A method for designing flow-through chromatography processes

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Abstract. Continuous manufacturing is expected to increase the productivity of the production of biologics such as protein drugs. However, it is not easy to build 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 method for separating two components as the flow is continuous. In FTC, a target protein is eluted from the chromatography column without adsorption whereas contaminants are strongly bound. Since at least two different modes of chromatography are needed in order to remove contaminants, two FTC columns have to be connected in order to build the 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 paper, we developed a method for designing FTC processes based on the mechanistic models that we have developed for linear gradient elution (LGE) of proteins. The distribution coefficient K as a function of salt concentration, I determined from LGE data were used for the simulation. It was found that the process is quite sensitive to a small change in I. This indicates that FTC processes are carefully monitored and controlled for the stable operation.

1 Introduction

Protein drugs such as monoclonal antibodies (mAbs) are known to be very effective drugs for cancer treatment and autoimmune diseases. mAbs are produced by the cell culture with mammalian cells such as CHO cells. After the cell culture (upstream process), mAbs have to be purified to a very pure form by using several chromatography and membrane systems. This process called downstream process (DSP) is known to be very costly because of several chromatography batch unit operations involved.

Continuous DSP is expected to increase the process efficiency. This willh reduce the cost of goods of mAb production, which may lower high price of mAb drugs.

Fig. 1 shows a typical platform downstream process (DSP). After the cell culture, cells are removed either by centrifugation or filtration in order to produce filtered cell culture supernatant (FCCS). FCCS is then applied to the first chromatography step called "capture step". Protein A chromatography (PAC) is commonly employed as the efficient capture step. However, since the final product must be purified to a very low impurity level, additional chromatography steps called "polishing steps" are needed. Various types of impurities such as viruses, host cell DNAs, host cell proteins (HCPs) and product-derived molecules (aggregates, fragments and charge variants) are included in FCCS. More than one chromatography step is needed as the impurities differ so much in terms of physical and biochemical properties. Ion-exchange chromatography (IEC) [anion exchange (AIEC) and cation exchange (CIEC)], hydrophobic-interaction chromatography (HIC) and mixed-mode chromatography (MMC) are commonly used for polishing steps **[2]**.

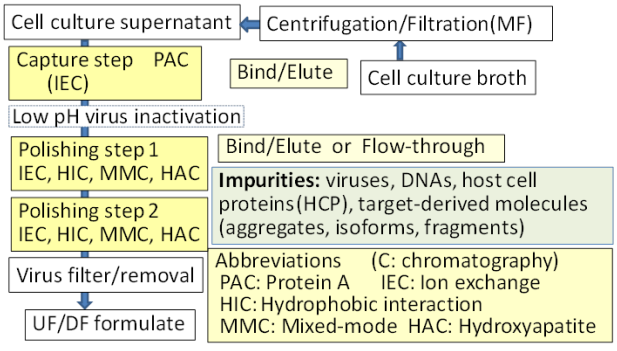

Fig. 1. Typical platform separation (downstream) process for monoclonal antibodies (mAbs).

2 Results and Discussion

2.1 Polishing chromatography steps

A typical operation of polishing chromatography by ion-exchange chromatography (IEC) is shown in **Fig. 2 [1]**.

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An IEC column is first equilibrated with a low salt buffer solution (mobile phase) at a fixed pH in order to bind proteins and impurities. After the sample loading, the column is washed to remove unbound or weakly bound impurities. Then, the elution buffer of high salt concentration is fed to the column to desorb (elute) the product (mAb). For the regeneration tightly bound impurities are stripped from the column with a very high salt concentration buffer and sodium hydroxide solution. The column is then re-equilibrated for the second run.

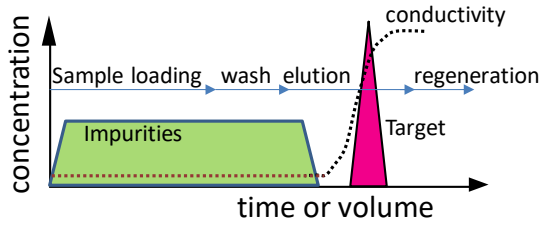

Fig. 2. Stepwise elution (bind/elute) chromatography. Dotted curve is the conductivity (salt concentration).

Proper choice of the buffer pH and salt concentrations for both adsorption (bind) and elution (elute) is very important. In addition, the sample load (feed) volume and the flow-velocity are important parameters for process optimization. However, in most cases they are determined by trial and error approaches.

The efficiency of typical DSP including three chromatography steps shown in **Fig. 1** is low. In order to increase the DSP efficiency various continuous chromatography operation methods have been proposed. For the capture step, multi-column switching techniques called "periodic counter-current (PCC)" chromatography are available. Although polishing steps can be operated by using PCC, it is not beneficial to use PCC for polishing since the purpose of polishing steps is to remove a small amount of impurities from the sample feed. Another method using negative adsorption operation mode called flow-through chromatography (FTC) is suitable for polishing step. FTC operation is shown in **Fig. 3**. While the sample feed is continuously fed to the column, the impurities are adsorbed and the flow-through stream from the outlet contains purified mAb in FTC. Although FTC is not a true continuous operation (the column has to be replaced after the breakthrough of the impurities), it is regarded as a pseudo-continuous operation.

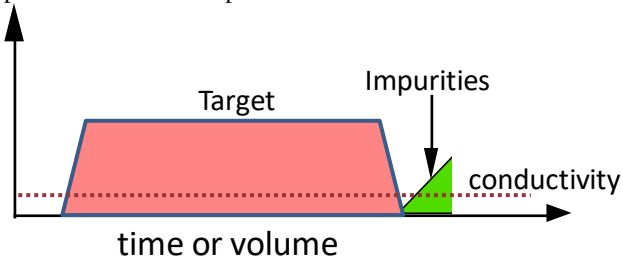

Fig. 3. Flow-through chromatography (FTC) operations.

2.2 Flow-through chromatography design method

The mobile phase condition (pH and salt concentration) where the target mAb is not adsorbed whereas the impurities are tightly bound must be found in order to design FTC processes. The retention of proteins in IEC is basically understood based on the concept shown in **Fig. 4.**

This can also be described by using *K-I* curves shown in **Fig. 5**. *K-I* curves can be obtained by 96-filter plate based batch adsorption methods. However, a method using LGE is simper and faster as shown below.

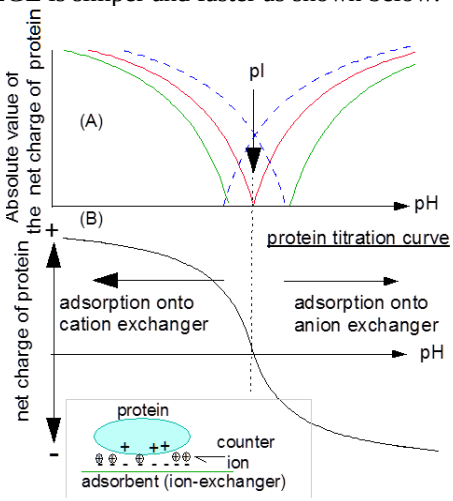

Fig. 4. Binding of proteins in ion-exchange chromatography **[1].**

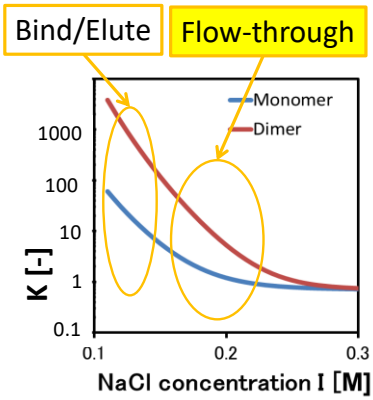

Fig. 5. Distribution coefficient *K* as a function of salt concentration of the mobile phase, *I*.

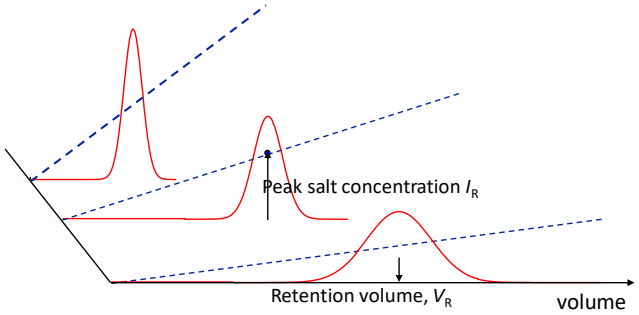

Fig. 6. LGE at different gradient slopes.

LGE experiments at different gradient slopes g(M/mL) are carried out to measure the salt concentration at the peak retention, I_R as shown in **Fig. 6**. Then, GH-I_R curves are constructed in a log-log scale based on Eq. (1). GH is the normalized gradient slope = $gV_oH = g\ (V_t\text{-}V_o)$ where V_t and V_o are the total and void volume of the packed bed column. $H=(V_t - V_o)/ V_o$ is the volumetric phase ratio.

$$GH = I^{(B+1)}/[A(B+1)] \quad (1)$$

Here B is the number of biding sites, and A is the parameter including the equilibrium coefficient.

Once A and B values are determined, the distribution coefficient K as a function of I can be calculated by **Eq. 2**.

$$K = AI^{-B} + K_C \quad (2)$$

Here K_C is the critical value of K (K at non-binding conditions, which is close to K of salt, K').

Suda et al. **[3]** successfully applied this method to designing FTC for removing aggregates.

In addition to the mobile phase condition several parameters have to be known for the actual operation such as flow-velocity and feed volume.

FTC is not a complete continuous system as the operation must be stopped before the breakthrough of the impurities as shown in **Fig. 3**. The breakthrough volume also depends on the flow velocity due to pore diffusion based band broadening. The calculation scheme for determining the maximum sample feed volume V_F is shown in **Fig. 7**.

V_{a1} and V_{a2} can be calculated by **Eqs. 3 – 6** where N_1 and N_2 are the plate number values for monomer and dimer, respectively. The plate number N is calculated from the Van Deemter type HETP equation, [**Eq. 7**, 1.4].

$$V_{a1}=V_{C1}(1+1.25/N_1^{0.5}) \quad (3)$$
$$V_{a2}=V_{C2}(1+1.25/N_2^{0.5}) \quad (4)$$
$$V_{C1}= V_o+(V_t-V_o)K_1 \quad (5)$$
$$V_{C2}= V_o+(V_t-V_o)K_2 \quad (6)$$
$$\text{HETP} = A^o + C^o u \quad (7)$$

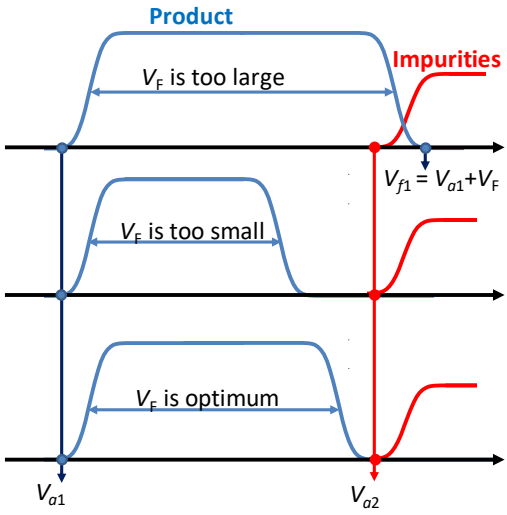

Fig. 7. Sample feed volume V_F for FTC

V_{a1} :Breakthrough (monomer), V_{f1} :End of monomer elution
V_{a2} Breakthrough (dimer), V_F Sample feed volume (CV basis)
CV=column volume = V/V_t

Here, A^o is the axial dispersion term and C^o is given by

$$C^o= (d_p^2/D_s)[HK/(1+HK)^2] \quad (8)$$

D_s is the stationary phase (pore) diffusion coefficient and d_p is the particle diameter.

As an example of this calculation procedure, effect of salt concentration is shown in **Fig. 8**. The elution curves were calculated by using a single zone spreading parameter model **[1,5]**. It is clearly shown that elution curves are very sensitive to salt concentration. By decreasing NaCl concentration from 0.2 to 0.18 M, the sample feed volume increased by a factor of three. This simulation also allows predicting the effect of flow velocity and particle diameter.

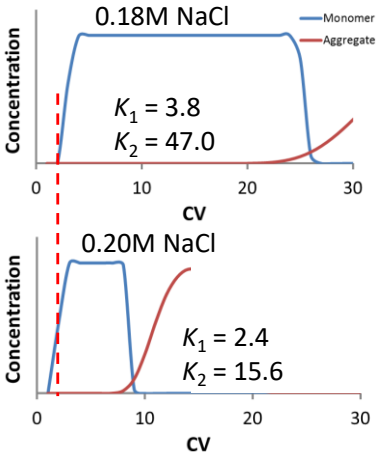

Fig. 8. Effect of salt concentration on FTC.

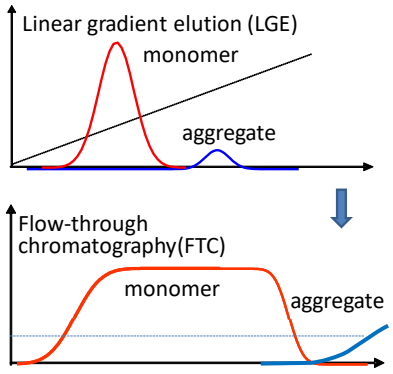

Fig. 9. Linear gradient elution (LGE) and FTC.

3 Conclusion

We have shown that flow through chromatography (FTC) can be designed based on the data obtained by linear gradient elution and isocratic elution at non-binding conditions (**Fig. 9**). The optimum conditions can be determined based on the separation time and the sample volume processed.

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