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Modeling of Counter Current Monoclonal Antibody Extraction using Aqueous Two-Phase Systems

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Abstract

Design of chemical processes is usually based on rigorous modeling of unit operations. Unfortunately, the use of physically grounded models in biotechnological applications is rare since their design is mainly based on heuristics and experiments. In this work a computer aided design method is presented for modeling of aqueous two-phase extraction of monoclonal antibodies (MAbs). A conventional counter current extractor is compared with a fractional extractor in terms of purity and concentration of MAbs in the extract. The purity of MAbs increased from 85% in the conventional to almost 100% in the fractional extractor.

Keywords

Monoclonal antibody, aqueous two-phase, extraction, purification

1. Introduction

The demand for pharmaceutical and biotechnological products is increasing as the world population is growing. Since 50-80% of the production costs for biochemical products are located in the downstream processing, optimizing the purification techniques can provide a great benefit. A viable way to improve the purification and reduce the processing costs may be Aqueous Two-Phase (ATP) extraction.
However, the design of processes and unit operations for biomolecule purification are still mainly based on heuristics, expert knowledge and extensive experiments. Simulation tools for process modeling of these processes are rare. This results on the one hand from lacking data on model parameters, on the other hand from insufficient on phenomena governing the process. Therefore, we have developed a model for the protein extraction, which is based on the simplified liquid-liquid equilibrium model.
In a case study the purification of monoclonal antibodies (MAbs) is investigated, which have already shown their potential to treat various diseases [1]. An artificial fermentation broth also containing the impurities Human Serum Albumin (HSA) and Myoglobin (Myo) is separated using an *ATP*-system based on Poly-Ethylen-Glycol (PEG) and mixed phosphate salts [2]. Sodium chloride is added to the system to increase the selectivity of the MAbs.

2. Extractor model

A fractional extractor is the combination of two conventional counter current extractors with the feed stream entering in the middle of the apparatus (Figure 1). It can be operated in two different ways. If the proteins enter the extractor with the phosphate rich raffinate stream the apparatus works like a conventional extractor. Co- current and counter-current flow can be applied. Operated as fractional extractor the stages below the feed are called stripping and above washing section. In the stripping section MAbs but also some impurities are extracted into the PEG rich extract stream. The impurities are back extracted into the phosphate phase in the washing section. All model equations have been implemented within the commercial software Aspen-Custom-Modeler™ (ACM), which provides the Solver methods. A user friendly interface has been established in-house that allows to transfer all input and output parameters like e.g. mode of operation or number of stages between Microsoft Excel™ and

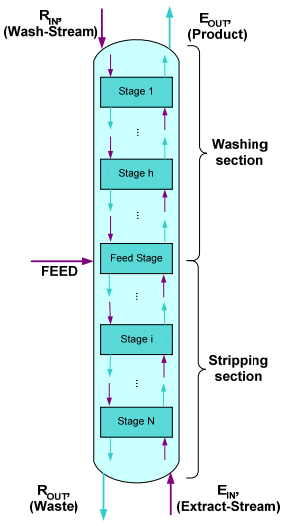

Figure 1: Fractional extractor

ACM with the help of Visual Basic for Applications (VBA)-Scripts.
In a system with n components 2n+2 equations are required to describe the biphasic Liquid-Liquid-Equilibrium on every stage in the extractor. These equations consist of a mass balance, n component balances, n equilibrium conditions and a summation criteria which is given in Eq. 1, were $w_{i,E}$ and $w_{i,R}$ stand for the weight fractions of component i in the top and bottom phase.

$$\sum_{i=1}^{n}\left(w_{i,E}-w_{i,R}\right)=0 \qquad \text{Eq. 1}$$

In the reported case study seven components are present (PEG{1}, phosphate {2}, NaCl{3} water {4}, Myo{5}, HSA{6} and MAbs {7}). Components 1-4 are regarded as the Phase Forming Components (PFC) and responsible for the formation of two liquid phases. The proteins (components 5-7) have a low concentration and it is assumed that these components have no effect on the phase equilibrium of the PFC.

2.1. Model of liquid-liquid equilibrium

In the selected PEG3400/phosphate system the phase diagrams at pH- values of 6, 7, 8 and 9.2 were measured in [3]. The pH is set by variation of the ratio of mono-potassium and di-potassium phosphate (0,5/pH6; 1,82/pH7; 15/pH8). Important is also the presence of NaCl which influences not only the selectivity of MAbs but also the phase equilibrium [2, 5]. However, data on the influence and distribution of NaCl are scarce and insufficient to be taken into account accurately. The partition coefficient of NaCl is set equal to that of water in the presented simulations, which is reasonable according to the data found in [5]. The partition coefficient (K) of PEG, phosphate and water is modeled according to the Setchenow equation Eq. 2 [6, 7]. These two model parameters a & α are dependent on the pH and determined from the experimental data by least squares method.

$$\log_{10}\left(\frac{w_i^E}{w_i^R}\right)=\log_{10}\left(K_i\right)=\log_{10}(\alpha_i)+a_i\cdot\left(w_i^R-w_i^E\right) \qquad \text{Eq. 2}$$

The partition coefficient of MAbs has been measured at pH 6, 7 and 8 at NaCl weight fractions of 0% and 15%, PEG concentrations between 12 and 14%, phosphates between 10-12% [2]. The initial concentrations of the proteins were 1g/l IgG, 10g/l HSA and 2g/l Myo. Since the equilibrium concentrations of NaCl are unknown, the model for the partition coefficient of the MAbs is based on the initial concentrations of the components NaCl, PEG and phosphate. Most vital here is the NaCl concentration, because in the absence of NaCl the MAbs and the impurities remain in the phosphate phase.

$$K_i=a\cdot\left(w_{PEG}^{INI}\right)^{\alpha}\cdot\left(w_{PO4}^{INI}\right)^{\beta}\cdot\left(w_{NaCl}^{INI}\right)^{\gamma}\cdot 10^{c\cdot pH} \qquad \text{Eq. 3}$$

In Eq. 3 it can be seen that the partition coefficient is written as a function of

pH, so the used parameters (a, α, β, γ and c) are pH independent.
The development of a correlation for the impurities Myo and HSA is not necessary because they remain mainly in the bottom phase [2]. The partition coefficients for these proteins are regarded in this paper as constant.

3. Results& discussions

The graphical representation of the developed model (Figure 2) for the phase diagrams at pH-values 6, 7 and 8 is based on the correlated parameters presented in Table 1. It can be used also to describe the phase diagram at a pH of 9,2 using the parameters determined for pH 8, because in this region the pH has almost no effect on the phase diagram.

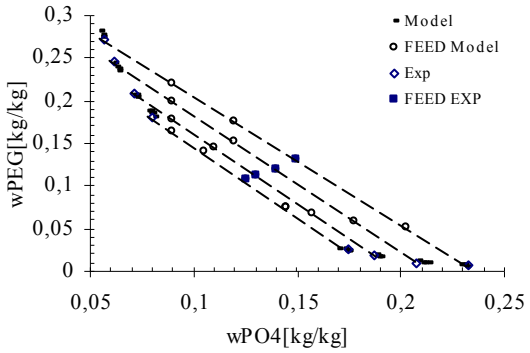

Table 1 Parameters at pH 6-8

	PEG	PO_4	water
		pH6	
a	-0,072	-0,034	-0,007
α	0,531	0,960	1,014
		pH7	
a	-0,067	-0,043	-0,007
α	0,586	1,004	1,022
		pH8	
a	-0,069	-0,044	-0,007
α	0,412	0,874	1,041

Figure 2: Simulation results for PEG3400 at pH6

It can be seen that the model accurately describes the equilibrium of the PFCs, the tie line length and slope in the complete two-phase region.

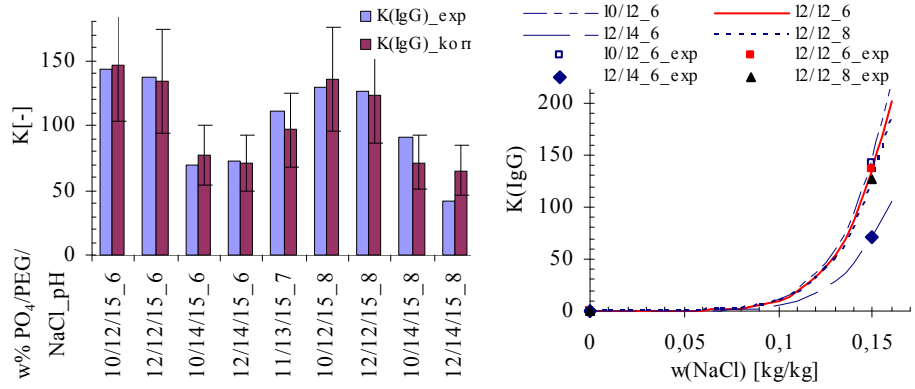

Figure 3: Simulation results for IgG at 15% NaCl, with a=0,651, α=–4,146, β=–0,505, γ=6,325, c=-0,017: Left Comparison with experiments. Right NaCl dependency

Figure 3 shows that the model can predict the experimental data within an error margin of 30% (left). Although the quantitative representation for the partition of MAbs is most reliable around 15% NaCl, the influence at lower concentrations can be studied (Figure 3 right).

A parametric study is performed for the simulation of a conventional and a fractional extractor with seven stages focused on the effect of NaCl. Extract (865kg/hr) and raffinate (635kg/hr) streams are fed as corresponding phases: They are in equilibrium so no transfer of the PFCs take place on each stage in the extractor. The proteins enter at the top of the extractor and the partition coefficients of Myo and HSA are fixed to 0,01.

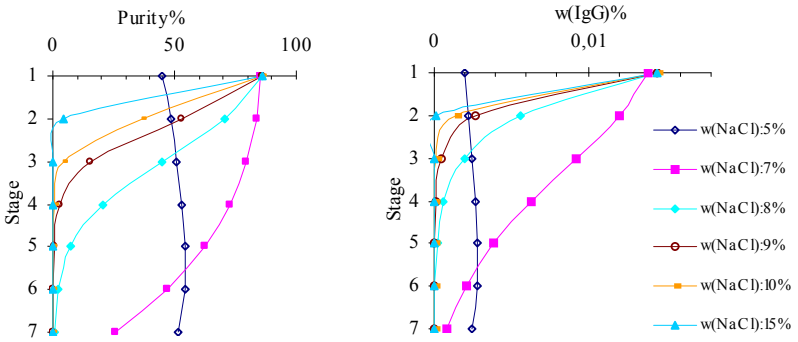

Figure 4: Purity and weight% MAbs in a conventional extractor pH 6; Phosphate 12%, PEG 12%

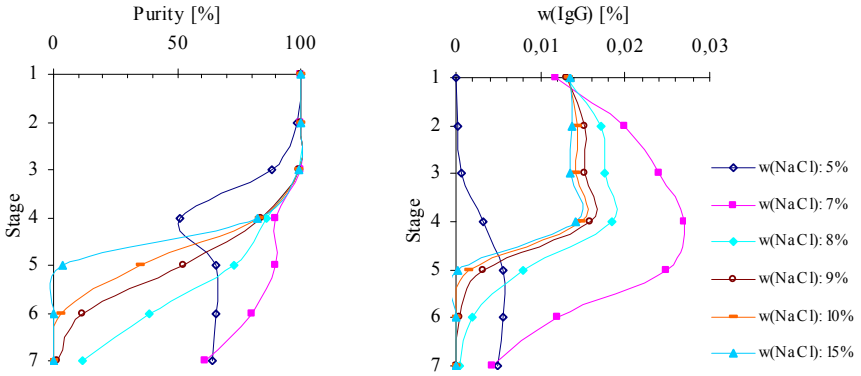

Figure 5: Purity and weight% MAbs in a fractional extractor: pH 6; Phosphate 12%, PEG 12%

In Figure 4 the concentration profiles at different NaCl weight fractions are plotted. The purity reaches about 85% at NaCl concentration higher then 7,5 weight%. If more NaCl is added fewer stages are required, but the purity is not increased. The fractional extractor operated under the same conditions and

number of stages but with the proteins fed in the middle of the extractor at stage 4. PEG and phosphate are added here as well to reduce changes of the composition. The total feed stream is 500kg/hr and accordingly, is the sum of the extract and raffinate streams reduced to 1000 kg/hr. It is observed that the purity is increased to almost 100%IgG (Figure 5). The maximum weight fractions of IgG are reached on the feed stage, a consequence of varying PEG and NaCl concentrations in the stripping and washing sections, as a result of the addition of feed stream.

4. Conclusion and Future work

The counter-current extraction of monoclonal antibodies by an aqueous two-phase system was modeled. The phase equilibrium is accurately described with the Setchenow equation. The protein distribution is mainly a function of the NaCl weight-fraction, and could be described by an exponential function. A fractional extractor with seven stages and operated with 8% NaCl can produce nearly 100% pure MAbs. More NaCl reduces the number of stages, but the purity is not changed significantly. The future work focuses at describing the phase equilibrium description with more fundamental thermodynamic models.

Acknowledgement

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References

1. Guadagni F, et. al. (1993). In Vivo. Nov-Dec 7-6B (591-9)
2. P.A.J. Rosa, A.M. Azevedo, M.R. Aires-Barros, Application of Central Composite Design to the Optimisation of Aqueous Two-Phase Extraction of Human Antibodies Journal of Chromatography A, accepted for publication
3. Lei X., Diamond A. D., Hsu J. T. (1990) Equilibrium Phase Behavior of the PEG/Potassium phosphate/ water System at 4°C J. Chem. Eng. Data;Vol.: 35 (420-423)
4. Mistry S. L., Kaul A., Merchuk J.C., J.A., Asenjo; (1996) Mathematical modelling and computer simulation of aqueous two-phase continuous protein extraction J. Chromatogr. A., Vol.:741, (151-163)
5. Andrews B. A., Nielsen S., Asenjo J.A.; (1996) Partitioning and purification of monoclonal antibodies in aqueous two-phase systems Journal: Bioseperatio Vol.: 6 (303-313)
6. Vainerman E. S., Ryashentsev V. Y., Rogozhin S. V. (1990); An approach to the description of the equilibrium of liquid two-phase three-component systems; Solvent Extraction and Ion Exchange; Vol.: 8 (361-370)
7. M. Setchenow (1892) Ann. Chirn. Phys., 25 226.