of chromatography are needed in order to remove various kinds of contaminants. Two FTC columns have to be connected in order to build the integrated continuous process. This is not an easy task since the mobile phase properties (pH, salt, buffer ions) are different for the two columns.

In this study, we investigated how FTC processes can be designed based on linear gradient elution (LGE) data by using our LGE model. As a first model separation system removal of BSA dimer from BSA monomer was chosen. The distribution coefficients of BSA monomer and dimer and the mass transfer data were obtained from LGE experimental data based on the model. Experimental breakthrough of BSA dimer was well predicted by the model simulation. The model simulation also showed that FTC is very sensitive to a small change in salt concentration and/or pH of the mobile phase as well as the mobile phase velocity (see Fig.1). The productivity calculation method was also developed. The second model system was to use two FTC columns for removing multiple contaminants. In this system the efficiency of FTC processes was examined in terms of impurity removal efficiency from the cell culture broth containing monoclonal antibody. It was found that two FTC (anion exchange chromatography and cation exchange chromatography) can remove impurities efficiently when the mobile phase pH and conductivity were properly chosen. It was also shown that the two columns can be connected as a pseudo continuous FTC operation.

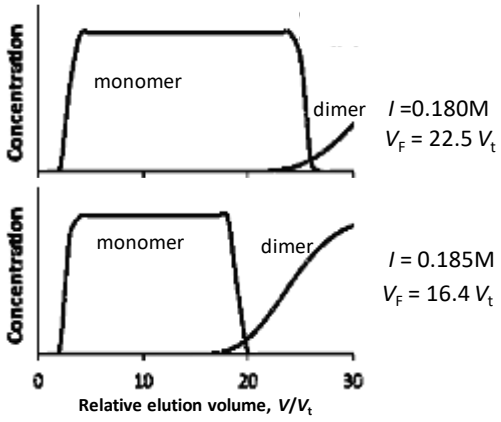

Figure 1 Effect of mobile phase salt concentration I on BSA dimer removable by flow-through chromatography with anion-exchange column (Q-Sepharose HP). V_t :column volume, V:volume, V_F: sample feed volume, I: salt (NaCl) concentration. The curves were calculated with the distribution coefficients as a function of I and mass transfer data determined by LGE experiments.

References

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