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Large-Scale Gradient Elution Chromatography

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The goal of this chapter is to provide practical strategies for large scale separations by gradient elution chromatography. A detailed model has been developed for gradient elution systems considering interference effect, longitudinal diffusion, film mass transfer, intraparticle diffusion, mixing mechanism of the mobile phases, Langmuir-type adsorption and desorption kinetics. This detailed model can be solved by an efficient and robust numerical procedure. Hence, the optimizaton strategy of gradient elution has been developed through the calculation using this detailed model. This detailed model can precisely predict the band position, profile and width at various gradient concentrations, gradient periods, flowrates, and column lengths, in fair agreement with the experimental results. As a result of optimization, an optimal column length may exist. All the input parameters in this model have been either experimentally measured or estimated through empirical correlations. An alternative instrument for large-scale production using gradient elution has been suggested compared with conventional gradient elution instrument. The tolerance of the gradient elution processes to the fluctuation of input parameters has also been discussed.

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List of Symbols

Symbol	Description
a	constant in Langmuir adsorption equation (–)
A	mobile phase component which has stronger affinity with the stationary phase
b	constant in Langmuir adsorption equation $(M)^{-1}$
B	mobile phase component which has weak affinity with the stationary phase
C	concentration (M)
C_b	eluate concentration (M)
CHY-A	a-chymotrypsinogen A
C_m	eluent concentration (M)
CYT-C	cytochrome C
d	molecular diameter (cm)
dp	pore diameter (cm)
D	Brownian diffusivity ($cm^2\ s^{-1}$)
D_b	axial dispersion coefficient ($cm^2\ s^{-1}$)
D_p	intraparticle diffusivity ($cm^2\ s^{-1}$)
F	flowrate ($ml\ s^{-1}$)
k	film mass transfer coefficient ($cm\ s^{-1}$)
k′	capacity factor (–)
L	column length (cm)
LYS	lysozyme
Mr	molecular weight (–)
Pe_L	Peclet number of axial dispersion, vLD_b^{-1} (–)
Re	Reynolds number, $2R_p\varepsilon_b v\rho\eta^{-1}$ (–)
RIB-A	ribonuclease A
R_p	particle radius (cm)
Sc	Schmidt number, $\eta r^{-1}D^{-1}$ (–)
t	time (s)
t_R	retention time (s)
t_W	band width (s)
v	interstitial velocity ($cm\ s^{-1}$)
V	liquid volume (ml)
V_m	internal volume of the mixer (ml)
V_s	specific volume ($ml\ g^{-1}$)
z	ZL^{-1} (–)
Z	axial coordinate (cm)
Z′	proportional coefficient (–)

Greek Letters

α	constant coefficient (–)

β	constant coefficient (–)
δ	standard deviation of the Gaussian band (–)
ε_b	bed void fraction (–)
ε_p	particle porosity (–)
η	viscosity of the mobile phase (g cm^{-1} s^{-1})
γ	constant coefficient (–)
λ	dd_p^{-1} (–)
ρ	density of the mobile phase (g ml^{-1})
τ	tvL^{-1} (–)
τ_{imp}	dimensionless time duration of the sample injection (–)

Subscripts

i	ith component
0	initial value

1 Introduction

This chapter is not intended to be a conventional review of gradient elution chromatography. Instead, the goal of this chapter is to provide practical strategies for large-scale separations using this method. Comprehensive reviews have provided its fundamentals and applications [1–4] for analytical purposes. In response to the increasing need for high purity bioproducts, advances in analytical liquid chromatography are being exploited for bioseparations [5]. Many of these bioproducts are proteins or other macro-molecules. However, most current theories and application strategies in gradient elution chromatography were developed for analytical purposes of small compounds, and they might not be appropriate for large-scale separations of macro-molecules, which will be generally described in this section and in detail in the remaining sections of this chapter.

Analyses are usually handled with small sample sizes and with dilute sample concentrations in the linear range of isotherms, with which the retention time and the band profiles of eluates are independent of the composition of the sample. By the same token, the elution bands in chemical analysis are usually treated as symmetrical Gaussian bands, whose band widths are always equal to 4δ, where δ is the standard deviation of the Gaussian band [4]. Under the assumption of Gaussian elution bands, it is a common belief that an increase in column length always improves separation performance. However, large-scale separations must be run with large sample sizes and/or with elevated sample concentrations, which have been shown to result in significant tailing of the bands with the concomitant loss of separation efficiency [6]. Thus, the nonlinearity of isotherms are often utilized in large-scale separations, in which the retention time and the band profiles of eluates, which are often asymmetrical, are dependent on the composition of the sample, which is called the interference effect [7]. For such asymmetrical elution bands of significant tailing, the common belief that an increase in column length always improves separation performance must be reexamined for large-scale separations. In an industrial scale operation, the greater length may mean an increased dispersion and thus affects the performance adversely. An effort to determine an "optimal" column length may be needed for large-scale separations.

The majority of current gradient elution theories emphasize the features regarding the chemical interaction between the stationary phase and the mobile phase [8–11]. Transport and kinetic problems in gradient elution systems are often overlooked, but can be significant in large-scale separations, especially for macro-molecules [12, 13]. Without considering the transport and kinetic effects, the band broadening and the band separation are difficult to elucidate [14]. Recently, the knowledge gained through studies in other fields of chemical engineering has been extended into the field of chromatographic separations. There is a large body of literature on band broadening due to the effects of transport and kinetics [15–22]. However, it is a challenge to develop a practical

and realistic optimization strategy for large-scale separations by gradient elution chromatography considering the transport and the kinetic effects.

Consideration of the transport and the kinetic mechanisms makes the mathematical modeling very complex. Analytical solutions are usually unavailable for such a complex model [23]. As a result most scale-up processes of gradient elution chromatography have been carried out empirically [24]. The plate theory [25–27] and the lumped method [28, 29] have long been used to simplify the mathematical model. On the other hand, the simplifications which do allow analytical solutions often fail to reflect the reality of the system. For instance, the plate theory is limited to symmetrical Gaussian bands, and the lumped method is incapable of predicting the dynamic dependence of the chromatographic behavior on the input parameters, such as the flowrate, the particle size and the column length. Therefore, a detailed mathematical model considering the interference effect, the transport and the kinetic mechanisms must be used in predicting optimization of large-scale gradient elution chromatography. Recently, an efficient and robust numerical procedure has been developed for the solution of the complex mathematical model [30]. In addition, band broadening phenomena may be caused by different mechanisms including transport, kinetics, thermodynamics and in-column reactions, and these are often difficult to distinguish from one another [31, 32]. In other words, a detailed model with many adjustable parameters is usually able to fit most of the band profiles. Hence, the controlling mechanism must be determined before the detailed model is used. Otherwise, any further extrapolation and conclusions drawn from such a complex model without validating the controlling mechanism may be unrealistic.

The existing gradient elution instrumentation and procedures were also developed for analytic purposes. The simple extension of analytical instrumentation and procedures may not be sufficient for large-scale separations. For instance, when two or more mobile phases are mixed in gradient elution chromatography, air bubbles are often formed and then captured in the closed mixer, which may lead to distortion of gradient shape [33]. In the laboratory, various methods, including heating, helium and nitrogen gas purging, decompression, ultrasonification and using special degassing devices, are employed for removal of air from the mobile phases. These degassing methods are impractical in large-scale separations. An alternative instrument of gradient elution chromatography must be developed for industrial separations to prevent problems with air bubbles. Furthermore, the proportioning of mobile phases in gradient elution chromatography must be precisely controlled, otherwise the gradient shape may be distorted [1, 2, 4]. However, a variety of other causes can also lead to the distortion of the gradient shape [1, 2, 4]. These causes include the inaccurate flowrate of the pump, poor mixing of the mobile phases, and large hold-up volume of the mixer, as well as a large volume between the mixer and the column inlet. As a consequence, there is no question that highly precise and accurate gradient shapes are difficult to reproduce, particularly on various gradient devices [4, 34]. The distortion of gradient shape can be more serious in

large-scale separations because industrial operations are usually not easily controlled as precisely as laboratory analyses. Therefore, the distortion of gradient shape must be solved in large-scale separations.

The retention relationship of the eluate concentrations and the eluent concentration describes how the eluent affects the retention of the eluates following the continual increase of the elution strength throughout the gradient period. Many conventional retention relationships developed for small molecules, such as the mass action law for small ions in ion exchange chromatography [35], have been extended to proteins. However, recent studies show the adsorption mechanism of proteins in ion exchange chromatography is not solely ion exchange [36–38]. One example is the significant hydrophobic interaction of macro-molecules in ion exchange chromatography, which has not been an important consideration for small compounds [39, 40]. Hence, Regnier has called the stoichiometric model as a non-mechanistic model and used the term electrochemical interaction chromatography (EIC) instead of ion exchange chromatography (IEC) for the adsorption of proteins in ion exchange systems [36]. Several empirical retention relationships of proteins have been developed [36, 39, 41].

The chromatographic procedures can be more precisely controlled in the laboratory than in an industrial setting. Therefore, the consistency of the gradient shape may not be easily achieved in industry. Other input parameters of chromatographic separations, such as feed concentrations, eluent concentration and pH value, can also vary from batch to batch in industrial operations. The tolerance of separation processes to the fluctuation of input parameters must also be considered in large-scale separations of gradient elution chromatography.

2 General Description

2.1 Overview

Gradient elution chromatography is a powerful tool for chemical analysis due to its broad range of retentivity, high peak capacity and short operation cycle [42]. The advantages of gradient elution chromatography are achieved by increasing elution strength during the gradient period, in contrast to the unchanged elution strength in isocratic elution chromatography. The continual increase of elution strength throughout the gradient period, known as a solvent gradient, is usually achieved by the proportioning of multiple mobile phases with a gradient former. Temperature gradient, flowrate gradient and column-material gradient or column switch (also called tandem columns) are alternatives to solvent gradient, but will not be discussed in this chapter. In solvent gradient, the gradient former programs the composition change of the mobile-phase mixture. Either the

pumps or the valves, which must be programmable, are controlled by the gradient former in order to proportion the mobile phases (detailed in Sect. 3). The commonly used binary gradients are formed by two mobile phases, a weak component, called mobile-phase A in this chapter, and a strong one, called mobile-phase B. However, ternary or more complex gradients are also used particularly with the aim of eliminating the demixing effect of the mobile phases, which is caused by the incompatibility of the mobile phases [1, 4]. A mixer is also needed to mix the mobile phases and can be either a dynamic mixer or a static mixer (detailed in Sect. 3). Furthermore, the change of the mobile-phase composition change with time is called gradient shape. Gradient shape can be simply classified as continuous gradient and stepwise gradient, shown in Fig. 1. The continuous gradient includes linear gradient, also known as linear solvent strength (LSS) gradient [4] (see Figs. 4a and 4b), and nonlinear gradient (see Fig. 5). The stepwise gradient is composed of multiple steps of isocratic elution. Displacement chromatography, which uses a step-up of the displacer solution to displace the pre-loaded sample compounds, can be classified as a stepwise gradient chromatography. However, a complex gradient, such as multi-stepwise linear gradient (also known as segmented linear gradient) [43] (see Fig. 6), can be composed of the various simple gradients as well as isocratic steps. Usually, the eluent concentration increases during the continuous gradient period; while it decreases in hydrophobic interaction chromatography [40] (see Fig. 4b). In this chapter, only the linear gradient and the stepwise gradient will be discussed and compared due to the inconvenient complexity of other gradient techniques. Before the gradient starts, the column is equilibrated with the starting mobile-phase. After the end of a previous gradient run, the column must be completely reequilibrated with its initial mobile-phase before the next injection, usually by switching to its initial mobile phase rather than by a reverse gradient [1]. Incomplete equilibrium with the initial mobile phase after the prior run will

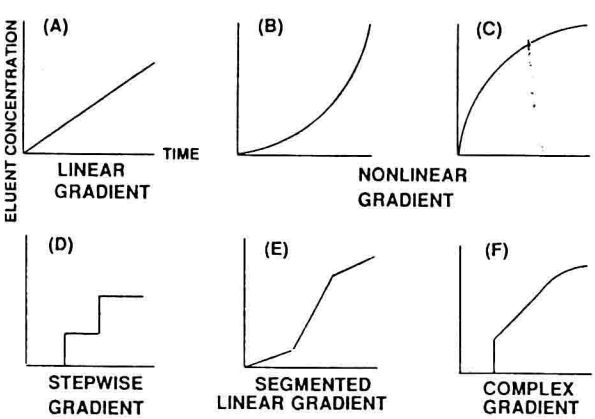

Fig. 1. Classification of gradient shapes

cause earlier elution and poor separation of the sample compounds in the next run. The sample compounds are usually dissolved in the initial mobile phase.

2.2 *Advantages*

In isocratic elution chromatography, the strongly retained sample compounds tend to tail and have late retention, shown in Fig. 2. To make these late-eluting bands sharper and elute faster with stronger elution strength, the weakly retained eluates might be poorly separated, shown in Fig. 3. However, in

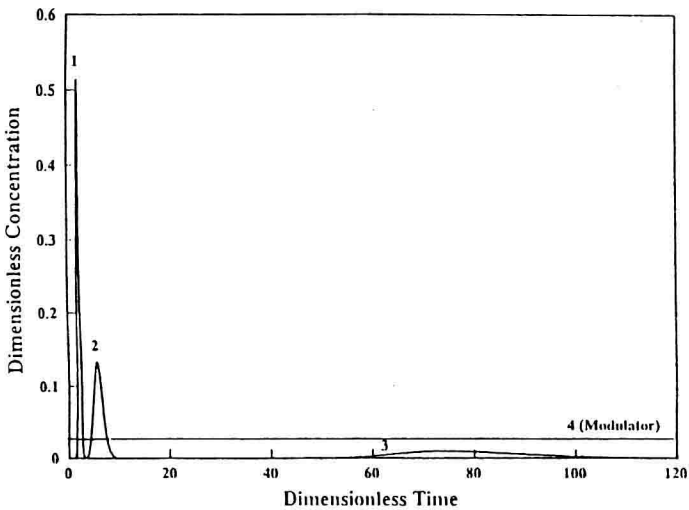

Fig. 2. Isocratic elution chromatogram calculated through the detailed model in the hydrophilic range of the retention relationship

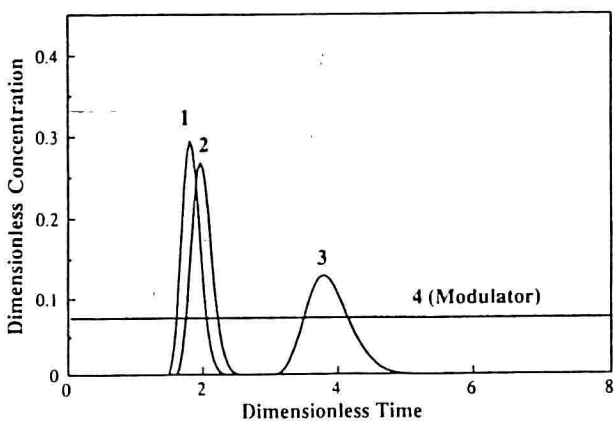

Fig. 3. Isocratic elution chromatogram at higher eluent concentration than in Fig. 2 calculated through the detailed model in the hydrophilic range of the retention relationship

gradient elution chromatography, the strongly retained eluates can be effectively stripped from the column by the continual increase of elution strength throughout the gradient, after the weakly retained eluates are well separated, as shown in Fig. 4. For this reason, the resulting bands are sharp, which means large peak capacity, and the separation cycle is short. Thus, gradient elution has great advantages versus isocratic elution in separating sample compounds which differ widely in retention on a chromatographic column, which is very common

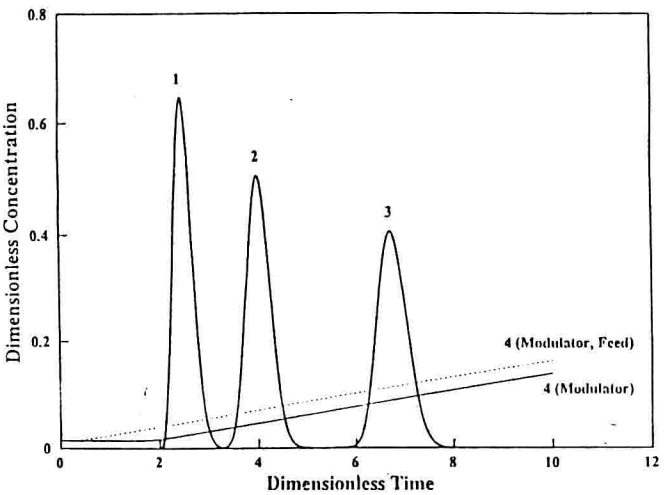

Fig. 4a. Linear gradient elution chromatogram calculated through the detailed model in the hydrophilic range of the retention relationship

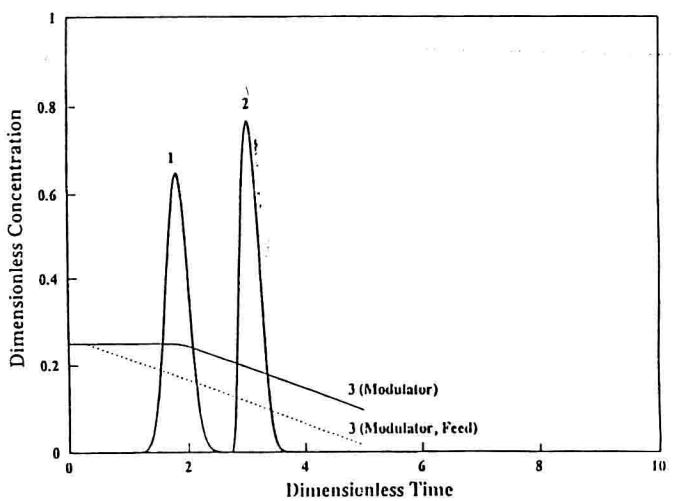

Fig. 4b. Linear gradient elution chromatogram calculated through the detailed model in the hydrophobic range of the retention relationship

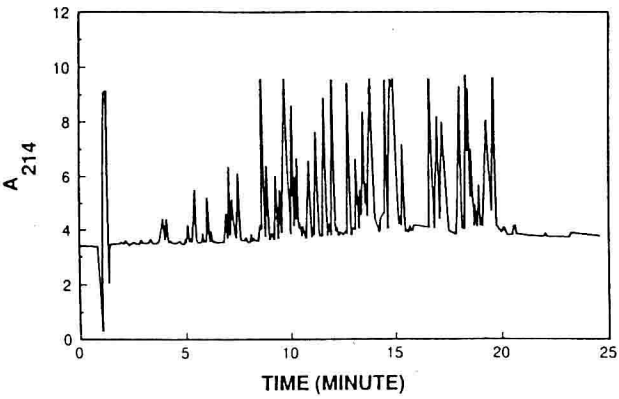

Fig. 5. Tryptic map of r-tissue plasminogen activator. 25 min linear gradient from 0 to 100% mobile phase (B); mobile phase (A): water/TFA, pH = 3, (B): acetonitrile: water = 60:40/TFA, pH = 3; column: Supelco RPC, LC18DB; detection: UV at 214 nm

in separations. Moreover, the gradient devices have been automated and commercially available. As a result, gradient elution chromatography has become a popular analytical technique in the laboratory. A case in point is the tryptic mapping of r-tissue plasminogen activator using gradient elution chromatography. Numerous peaks can be obtained in a single tryptic mapping chromatogram, shown in Fig. 5. Consequently, gradient elution chromatography provides fast and highly resolved separations, which also implies high loading capacity.

2.3 Disadvantages

Gradient elution chromatography is not a simple technique. The difficulties of reproducing the results, optimizing the conditions as well as scaling-up gradient elution separations are well known [34]. These difficulties occur because of both the theoretical and practical limitation of gradient elution. The theoretical calculation of gradient elution was limited by lack of the analytical solutions to the detailed model of gradient elution systems, that consider interference, transport and kinetic effects. Hence, further simplification is necessary in order to derive analytical solutions. The major simplifications include the assumption of Gaussian elution bands, linear chromatographic behavior with small sample sizes and dilute sample concentrations, simple retention relationships, and neglect of the transport and kinetic effects for the comparison of the existing models on gradient elution chromatography. However, as mentioned in Sect. 1, these simplifications have not been validated for large-scale separations, particularly of proteins. Several workers considered the transport or kinetic effect, but used lumping techniques to simplify the model and obtain analytical solutions, and ignored the interference effect [28, 29]. Likewise in gradient elution, isocratic elution also has the same theoretical limitation. However, it is much more difficult to calculate effluent profiles for gradient elution than for isocratic elution due to the complication of line-dependent mobile phase