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Optimization of monoclonal antibody purification by ion-exchange chromatography☆ Application of simple methods with linear gradient elution experimental data

Takashi Ishihara[a,*], Shuichi Yamamoto[b]

[a] *Kirin Brewery Co. Ltd., Takasaki, Gunma 370-0013, Japan*
[b] *Department of Chemical Engineering, Yamaguchi University Tokiwadai, Ube 755-8611, Japan*

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Abstract

Simple methods for the optimization of ion-exchange chromatography of proteins in our previous papers were applied to cation-exchange chromatography purification of monoclonal antibodies (Mab). We carried out linear gradient elution experiments, and obtained the data for the peak salt concentration and the peak width. From these data, the distribution coefficient as a function of salt concentration, and the height equivalent to a theoretical plate (HETP) as a function of mobile phase velocity were calculated. The optimized linear gradient elution conditions were determined based on the relationship between buffer consumption and separation time. The optimal stepwise elution conditions were determined based on the relationship between the distribution coefficient and the salt concentration.

Keywords: Antibodies; Cation-exchange chromatography; Ion-exchange chromatography; Protein separation; Chromatography models

1. Introduction

Ion-exchange chromatography (IEC) is a major unit operation in protein drug purification processes [1–5]. Both cation- and anion-exchange chromatography steps are generally involved in monoclonal antibody (Mab) purification processes [5]. However, in designing such processes many parameters must be considered such as mobile phase (pH, salt concentration, etc.), stationary phase (type of ion-exchange group, ion-exchange capacity, particle diameter, pore structure, pore size distribution, base matrix property, etc.), column parameters (length, diameter, etc.) and operating variables (flow rate, gradient slope, sample loading, etc.). Therefore, optimization of IEC is labor-intensive and time-consuming.

For example, in linear gradient elution IEC the gradient slope and the flow rate as well as the column length affect the separation behavior in a complicated way [3]. So if the process is not well understood, it is not easy to choose the right conditions, which provide the required resolution, the allowable process time and the desired buffer consumption. Another typical elution method, stepwise (or step gradient) elution IEC, is commonly employed for process IEC. In this elution method, the concentration of the elution buffer is the key variable, which is usually determined by a trial-and-error approach. In addition, the sensitivity of the elution buffer compositions to the separation behavior must be carefully considered [6].

Therefore, rapid and effective optimizing methodologies for the purification process are much expected in process development. Various chromatography models incorporating economics have been developed, and explained in detail by Guiochon and co-workers [4,7].

In this study, we optimized cation-exchange chromatography processes for recombinant Mab purification by using

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* Corresponding author. Tel.: +81 27 353 7385; fax: +81 27 353 7400.
E-mail address: t-ishihara@kirin.co.jp (T. Ishihara).

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simple methods developed in our previous papers [3,8]. Linear gradient elution experiments were carried out in order to obtain the data on the peak salt concentration and the peak width as a function of gradient slope and/or the flow velocity. Based on the information thus obtained, linear gradient elution and stepwise elution were optimized. The peak shape, the recovery and the purity of the optimized peaks were examined in order to verify the proposed method.

2. Experimental

2.1. Chromatography media and column

HiTrap SP Sepharose FF column (6% cross-linked agarose, sulfopropyl group, particle diameter ca. 100 μm, column size 25 mm × 7.0 mm i.d., total bed volume V_t = 0.96 mL, Amersham Biosciences, Uppsala, Sweden) was used as a cation-exchange chromatography column and media.

2.2. Materials

The model recombinant human monoclonal antibodies Mab A [IgG_1, VH1, Vκ4, isoelectric point (p*I*) ca. 8, M_r ca. 150 000] and Mab B (IgG_1, VH3, Vκ1, p*I* ca. 8, M_r ca. 150 000) used in this study were produced at Kirin (Takasaki, Japan). Other reagents used in these studies were of analytical grade.

2.3. Chromatography apparatus

Most experiments were performed on a fully automated liquid chromatography system ÄKTA explorer 100 (Amersham Biosciences).

2.4. Linear gradient elution experiment

The SP Sepharose FF column was equilibrated with a starting buffer (buffer A: 20 mM sodium phosphate pH 7.0). The Mab sample loading was fixed as 1-mg/mL gel bed. Elution was performed with the final elution buffer (buffer B: 20 mM sodium phosphate pH 7.0 containing 0.5 M NaCl). The linear gradient elution was performed by changing the buffer composition linearly from buffer A to buffer B with time. Namely, the NaCl concentration was increased with time at a fixed pH and buffer compositions. The gradient slopes g (M/mL) were chosen so that gradient volume was 10, 20, 30, or 40 column bed volume. The volumetric flow rate F was 0.5, 1.0, 1.5, or 2.0 mL/min. The linear mobile phase velocity u was calculated with the cross-sectional area A_c and the column bed void fraction ε as $u = F/(A_c\varepsilon)$. The column bed void fraction ε was determined from the peak retention volume of Dextran T 2000 pulses. The experiments were performed at room temperature.

2.5. Size-exclusion chromatography

Analytical size-exclusion chromatography was conducted using a G3000SWXL column (Tosoh, Japan). The mobile phase was a 20 mM sodium phosphate buffer solution (pH 7) containing 0.25 M NaCl. Flow rate was 0.5 mL/min. Sample volume was 20 μL and the sample concentration was 1 mg/mL.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was conducted on 4–20% polyacrylamide gels commercially available from Daiich Pure Chemicals, Japan. Mab sample was loaded (5 μg/lane) gels were stained with silver for detection.

3. Model and calculation

3.1. Linear gradient elution model

We proposed and experimentally verified the method, by which the distribution coefficient K as a function of salt concentration I is determined from the protein peak salt concentration I_R in linear gradient elution. Below the method is explained briefly. The normalized gradient slope GH in linear gradient elution [3,6,8–10] is defined by the following equation:

$$GH = (gV_0)\left(\frac{V_t - V_0}{V_0}\right) = g(V_t - V_0) \quad (1)$$

where V_t is the total bed volume, V_0 the column void volume, $G = gV_0$ and $H = (V_t - V_0)/V_0$ is the phase ratio. g (M/mL) is the gradient slope of the salt, which is defined by the following equation:

$$g = \frac{I_F - I_0}{V_g} \quad (2)$$

where I_F is the final salt concentration, I_o the initial salt concentration, and V_g is the gradient volume. Linear gradient elution experiments are performed at different gradient slopes (GH values) at a fixed pH. The salt concentration at the peak position I_R is determined as a function of GH. The GH–I_R curves thus constructed do not depend on the flow velocity, the column dimension, the sample loading at non-overloading conditions, or the initial salt concentration I_o provided that the sample is initially strongly bound to the column [3,8]. The experimental GH–I_R data can usually be expressed by the following equation [3,8–10]:

$$GH = \frac{I_R^{(B+1)}}{[A(B+1)]} \quad (3)$$