541d Systematic Design of Aqueous Two-Phase Extraction for Protein Separation

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In high-value protein production, there are three crucial factors that determine its commercial success: time to market, quality (in terms of purity, activity, dependability or flexibility) and cost [1]. Technologists are in search of and the industry is in demand of efficient yet inexpensive large-scale separation techniques with short process development times. Packed-bed chromatography, as traditionally applied is the most common bioseparation technique but, it is expensive [2] and operated in batch-mode, has low throughput and has complex scale-up. More systematic alternatives and approaches are needed to make further progress in downstream bioprocessing.

Aqueous Two-Phase Extraction (ATPE) systems represent one class of downstream processing unit operations that are responsive to these criteria and for which first principles systematic design approaches can potentially be developed. ATPEs have been explored by industry for the extraction of recombinant proteins, for instance in a purification process developed by Genentech, Inc. for insulin-like growth factor-I [3,4]. Besides allowing continuous steady-state operation with high capacity (up to 40g/L using affinity partitioning systems) [5] and easy scale-up [6], the key motivation for using ATPE is the ability to directly treat very crude mixtures, such as cell homogenates [7]. This opens a new area for ATPE application: integration of cell disruption and product recovery [8]. However, this method has not been extensively adopted in process plants. Among the governing factors are the cost of conventional phase-forming polymers and limited available design approaches.

Our work focuses on designing flowsheets that exploit a two-stage ATPE as downstream protein separation operation. We are developing a thermodynamic framework that is applicable to model the liquid-liquid equilibrium behaviors of multicomponent ATPE systems using Flory-Huggins theory [9,10]. In our work, we model and simulate the phase separation behavior in aqueous polymers and polymer-salt systems. We then use the calculation to initialize the equilibrium and partitioning calculations for the systems containing target proteins and lumped contaminants. We include simplified calculations of the performance and phase separation kinetics effects into the framework and apply them to design ATPE as a two-stage continuous operation.

Here we demonstrate our work through a complete flowsheet of a two-stage extraction unit for the separation of a specified target protein in a specified ATPE system and operating conditions. We simulate the behaviors in a first extraction stage as a polyethylene glycol-dextran system and in the second as a polyethylene glycol-sodium phosphate system. We address the partitioning properties of polymers, salt and protein, and evaluate the performance of a two-stage setup in terms of yield and purity of target proteins phosphofructokinase and ovalbumin, with simplifying assumptions on the behavior of the contaminant partitioning.

References

[1] S.M. Wheelwright. Protein purification: Design and scaleup of downstream processing. Hanser, 1991.

[2] H.L. Levine. Economic Analysis of Biopharmaceutical Manufacturing. 2002 BIO International Biotechnology Convention and Exhibition, Canada, June 2002.

[3] S. Builder, R. Hart, P. Lester, J. Ogez and D. Reifsnyder. Aqueous multiple-phase isolation of polypeptide. U.S. Patent 5,407,810, April 1995.

[4] R.A. Hart, P.M. Lester, D.H. Reifsynder, J.R. Ogez and S.E. Builder. Large scale, in situ isolation of periplasmic IGF-I from *E.coli*. Biotechnology, 12:1113-1117, 1994.

[5] M.P. Brocklebank. Downstream processing plant and equipment. In J.A. Asenjo, editor, Separation Processes in Biotechnology, 9:617-740, Dekker, 1990.

[6] P.A. Albertsson. Partition of cell particles and macromolecules. Wiley Interscience, New York, 1986.

[7] C. Kepka, E. Collet, J. Persson, A. Stahl, T. Lagerstedt, F. Tjerneld and A. Veide. Pilot-scale extraction of an intracellular recombinant cutinase from *E.coli* cell homogenate using a thermoseparating aqueous two-phase system. J. Biotechnol., 103:165-81, 2003.

[8] M. Rito-Palomares and A. Lyddiatt. Process integration using aqueous two-phase partition for the recovery of intracellular proteins. Chem. Eng. J., 87:313-319, 2002.

[9] P.J. Flory. Thermodynamics of high-polymer solutions. J.Chem.Phys., 10:51, 1942.

[10] M.L. Huggins. Some properties of solutions of long-chain compounds. J.Phy.Chem., 46:151-158, 1942.