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Mathematical Modeling
and Scale-up
of Liquid Chromatography

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With 75 Figures

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Preface

Liquid chromatography is no longer limited to chemical analysis. It has become an indispensable tool for the preparative- and large-scale purifications of proteins and other fine chemicals. So far, the scale-up of liquid chromatography relies mostly on trial and error and a few scaling rules that are more of a rule-of-thumb nature.

This book provides numerical solutions to a series of general multicomponent rate models for liquid chromatography. The models consider dispersion, interfacial film mass transfer, intraparticle diffusion, and nonlinear multicomponent isotherm, or the second order kinetics. The models can be used to simulate various chromatographic operations. They provide more realistic descriptions of preparative- and large-scale liquid chromatography than the equilibrium theory and plate models because various mass transfer mechanisms are included.

The applications of the Fortran 77 codes for the models are explained. Parameter estimation for the models is discussed. The codes should be helpful in both the understanding of the dynamics of liquid chromatography and its scale-up. The codes are available to readers upon request by a letter, or preferably an electronic mail (to guting@ent.ohiou.edu).

Most of this book is based on the theoretical part of the author's Ph.D. thesis work at Purdue University, West Lafayette, Indiana, U.S.A. I am deeply indebted to my former advisor, Prof. George T. Tsao of the Laboratory of Renewable Resources Engineering at Purdue.

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1 Introduction

High Performance Liquid Chromatography (HPLC) is undoubtedly one of the most important tools in chemical analysis. It has become increasingly popular at preparative- and large-scales, especially in purifying proteins. At such scales, larger particles are often used to pack the columns in order to reduce column pressure and facilitate column packing. Unlike small scale analytical HPLC columns that may give near plug flow performances, in large HPLC and lower pressure liquid chromatographic columns, dispersion and mass transfer effects are often important.

At smaller scales, the scale-up of liquid chromatography columns can usually be carried out by trial and error. Success depends to a large extent on the experience of the researcher. A failure is often not detrimental. The situation changes where large-scale columns are concerned. Such columns may easily cost thousands of dollars. There is much at stake in scale-up. A wrong estimation will render a purchased, or self-manufactured column, unsuitable for a particular application because of either insufficient resolution or low sample loading capacity. When an appropriate mathematical model is applied, it can be very helpful in supplementing the researcher's experience during scale-up.

There are several kinds of mathematical models for liquid chromatography with different complexities. A brief review of the models is given in Chap. 2. The equilibrium theory and plate models are generally not adequate for the realistic modeling and scale-up of multicomponent liquid chromatography, because of their inability to detail mass transfer mechanisms involved in preparative- and large-scale chromatographic separations.

The comprehensive mathematical models for liquid chromatography are often called the general multicomponent rate models, since they consider axial dispersion, interfacial mass transfer between the mobile and the stationary phases, intraparticle diffusion, and multicomponent isotherms. They provide an attractive alternative to the equilibrium theory and plate models for the modeling and scale-up of multicomponent chromatography. In the past, such a model was difficult to solve numerically on a computer. Due to tremendous advances in computer hardware, the model can now be solved using a common minicomputer. Because the general rate model considers different mass transfer mechanisms in a column, it is suitable for the realistic modeling of preparative- and large-scale chromatography. Computer simu-

lation using the model also provides an excellent tool for studying many chromatographic phenomena without doing actual experiments.

In Chap. 3 of this book, a robust and efficient numerical procedure is presented to solve a general nonlinear multicomponent rate model that considers axial dispersion, external film mass transfer, intraparticle diffusion and complicated nonlinear isotherms. It uses quadratic finite elements for the discretization of the bulk-fluid phase partial differential equation (PDE) and the orthogonal collocation method for the particle phase PDE in the model, respectively. The resulting ordinary differential equation (ODE) system is solved by Gear's stiff method [1]. The model has been extended to include second order kinetics and the size exclusion effect. An alternative boundary condition at the column exit to the Danckwerts boundary condition is also analyzed.

The theoretical study of mass transfer in liquid chromatography in Chap. 4 points out the effects of mass transfer on multicomponent chromatographic separations. The influence of mass transfer related dimensionless parameters in the general multicomponent rate model is demonstrated by simulation. Also shown is an interesting case in which a component with an unfavorable isotherm does not show the expected anti-Langmuir peak shape with a diffused front and sharpened tail. Instead, it gives a peak with a tail more diffused than the peak front because of slow mass transfer rates.

In Chap. 5, a unified approach to a better understanding of multicomponent interference effects under mass transfer conditions is proposed. It has been shown that a displacement effect can be used to explain the dominating interference effects arising from the competition for binding sites among different components in multicomponent chromatography. It has been concluded that the concentration profile of a component usually becomes sharper due to the displacement effect from another component, while the concentration front of the displacer is often diffused as a consequence. Five factors stemming from equilibrium isotherms, which tend to escalate the displacement effect in multicomponent elutions, have been investigated. They have important implications for interference effects in multicomponent elutions under column-overload conditions.

In multicomponent elutions, competing modifiers are sometimes added to the mobile phase to compete with sample solutes for binding sites in order to reduce the retention times of strongly retained sample solutes [2]. Peaks in the chromatogram corresponding to a modifier are called system peaks [3]. Studies of system peaks may provide useful information on the effect of modifiers on the sample solutes and interpretation of some chromatograms. In Chap. 6, system peaks are studied systematically using the general multicomponent rate model. Systems peak patterns have been summarized for binary elutions with one competing modifier in the mobile phase involving samples that are either prepared in the mobile phase or in an inert solution. Binary elutions with two competing modifiers have also been investigated briefly.

A methodology is presented in Chap. 7 for the development of kinetic and isotherm models for multicomponent adsorption systems with uneven saturation capacities for different components, which are either physically induced or due to chiral discrimination of binding sites. The extended multicomponent Langmuir isotherm derived with this methodology, which is thermodynamically consistent, has been used successfully to explain isotherm crossovers and to demonstrate the peak reversal phenomenon under column-overload conditions.

In Chap. 8, the kinetic and mass transfer effects are discussed. The rate-limiting step in chromatography is investigated. The general multicomponent rate model has been modified to account for a reaction in the fluid phase between macromolecules and soluble ligands for the study of affinity chromatography. The adsorption, wash and elution stages in affinity chromatography have been simulated and analyzed.

Chap. 9 presents a general rate model for multicomponent gradient elution. The semi-empirical relationship between the modulator concentration and eluite affinity with the stationary phase developed by Melander et al. [4] is used in the model. Examples of multicomponent elution with linear and nonlinear gradients are demonstrated using computer simulation.

A general multicomponent rate model for radial flow chromatography (RFC) has been solved in Chap. 10 using the same numerical approach as that for conventional axial flow chromatography (AFC). The radial dispersion and external film mass transfer coefficients are treated as variables in the model for AFC. The extension of the general multicomponent rate model for RFC to include second order kinetics, the size exclusion effect and liquid phase reaction for the study of affinity RFC is also mentioned.

In Chap. 11, methods and correlations useful for the estimation of mass transfer and isotherm data are reviewed. The general approach and practical considerations for the scale-up of liquid chromatography using the general multicomponent rate models have been discussed.

Fortran 77 computer codes for the numerical solutions to all the rate models discussed in this book are available to the reader by sending a letter or preferably e-mail (guting@ent.ohiou.edu) to the author. They can be used with a Unix computer or a high-end IBM-compatible personal computer with sufficient RAM. The International Mathematical and Statistical Libraries (IMSL) from IMSL, Inc. (Houston, Texas) is required for the codes. The usage of the codes is demonstrated where they are first introduced in their respective chapters. If the IMSL is not available, the user may find a substitute from a public domain on the Internet for non-commercial applications.

2 Literature Review

2.1 Theories for Nonlinear Multicomponent Liquid Chromatography

Many researchers have contributed to the modeling of liquid chromatography. There exist a dozen or more theories of different complexities. A comprehensive review on the dynamics and mathematical modeling of isothermal adsorption and chromatography has been given by Ruthven [5] who classified models into three general categories: equilibrium theory, plate models, and rate models.

2.1.1 Equilibrium Theory

According to Ruthven, the equilibrium theory of multicomponent isothermal adsorption was first developed by Glueckauf [6]. The interference theory by Helfferich and Klein [7] that is mainly aimed at stoichiometric ion-exchange systems with constant separation factors, and the mathematically parallel treatise for systems with multicomponent Langmuir isotherms by Rhee and coworkers [8, 9] are both extensions of the equilibrium theory.

Equilibrium theory assumes a direct local equilibrium between the mobile phase and the stationary phase, neglecting axial dispersion and mass transfer resistance. It effectively predicts experimental retention times for chromatographic columns with fast mass transfer rates. It provides general locations, or retention times of elution peaks, but it fails to describe peak shapes accurately if mass transfer effects are significant. Equilibrium theory has been used for the study of multicomponent interference effects [7] and the ideal displacement development [9]. Many practical applications have been reported [7, 10-14].

2.1.2 Plate Models

Generally speaking, there are two kinds of plate models. One is directly analogous to the tanks in series model for nonideal flow systems [5]. In such a model, the column is divided into a series of small artificial cells, each with

complete mixing. This gives a set of first order ODEs that describe the adsorption and interfacial mass transfer. Many researchers have contributed to this kind of plate model [5, 15-17]. However, plate models of this kind are generally not suitable for multicomponent chromatography since the equilibrium stages may not be assumed equal for different components.

The other kind of plate model is formulated based on the distribution factors that determine the equilibrium of each component in each artificial stage. The model solution involves recursive iterations rather than solving ODE systems. The most popular are the Craig distribution models. By considering the so-called blockage effect, the Craig models are applicable to multicomponent systems. Descriptions of Craig models were given by Eble et al. [18], Seshadri and Deming [19], and Solms et al. [20]. The Craig models have been used for the study of column-overload problems [18, 21]. Recently, Velayudhan and Ladisch [22] used a Craig model with a corrected plate count to simulate elutions and frontal adsorptions.

2.1.3 Rate Models

Rate models refer to models containing a rate expression, or rate equation, which describes the interfacial mass transfer between the mobile phase and the stationary phase. A rate model usually consists of two sets of differential mass balance equations, one for the bulk-fluid phase, the other for the particle phase. Different rate models have varying complexities [5].

2.1.3.1 Rate Expressions

The solid film resistance hypothesis was first proposed by Glueckauf and Coates [23]. It assumes a linear driving force between the equilibrium concentrations in the stationary phase (determined from the isotherm) and the average fictitious concentrations in the stationary phase. This simple rate expression has been used by many researchers [5, 24-26] because of its simplicity, but this model cannot provide details of the mass transfer processes.

The fluid film mass transfer mechanism with a linear driving force is also widely used [5]. The driving force is the concentration difference of a component between that on the surface of a particle and that in the surrounding bulk-fluid. It is assumed that there is a stagnant fluid film between the particle surface and the bulk-fluid. The fluid film exerts a mass transfer resistance between the bulk-fluid phase and the particle phase, often called the external mass transfer resistance. If the concentration gradient inside the particle phase is ignored, the chromatography model then becomes a lumped particle model, which has been used by some researchers [27-29]. If the mass transfer Biot number, which reflects the ratio of the characteristic rate of film mass

transfer to that of intraparticle diffusion, is much larger than one, the external film mass transfer resistance can be neglected with respect to intraparticle diffusion.

In many cases, both the external mass transfer and the intraparticle diffusion must be considered. A local equilibrium is often assumed between the concentration in the stagnant fluid phase inside macropores and the solid phase of the particle. Such a rate mechanism is adequate to describe the adsorption and mass transfer between the bulk-fluid and particle phases, and inside the particle phase in most chromatographic processes. The local equilibrium assumption here is different from that made for the equilibrium theory. The equilibrium theory assumes a direct equilibrium of concentrations in the solid and the liquid phases without any mass transfer resistance.

If the adsorption and desorption rates are not sufficiently high, the local equilibrium assumption is no longer valid. A kinetic model must be used. Some kinetic models were reviewed by Ruthven [5] and Lee et al. [30, 31]. Second order kinetics has been widely used in kinetic models for affinity chromatography [32-39].

2.1.3.2 *Governing Equation for the Bulk-Fluid Phase*

The governing partial differential equation for the bulk-fluid phase can be easily obtained from a differential mass balance of the bulk-fluid phase for each component. Axial dispersion, convection, transient, and the interfacial flux terms are usually included. Such equations themselves are generally linear if physical parameters are not concentration dependent. They become nonlinear when coupled with a rate expression involving nonlinear isotherms or second order kinetics.

For some rate models, such as models for isothermal, single component systems with linear isotherms, analytical solutions may be obtained using the Laplace transform [5, 31]. For more complex systems, especially those involving nonlinear isotherms, analytical solutions generally cannot be derived [5]. Numerical methods must be used to obtain solutions to complex rate models that consider various forms of mass transfer mechanisms [40]. Detailed rate models are becoming increasingly popular, especially in the study of preparative- and large-scale chromatography.

2.1.3.3 *General Multicomponent Rate Models*

A rate model that considers axial dispersion, external mass transfer, intraparticle diffusion and nonlinear isotherms, is called a general multicomponent rate model. Such a model is adequate in most cases to describe the adsorption and mass transfer processes in multicomponent chromatography.

In some cases, surface adsorption, size exclusion and adsorption kinetics may have to be included to give an adequate description of a particular system. Several groups of researchers have used different numerical procedures to solve various general multicomponent rate models [40-43].

2.1.3.4 Numerical Solutions

A general multicomponent rate model consists of a coupled PDE system with two sets of mass balance equations, one for the bulk-fluid and one for the particle phases for each component, respectively. The finite difference method is a simple numerical procedure that can be directly applied to the solution of the entire model [42, 44]. This often requires a huge amount of computer memory during computation, and its efficiency and accuracy are not competitive compared with other more advanced numerical methods, such as the orthogonal collocation (OC), finite element, or the orthogonal collocation on finite element (OCFE) methods.

For the particle phase governing equation, the OC method is the obvious choice. It is a very accurate, efficient and simple method for discretization. It has been widely used with success for many particle problems [45, 46]. The formulation of the OC method for particles is readily available from Finlayson's book [46].

Unfortunately, concentration gradients in the bulk-fluid phase can be very steep, thus the OC method is no longer a desirable choice, since global splines using high order polynomials are very expensive [46] and sometimes unstable. The method of OCFE uses linear finite elements for global splines and collocation points inside each element. No numerical integration for element matrices is needed because of the use of linear elements. This discretization method can be used for systems with stiff gradients [46].

The finite element method with higher order of interpolation functions (typically quadratic, or occasionally cubic) is a very powerful method for stiff systems. Its highly streamlined structure provides unsurpassed convenience and versatility. This method is especially useful for systems with variable physical parameters, as in radial flow chromatography and nonisothermal adsorption with or without chemical reactions. Chromatography of some biopolymers also involves a variable axial dispersion coefficient [47].

2.1.3.5 Solution to the ODE System

If the finite element method is used for the discretization of the bulk-fluid phase PDE and the OC method for the particle phase equations, an ODE system is produced. The ODE system with initial values can be readily solved using an ODE solver such as subroutine "IVPAG" of the IMSL [48] software package, which uses the powerful Gear's stiff method [40].

2.2 Scale-Up of Liquid Chromatography

Currently, scale-up of liquid chromatography is carried out largely based on trial-and-error and experience, with the help of some general scale-up rules that are not necessarily accurate. Some of these rules were discussed by Snyder et al. [2], and Pieri et al. [49]. They are mostly empirical or semi-empirical relationships about particle size, flow rate, column length, and resolution. The correlations are more of a "rule of thumb" nature when they are used for scale-up. Knox and Pyper [50] did an extensive study on column-overload. Some of their results on concentration and volume overload are also helpful in the scale-up of liquid chromatography. There are many papers in this area. They are not the focus of this book.

Instead of following these scale-up rules, a rate model can be used to simulate chromatograms of a larger column before it is built or purchased. The model uses only a few experimental data from a small column with the same packing as a large column. Although rate models have great potential in more accurate scale-up of liquid chromatography, most applications have been on the simulation of smaller columns to match experimental chromatograms. Practical examples involving larger columns have not been reported in the literature.

3 A General Multicomponent Rate Model for Column Liquid Chromatography

3.1 Model Assumptions

For the modeling of multicomponent liquid chromatography, the column is divided into the bulk-fluid phase and the particle phase. The anatomy of a fixed-bed axial flow chromatography column is given in Fig. 3.1. To formulate a general rate model, the following basic assumptions are required.

(1) The chromatographic process is isothermal. There is no temperature change during a run.
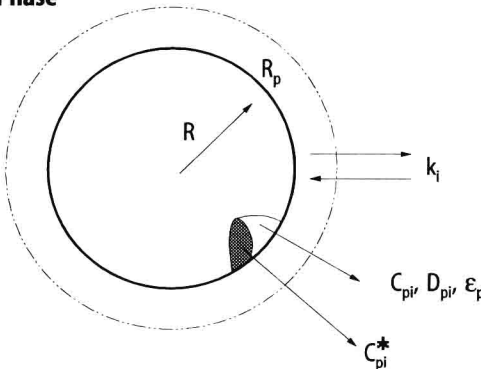
Bulk-Fluid Phase
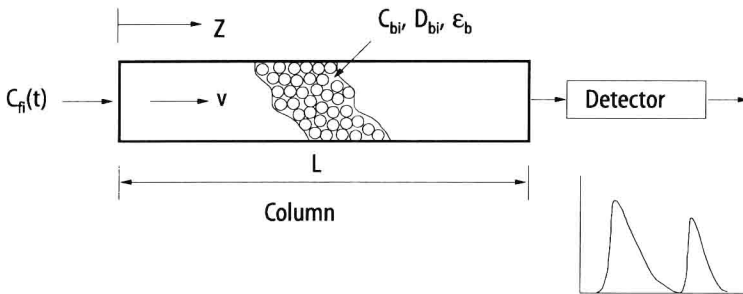
Particle Phase

Fig. 3.1. Modeling of fixed-bed axial flow chromatography

(2) The porous particles in the column are spherical and uniform in diameter.
(3) The concentration gradients in the radial direction are negligible.
(4) The fluid inside particle macropores is stagnant, i.e., there is no convective flow inside macropores.
(5) An instantaneous local equilibrium exists between the macropore surfaces and the stagnant fluid inside macropores of the particles.
(6) The film mass transfer mechanism can be used to describe the interfacial mass transfer between the bulk-fluid and particle phases.
(7) The diffusional and mass transfer parameters are constant and independent of the mixing effects of the components involved.

3.2 Model Formulation

Based on the assumptions above, the governing equations can be obtained from differential mass balances of the bulk-fluid phase and the particle phase, respectively, for component i. The following equations can also be derived from equations of continuity provided by Bird et al. [51]:

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2} + v\frac{\partial C_{bi}}{\partial Z} + \frac{\partial C_{bi}}{\partial t} + \frac{3k_i(1-\varepsilon_b)}{\varepsilon_b R_p}\left(C_{bi} - C_{pi,R=R_p}\right) = 0 \tag{3-1}$$

$$\left(1-\varepsilon_p\right)\frac{\partial C_{pi}^*}{\partial t} + \varepsilon_p\frac{\partial C_{pi}}{\partial t} - \varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right] = 0 \tag{3-2}$$

with the initial and boundary conditions

$$t = 0, \quad C_{bi} = C_{bi}(0,Z); \quad C_{pi} = C_{pi}(0,R,Z) \tag{3-3,4}$$

$$Z = 0, \quad \frac{\partial C_{bi}}{\partial Z} = \frac{v}{D_{bi}}\left(C_{bi} - C_{fi}(t)\right); \quad Z = L, \quad \frac{\partial C_{bi}}{\partial Z} = 0 \tag{3-5,6}$$

$$R = 0, \quad \frac{\partial C_{pi}}{\partial R} = 0; \quad R = R_p, \quad \frac{\partial C_{pi}}{\partial R} = \frac{k_i}{\varepsilon_p D_{pi}}\left(C_{bi} - C_{pi,R=R_p}\right) \quad . \tag{3-7,8}$$

Defining the following dimensionless constants,

$$c_{bi} = C_{bi}/C_{0i}, \quad c_{pi} = C_{pi}/C_{0i}, \quad c_{pi}^* = C_{pi}^*/C_{0i}, \quad \tau = vt/L, \quad r = R/R_p$$

$$z = Z/L, \quad Pe_{Li} = vL/D_{bi}, \quad Bi_i = k_i R_p/(\varepsilon_p D_{pi}), \quad \eta_i = \varepsilon_p D_{pi}L/(R_p^2 v)$$

$$\xi_i = 3Bi_i\eta_i(1-\varepsilon_b)/\varepsilon_b$$

the model equations can be transformed into the following dimensionless equations:

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2} + \frac{\partial c_{bi}}{\partial z} + \frac{\partial c_{bi}}{\partial \tau} + \xi_i\left(c_{bi} - c_{pi,r=1}\right) = 0 \tag{3-9}$$