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Review

Preparative liquid chromatography

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Abstract

The status of the theory and the main methods of implementation of preparative liquid chromatography are reviewed. On the theory front, the focus has recently shifted. The theory of non-linear, non-ideal chromatography has given rise to numerous models whose advantages, disadvantages and ranges of application are now well understood. Interest now resides in investigating the equilibrium thermodynamics of complex new systems, in the study of the kinetics of mass transfers in conventional chromatographic systems, and in the application of the various models of chromatography to optimize the experimental conditions. Progress in computer technology allows the use of sophisticated models, provided their parameters can be measured. This allows the detailed investigation of separations for which the mass transfer kinetics is slow such as chiral separations, the purification of basic compounds, and the extraction of recombinant proteins. On the applied front, in addition to numerous incremental improvements in reliability and economic performance, a few essential new features should be noted, i.e. the availability of instruments for simulated moving bed separations at the scale needed for preparative chiral separations, the use of expanded beds for the extraction of recombinant proteins from fermentation broths, and the attention given to improvements in the performance of packed beds. A survey of the literature dealing with practical applications and recent meetings shows that preparative chromatography is becoming a well established separation and purification method in the pharmaceutical industry.

Keywords: Reviews; Preparative liquid chromatography; Non-linear chromatography; Theory of chromatography; Models of chromatography; Industrial chromatography

Contents

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1. Introduction

Chromatography is a powerful separation method that was developed initially for the extraction and purification of complex mixtures of vegetal origin [1]. Collected fractions were analyzed, identified, and used for further applications, notably synthesis of derivatives. Later, chromatography became an ubiquitous analytical method. Its separation power kept attracting the attention of those interested in producing pure chemicals in large amounts for a variety of purposes. Most implementations of chromatography were developed for analytical purposes: thin-layer chromatography, gas–liquid and gas–solid chromatography, low- and high-performance column liquid chromatography. All have also been used for preparative applications. The elution, displacement, and frontal analysis modes were applied with various degrees of success. Nearly all conventional phase systems, NPLC and RPLC, liquid–liquid, liquid–solid, size-exclusion, ion-exchange, bioaffinity, HIC and MECC, have been used. Complex instruments, coupling two or several columns, have been described and applied to solve either analytical or preparative separation problems. The use of wide-bore, standard bore, small bore, and narrow bore columns has been described. Actually, this observation points to the essential difference between analytical and preparative chromatography. It is not in the size of the instrument used, it is in the chemist's goal.

Analytical chromatography aims at separating complex mixtures to identify and quantitize the components of mixtures, simple or complex. Once

the required signals have been acquired for a component, the chemical is discarded. This aim may involve the use of simple (e.g. UV, FID, etc.) detectors or on-line coupling with the most complex detectors (e.g. multiple MS, high field strength NMR, etc.). The purpose remains the rapid determination of the structure of the component, through the direct acquisition of the proper information, and the calculation of its concentration, through calibration of the detector signal. Preparative chromatography aims at isolating a certain amount of a purified component and using it for a further goal. However, the exact amount desired has only a secondary effect on the difference between the perspectives followed by the chemist. Whether the amount of purified compound isolated is going to be used for off-line spectral acquisition (an application sometimes still necessary in spite of the tremendous progress made these last few years in HPLC–NMR) or for sale as a reagent or a pharmaceutical, some concerns remain identical, among them the need to produce as concentrated a fraction as possible, to collect and transfer it without pollution, and to do the separation as quickly and cheaply as possible (admittedly, the cost estimates are very different depending on whether the separation is made once or on a routine basis, at a low or a high production level).

As soon as the chemist needs to produce and recover even a modest amount of a fraction contained in a more complex mixture, she feels a need to inject a larger sample. Soon, she comes to realize that retention times, band profiles, and hence recovery yield and production rate depend heavily on the sample size and the concentration of the sample. She hits the conceptual wall of non-linear chromatography, with the consequence that a dramatic change in perspective is required. Chromatograms are no longer a series of more-or-less well-resolved more-or-less Gaussian-shaped peaks that change in size but not in shape with increasing sample size. They are complex assemblies of bands that change shape and seem to interact with their neighbors when the sample size is increased. They do so because, at least to some extent, retention mechanisms are never linear nor independent. The amount of a compound adsorbed on or dissolved in the stationary phase, at equilibrium with a certain concentration in the mobile phase depends (i) on this concentration, and (ii) on the nature and concentration of all the other components locally present, be they mobile phase additives or sample components eluted closely to the component considered [2]. Failure to realize the importance of the isotherm curvature at the origin and of the competitive nature of isotherms explains many initial failures in the scale-up of chromatography for preparative applications. In part because of the complexity of the physical chemistry aspect of the problem, in part because of the considerable economic interests involved in its successful solution, theory has played an important role in the development of the preparative applications of chromatography.

Indeed, the engine behind the considerable development that has taken place in preparative chromatography during the last 10 years was the recognition by the pharmaceutical industry that chromatography is the only general separation method for the purification of the drug intermediates and the pharmaceuticals that it produces. It is not generally recognized how this industry is profoundly different from others in two critical aspects. Its price are not elastic. Drugs are prescribed and purchased because of an urgent need. Reducing their price barely affects the sale volume. The cost of a drug at the counter is heavily influenced by QA/QC (quality assurance and quality control), packaging, distribution, and advertisement. The use of a separation method as expensive as chromatography is acceptable if it is required to achieve production of a marketable product. On the other hand, in contrast to all other industries, the pharmaceutical industry is not free to adjust the specification of its products to achieve maximum profit. These specifications must be accepted by the regulatory agency, the FDA. In many cases, preparative chromatography is the method needed to satisfy the purity specifications required on a routine basis.

This review addresses the main aspects of preparative chromatography in connection with the current applications in the pharmaceutical industry. It includes considerations on the theory that should guide method development and the selection of optimum experimental conditions, on the design and preparation of the columns, and on the instrument.

2. Theory of non-linear chromatography

This section begins with a brief history of the

development of the theory. This issue was addressed in more detail elsewhere [2], but history always provides useful lessons for those who know it. It will be followed by a presentation of the two approaches used, the extension of linear chromatography to the realm of non-linear chromatography through various implementations of perturbation theories and the direct investigation of full-fledged non-linear chromatography. This will be completed by a discussion of the models available, their advantages and disadvantages, and by a presentation of results obtained in the study of optimization strategies.

2.1. History of non-linear chromatography

Bohart and Adams [3] derived the equation of the equilibrium–dispersive model (see below) as early as 1920, but it does not seem that they attempted any calculations based on this model. Wicke [4,5] derived the mass balance equation of this same model in 1939 and discussed its application to gas chromatography on activated charcoal. There were not many applications of this work either. The model has no analytical solutions. Numerical solutions were too long and complex to calculate before powerful computers became available. It was only around 1987 that the calculation of the band profiles of single compounds and binary mixtures began to become accessible [6].

The theory of linear chromatography began later but progressed faster because no complex numerical calculations are needed to apply it. It developed along three different axes, leading to the three broad classes of models which are still used to describe and predict elution profiles. These classes are: (i) the plate or ''tank in series'' models of Martin and Synge [7] and Craig [8]; (ii) the solutions of systems of differential equations that are similar to those derived by Bohart and Adams [3] and by Wicke [4,5], that describe the mass balance and the mass transfer kinetics, and that were the basis of the fundamental work of Wilson [9], DeVault [10], Lapidus and Amundson [11] and van Deemter et al. [12]; and (iii) the statistical models, first developed by Giddings and Eyring [13].

The plate models divide the column into a number of identical equilibrium stages, or theoretical plates, that are placed in series. The mobile phase percolates from one plate to the next after equilibrium is achieved between the mobile and the stationary phases. This division of the column into a series of plates is arbitrary, so plate models are, in essence, approximate models because they depict a continuous column of length L as a discrete number of well-mixed cells in series. Although any mixing mechanism is clearly absent from the actual physical system, plate models have been used successfully to define the column operation physically and to describe it in mathematical terms. However, by nature, plate models are empirical ones, which cannot be related to first principles. Their parameters are empirically correlated, after the facts, with the column parameters. These models have no predictive value and do not allow the development of a reliable theory of linear chromatography [2]. They are described in sufficient, often excessive detail in all elementary books on chromatography and no further elaboration is needed here.

As clearly shown by Giddings [14], chromatography is an F($\perp$)d separation method, i.e. a method combining a chemical potential discontinuity in a direction perpendicular to that of a convective transport. The presence of the chemical potential discontinuity ensures some degree of separation of the sample components between the two phases. The flow of the stream of mobile phase in the direction perpendicular to that of the discontinuous gradient considerably magnifies the result of even a small degree of separation [14]. This model introduces naturally the mass balance approach which was used very early [3–5,9–12], and most extensively [2], to calculate the chromatographic response to a given input function (i.e. injection conditions or profile). This approach is based on the use of an equation of motion. In this method, we search for the mathematical solution of the set of partial differential equations describing the chromatographic process, or more precisely, the differential mass balance of the solute in a slice of column and its kinetics of mass transfer in the column. Various mathematical models have been developed to describe the chromatographic process. Provided the proper parameters (isotherm and mass transfer kinetics models and their parameters, experimental conditions) are entered into the calculations, these models have great predictive value [2,15]. The most important of these models are

the ideal model, the equilibrium–dispersive model, the lumped kinetic model, the pore model, and the general rate model of chromatography. We discuss these models in more detail in the next subsections.

A ''microscopic statistical'' method can be used to model linear chromatography. It consists in deriving the probability density function at position x and time t of a single molecule of solute. The ''random walk'' approach [16] is the simplest method of that type. It was used for the simple calculation of estimates of the variance of chromatographic band profiles and to study the mechanism of band broadening [16]. Giddings and Eyring [13] introduced a more sophisticated probabilistic approach, the stochastic model, for the description of the molecular migration in chromatography. Their molecular dynamic approach is based on statistical ideas and treats the chromatographic process as a Poisson distribution process. These authors considered the random migration of a single solute molecule along a chromatographic column. They derived an expression for the elution profile, or residence time distribution of the molecules of the sample in the column, assuming random adsorption–desorption processes, with a single type of site or two different sites on the stationary phase and impulse injection. They ignored the axial dispersion and approximated the mass transfer kinetics in the mobile phase by a random walk model. Later, McQuarrie [17] extended the stochastic theory to the case of multiple adsorption sites and to a column with a single type of site, but with various input distribution functions, by means of the theory of the Laplace transform. Over the years, Dondi and his group made important contributions to the development of the stochastic theory of chromatography [18], using the characteristic function method. This group has recently obtained remarkable results in the extension of this model to non-linear chromatography [19]. The enormous difficulty in this last case was that of accounting, in a stochastic theory, for the interactions between the molecules of one or two different solutes, interactions that cause the non-linear isotherm and their competition, respectively.

2.2. Models of non-linear chromatography

We will consider here five models of uneven importance. These models are the ideal model, the equilibrium–dispersive model, the lumped kinetic model, the pore model and the general rate model of chromatography. They can be applied to both linear and non-linear chromatography.

2.2.1. The ideal model

The ideal model assumes that the column has an infinite efficiency. Accordingly, the band profile arises only from the characteristics of the equilibrium thermodynamics. If the isotherm is linear, the elution profile is merely the injection profile shifted by a time equal to the hold-up time, t_0. This model has the advantage of its simplicity and of predicting the best possible results: thermodynamics cannot be improved upon. All other models are non-ideal and use different approaches to account for the finite column efficiency.

2.2.2. The equilibrium–dispersive model

The equilibrium–dispersive model still assumes that mass transfer across the column is infinitely fast but it accounts for a finite extent of axial dispersion. Further, it treats the finite rate of the mass transfer kinetics as another contribution to axial dispersion. Thus, it relates apparent axial dispersion and column HETP. This approach is valid when the column efficiency is high, as it is in the RPLC of small molecules of moderate polarity. It is not correct in most cases of enantiomeric separations nor for the elution of many proteins. Because this model considers the influence of a finite column efficiency as a small correction, it should not be applied when this efficiency is poor.

2.2.3. The lumped kinetic model

The lumped kinetic model completes the mass balance of the ideal model by a kinetic equation that relates the rate of variation of the local concentration of solute in the stationary phase and the extent of the local deviation from equilibrium. This is a simplistic model of the mass transfer kinetics. Accordingly, it is valid only when it provides a correction for this kinetics, when the rate of this kinetics is fast, something that the equilibrium–dispersive model does more easily. At low rates of the mass transfer

kinetics, the model may account for the experimental results but it introduces model errors that can be considerable. This means, in practice, that it may be difficult to use the values of the rate coefficients to derive useful information regarding the mass transfer mechanisms and the kinetics of its different steps. For example, the model error may explain the often observed dependence of the lumped mass transfer coefficient on the solute concentration [20,21].

2.2.4. The general rate model

The general rate model attempts simultaneously to account for all the possible contributions to the mass transfer kinetics arising in chromatography. It does this by including their contributions in the system of partial differential equations which states mass conservation and transport. Thus, there are almost as many versions of the general rate model as there are specific cases of chromatography and each author tends to write his own. The first problem in writing a version of the model is to make a complete census of all the contributions of significance. Usually, axial dispersion as obtained by combining axial diffusion, tortuosity and constriction of the channels between bed particles and eddy diffusion is included. Also included are the external film mass transfer resistance and the intraparticle or pore diffusion. Surface diffusion and the rate of adsorption–desorption are sometimes also included but often neglected. Because this model considers separately the stagnant mobile phase, inside the particle, and the percolating mobile phase, outside the particles, two mass balance equations are written for the solute. This allows the coexistence of a homogeneous stream of mobile phase and a non-homogeneous bed of particles, the concentration in the particles increasing from the surface to the center when the front of the peak passes, decreasing on the peak tail [21].

2.2.5. The lumped pore model

The lumped pore model is a simplified version of the general rate model [22,23]. Its use is mainly recommended when the mass transfer kinetics is moderately fast and the run time of the programs is critical.

2.3. The equations of the models of chromatography

Since the character, linear or non-linear, of chromatography arises only from the behavior of the equilibrium isotherm, the models of chromatography and their equation systems are not specific of this character. All the models described in the previous section can be studied and used in combination with any isotherm model. Obviously, the level of mathematical difficulty encountered depends much on the nature of the isotherm. It increases very steeply from the linear to the Langmuir isotherm which will often be used later:

$$= \frac{aC}{1 + bC} = \frac{bq_s C}{1 + bC} \tag{1}$$

(with q and C, concentrations in the solid and liquid phase in equilibrium, a, b, and q_s, numerical parameters) and then again from the Langmuir to any more complex isotherms. Note that in this equation, the product bC is a measure of the deviation of the isotherm from linear behavior. If it is small compared to unity, the isotherm is practically linear.

We first present the equations of the general rate model, then explain how these equations can be progressively simplified for the other different models. The equations of the model are partial differential equations. These equations are combined with the isotherm equations and with proper initial and boundary conditions. These conditions are the translation into mathematical terms of the description of the experiment calculated (see below). In all cases, we make the following assumptions. The chromatographic process is isothermal. The mobile phase velocity remains constant during a run. The compressibility of the mobile phase is negligible. The packing material is made of porous particles that are spherical and uniform in size. The bed is homogeneous and the concentration gradient in the radial direction of the bed is negligible. There is local equilibrium for each component between the pore surface (monolayer) and the stagnant fluid phase inside the macropores. The dispersion coefficients of all components are constant (independent of the concentrations).

There are two mass balance equations for each

component, one for the mobile phase percolating through the bed of particles, the other for the interior of the particles, involving the stagnant mobile phase and the adsorbed monolayer.

2.3.1. Mass balance of component i in the mobile phase

This mass balance is written for a fluid percolating through a bed of spherical particles of radius R_p:

$$\varepsilon_e \frac{\partial C_i}{\partial t} + u \frac{\partial C_i}{\partial z} = \varepsilon_e D_L \frac{\partial^2 C_i}{\partial z^2} - (1 - \varepsilon_e) k_{exp,i} a_p \times [C_i - C_{p,i}(r = R_p)] \quad (2)$$

where C_i and $C_{p,i}$ are the concentration of component i in the mobile phase and its concentration in the stagnant fluid phase (in the particle pores), respectively, z and t are the abscissa and time, respectively, ϵ_e is the external or intersticial porosity, u is the mobile phase velocity, D_L is the coefficient of axial dispersion, k_{exp} the external mass transfer coefficient, and a_p the external surface area of the adsorbent particles.

2.3.2. Mass balance of component i in the solid phase

The mass balance inside the particles is written:

$$\varepsilon_p \frac{\partial C_{p,i}}{\partial t} + (1 - \varepsilon_p) \frac{\partial q_i}{\partial t} = D_{eff} \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_{p,i}}{\partial r} \right) \quad (3)$$

where ϵ_p is the internal porosity of the particles, q is the concentration of the studied component in the adsorbed phase, and D_{eff} is the effective diffusion coefficient.

2.3.3. The initial conditions

The initial condition describes the status of the column at the beginning of the experiment. Usually (but not in the simulated moving bed process, SMB), it contains the mobile phase in equilibrium with the stationary phase, the concentration being constant (and often zero) all along the column. There are two initial conditions, one for each partial differential equation. The following conditions correspond to those encountered in step-wise frontal analysis:

$$C_i(0,z) = C_i^0 \quad (4)$$

$$C_{p,i}(0, r, z) = C_{p,i}^0(r, z);$$
$$q_i(0, r, z) = q_i^0(r, z); \quad \text{for } 0 < z < L; \quad 0 < z < R_p \quad (5)$$

In most elution experiments, $C^0 = 0$.

2.3.4. The boundary conditions for Eq. (1)

We have two boundary conditions, one at the column inlet (a Danckwerts condition [2]), and the other at the column exit. The condition for $t > 0$ and $z = 0$ is

$$u_f C'_{fi} - u(0)C(0) = -\varepsilon_e D_L \frac{\partial C_i}{\partial z}$$
$$C'_{fi} = C_{fi} \quad \text{for} \quad 0 < t < t_p \quad (6)$$
$$C'_{fi} = 0 \quad \text{for} \quad t_p < t$$

The condition for $t > 0$ and $z = L$ is

$$\frac{\partial C_i}{\partial z} = 0 \quad (7)$$

2.3.5. The boundary conditions for Eq. (2)

There are again two boundary conditions, one for $t > 0$ and $r = R_p$:

$$D_{eff} \frac{\partial C_{p,i}(t,r)}{\partial r} = k_{ext,i}[C_i - C_{p,i}(t,r)] \quad (8)$$

and the other for $t > 0$ and $r = 0$:

$$\frac{\partial C_{p,i}(t,r)}{\partial r} = 0 \quad (9)$$

2.3.6. The isotherm model

An isotherm model, relating the concentration q_i of component i in Eq. (2), its mobile phase concentration and the other parameters of the system (including the local concentrations of all the other components of the system) is needed to complete the set of equations that constitute the mathematical translation of the general rate model. The equations of models suitable to account for the adsorption data of multicomponent mixtures in HPLC were reviewed recently [24,25]. Several considerations are important in selecting models and fitting experimental data to the equations of these models.

First, it is important to discriminate between models having a Henry constant and those which do not. For the former, the ratio q/C tends toward a finite limit when the mobile phase concentration tends toward 0, hence the component will have a finite retention factor. All materials used in HPLC should give isotherms that have this property. If there is no finite Henry constant, the ratio q/C tends toward infinity and the corresponding components have an infinite retention time at infinite dilution. Although this fact may make sense from a physical chemistry point of view, this property defeats the purpose of the separation scientist. A packing material having this property will be nearly impossible to use to perform any practical separation. Chromatographers rightly refrain from using any material that exhibits a Freundlich-type isotherm behavior. Secondly, whenever possible, isotherms the equation of which is explicit, i.e. can be written as $q = f(C)$, not as $f(q,C) = 0$ (an equation not solvable for q) should be preferred because they lead to easier, much faster calculations of band profiles. Although it has been shown that this is not a necessary condition for performing calculations of band profiles [26], this remains useful as it reduces the computing time.

Finally, we must admit that most isotherm models are arbitrary constructions which attempt to account for the most important features of liquid–solid interactions but cannot account for them all. Even in the simplest of cases, liquid–solid adsorption is always a competitive process: a single compound dissolved in a pure solvent competes with this solvent for adsorption on the surface. Therefore, excess isotherms should be used [27]. The simplification of using apparent isotherms instead, as is generally done, does not seem to cause significant errors. Furthermore, a variety of energetic and steric considerations are involved in adsorption that are too complex to be easily cast into an equation having few coefficients. It might be that one day we will be able to use molecular modeling and calculate ab initio competitive isotherms for a pair of structurally defined compounds on a stationary phase, but today this seems a rather remote possibility. In the meantime, we need isotherm models simple enough for accurate estimates of their coefficients to be derived from a fit of the experimental data to these models. Only with such isotherms can we account for the profiles of high concentration zones [2]. So, we should expect our results to suffer from some model errors.

2.3.7. Simplification of the equations of the general rate model. I—Pore model

The first two equations of the model are simplified by considering that the particles are homogeneous [22,23,28]. They are now written as follows:

$$\varepsilon_e \frac{\partial C_i}{\partial t} + u \frac{\partial C_i}{\partial z} = \varepsilon_e D_L \frac{\partial^2 C_i}{\partial z^2} - (1 - \varepsilon_e) k_i a_p (C_i - \bar{C}_{p,i}) \quad (10)$$

$$\varepsilon_p \frac{\partial \bar{C}_{p,i}}{\partial t} + (1 - \varepsilon_p) \frac{\partial \bar{q}_i}{\partial t} = k_i a_p (C_i - \bar{C}_{p,i}) \quad (11)$$

where $\bar{c}_p$ and $\bar{q}_i$ denote the particle average concentrations. The overall mass transfer coefficient k_i in the second equation is given by the following relationship:

$$k_i = \left[\frac{1}{k_{ext}} + \frac{1}{k_{int}} \right]^{-1} \quad (12)$$

where k_{ext} and k_{int} are the external and the internal mass transfer coefficients, respectively. The internal mass transfer coefficient is calculated from the following two relationships:

$$k_{int} = \frac{10 D_{eff}}{d_p} \quad D_{eff} = \frac{\varepsilon_p D_m}{\gamma} \quad (13)$$

where D_m is the molecular diffusivity and γ is the internal tortuosity factor of the particle. The initial and the boundary conditions are similar to those used in the general rate model.

2.3.8. Simplification of the equations of the general rate model. II—The equilibrium–dispersive and the lumped kinetic models

The equilibrium–dispersive model is easily derived from the pore model by assuming that the mass transfer kinetics between the mobile phase percolating through the bed and the particles is infinitely fast. Thus, $C_i = C_{p,i}$, and we may eliminate from both Eqs. (9) and (10) the term $k_i a_p (C_i - \bar{C}_{p,i})$. This gives the following form of the mass balance equation for the equilibrium–dispersive model:

$$\varepsilon_T \frac{\partial C_i}{\partial t} + (1 - \varepsilon_T) \frac{\partial q_i}{\partial t} + u \frac{\partial C_i}{\partial z} = \varepsilon_e D_L \frac{\partial^2 C_i}{\partial z^2} \quad (14)$$

This form is similar to the one found in the literature [2]. The only difference is in the replacement of the total porosity in the RHS of Eq. (13) by the external porosity. The initial and boundary conditions are adapted from those used in the general rate model. As in all the models, q_i is calculated from the appropriate isotherm equation.

In cases in which the mass transfer resistances, without being large, cannot be entirely neglected, it has become common to use the transport–dispersive model [29–38]. This model consists of Eq. (13) completed by the following kinetic equation:

$$\frac{\partial q_i}{\partial t} = k_{f,i}(q_i^* - q_i) \quad (15)$$

in which q_i^* is the concentration in the adsorption monolayer at the adsorbent surface in equilibrium with the concentration C_i in the mobile phase, i.e. is given by the equation of the equilibrium isotherm (while in the equilibrium–dispersive model, q_i is given by the isotherm equation).

2.3.9. Simplification of the equations of the general rate model. III—The ideal model

In the ideal model, mass transfer between mobile and stationary phase is assumed to be instantaneous and there is no axial dispersion [2]. So the mass balance equation simplifies to the hyperbolic first order partial differential equation:

$$\frac{\partial C_i}{\partial t} + \frac{1 - \varepsilon_T}{\varepsilon_T} \frac{\partial q_i}{\partial t} + u \frac{\partial C_i}{\partial z} = 0 \quad (16)$$

In contrast to the mass balance equations written earlier, which are parabolic equations, Eq. (15) can propagate discontinuities [2]. From this property originate some of the unusual features of the ideal model of chromatography.

2.4. Solutions of the equations of the models of chromatography

These models can be sorted into two groups, the equilibrium or ideal model [Eq. (15)] and the other models. In the elution case, the equation of the ideal model for a single component has analytical solutions for all isotherms; it has simple algebraic solutions in many cases [2,39–41]. The system of equations for a binary mixture has an analytical solution in the case in which the competitive Langmuir isotherm accounts for the isotherm behavior [2,42]. The lumped kinetic model has also an analytical solution in the case of a single component with a Langmuir isotherm [43–45], but this solution is most complex and it takes as long to calculate numerically as the solution of the PDE itself. In the case of single component breakthrough curves and a Langmuir isotherm, however, a most simple result is obtained (see shock layer in Section 2.4.2) [46–49].

In all other cases, there are no analytical, let alone algebraic, solutions. The current availability of high computing power on every scientist's desk, however, makes rather easy the calculation of numerical solutions of all models, in all cases. The only cases in which computing time may still be a problem would be when numerical solutions of the general rate model would be required for SMB systems (steady-state is often approached sufficiently closely only after over one or two hundred cycles) or for complex optimization problems (SMB with high efficiency columns or with complex models). A few more detail on these results are given below.

2.4.1. Algebraic solution of the ideal model for single components

The study of the mathematical properties of Eq. (15) shows that to each mobile phase concentration, C, is associated a migration velocity, u_z, given by:

$$u_z = \frac{u}{1 + F \dfrac{dq}{dC}} \quad (17)$$

The velocity u_z depends only on the concentration C with which it is associated and on the local curvature of the isotherm. Thus, the velocity associated with a given concentration is constant, and each concentration propagates along the column at a constant velocity [2,50]. The point representing this concentration in a (t, z) graph moves along a straight line called characteristic line. It follows that, in elution, one side of the band tends to spread and become increasingly diffuse because the velocity associated with a concentration increases with increasing concentration and the profile spreads. For a Langmuir

isotherm (or for any other isotherm having the same curvature), this is the rear side. In contrast, the front becomes increasingly steep. However, it is physically impossible for the high, hence faster, concentrations to pass the low, slower concentrations. This is why a concentration discontinuity or shock tends to build up.

This concentration shock migrates at a velocity given by:

$$U_s = \frac{u}{1 + F\dfrac{\Delta q}{\Delta C}} \tag{18}$$

where Δq and ΔC are the amplitudes of the concentration shock in the stationary and the mobile phases, respectively [2]. Comparing Eqs. (16) and (17), one can see that the velocity of a shock of amplitude 0 to C (in the mobile phase) is lower than the velocity associated with a concentration C on a continuous profile. So, during the migration of a band, the shock erodes constantly, hence its velocity decreases continuously. So, Eq. (17) cannot be used without further elaboration to calculate the retention time of the shock.

Now, assume that a rectangular profile of width t_p is injected on a column of length L. The rear discontinuity is unstable and, from Eq. (16), it follows that the equation of the rear of the elution profile (in the case of Langmuirian isotherms) is:

$$t(C) = t_p + \frac{L}{u}\left(1 + F\frac{dq}{dC}\right) \tag{19}$$

Eq. (19) provides a means to derive isotherms from the rear of a high concentration injection. It is the equation used in ECP [51], a method that is based on the use of the ideal model of chromatography, and hence suffers from a model error when used with columns of low efficiency [52]. To complete the prediction of the elution chromatogram obtained, the retention time of the front discontinuity is required and it is simply derived by observing that the area of the profile, proportional to the mass of compound, is constant [41]. Hence, by integration, the maximum concentration of the band is:

$$\left| q - C_M\frac{dq}{dC} \right| C = C_M = \frac{n}{F_v t_0 F} \tag{20}$$

where $q = q(C_M)$ and $dq/dC(C_M)$ are equations given by the isotherm model. Introducing the solution of Eq. (18) into Eq. (17) gives the retention time of the maximum concentration on the rear diffuse part of the profile, hence the retention time of the front shock. A closed-form or algebraic solution of Eq. (18) requires knowledge of the equilibrium isotherm. Solutions are easily obtained for the Langmuir, the biLangmuir, and the Freundlich isotherms [2].

The band profiles obtained with some important isotherm models are illustrated in Fig. 1. The models selected are the Langmuir, the bilangmuir and the Freundlich models (see the model equations in the figure caption). Fig. 2 illustrates the excellent agreement observed between the experimental profiles and the profiles calculated with the ideal model, using the equilibrium isotherm (Langmuir in this case) derived from independent measurements. The agreement between the two profiles improves with increasing loading factor. The steepness of the front part of the profiles increases with increasing sample size. The front becomes nearly vertical for loading factors in excess of a few percent. In the low concentration range, the rear parts of the profiles are more strongly diffuse than predicted by the model and tail indefinitely because of the finite column efficiency. The curvature of this rear part is stronger for the experimental profiles than for the calculated ones. This might be explained by a slight isotherm model error.

2.4.2. Algebraic solution of the ideal model for a binary mixture

This is the most realistic application of the ideal model because chromatography is foremost a separation method, not the study of relationships between band profiles and the physico-chemical parameters characterizing the thermodynamics and kinetics of phase equilibrium in a biphasic system. In the case of two compounds exhibiting competitive Langmuir isotherm behavior in the phase system used, the two individual band profiles can be calculated completely, using algebraic equations. Only the retention time of the first compound has to be calculated by solving numerically an integral [42]. Two examples of the algebraic solution are shown in Fig. 3. They correspond to two different relative compositions of the binary mixture. The equations giving the values of the different characteristics of the solution (coordi-

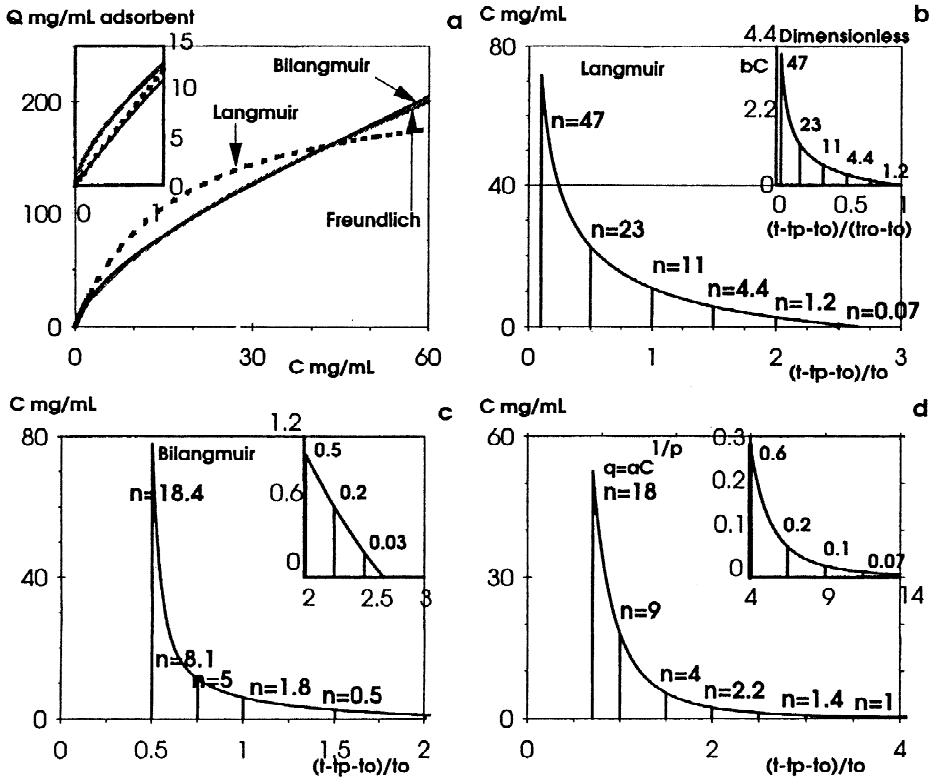

Fig. 1. Band profiles obtained with the Langmuir (1, $q = aC/[1 + bC]$), the biLangmuir (2, $q = a_1C/[1 + b_1C] + a_2C/[1 + b_2C]$) and the Freundlich (3, $q = aC^{1/n}$, $n>1$) isotherm models as solutions of the ideal model. Elution profile for a narrow injection. Conditions for the calculations: phase ratio: $F=0.25$; column length 10 cm, diameter 0.46 cm; flow-rate: $F_v=1$ ml/min; retention factor: $k'_0 = Fa = 6.0$; isotherm coefficients: $a = 13.1$; $b = 0.058$; $a_1 = 10.4$; $b_1 = 0.192$; $a_2 = 2.7$; $b_2 = 0.007$; $n = 0.66$; sample size: 66.7 μmol. Reproduced with permission from G. Guiochon, S.G. Shirazi and A.M. Katti, ''Fundamentals of Preparative and Nonlinear Chromatography,'' 1994, p. 270 (Fig. 7.6), Academic Press, Boston, MA, USA.

nates of the points and equations of the curves) are available in the literature [2,42]. The two chromatograms illustrate the effects arising in chromatography from the competition between the two compounds for interaction with the stationary phase. Although the quantitative values depend on the parameters of the isotherm equations, the qualitative nature of these interactions would be the same for most isotherm models.

The competitive Langmuir isotherm model is written:

$$q_i = \frac{b_i q_s C_i}{1 + b_1 C_1 + b_2 C_2} \tag{21}$$

where b_1, b_2 and q_s are numerical coefficients. In numerous cases, the values of q_s fitting best the experimental data are different for the two compounds. In this case, the isotherm model is not thermodynamically consistent and must be corrected [2]. It is obvious from the isotherm equation that an increase in the concentration (C_1) of the first component at constant value of the concentration of the second component (C_2) results in a decrease in the amount of first component adsorbed at equilibrium. This explains why the concentration of the first component decreases when the second one begins to elute (Fig. 3).

An important property of the ideal model is that the concentration signals in the elution profiles end after a finite time. This is easily explained by the lack of axial dispersion and by the infinitely fast mass transfer that is assumed between the two

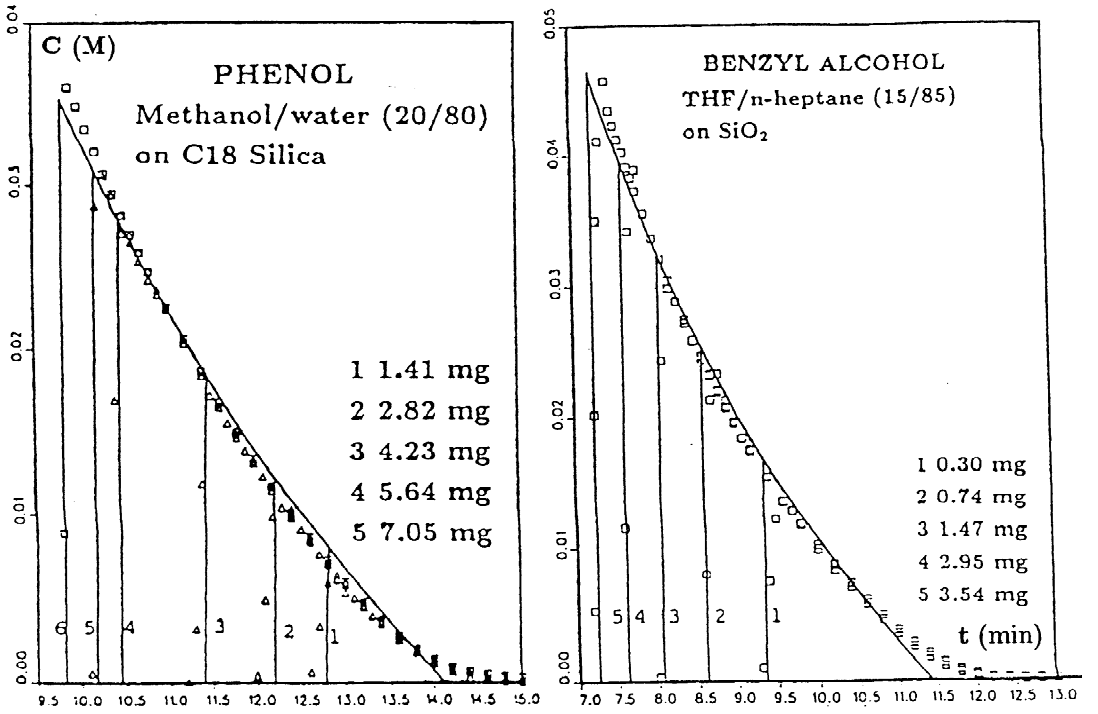

Fig. 2. Comparison between the solution of the ideal model and experimental band profiles. Samples: phenol on C_{18} silica, mobile phase 20:80 methanol–water mixture, loading factors (%): 1, 2.1; 2, 4.3; 3, 6.4; 4, 8.5; and 5, 10.7; benzyl alcohol on silica, mobile phase 15:85 THF/*n*-heptane mixture, loading factors (%): 1, 0.47; 2, 0.95; 3, 1.9; 4, 3.8; 5, 4.6; and 6, 5.7. Reproduced with permission from Golshan-Shirazi et al. [60] (Figs. 1 and 2), ©1989 American Chemical Society.

phases. It is an important property of the first order PDE [Eq. (16)]. Another important property, as seen in Fig. 3, is that the front of the elution profiles of both components are concentration discontinuities. A negative discontinuity in the elution profile of the first component takes place at the same time as a positive discontinuity of the concentration profile of the second component. This is the displacement effect of the first component by the second one [42]. This effect explains why the separation achieved is often better than the chromatographer used to linear separations would tend to suppose. A second effect is observed in Fig. 3, particularly in Fig. 3b. The eluent concentration of the second component remains constant for a certain time after completion of the elution of the first component. This is because the velocity associated with a concentration of the pure second component is always lower than the limit at infinite dilution of the first component of the velocity associated with the same concentration of second component but in the presence of the first one. This effect was named the tag-along effect [42]. It explains the difficulties encountered in the purification of a compound from later eluted impurities.

Fig. 4 illustrates the agreement between the profile predicted by the ideal model and experimental data. It is excellent for the prediction of band profiles needed for optimization purposes. Nevertheless, it is not good enough for the solution of the inverse problem. Note that the profiles were calculated assuming that the competitive adsorption of the two components follows Langmuir isotherm behavior. Although the adsorption of each component is well accounted for by the Langmuir model, this assumption usually introduces a certain amount of model error [2].

2.4.3. Algebraic solutions of the lumped kinetic model

A particular case of this model is the Thomas

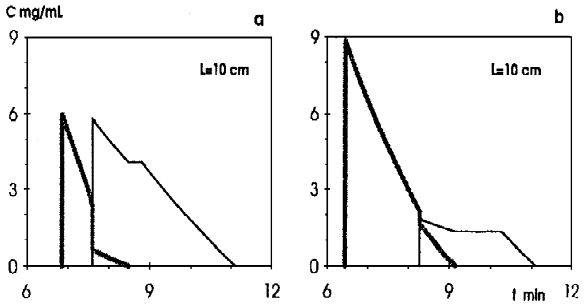

Fig. 3. Schematics of the solution of the ideal model in the case of two components and competitive Langmuir isotherms. Individual elution profiles of the two components of a binary mixture for a wide rectangular injection pulse. Thick solid line, first component; thin solid line, second component; left (a), 1:3 mixture; right (b), 3:1 mixture. Conditions for the calculations: phase ratio: $F=0.25$; column length 10 cm, diameter 0.46 cm; flow-rate: $F_v=1$ ml/min; separation factor $\alpha=1.2$; retention factor: $k'_{0,1}=6.0$; other isotherm coefficients: $b_1=6$; $b_2=7.2$; sample size: 66.7 μmol; injection volume: 2.5 ml. Reproduced with permission from S. Guiochon, S.G. Shirazi and A.M. Katti, ''Fundamentals of Preparative and Nonlinear Chromatography,'' 1994, p. 270 (Fig. 8.5), Academic Press, Boston, MA, USA.

model which assumes that the lumped mass transfer kinetics is given by the Langmuir model (desorption rate proportional to the concentration in the adsorbed phase, adsorption rate proportional to the difference between monolayer capacity and concentration in the adsorbed phase). In this case, the isotherm is also given by the Langmuir model. Then, the model has an analytical solution for the elution problem [43–45]. This solution, however, has not proven to be practical. It needs Bessel functions in its formulation and the numerical calculation of these functions is neither convenient nor fast.

The algebraic solution of the lumped kinetic models for the boundary conditions corresponding to the breakthrough curves is of far greater importance. In this case, the mathematical problem is considerably simpler because the concentration increases (or decreases) uniformly from a constant state (initial condition, $C=C_i^0$) to a constant final state ($C=C_f^0$) [46,47]. Rhee and Amundson [46,47] showed that, under non-ideal conditions, with a positively curved isotherm (i.e. an isotherm that is convex upward), the breakthrough curve moves along the column at the velocity of the shock, u_s [Eq. (17)], independently of the column efficiency (as long as it is not very

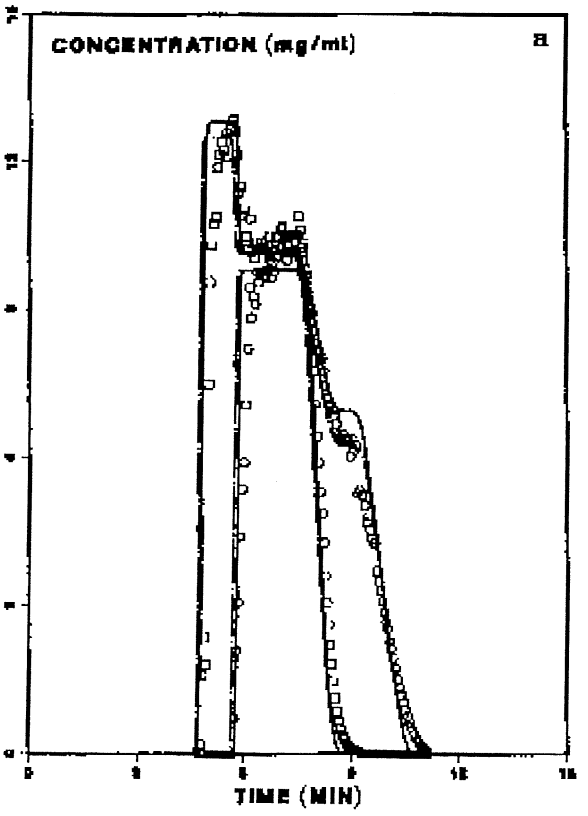

Fig. 4. Comparison between experimental and calculated band profiles for a wide pulse injection. Individual elution profiles of 2-phenylethanol (□) and 3-phenylpropanol (○) in a 1:1 mixture. Profiles calculated assuming competitive Langmuir isotherms with numerical parameters derived from the single component isotherms measured by frontal analysis (solid lines) [89] (Fig. 7). Reproduced by permission of The American Institute of Chemical Engineers. ©1990 AIChE. All rights reserved.

low). They derived an algebraic solution that relates the shock layer thickness (SLT) and the column efficiency. The SLT is approximately proportional to the column HETP. Zhu et al. [48,49] have shown that, in the case of a Langmuir isotherm, the solution of Rhee and Amundson becomes a simple algebraic equation. This establishes a simple relationship between the slope of the inflection tangent to the breakthrough curve and the rate coefficient of the lumped kinetic model. This approach was used to acquire simultaneously adsorption equilibrium and kinetic data by recording breakthrough curves and to investigate the mass transfer kinetics [20,29,53].

As explained in the previous section, in the case of

a Langmuirian isotherm, high concentrations move faster than low concentrations, hence the front of a breakthrough curve is self-sharpening. However, for an actual column, axial dispersion and the finite rate of the mass transfer kinetics tend to broaden the band. For a sharp front, the concentration gradient is steep. Dispersion becomes fast. Even rapid, mass transfer kinetics can hardly lead to equilibrium between the two phases when the concentration gradient is steep. Band broadening tends to become more intense when the band front becomes sharper and a steady-state is reached when the band propagates without changing shape in a coordinate reference system moving at the velocity of the band [54]. This is the concept of shock layer, also found in aerodynamic (the PDE of chromatography is the same as that of aerodynamic [2]). Rhee and Amundson [46] defined the shock layer thickness as the length distance between the two points where the concentrations are the fractions $1-\theta$ and θ of the amplitude of the breakthrough curve (Fig. 5). The value of $\theta=0.02$ is usually chosen. If we assume a transport–dispersive model in which axial dispersion arises from axial diffusion and eddy dispersion and the mass transfer resistance from a finite rate coefficient of mass transfer between the mobile and the stationary phase, we obtain [46]:

$$\Delta x = \frac{1+K}{K}\left(\frac{D_a}{u}+\frac{Ku}{(1+K)^2 k_f}\right) \times \frac{bC_0+2}{bC_0}\ln\left|\frac{1-\theta}{\theta}\right| \quad (22)$$

where Δx is the SLT, D_a the dispersion coefficient, k_f the lumped rate coefficient, b the second coefficient of the Langmuir isotherm and $K=k_0'/(1+b\ C_0)$. A detailed study of the property of the SLT allowed a realistic estimate of the performance limits of displacement chromatography [49].

Fig. 6 compares a staircase series of experimental breakthrough curves with the curve calculated [38]. Note that the isotherm of the compound studied is S-shaped, which explains why the first few ascending steps are diffuse while the last descending steps are self-sharpening. Again, the agreement is excellent.

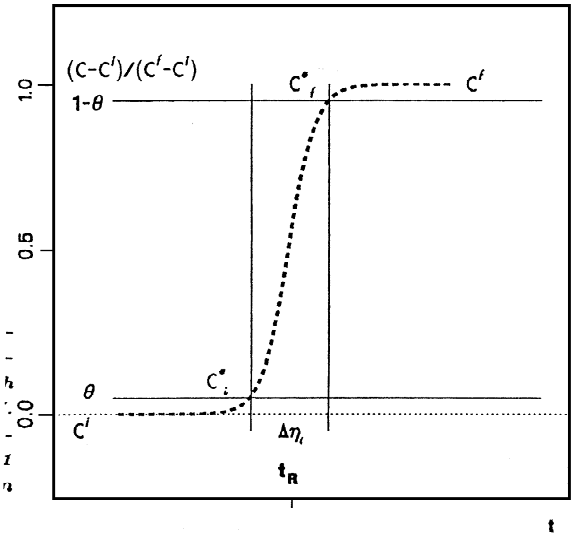

Fig. 5. Schematic of the shock layer profile illustrating the definition of the shock layer thickness [45–49]. Reprinted with permission from Zhu et al. [48] (Fig. 1). ©1993, American Chemical Society.

2.4.4. Numerical solutions of the equilibrium–dispersive model

As explained earlier, the non-ideal behavior of actual columns originates in the axial dispersion and the finite rate of mass transfer between the two phases of the chromatographic system. The former is due to a combination of the axial diffusion in the complex network of anastomized pores through which the mobile phase percolates along the bed and of the effects of the complex velocity distributions in these channels [2,16]. It is accounted for in most models of chromatography by either a coefficient of axial dispersion or by Giddings' coupled term [16] that takes into account the combination of eddy dispersion and radial diffusion in the eluent stream.

Formally, the equilibrium–dispersive model can be presented as a model in which we assume that the rate of mass transfer between the phases of the system is infinite and we account for its effects on the axial dispersion of the front and rear of the band profile by no longer using the coefficient of axial dispersion but an apparent or lumped dispersion coefficient related to the column HETP by:

$$D_L = \frac{\sigma^2}{2t} = \frac{Hu}{2} \quad (23)$$

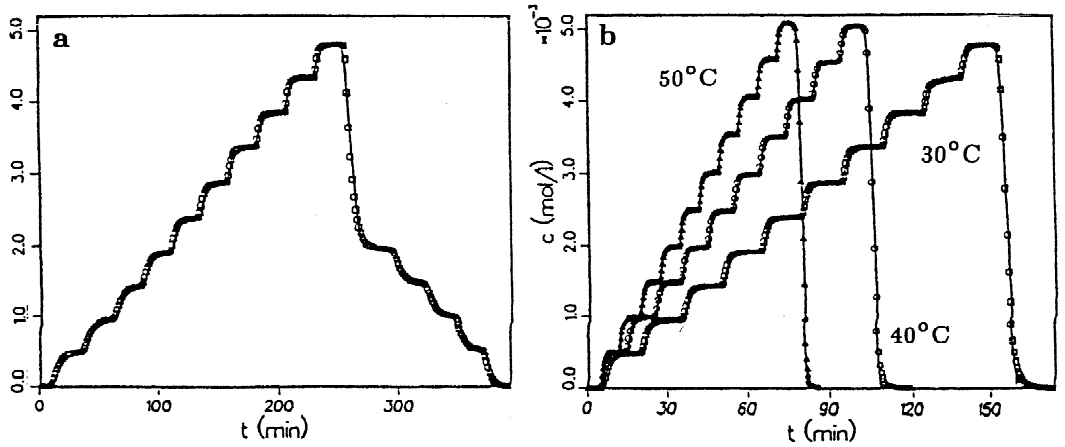

Fig. 6. Comparison between experimental data (symbols) and a breakthrough curve calculated with the equilibrium–dispersive model. Staircase frontal analysis of (+)- and (−)- Tr\"oger's base. (a) (+) enantiomer, 30 °C, positive and negative steps. Column: 0.46×10 cm, packed with 15–25-μm microcrystalline cellulose triacetate; porosity, 0.602; $F_v = 0.5$ ml/min, mobile phase 100% ethanol. Reproduced with permission from Seidel-Morgenstern et al. [38] (Figs. 3, 8 and 9).

where σ is the variance of the band under linear conditions [2]. However, the model is actually based on a demonstration by Giddings [16] that, provided that the column efficiency is sufficiently high, as it is in most current applications of HPLC, there is an equivalence between the band broadening effects of a finite rate of mass transfer and those of axial dispersion. This work was based on the demonstration by van Deemter et al. [12] that, if the mass transfer kinetics is fast enough, the band profile obtained as a response to the injection of an infinitely narrow rectangular pulse injection is a Gaussian-shaped band with a standard deviation related to the column HETP by $\sigma^2 = HL$. This work provided also the van Deemter equation, the first HETP equation (Section 2.5.4).

When applying the equilibrium–dispersive model to non-linear chromatography problems, it is assumed that the apparent axial dispersion coefficient is independent of the concentration. This is reasonable because, in this model, the influence of the finite column efficiency is considered to be small and is treated as a correction, the non-linear thermodynamics of phase equilibrium being the essential cause of the shape of the band profiles observed. The correction accounts for the difference between the profiles predicted by the ideal model and those obtained with actual columns that have a finite efficiency. All investigations have proven the validity of the method, as long as the efficiency is high and the mass transfer kinetics is fast [2].

Under non-linear conditions, however, there are no analytical solutions for the equilibrium–dispersive model. Solutions must be calculated numerically. Several different approaches have been proposed, using finite differences [6], collation on finite elements [55] or more advanced algorithms [56]. Among the possible calculation schemes based on finite differences, the forward–backward scheme proposed by Rouchon et al. [57] has the great advantage that the calculation of a mass balance in each differential slice of the column is not necessary to obtain the new values of the solid and liquid concentrations, dramatically cutting the run time. Furthermore, the proper selection of the values of the integration elements allows the use of numerical dispersion to simulate the effects of the actual, physical dispersion and to account for the effects of a finite column efficiency [2,6]. This correspondence is exact under linear conditions, not under non-linear conditions [58], but the approximation remains acceptable in almost all cases, at least for single component profiles [2]. For binary mixtures, there may be, in some cases, a small but noticeable

difference between profiles calculated using the Rouchon method and those obtained with a finite element program [59].

Possibly the most fundamental result of our investigations of the equilibrium–dispersion model was the introduction of the Shirazi number, m [2,60,61]. This number is defined by:

$$m = N\left(\frac{k'_0}{1+k'_0}\right)^2 L_f = N\left(\frac{k'_0}{1+k'_0}\right)^2 \frac{nb}{\varepsilon_T SLk'_0} \qquad (24)$$

where N is the column efficiency, L its length, S its cross-section area, ϵ_T its total porosity, n the amount of compound injected, b the second coefficient of the Langmuir isotherm (in such units that the product nb is a volume), k'_0 the retention factor of the compound at infinite dilution, and L_f the column loading factor. The later is the sample size expressed as the fractional amount of a monolayer. It is given by the following equation:

$$L_f = \frac{n}{(1-\varepsilon_T)SLq_s} = \frac{nb}{\varepsilon_T SLk'_0} \qquad (24a)$$

Thus, the Shirazi number is a reduced sample size. Its value indicates the degree of closeness of the actual band profile to the one predicted by the ideal model. In practice, k'_0 is between 1 and 10, so the term $[k'_0(1+k'_0)]^2$ is between 0.25 and 0.83. This range is too wide for this term to be neglected when estimating the Shirazi number or in its discussion. When $m > 50$, there is a very good agreement between actual profiles and those predicted by the ideal model, but for a finite slope of the very steep front observed. The agreement is reasonable for values larger than 20–30. Below 10, it rapidly becomes very poor.

The same numerical methods apply to the calculation of the individual band profiles for multicomponent mixtures [2]. As for single components, an excellent agreement is observed between experimental profiles and profiles calculated with the equilibrium–dispersive model in a wide range of experimental conditions, including concentrations, as long as the column efficiency is good. When the mass transfer kinetics is slow, more complex models are required. Although the lumped kinetic model is often considered in the literature as an acceptable approach to tackle problems in which the mass transfer kinetics is moderately slow, e.g. in enantioseparations or in the elution of proteins in ion-exchange chromatography, recent results have shown that this is not true [21]. The more complex pore model or general rate model should be considered when the mass transfer kinetics is slow and the equilibrium–dispersive model fails.

Figs. 7–9 compare experimental data obtained in various separations, under widely different sets of conditions (gradient elution (Fig. 7), system peaks (Fig. 8) and SMB (Fig. 9)), with the band profiles calculated using the equilibrium–dispersive model. The agreement is generally excellent. Exceptions to this rule are found when the kinetics of mass transfer is slow, e.g. in the separation of proteins. In this case, the general rate model gives excellent results [21].

2.5. Perturbation theories—from linear to nonlinear chromatography

Most chromatographers are essentially analysts and are quite familiar with linear chromatography. Many models have been proposed for this particular case and many have solutions. These solutions are identical, for all practical purposes when the column efficiency exceeds 50–100 theoretical plates [2,61]. A variety of solutions were derived for the general rate model in linear chromatography and are reviewed in detail elsewhere [2]. An ingenious solution of the general rate model in the linear case, due to Carta [62], is worth noting. The model assumes that identical injections are performed periodically. However, the period can be chosen as long as needed for the concentration of the compound in the effluent to become practically negligible at the end of the period. Solutions of two simpler models are more interesting for our purpose here. First is the solution of the equilibrium–dispersive model which introduced the Gaussian profile into chromatography. Second, of great seminal importance, is the solution that was derived by Lapidus and Amundson for the lumped kinetic model [11]. Later, van Deemter et al. [12] showed that this rather formidable solution simplifies into a Gaussian profile when the mass transfer kinetics is reasonably fast. This later work is

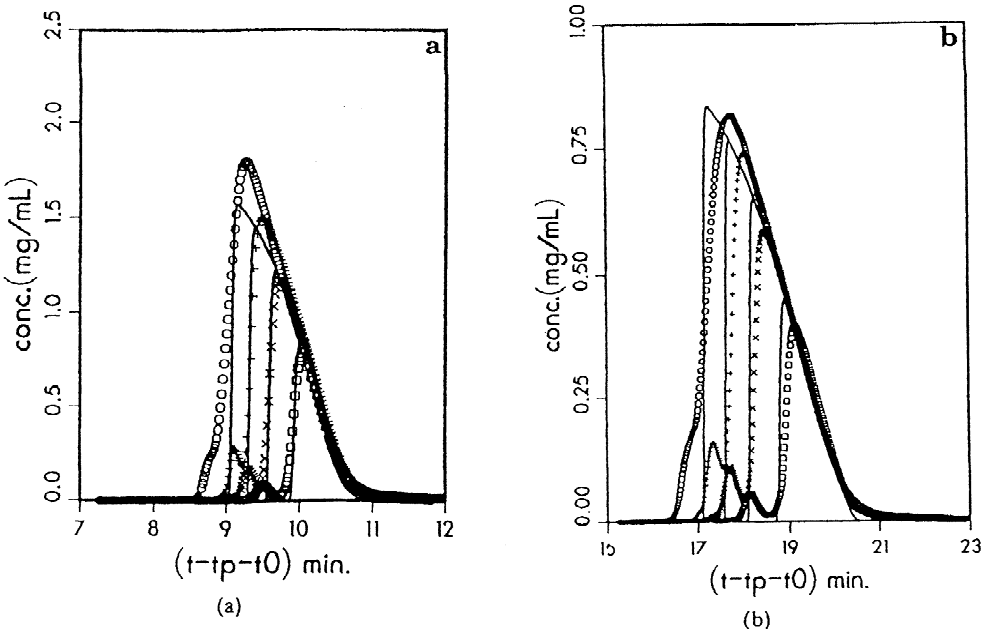

Fig. 7. Comparison between experimental data (symbols) and chromatograms (solid lines) calculated with the equilibrium–dispersive model. Increasing sample sizes of lyzozyme on a Vydac C_{18} column. Sample size: □, 1 ml (0.40 mg, $L_f = 2.9\%$); ×, 2 ml (0.81 mg, $L_f = 5.8\%$); +, 3 ml (1.21 mg, $L_f = 8.7\%$); and ○, 4 ml (1.62 mg, $L_f = 11.6\%$). Solution concentration: 0.405 mg/ml. Mobile phase composition: initial acetonitrile/water, 25:75 (0.1% TFA); gradient rate, (a) 1% ACN/min, (b) 0.5% ACN/min. Reproduced with permission from M.Z. El Fallah and G. Guiochon, Biotechnol. Bioeng. 39 (1992) 877 (Fig. 6).

the theoretical basis of all work done later on the HETP equations.

Numerous attempts were made to extend toward high concentrations some of these solutions. This approach is often considered in the physical sciences and is known as the perturbation method. It often supplies interesting results. It turned out, however, that the results that it gives in chromatography are not acceptable in a wide enough concentration range to satisfy the needs of the modeling of preparative chromatography. This is because perturbation methods cannot take into account the strong concentration dependence of the velocity associated with a concentration.

2.5.1. Solution of the equilibrium–dispersive model in linear chromatography

The simplest solution of this model assumes that a Dirac pulse injection is made at point $z = 0$, in an infinitely long column stretching from $-\infty$ to $+\infty$. The solution was derived long ago by Lapidus and Amundson [11]:

$$C_d(z_d, t_d) = \sqrt{\frac{N}{2\pi t_d}}\, e^{-\frac{N}{2t_d}(z_d - t_d)^2} \quad \text{with} \quad z_d = \frac{z}{L} \quad \text{and} \quad t_d = \frac{t}{t_R} \tag{25}$$

Thus, the concentration profile along the column ($t_d = \text{constant}$) is Gaussian while the elution profile ($z_d = \text{const}$) is not. It may surprise analytical chemists to know that solutions that are formally very different (and no longer Gaussian) are obtained for seemingly nearly identical problems of a Dirac injection made in space or for columns extending from only $z = 0$ (where the injection is done) to $z = +\infty$ or from $z = 0$ to $z = L$. This has to be so because the mathematical problems are different. It is reassuring, however, that the differences between these profiles under any set of realistic conditions are negligible [2].

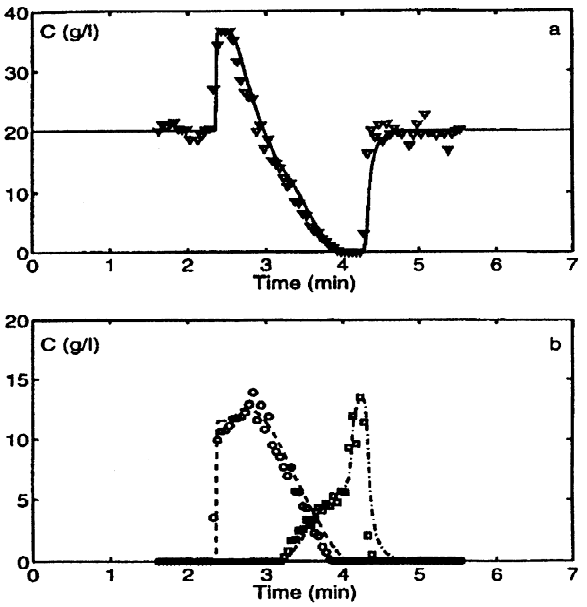

Fig. 8. Comparison between experimental data (symbols) and chromatograms (solid lines) calculated with the equilibrium–dispersive model. Band profiles corresponding to the additive system peaks (a) and solute peaks (b) corresponding to the separation of a binary solute mixture of benzyl alcohol (BA) and 2-phenylethanol (PE) (2:1, w/w) with a total solute concentration of 30 g/l. The mobile phase contains 20 g/l of 2-methylbenzyl alcohol (MBA) in a mixture of methanol and water (1:1, v/v). Flow rate, 0.8 ml/min, volume injected, 0.5 ml. Symbols: BA (○ and dashed line), PE (□ and dash-dotted line), and MBA (△ and solid line). Reproduced with permission from Quiñones et al. [15] (Fig. 12), ©2000 American Chemical Society.

2.5.2. Solution of the transport–dispersive model in linear chromatography

The equation system of the lumped kinetic model in its transport–equilibrium version:

$$u\frac{\partial C}{\partial x}+\frac{\partial C}{\partial t}+F\frac{\partial q}{\partial t}=D\frac{\partial^2 C}{\partial x^2}$$
$$\frac{\partial q}{\partial t}=-k_f[q-f(C)] \qquad (26)$$

where $f(C)$ is the isotherm, with $f(C)=aC$ in the case of a linear isotherm. This system of PDE is easy to solved in the Laplace domain. The difficulty, as always with this method, is in calculating the inverse of the solution, in order to return with it from the Laplace domain into the real domain. Lapidus and Amundson [11] have discussed this inversion in detail, for both the breakthrough curve and the elution profile. It would not be useful to reproduce their solution that is formidable in its complexity. It includes a Bessel modified integral of the first kind and of order 1, a function known to be difficult and long to calculate numerically, so the calculation of a profile in a specific case takes longer if it is done from the analytical solution than by numerical integration of the PDE system in Eq. (26).

Nevertheless, this solution has great seminal importance because it was at the origin of the two most fruitful lines of investigations in fundamental chromatography that developed since its publication. First, van Deemter et al. [12] showed that this solution can be simplified into a Gaussian profile whenever the column efficiency is not very small, a case that includes all the practical applications of analytical and preparative chromatography. From this work arose the entire field of HETP investigations. Second, we observe that, for all practical purposes, the important results are the values of the parameters that define the peak position, its width, and its asymmetry. This information is easily derived, at least in linear chromatography, from the values of the peak moments, values that can be measured from the elution curve or derived from the solution of the Laplace transform. Thus was moment analysis of band profiles generated.

2.5.3. The HETP equations

The original van Deemter equation [12] was:

$$H=\frac{2D_L}{u}+2\left(\frac{k_0'}{1+k_0'}\right)^2\frac{u}{k_0'k_m} \qquad (27)$$

where k is the lumped rate coefficient of the mass transfer kinetics. This equation became popular in chromatography under the conventional form:

$$H=A+\frac{B}{u}+Cu \qquad (28)$$

and, later, in HPLC, under the name of the Knox equation [63]:

$$H=\frac{B}{u}+Au^{1/3}+Cu \qquad (29)$$

where A, B, and C are empirical coefficients. These last two equations are purely empirical and their coefficients have no real physical meaning. They are

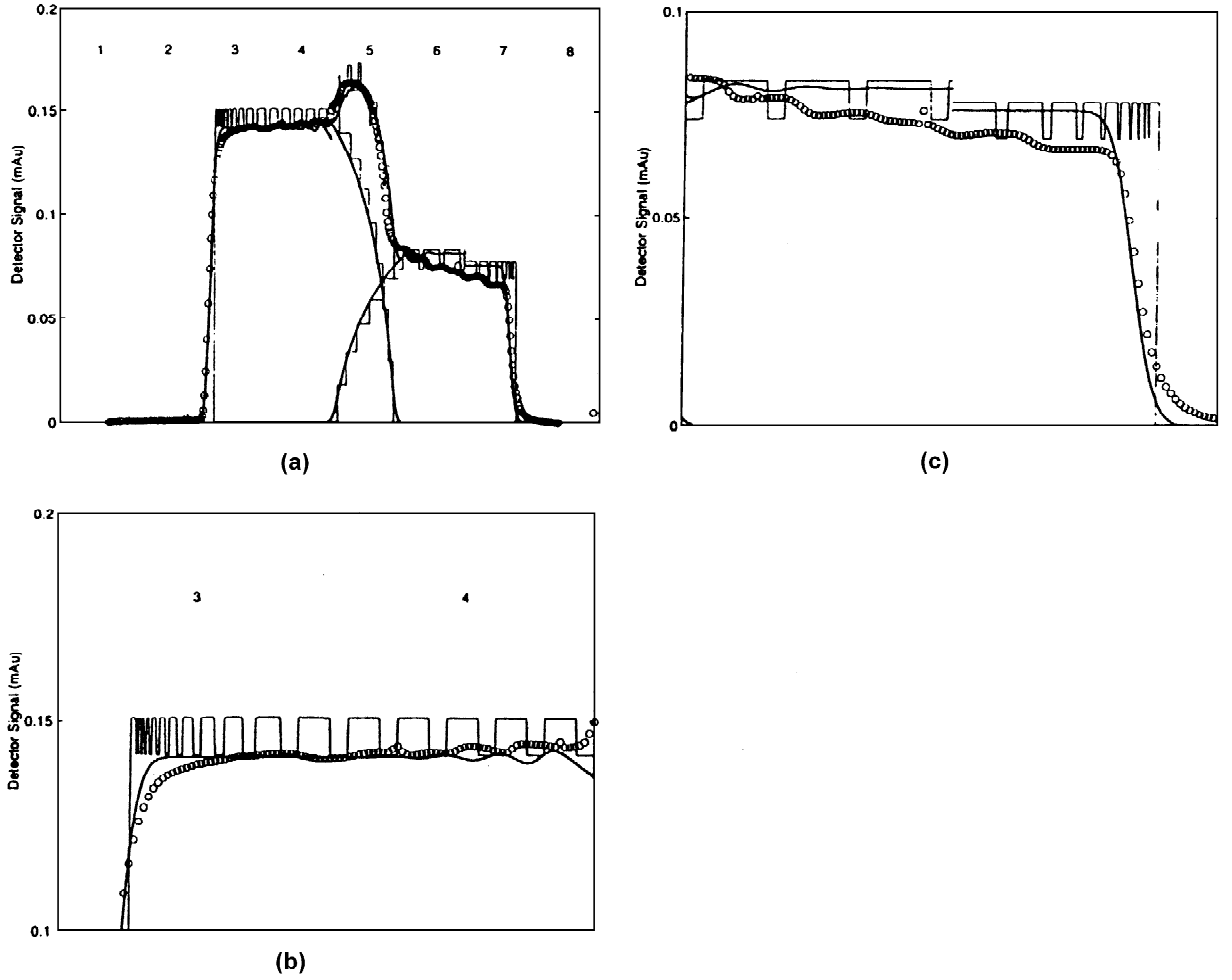

Fig. 9. Schematics of the SMB principle.

not predictable. Somewhere, analysts lost touch with theoreticians and stopped paying attention to the actual meaning of the van Deemter equation. Admittedly, it is difficult to measure by non-chromatographic methods the various parameters of the original van Deemter equations or those of the far more complex equations derived from a moment analysis of the general rate model:

$$H = \frac{2D_L}{u} + 2\left(\frac{k_1}{1+k_1}\right)^2 \times \left[\frac{ud_p^2}{60FD_p} + \frac{ud_p}{6Fk_f} + \left(\frac{k_p}{1+k_p}\right)^2 \frac{u}{Fk_{ads}}\right] \quad (30)$$

or those contained in more advanced models integrating, for example, the contribution of surface diffusion [64]. In non-linear chromatography, however, the very concept of HETP is not useful. The numerical calculations of band profiles using the equilibrium–dispersive model requires the HETP under linear conditions. Its mobile phase dependence is useful for optimization studies. Detailed investigations of the dependence of the HETP of a phase system on the different parameters involved supply information on the nature and relative intensity of the corresponding phenomena and should help is selecting the major contributions to use in applying the general rate model to a specific case.

We have so far neglected the extra-column contributions to the plate height that originate from the injector, the connecting tubes and the detector. These contributions must be properly accounted for. We neglected also the influence of the deviation of the packed bed from homogeneity. Experimentally, it was shown that the column efficiency measured by classical chromatographic methods (i.e. from the standard deviation of the distribution of the residence time of the sample molecules in the column) is twice as large as the efficiency derived from the apparent dispersion of the molecules measured by NMR during a short period of time [65]. Theoretical investigations have shown that this effect of a radial distribution of the bed properties is difficult to correct [66–68]. The plate height derived by Giddings [16] is written:

$$H = \frac{2\gamma D_m}{u} + \Sigma \frac{\lambda}{1 + \omega u^{-1}} + Cu \tag{31}$$

where γ, λ, and ω are geometrical parameters of the bed and the packing material that are also related to the size and internal porosity of the particles. The number of parameters in the proper combination of Eqs. (30) and (31) is staggering, making is extremely difficult to determine them by model identification. This is why HETP investigations have lead to relatively little progress in the detailed understanding of the numerous phenomena involved in the broadening of chromatographic bands.

2.5.4. Perturbation solutions

As shown earlier, there are algebraic solutions for only two chromatographic models, the linear non-ideal model and the non-linear ideal model. Thus, it is tempting to use one of the classical perturbation methods available and to apply it to either of these two solutions and extend this solution into the non-ideal, non-linear range. The solutions derived by Houghton [69] or by Haarhoff and Van der Linde [70] that are discussed in the next section are examples of perturbation solutions of the linear, non-ideal model using a two-term expansion of the isotherm. These solutions account for what happens when the concentration of the injected band is such that the equilibrium isotherm is moderately non-linear. Their study informs us on what happens at the onset of column overloading. In contrast, the perturbation of the ideal, non-linear model, the solution of which contains a shock or discontinuity (Section 2.4.1), is entirely different from that of a continuous solution, such as that of the non-ideal linear model. The mathematical methods of perturbation to be used are different. The shock layer theory is, in fact, a perturbation solution of the ideal model. The limit of the shock layer at zero dispersion coefficient is a true concentration shock or discontinuity. In the shock layer, the diffusion term of the mass balance PDE is very large and the effect of diffusion on the profile is considerable. It replaces the shock, that is infinitely narrow, with a shock layer of finite thickness.

If the deviation of the isotherm from linear behavior is moderate and if the mass transfer kinetics is reasonably fast, a perturbation solution may be found. This is a classical approach in the physics or physical chemistry of non-linear phenomena. The kinetic equation in Eq. (26) can be rewritten:

$$q = f(C) - \frac{1}{k_f}\frac{\partial q}{\partial t} \tag{32}$$

When the rate coefficient increases indefinitely, the second term of the RHS of the equation tends toward 0. The zeroth and first order approximation are thus:

$$q = f(C)$$
$$q = f(C) - \frac{1}{k_f}\frac{\partial f(C)}{\partial t} \tag{33}$$

Since we are investigating the consequences of moderate deviations of the isotherm from linear behavior, we consider a two term expansion of the isotherm:

$$f(C) = aC + a_2C^2 \tag{34}$$

where a and a_2 are constant (and with $a_2C << a$). Combining these equations gives:

$$u\frac{\partial C}{\partial x} + \frac{\partial C}{\partial t} - D\frac{\partial^2 C}{\partial x^2} + Fa_1\frac{\partial C}{\partial t} + Fa_2\frac{\partial C^2}{\partial t^2} - \frac{Fa_1}{k_f}\frac{\partial^2 C}{\partial t^2} - \frac{Fa_2}{k_f}\frac{\partial^2 C^2}{\partial t^2} = 0 \tag{35}$$

Neglecting the last term, that is a second order term, and simplifying, we obtain:

$$u\frac{\partial C}{\partial x} + (1 + Fa_1)\frac{\partial C}{\partial t} - D\frac{\partial^2 C}{\partial x^2} + Fa^2\frac{\partial C^2}{\partial t} - \frac{Fa_1}{k_f}\frac{\partial^2 C}{\partial t^2} = 0 \quad (36)$$

The solution of this equation is a concentration distribution, $C(x, t)$, along the column length that is a function of time, i.e. migrates along the column. It can be written as the sum of two terms

$$C = C_l + C_p \quad (37)$$

where C_l is the solution of the linear, zero-order, approximation, i.e. the solution of Eq. (26) with $q = FaC$, and C_p is a perturbation term which tends toward 0 with a_2C^2. Combination of these equations gives a PDE that allows the determination of C_p [56]. Although this calculation is simpler than that of C_l, the complexity of the linear solution (Section 2.5.2) precludes its practical use.

2.5.5. Approximate analytical solutions of the equilibrium–dispersive model under non-linear conditions

Two approximate solutions of the equilibrium dispersive model in the case of a parabolic isotherm are well known, those of Houghton [69] and of Haarhoff and Van der Linde [70]. The second of these solutions is better than the first one. It conserves the peak area, i.e. the mass of component contained in the band. Accordingly, we will restrict this discussion to this second solution. As long as the concentrations considered are low enough and the equilibrium isotherm does not deviate much from linear behavior, it is legitimate to replace the isotherm by its second order Taylor expansion around the origin:

$$q = aC(1 - bC) \quad (38)$$

where a and b are the coefficients of a classical Langmuir isotherm. Introduced in the mass balance equation, this expression gives a PDE which has no solutions. It was simplified by replacing some terms that are functions of the concentration by their value derived from the ideal model, for the same isotherm [71]. A solution correct at the first order, hence conserving mass is obtained with:

$$X = \left| \frac{e^{-\tau^2/2}}{\sqrt{2\pi}\coth m + erf\dfrac{\tau}{\sqrt{2}}} \right| \quad (39)$$

$$X = bC\frac{k_0'}{1 + k_0'}\sqrt{N}$$
$$\tau = \sqrt{N}\frac{k_0'}{1 + k_0'}\frac{t_{R,0} - t}{t_{R,0} - t_0} \quad (40)$$
$$m = N\left[\frac{k_0'}{1 + k_0'}\right]^2 L_f$$

(see Eq. (24) for the definition of L_f). In spite of the simplifications made in the calculations, the profile obtained is essentially correct when the product $bC_{Max} < 0.05$ (C_{Max} is the maximum concentration in the band) and is reasonably close to the correct one when $bC_{Max} < 0.1$ [70,71]. Note that preparative liquid chromatography is often carried out with values of bC_{Max} exceeding 0.20–0.30. Note that the main deviations between correct and approximate solutions arise from the incorrect approach used to solve the problem (perturbation method), not from the simplification made when replacing the true isotherm [Eq. (1)] by a parabolic one [Eq. (38)].

There is an interesting relationship between the retention time of the maximum of the band (t_M) and its concentration (C_{Max}) [2]:

$$t_M = t_{R,0} - 2bC_{Max}(t_{R,0} - t_0) \quad (41)$$

This equation allows the derivation of the maximum concentration for which the isotherm behavior can be considered as linear, taking as definition of linear behavior a change in retention that is insignificant within the precision of the measurements.

2.5.6. Empirical perturbation solutions

Many chromatographers, interested in preparative applications, studied in the late 1980s the dependence of the band broadening on the sample size. Interpreting as a dispersive contribution to band broadening what is fundamentally a thermodynamic effect can only lead to empirical investigations. Never in the history of chromatography were the viewpoints of the leading analysts more at odds with those of the chemical engineers. Unfortunately, if the second were right, their solution was far heavier and

more complex than the one the former were hoping for.

Careful experiments may lead to useful empirical correlations and interesting insights, provided the investigators keep in mind that, although the most efficient columns are the first to exhibit significant band broadening when the sample size is increased, they always remain the most efficient. Usually, the conventional expression relating plate number and band width can be applied to this study [72], with the new definition:

$$H = \frac{L}{N_{ap}} = \frac{L}{16}\left(\frac{w_{1/2}}{t_R}\right)^2 \quad (42)$$

where N_{ap}, t_R and $W_{1/2}$ are the apparent plate number (under load), the retention time of the band maximum and the baseline bandwidth, respectively. These three parameters are functions of the sample size. Poppe and Kraak [72] showed even that the apparent plate number is a function of the loading factor, m [Eq. (40c)] and is given by:

$$N_{ap} = Nf(m) = Nf\left(N, \left[\frac{k'_0}{1+k'_0}\right]^2, L_f\right) \quad (43)$$

This equation was also used by Knox and Pyper [73] and by Eble et al. [74]. It derives directly from the Haarhoff–Van der Linde equation [70]. Unfortunately, there is no similar equation for the apparent retention factor. Eble et al. [74] suggested an equation similar to Eq. (42):

$$k' = k'^0\, g(\sqrt{m}) \quad (44)$$

However, this relation is empirical and is not equivalent to Eq. (41). It is valid only in the same range as the Haarhoff–Van der Linde equation, for very low values of the loading factor. The solution cannot be obtained simply by applying axial dispersion to the profile since dispersion broadens the profile but, as a consequence, also makes it shorter. There is no easy way to derive the new height [73]. Besides, experiments show that the profiles become strongly unsymmetrical with increasing sample size and that their retention times usually decrease. The suggestion to use Eq. (41) [75] did not attract attention and was not pursued by their authors. The approach by Knox and Pyper [73], based on the rule of variance addition, had a similar fate. It is valid only at very low concentrations since, under non-linear conditions, the convolution of the thermodynamic band profile by apparent axial dispersion is shift-variant [76].

Nevertheless, in the range of low concentrations within which the onset of column overloading takes place and the band profiles begin to be affected by the sample size, we may assume the rule of variance addition, and hence write that the HETP is given by the sum of two contributions, one of thermodynamic, the other of kinetic origin. The second one is derived from the column efficiency at infinite dilution. The first one may be calculated from the band profile given by the analytical solution of the ideal model [2]. The result of the exercise is merely a more precise form of Eq. (42) [2]. The work of Eble et al. [74,77–80] was carried out using an algorithm based on the Craig model and the Langmuir isotherm to calculate the band profiles. Accordingly, the profiles obtained were relatively accurate numerical solutions of the equilibrium–dispersive model, calculated with the backward–forward finite difference scheme [2,81]. These authors proceeded then to derive empirical correlations for these correct profiles, correlations that were satisfactory at low concentrations but could not hold at higher concentrations because the strong influence of the curvature of the equilibrium isotherm, an influence that has no equivalent in other fields of chromatography, was largely overlooked. Furthermore, this empirical model encountered serious difficulties in accounting for the influence of isotherm competition on the individual band profiles [78–80]. An approach that could have been useful if developed 10 years earlier when the derivation of numerical solutions of the simplest model of non-linear chromatography was impractical had to be abandoned.

2.6. Optimization problems

The literature on optimization contains numerous reports on empirical investigations and a few theoretical studies. Usually, the former discuss too few specific cases to allow for the derivation of reasonably broad rules. Only theoretical discussions can place such empirical observations into proper perspective. Thus, the systematic use of computer programs implementing the different models of

chromatography greatly facilitates theoretical investigations of optimization problems. In practice, to apply this method to the determination of the optimum conditions for the separation of a specific feed and the production of a certain purified compound, the relevant physico–chemical data must be acquired in the range of conditions (e.g. temperature, flow-rate) that are considered to be practical. Depending on the rate of the mass transfers, the proper model must be chosen, following simple rules [21,28]. Thus, this approach is heavy and expensive and cannot be used for the optimization of separations made at the laboratory scale. For obvious reasons, few actual examples of application of this powerful approach are available in the literature.

Any optimization problem begins by the selection of the objective function. The best objective function in industrial preparative chromatography would obviously be the product cost. However, reasonable estimates of the cost figures are usually not available for fundamental or general investigations. The objective function generally used in academic studies is the production rate [2]. Maximizing it is realistic only in those cases in which capital costs largely dominate the product costs. In practice, the operation costs, particularly those of the solvent used and of the wasted feedstock (recycling it is a possible option that is not easy to implement), often account for a large fraction of the production costs. Felinger proposed as objective function the product of the production rate and the recovery yield and showed that, in practice, the use of this function avoids extreme ways of maximizing the production rate and delivers optimum conditions for which the recovery yield remains reasonable and the production rate is not far below its absolute maximum [82].

Separations carried out in the laboratory, in the course of an investigation program, usually require the rapid development of separation schemes which are easy to implement rapidly. The time spent by chemists to develop it is usually the main cost factor. Empirical rules are useful. Calculations requiring the prior acquisition of a large amount of data are often ruled out. In contrast, industrial scale preparative chromatography requires careful minimization of the costs but is performed under conditions that allow more time and greater means to develop the exact process.

2.6.1. Optimization of industrial-scale separations

A systematic approach is recommended to optimize industrial separations. It involves the following steps that are justified elsewhere [2].

(1) First, the best combination of a stationary phase and a mobile phase solution must be selected. Systematic experiments are required to compare the packing materials which are commercially available in sufficient amounts. (Serious difficulties may be encountered when several batch of a ''designer'' stationary phase must be combined to pack a large column [83].) The separation factor must be large and the retention factors relatively small [82,84]. More importantly still, the feedstock must be sufficiently soluble in the mobile phase and the saturation capacity of the adsorbent must be large. These last two factors are usually of little concern in the development of an analytical separation. They are of primary concern for the economics of a preparative separation.

(2) Accurate measurements of the physico-chemical parameters of the required separation in the phase system selected are necessary to determine satisfactory estimates of the optimum experimental conditions. This includes the measurement of equilibrium isotherm data, the selection of the proper competitive isotherm model, the derivation of accurate estimates of its parameters for all the feed components of importance, and the measurement of the parameters that characterize the mass transfer kinetics. Depending on the specifics of the case, the latter include the column efficiency as a function of the mobile phase velocity (if the equilibrium–dispersive model can be applied) or other mass transfer coefficients, e.g. pore diffusion, and the viscosity of the feed solutions in the mobile phase because the inlet pressure at constant flow-rate depends on this viscosity and may vary markedly during a cycle [85]. Caution is necessary to extrapolate to large size columns the results of measurements carried out on analytical size columns. The influence of a difference in the packing density of the columns used in the laboratory and the plant is often overlooked, with costly consequences.

(3) Once this information is available, a computer program can be used to derive the optimum experimental conditions for the required production, using the proper objective function and taking into

account the important cost components, the required purity, and the various constraints (e.g. minimum yield, maximum inlet pressure). The cost of various economic strategies can be easily compared [86].

(4) Finally, the results of these calculations must be validated, by making an appropriate series of experiments, preferably at the pilot scale.

2.6.2. Optimization of laboratory-scale separations

In the laboratory, separations are usually carried out at a much lower scale than in production, a short development time is of the essence, and manpower is the main if not the essential cost factor. So, the determination of the parameters of the thermodynamics and the kinetics of the feed separation cannot be as comprehensive as for an industrial separation. The potential cost savings would not justify such an approach. Nevertheless, some measurements are required, e.g. to select the best phase system. A systematic approach, applying some simple rules, may save much time. The following set of practical rules appears as a good compromise between the opposite requirements of a fundamental and a practical approach.

(1) The search for the best chromatographic system remains a necessary first step. This system should give: (i) the maximum possible resolution between the critical feed components, i.e. the component of interest and its nearest neighbors; (ii) a retention factor for the first component not exceeding 2; (iii) a reasonable or large saturation capacity for the main components of the feedstock; and (iv) a good solubility of the feed in the mobile phase. This last condition is critical, but is not always possible to satisfy. A good example of the difficulties in finding a ''good'' solvent of the feed and of the potential pay-off is to be found in the case of the purification of the fullerene C_{60} [87]. It is important that the search for the best chromatographic system be limited to those phases that can be used in preparative applications. The rest of the development work should be done with a sample of the phase which has been selected (including same particle size).

(2) A second step is the optimization of the column length and the particle size for maximum production rate. In practice, there is an optimum for the ratio d_p^2/L but no separate optima for L and d_p [2]. A satisfactory approximation of the optimum ratio d_p^2/L is given by the equation below. It was derived assuming a simple plate height equation and neglecting the effects of adsorption competition between the feed components:

$$\left(\frac{d_p^2}{L}\right)_{opt} = \frac{1}{4}\frac{\alpha-1}{\alpha}\frac{k'_{0,2}}{1+k'_{0,2}}\sqrt{\frac{D_m\eta}{3k_0 c\,\Delta P}} \tag{45}$$

where $k'_{0,2}$ is the retention factor of the second component of the main pair, ΔP is the maximum pressure at which the equipment available can be operated safely on a routine basis, and c is the numerical coefficient of the third term in Knox plate height equation [Eq. (29)]. Thus, the most convenient average particle size can be chosen among the different grades available for the selected stationary phase. Then, the column of optimum length can be packed or a commercial column having a value of d_p^2/L close to the optimum can be selected.

(3) After the column is equilibrated with the mobile phase, the flow-rate is optimized by injecting small samples and increasing the flow-rate until the resolution obtained is approximately equal to 1.7–2 if the separation must be carried out under touching bands condition (i.e. with a 100% recovery yield), or a resolution near unity if the separation is to be done under overlapping bands condition (which gives a higher production rate at the cost of a yield loss).

(4) An estimate of the optimum sample size is given by the following equation giving the loading factor in the case of a touching bands strategy:

$$L_{f,2,t} = \frac{(\alpha-1)^2}{\alpha^2(1+r)} \quad \text{with} \quad r = \frac{L_{f,1}}{\alpha L_{f,2}} \tag{46}$$

(where α is the separation factor of the two components and $L_{f,i}$ their loading factor, Eq. (24a)) and by a similar but more complex equation in the case of overlapping bands strategy [2]. The derivation of these equations is based on the assumption of Langmuir adsorption behavior. In the many cases in which deviation from this behavior is modest, the best approach for the rapid determination of sufficiently precise estimates of these parameters is the retention time method [88].

Experiments carried out with an analytical column having the optimum geometry permit an empirical determination of the optimum sample size for either

approach and a fine tuning of the optimum conditions.

In analytical applications, the injection bandwidth is usually tiny compared to that of the elution peak, so the shape of the injection profile is not of great concern, as long as it does not tail badly. In all preparative applications, however, wide injections, as wide or wider than the peaks at infinite dilution, are often made. The injection profiles at the column inlet should be close to rectangular. Otherwise, serious differences may arise between calculated and recorded band profiles [89,90]. Extra-column band broadening often causes yield losses or affects the purity of the product. The location of the cut points is also changed. A well-designed system can avoid such effects and permits a more economical process. This observation applies also to SMB [91].

The procedure just described usually gives rapid and excellent results. The production rate may often be increased further, if needed, by increasing the sample size, reducing the retention factor, and increasing the mobile phase velocity. This requires further experiments. Examples of applications of the method were provided by Newburger et al. [92,93].

3. Instruments and practical issues

The instrumentation and methodology used in preparative liquid chromatography has not change much in the last 10 years. In contrast, the quality of the packing materials has markedly improved. Compressed bed columns have become widely accepted as giving highly reproducible results but we still do not fully understand the compression process [94]. Radial and annular compression have nearly disappeared in favor of axial compression, although the properties of radially compressed columns have not been as seriously investigated as those of axially compressed columns. Investigations on the behavior of particle beds under mechanical stress have provided useful information on what is actually happening during the packing of a chromatographic column [94–96]. However, these clues have not yet led to significant progress in column efficiency. In the same field, the use of expanded beds has attracted interest. This new technique allows percolation, without fouling the column, of the raw feedstocks from the biotechnology industry, feedstocks containing rather large amounts of cell debris in suspension. This change in the conventional flow-sheet allows a rapid pre-clean-up of the effluent of the fermentors and the extraction of a coarse fraction containing the compounds of interest, for further processing [97].

Most implementations of preparative chromatography use overloaded elution. This batch process consists in periodically injecting a large amount of feedstock and in collecting purified fractions. Its advantage is its simplicity, its disadvantages are that it is a dilution process and that it forces a compromise between production rate and recovery yield. A mixed fraction is collected that must be discarded or recycled. In spite of much effort by Horváth and his group [98–101], displacement chromatography has been unable to get a foothold. In this process, a stream of a solution of a strongly adsorbed compound, the displacer, flushes the column after injection of the feed. With this method, a larger feed amount can be injected and the collected fractions are more concentrated than with elution. However, the need to regenerate the column lengthens the batch period, mixed fractions are also collected, and the need to use a displacer causes additional problems with a regulatory aspect that is often hard to contemplate. Various recycling procedures have been investigated extensively for over 30 years [102,103]. It does not seem, however, that their major advantage, the elimination of the waste of the intermediate, mixed fraction at practically no production rate cost can compensate for the disadvantages of their higher cost and complexity, at least in the mind of the users. In contrast, the simulated moving bed process (SMB) has become the process of choice for the separation and purification of enantiomers from a feed composed of the racemic mixture or resulting from an incompletely selective stereochemical synthesis [104]. While very high enantiomeric purities cannot be achieved economically with SMB, this process appears as a key intermediate for the production of drug intermediates. Its detailed review, however, is beyond the scope of this work.

3.1. Axial, radial, and annular dynamic compression columns

It has long been recognized that packing large diameter columns using the traditional procedures requires extreme means [105] or generates beds that

are unstable in the long run, causing severe losses of performance in operation and requiring difficult and costly interventions. The need to prepare stable beds has led to the development of several compression processes. The bed can be compressed axially [106], as in a syringe, radially [107], by using a plastic or rubber wall column contained in a steel chamber and keeping a fluid under pressure between these two walls, or in an annular fashion [108] by moving a conical rod along the axis of the bed. Little experience is available with this third approach. As far as we know, it was used mainly for laboratory scale applications and few units were sold before its manufacture stopped. The axial compression process dominates the market. Columns with diameters ranging between ~2.5 and 80 cm (at least) are available commercially. On the other hand, the radial compression process has declined to almost nothing, both at the laboratory and the preparative level, largely because it has not been promoted.

Detailed investigations of the properties of dynamic axial [109–111] and radial [112–114] compression columns were published. The goal of these methods is the preparation of homogeneous beds. They succeed in preparing stable, dense beds that give columns with good efficiencies [109–114]. However, these beds are not homogeneous (see Section 3.2). The friction of the bed against the column wall during the axial compression causes the packing density to be higher against the wall than in the core region. Similarly, because mechanical stress does not convey homogeneously in particulate beds, radial compression also gives beds that are denser against the wall than in the core region. The packing density also varies along the column, in the axial directions. However, the effects on the column efficiency of a systematic variation of the packing density in the axial direction are much less important than those of its systematic variation in the radial direction.

3.2. Properties of column beds

Column beds are not homogeneous. They cannot be. There are no processes in a column that can let particles move from the position that they occupy at the end of the packing process, as illustrated in Table 1. The potential energy of a particle occupying a metastable position in a column bed is several orders of magnitude larger than its thermal energy ($kT = 6.1 \times 10^{-14}$ erg). These two energies become comparable only for a value of $\Delta h = 10$ nm, more than two orders of magnitude smaller than the particle diameter. Certainly, in a free suspension, given the buoyancy of the solvent, particles of this size scatter and their density decreases by a factor 2 for an increase in altitude of ~0 μm. However, in a densely packed bed, a particle would need a certain activation energy to change position, i.e. to diffuse. It cannot find this energy and remains stuck forever at the same position, unless a catastrophic event changes the bed structure [94,95,111]. These events take place during periods of consolidation, when a mechanical stress is applied to the bed [95].

So, not only particles do not migrate inside packed beds, but most heterogeneity in the particle distribution tends to last indefinitely. This is certainly true for heterogeneities in the radial distribution of the particle size. Dry packing methods tend to segregate large particles close to the wall, fine ones in the center of the bed. This was at the origin of the poor efficiency observed for the first wide-diameter columns packed for preparative gas chromatography [115]. Drastic methods of packing were developed to remedy these effects [105,116]. This phenomenon does not take place to a great extent in the slurry packing process used in the preparation of columns for liquid chromatography. On the contrary, the packing density of the columns is found to be higher close to the column wall and lower in the core region. The extent of the radial variation of the mobile phase velocity across the column may be of several percent in good columns, corresponding to a radial variation of their packing density of the order of 1%. Various attempts at altering the classical methods of column preparation have not produced any significant improvements in these performances [94]. However, NMR studies of the axial dispersion in chromatographic beds have shown that radial heterogeneity of the distribution of the mobile phase velocity contributes to half the band broadening in excellent columns, probably much more in fair ones [117,118].

It was shown that the source of a radial distribution of the packing density of column beds is the friction of this bed along the wall and the differential

Table 1
Properties of particles of packing materials

Particle diameter (μm)	10	5	3
Particle volume ($\pi d^3/6$) (pl)	0.52	6.5×10^{-2}	1.4×10^{-2}
Particle mass[a] (ng)	0.81	0.10	0.022
Potential energy of one particle for $\Delta h = 1$ μm (erg)	7.9×10^{-11}	9.7×10^{-12}	2.2×10^{-12}
Thermal energy of one particle (kT) at 293 K (erg)[b]	6.1×10^{-14}	6.1×10^{-14}	6.1×10^{-14}
Ratio potential/thermal energy	1300	160	36
Number of particles in 1 g of packing material (billions)	1.25	10	45

The ratio of the potential to the thermal energy precludes the possibility of any significant movement of one particle in respect of its neighbors. It could be argued that the buoyancy of the particles in the mobile phase and the Brownian motion could combine and help the particles in moving around. This does not happen. Even in a dilute slurry (in which the interactions between particles are negligible and their relative migration easy), the extent of Brownian motion is nil. The ratio of the equilibrium concentrations of 3-μm particles in a dilute slurry, 1 μm above the bottom of the flask and at its bottom is [14]

$$K = e^{\frac{F_p \Delta x}{kT}}$$

where F is the force acting on the particles (their weight minus their buoyancy), $\Delta x = 1$ μm, k is Boltzman constant and T the temperature (K). With the numbers above, the apparent particle weight is 8 pg, assuming a density of the slurry solvent of 1, and we obtain $\log K = -12.9$, a negligible ratio.

[a] Assuming $\epsilon_i = 0.40$ and the density of silica, $\rho = 2.6$.

[b] $3/2\ kT$.

migration of the particles in the direction parallel to the column axis in the last instants of the bed consolidation, whether under axial mechanical stress (axial compression columns) or under hydrostatic stress (slurry packing) [95,96]. It does not seem, however, that packing materials that have higher friction coefficients with stainless steel give lower column efficiencies [96]. Although the properties and the behavior of column beds are beginning to be better understood, we still have to translate this new knowledge into improved methods for the packing of more efficient columns.

3.3. Properties of expanded beds

One of the important applications of preparative liquid chromatography is the extraction of recombinant proteins from cell cultures and their preparation. The first step of the recovery of the proteins consists of lysing the cells and separating the cell debris from the desired protein. The careful clean-up by filtration, centrifugation, and/or other methods of the effluents of these units is required prior to introducing them into conventional chromatography columns, otherwise, the suspended cell debris would rapidly clog these columns. Anspach et al. [97] have shown that expanded beds can be used to trap proteins from fermentation broths or cell homogenates. These beds are obtained by filling a column with the appropriate suspension of packing material and not closing it with a top frit that constrains the bed and prevents it from moving upward when the mobile phase begins to percolate through it. The expansion of the bed by a factor 2–3 is allowed. Provided that the velocity of the stream of mobile phase percolating through the bed is such that the settling velocity of the particulates in suspension in the feedstock is much lower than that of the packing particles, the proteins are adsorbed in the bed and the cells or debris in suspension in the feed leave the column.

When the mobile phase velocity increases, the bed expands and the pressure drop required to achieve the increasing flow-rate increases less rapidly and tends toward a limit corresponding to the expanded

bed state [97]. The extent of the transition range depends on the particle size distribution of the packing material used. A maximum velocity is determined by the settling velocity of the packing particles and the operation must be carried out in a range of flow-rate which ensures bed expansion while avoiding fluidization. This method should find applications in the recovery of recombinant proteins.

3.4. Recycling processes in preparative chromatography

Since the beginning of preparative chromatography, when gas chromatography was the main method, recycling schemes have attracted attention [102,103]. Initially, the main advantage of the method appeared to be that it allowed the achievement of difficult separations while using poorly efficient columns. Column efficiency increased considerably in the last 30 years and preparative columns are as efficient as their analytical relatives. More recently, the principle has attracted interest for other reasons, to increase the production rate and to reduce or eliminate the loss of feed. In the common process of overloaded elution, it is not possible to approach the maximum production rate and, in the same time, to experience a high recovery yield. Mixed zones form before and after the band of the compound to be purified, containing the bands of its impurities. These zones must be either collected for further processing or discarded. However, the collected mixed zones have a composition that is different from that of the feed. Discarding this product is costly and the resulting yield loss may be quite significant. Reprocessing it is also costly because this separation is different from that of the initial feed and it must be optimized separately, resulting in two choices between discarding the new mixed products or reprocessing them again.

Recycling procedures may achieve production rates comparable to those obtained under straightforward conditions and eliminate the yield loss. Several strategies have been developed and compared [119–123]. Some consist in injecting a feed sample, shaving fractions containing the interfering components at the end of the column and recycling the core. The sample size decreases but the separation improves and the purity of the product increases at each cycle but the production rate decreases with increasing number of cycles. Alternately, purified fractions are collected and a new feed sample is injected after each cycle. The band profiles, the amount of feed in the system, and the amounts of purified fractions collected after each cycle change progressively, until steady-state is achieved, much as in SMB. Under favorable conditions, there are no yield losses and the production rate is competitive with that obtained under conventional conditions [124]. The process could be attractive in the extraction and/or purification of a major component from a complex feed or when several impurities eluted both before and after the main component must be eliminated, cases that are not conducive to the most effective use of SMB.

Belonging to the same type of approach as recycling, is the intriguing concept of flip-flop chromatography. Two independent reports [125,126] were published with a 20-year interval, developing what is essentially the same approach but with different goals, the first one [125] in analytical chromatography, attempting to concentrate trace impurities, the second one [126] in preparative chromatography, aiming at shortening the cycle time. In both cases, flow reversal takes place before the complete elution of the feed components has been achieved. In the first case, no component is let out of the column but a second sample is injected. A progressive increase of the concentrations of the various components in the column takes place. Trace components are ultimately eluted at the end of the operation, by eluting the whole column content. The main problem appears to originate in the non-linear behavior of the isotherms of the main components of the feed. This would limit the extent of feed concentration that can be achieved. In the second approach, flow reversal takes place later, after the end of the elution and the collection of the purified compound, just at the time of the second cut-point. Then, column backflush takes place for a certain time after which a second feed sample is injected. The delay is adjusted so that the compound of interest begins to elute (i.e. the first cut-point takes place) just after the elution of its retained impurities contained in the previous injection and which were backflushed after the flow reversal. Reductions in cycle time and hence com-

parable increases in the production rate of 40% were documented [126].

In conclusion, it appears that there are many possibilities of manipulating the parameters of preparative chromatography in the elution mode that could be used to markedly improve its production rate. Unfortunately, chemical engineers are extremely reluctant at using transient processes, even in the simplest cases such as conventional elution chromatography, and are obsessed with the use of steady-state processes. The non-linear behavior of chromatography complicates still further its implementation. The use of complex cycles, even when the proper use of computers allows the straightforward control of the operations, is rarely considered.

3.5. Simulated moving bed chromatography (SMB)

There are books [127] and numerous reviews dedicated to SMB [128–131]. This process has acquired considerable importance in recent years because it is an excellent implementation of chromatography for a general application of great economic importance, the preparation of purified enantiomers of drug intermediates [104]. SMB is a continuous process, which greatly pleases chemical engineers and is one of the important reasons for its success. Liquid–liquid countercurrent separations have been known for a long time and are widely used, e.g. for metal extraction. The attempts at countercurrent solid–liquid separations or countercurrent gas or liquid chromatography faltered on the practical impossibilities of properly flowing the solid phase. Broughton [132,133] developed a process replacing the continuous movement of the solid phase by an abrupt, step movement of finite length obtained by valve switching. The column used is divided into a number (usually eight to 16) of identical columns that are arranged in four successive sections (which can have the same or a different number of columns). The liquid phase and the feed are injected between the first and fourth section and between the second and third section, respectively. Streams of pure products (those migrating with the liquid and with the solid phase, respectively) exit between the first and second section and between the third and fourth section, respectively (Fig. 10). The movement of the solid phase is simulated by periodically switching the positions between two successive columns where these different external streams connect with the column train [131].

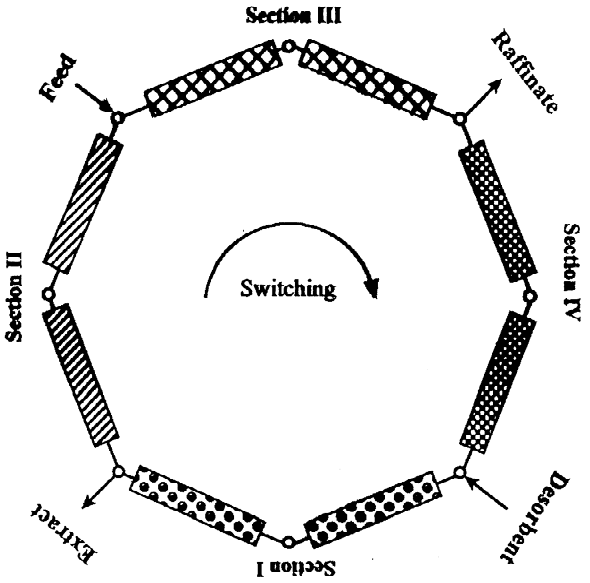

Fig. 10. Comparison between experimental data (symbols) and concentration profiles (lines) calculated with the equilibrium–dispersive model. Concentration profiles at the intermediate nodes of an eight-column simulated moving bed separator. Thick solid lines, detector signal derived from the calculated concentration profiles and the detector response factors (in panel 3, the hypothetical detector trace for the individual compounds is also shown). Thin solid lines, same but profiles calculated with the algebraic solution of the ideal model. Experimental conditions: average isotherm coefficients, $a_1 = 1.40$, $a_2 = 2.40$; safety factor, $\beta = 1.12$; column porosity, $\epsilon_T = 0.57$; cycle time, $t^* = 6.31$ min; column flow-rates: in section I, $Q_I = 2.10$ ml/min; in section II, $Q_{II} = 1.52$ ml/min; in section III $Q_{III} = 1.82$ ml/min; in section IV, $Q_{IV} = 1.35$ ml/min; feed concentrations, 0.112 mg/ml of 2-phenylethanol and 0.56 mg/ml of 3-phenyl-propanol. (a) Comparison of the elution profiles from all four positions. (b) Expansion of the concentration shoulder of the extract in panel 2. (c) Expansion of the concentration shoulder of the raffinate in panel 4 [141]. Reproduced by permission of The American Institute of Chemical Engineers. ©1997 AIChE. All rights reserved.

Earlier implementations of SMB were large separation units used in the food and petrochemical industries [133]. They used phase systems that operated under nearly linear behavior of the equilibrium isotherm (e.g. sugars on ion-exchange resins). Recently, however, a new field of applications has appeared, the industrial separation of enantiomers,

using chiral stationary phases [104]. The major drawback of SMB as an implementation of HPLC is that it can easily deliver only two streams of products. This is not a drawback in the purification of enantiomers. On the other hand, because of the limited saturation capacity of chiral phases, these separations must be carried out under non-linear conditions. The development of a new separation by SMB that is relatively simple under linear conditions becomes much more complex and so is the start-up operation. Because the velocity associated with a concentration (see Section 2.4.1, Eq. (17)) is a function of this concentration, the zone in the column train where the concentration fronts of the two components oscillate during a cycle of the SMB vary. The velocity usually increases with increasing concentration. The switching period must be adjusted accordingly. An improved understanding of the SMB behavior is acquired by careful consideration of the effects of the different parameters on the band profiles along the columns of the SMB and of the concentration histories at its two outputs. The use of modeling is absolutely necessary to succeed in this matter.

SMB has been the object of intense theoretical investigations. Its operation under linear conditions was studied by Ruthven et al. [128,129], by Wankat [130], and by the group of Mazzotti, Morbidelli and Storti [134–139] who also investigated the optimization of the experimental parameters. It was reviewed by Zhong and Guiochon [131]. An interesting algebraic solution of the linear ideal model [140] allows a prediction of SMB results that is very close to the experimental results at high concentrations, even with the columns of mediocre efficiency that are commonly used in SMB [141]. Later, the successful modeling of SMB under non-linear conditions has had major consequences on the development of the process in the development and operation of which it has played a major role [22,138,139,142–145]. Among many others, an example of the use of SMB modeling under non-linear conditions can be found in Ref. [146].

4. Conclusions

Preparative liquid chromatography 15 years ago was an unchartered field offering alluring perspectives and numerous fundamental and practical difficulties. It has now become a well known, useful technique, that is applied to the preparation of numerous purified compounds, mainly in the pharmaceutical industry and in other areas of the life science businesses. Meetings dedicated to this area of chromatography attract a large attendance, a sure sign of maturity [147]. The process is now well understood, as demonstrated by its successful modeling under a wide variety of experimental conditions and the excellent agreement observed between the model predictions and the experimental results [2,146]. The development of new separations can be considerably helped by the use of various computer programs. Now, the theory of chromatography is essentially complete. The important unsolved issues that remain in our field are actually upstream. They are: (i) in the thermodynamics of equilibrium:

- how can we rapidly and accurately measure equilibrium isotherms?
- how can we simply and accurately model these isotherms?
- how can we use molecular modeling to calculate these isotherms?
- more specifically, how can we predict the competitive isotherms of for example two enantiomers on a chiral phase

and (ii) in the mass transfer kinetics across the bed of a chromatographic column:

- what are the key parameters controlling diffusion through a particle?
- how can we accelerate this rate?

The necessary instrumentation is available to perform preparative chromatography at about any scale required by industry. Efficient columns can be readily packed. Studies on the rheology of particulate beds suggest that higher column efficiencies, probably 30–50% higher than those currently achieved under favorable circumstances and possibly twice as large, could be obtained. However, improved packing methods using our better understanding of the behavior of column beds have yet to be developed. Recent results suggest that a dramatic reduction in the reduced HETP of packed columns is a definite possibility [149]. We may regret the relative lack of competition between possible packing techniques and between the manufacturers of column skids.

Packing materials with a wide variety of properties can easily be procured from numerous sources. It is often possible to find a solid phase that, combined with a suitable solution, gives a sufficient separation factor between the components of the feed that need to be resolved. However, the dependence of the production rate of the separation factor is so large that a small improvement can translate into a meaningful gain. The main practical difficulties reside in the relative lack of rules for the rapid selection of a chiral phase suitable for the separation of a given pair of enantiomers and in the problems associated with the packing of a homogeneous bed with certain designer stationary phases that are produced in batches smaller than the column to be packed.

A variety of implementations are available, from straightforward overloaded elution to SMB. Their most important advantages and disadvantages relative to other possible implementations and to other separation methods are well known. The reasons to chose one or the other are clear. Modeling can be done under any set of experimental conditions, provided the physico-chemical parameters of the thermodynamics and kinetics of the separation considered are known accurately. Using available models, the design and operation parameters can be optimized and the production costs calculated. At this stage, the only missing link seems to be a careful comparison of the performance achieved with several different implementations (e.g. overloaded elution, recycling, SMB) under carefully optimized conditions, conditions that will be markedly different for each implementation.

So, we should now recognize the intellectual pioneers who began the adventure, Poppe and Kraak [72], Knox and Pyper [73], Snyder and his associates [74,77–80], those who knew the powerful ability of chromatography at separating closely related compounds and its extreme flexibility that allows its use to separate nearly all binary possible mixtures. The critical concepts of nonlinear chromatography were unfortunately foreign to them. After the work of Wicke [4,5], DeVault [10], Wilson [9], Lapidus and Amundson [11], van Deemter et al. [12], Rhee and Amudson [46,47,50], and Helfferich and Klein [148], chemical engineers knew nearly all about non-linear chromatography that was needed to implement it on a large scale. By inventing the SMB, Broughton and Gerhold [132] had demonstrated the possibility of a process based on a refined implementation of linear chromatography and applied on a grand scale. Engineers, however, considered chromatography as an adsorption process of little interest for industrial applications and, with a few remarkable exceptions, they neglect it to this day.

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References

[1] M.S. Tswett, Tr. Protok, Varshav. Obshch. Estestvoistpyt., Otd. Biol. 14 (1903, publ. 1905) 20. Reprinted and translated in G. Hesse, H. Weil, Michael Tswett's erste chromatographische Schrift, Woelm, Eschwegen, 1954.
[2] G. Guiochon, S.G. Shirazi, A.M. Katti, Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.
[3] G.S. Bohart, E.Q. Adams, J. Am. Chem. Soc. 42 (1920) 523.
[4] E. Wicke, Kolloid Z. 86 (1939) 295.
[5] E. Wicke, Kolloid Z. 90 (1940) 156.
[6] G. Guiochon, S. Golshan-Shirazi, A. Jaulmes, Anal. Chem. 60 (1988) 1856.
[7] A.J.P. Martin, R.L.M. Synge, Biochem. J. 35 (1941) 1358.
[8] L.C. Craig, J. Biol. Chem. 155 (1944) 519.
[9] J.N. Wilson, J. Am. Chem. Soc. 62 (1940) 1583.
[10] D. DeVault, J. Am. Chem. Soc. 65 (1943) 532.
[11] L. Lapidus, N.L. Amundson, J. Phys. Chem. 56 (1952) 984.
[12] J.J. van Deemter, F.J. Zuiderweg, A. Klinkenberg, Chem. Eng. Sci. 5 (1956) 271.
[13] J.C. Giddings, H. Eyring, J. Phys. Chem. 59 (1955) 416.
[14] J.C. Giddings, Unified Separation Science, Wiley, New York, 1990.
[15] I. Quiñones, J.C. Ford, G. Guiochon, Anal. Chem. 72 (2000) 1495.
[16] J.C. Giddings, Dynamics of Chromatography, Marcel Dekker, New York, 1960.
[17] D.A. McQuarrie, J. Chem. Phys. 38 (1963) 437.
[18] F. Dondi, M. Remelli, J. Phys. Chem. 90 (1986) 1885.
[19] F. Dondi, P. Munari, M. Remelli, A. Cavazzini, Anal. Chem. 72 (2000) 4353.
[20] P. Sajonz, H. Guan-Sajonz, G. Zhong, G. Guiochon, Biotechnol. Prog. 13 (1997) 170.
[21] K. Kaczmarski, D. Antos, H. Sajonz, P. Sajonz, G. Guiochon, J. Chromatogr. A 925 (2001) 1.

[22] M. Morbidelli, A. Servida, G. Storti, S. Carra, Ind. Eng. Chem. Fundam. 21 (1982) 123.
[23] M. Morbidelli, G. Storti, S. Carra, G. Niederjaufner, A. Pontoglio, Chem. Eng. Sci. 39 (1984) 383.
[24] I. Quiñones, G. Guiochon, Langmuir 12 (1996) 5433.
[25] I. Quiñones, G. Guiochon, J. Chromatogr. A 796 (1998) 15.
[26] K. Kaczmarski, D. Antos, J. Chromatogr. A 862 (1999) 1.
[27] E. sz Kovats, in: F. Bruner (Ed.), The Science of Chromatography, Elsevier, Amsterdam, 1990, p. 205.
[28] K. Kaczmarski, D. Antos, J. Chromatogr. A 756 (1996) 73.
[29] P. Sajonz, H. Guan-Sajonz, G. Zhong, G. Guiochon, Biotechnol. Prog. 12 (1997) 170.
[30] K. Miyabe, G. Guiochon, Biotechnol. Prog. 15 (1999) 740.
[31] K. Miyabe, G. Guiochon, J. Chromatogr. A 866 (2000) 147.
[32] M. Friedrich, A. Seidel, D. Gelbin, Chem. Eng. Process. 24 (1988) 33.
[33] K. Lederer, I. Amtmann, S. Vijaykumar, J. Billiani, J. Liq. Chromatogr. 13 (1990) 1849.
[34] D. Leaist, L. Hao, J. Chem. Soc., Faraday Trans. 89 (1993) 2775.
[35] S. Gibbs, A. Chu, E.N. Lightfoot, T. Root, J. Phys. Chem. 95 (1991) 467.
[36] B. Al-Duri, G. McKay, J. Chem. Tech. Biotechnol. V 55 (1992) 245.
[37] W. Gallagher, C. Woodward, Biopolymers 28 (1989) 2001.
[38] A. Seidel-Morgernstern, S.C. Jacobson, G. Guiochon, J. Chromatogr. 637 (1993) 19.
[39] F. James, Sur la modélisation mathématique des équilibres diphasiques et des colonnes de chromatographie, Ph.D. dissertation, Ecole Polytechnique, Palaiseau, France, 1990.
[40] S. Golshan-Shirazi, G. Guiochon, Anal. Chem. 60 (1988) 2364.
[41] S. Golshan-Shirazi, G. Guiochon, J. Phys. Chem. 94 (1990) 495.
[42] S. Golshan-Shirazi, G. Guiochon, J. Phys. Chem. 93 (1989) 4143.
[43] S. Goldstein, Proc. R. Soc. Lond. A219 (1953) 151.
[44] F.H. Arnold, H.W. Blanch, J. Chromatogr. 355 (1986) 13.
[45] J.L. Wade, A.F. Bergold, P.W. Carr, Anal. Chem. 59 (1987) 1286.
[46] H.-K. Rhee, B.F. Bodin, N.R. Amundson, Chem. Eng. Sci. 26 (1971) 1571.
[47] H.-K. Rhee, N.R. Amundson, Chem. Eng. Sci. 27 (1972) 199.
[48] J. Zhu, Z. Ma, G. Guiochon, Biotechnol. Prog. 9 (1993) 421.
[49] J. Zhu, G. Guiochon, AIChE J. 41 (1995) 45.
[50] H.-K. Rhee, R. Aris, N.R. Amundson, First-Order Partial Differential Equations. II—Theory and Application of Hyperbolic Systems of Quasilinear Equations, Prentice Hall, Englewood Cliffs, NJ, 1989.
[51] E. Cremer, G.H. Huber, Angew. Chem. 73 (1961) 461.
[52] H. Guan, B.J. Stanley, G. Guiochon, J. Chromatogr. A 659 (1994) 27.
[53] G. Zhong, P. Sajonz, G. Guiochon, Ind. Eng. Chem. Res. 36 (1997) 506.
[54] B.-C. Lin, S. Golshan-Shirazi, Z. Ma, G. Guiochon, Anal. Chem. 61 (1988) 2647.
[55] Z. Ma, G. Guiochon, Comput. Chem. Eng. 15 (1991) 415.
[56] B. Lin, G. Guiochon, Mathematics of Chromatography (in press).
[57] P. Rouchon, M. Schonauer, P. Valentin, G. Guiochon, Sep. Sci. Technol. 22 (1987) 1793.
[58] B. Lin, G. Guiochon, Sep. Sci. Technol. 24 (1988) 31.
[59] A.M. Katti, M. Czok, G. Guiochon, J. Chromatogr. 556 (1991) 205.
[60] S. Golshan-Shirazi, G. Guiochon, Anal. Chem. 61 (1989) 462.
[61] S. Golshan-Shirazi, G. Guiochon, J. Chromatogr. 506 (1990) 495.
[62] G. Carta, Chem. Eng. Sci. 43 (1988) 2877.
[63] J.H. Knox, M. Saleem, J. Chromatogr. Sci. 7 (1969) 614.
[64] K. Miyabe, G. Guiochon, Adv. Chromatogr. 40 (2000) 1.
[65] U. Tallarek, K. Albert, E. Bayer, G. Guiochon, AIChE J. 42 (1996) 3041.
[66] K. Miyabe, G. Guiochon, J. Chromatogr. A 830 (1999) 29.
[67] K. Miyabe, G. Guiochon, J. Chromatogr. A 830 (1999) 263.
[68] K. Miyabe, G. Guiochon, J. Chromatogr. A 857 (1999) 69.
[69] G.J. Houghton, J. Phys. Chem. 67 (1963) 84.
[70] P.C. Haarhoff, H.J. Van der Linde, Anal. Chem. 38 (1966) 573.
[71] S. Golshan-Shirazi, G. Guiochon, J. Chromatogr. 506 (1990) 495.
[72] H. Poppe, J.C. Kraak, J. Chromatogr. 255 (1983) 395.
[73] J.H. Knox, H.M. Pyper, J. Chromatogr. 363 (1986) 1.
[74] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 384 (1987) 25.
[75] A. Jaulmes, C. Vidal-Madjar, A. Ladurelli, G. Guiochon, J. Phys. Chem. 88 (1984) 5379.
[76] E.V. Dose, G. Guiochon, Anal. Chem. 62 (1990) 1723.
[77] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 384 (1987) 45.
[78] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 405 (1987) 1.
[79] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 405 (1987) 31.
[80] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 405 (1987) 51.
[81] M. Czok, G. Guiochon, Anal. Chem. 62 (1990) 189.
[82] A. Felinger, G. Guiochon, J. Chromatogr. A 796 (1998) 59.
[83] M.S. Smith, G. Guiochon, J. Chromatogr. A 827 (1998) 241.
[84] A. Felinger, G. Guiochon, Biotechnol. Bioeng. 41 (1993) 134.
[85] A. Felinger, G. Guiochon, Biotechnol. Prog. 9 (1993) 450.
[86] A. Felinger, G. Guiochon, AIChE J. 40 (1994) 594.
[87] M. Kele, R.N. Compton, G. Guiochon, J. Chromatogr. A 786 (1997) 31.
[88] S. Golshan-Shirazi, G. Guiochon, Anal. Chem. 61 (1989) 462.
[89] A.M. Katti, Z. Ma, G. Guiochon, AIChE J. 36 (1990) 1722.
[90] I. Quiñones, C.M. Grill, L. Miller, G. Guiochon, J. Chromatogr. A 867 (2000) 1.
[91] C. Migliorini, M. Mazzotti, M. Morbidelli, AIChE J. 45 (1999) 1411.
[92] J. Newburger, G. Guiochon, J. Chromatogr. 523 (1990) 63.

[93] J. Newburger, B. DeLange, G. Guiochon, J. Chromatogr. A 684 (1994) 1.
[94] G. Guiochon, T. Farkas, H. Guan-Sajonz, J.-H. Koh, M. Sarker, B.J. Stanley, T. Yun, J. Chromatogr. A 762 (1997) 83.
[95] G. Guiochon, E. Drumm, D. Cherrak, J. Chromatogr. A 835 (1999) 41.
[96] D.E. Cherrak, G. Guiochon, J. Chromatogr. A 911 (2001) 147.
[97] F.B. Anspach, D. Curbelo, R. Hartmann, G. Garke, W.-D. Deckwer, J. Chromatogr. A 865 (1999) 129.
[98] Cs Horváth, A. Nahum, J.H. Frenz, J. Chromatogr. 218 (1981) 365.
[99] J.H. Frenz, Ph.D. Thesis, Yale University, New Haven, CT, 1983.
[100] S. Cramer, Cs Horváth, J. Prep. Chromatogr. 1 (1988) 29.
[101] G. Viscomi, S. Lande, Cs Horváth, J. Chromatogr. 440 (1988) 157.
[102] K.J. Bombaugh, W.A. Dark, R.F. Levangie, J. Chromatogr. Sci. 7 (1969) 42.
[103] I. Duvdevani, J.A. Biesenberger, M. Tan, Polymer Lett. 9 (1971) 429.
[104] S.C. Stinson, Chem. Eng. News 73 (Oct. 9) (1995) 44.
[105] B. Roz, R. Bonmati, G. Hagenbach, P. Valentin, G. Guiochon, J. Chromatogr. Sci. 14 (1976) 367.
[106] Roussel-Uclaf, Romainville, France, US Patent 3,966,609 (June 17, 1976).
[107] Waters Assoc., Milford, MA, USA, US Patents 4,211,658 (1980), 4,228,007 (1980), and 4,250,035 (1981).
[108] Separations Technology, Wakefield, RI, USA, US Patent 4,737,292 (April 12, 1988).
[109] M. Sarker, G. Guiochon, J. Chromatogr. A 702 (1995) 27.
[110] M. Sarker, G. Guiochon, J. Chromatogr. A 709 (1995) 227.
[111] M. Sarker, A.M. Katti, G. Guiochon, J. Chromatogr. A 719 (1996) 275.
[112] G. Carta, W.B. Stringfield, J. Chromatogr. A 658 (1994) 407.
[113] M. Sarker, G. Guiochon, J. Chromatogr. A 683 (1994) 293.
[114] M. Sarker, T. Yun, G. Guiochon, J. Chromatogr. A 728 (1996) 3.
[115] E. Bayer, K.P. Hupe, H.G. Witsch, Angew. Chem. 73 (1961) 525.
[116] M. Verzele, C. Dewaele, Preparative High Performance Liquid Chromatography, TEC, Gent, Belgium, 1986, Chapter I.
[117] U. Tallarek, E. Baumeister, K. Albert, E. Bayer, G. Guiochon, J. Chromatogr. A 696 (1995) 1.
[118] U. Tallarek, K. Albert, E. Bayer, G. Guiochon, AIChE J. 42 (1996) 3041.
[119] M. Bailly, D. Tondeur, Chem. Eng. Process. 18 (1984) 293.
[120] A. Seidel-Morgenstern, G. Guiochon, AIChE J. 39 (1993) 809.
[121] F. Charton, M. Bailly, G. Guiochon, J. Chromatogr. A 687 (1994) 13.
[122] C. Heuer, A. Seidel-Morgenstern, P. Hugo, Chem. Eng. Sci. 50 (1995) 1115.
[123] C. Heller, P. Hugo, A. Seidel-Morgenstern, Sep. Sci. Technol. 34 (1999) 173.
[124] I. Quiñones, C.M. Grill, L. Miller, G. Guiochon, J. Chromatogr. A 867 (2000) 1.
[125] A.J.P. Martin, I. Halasz, H. Engelhardt, P. Sewell, J. Chromatogr. 186 (1982).
[126] H. Colin, P. Hilaireau, M. Martin, J. Chromatogr. 557 (1991) 137.
[127] G. Ganetsos, P.E. Barker, Preparative and Production Scale Chromatography, Marcel Dekker, New York, 1994.
[128] C.B. Ching, D.M. Ruthven, Chem. Eng. Sci. 40 (1985) 877.
[129] D.M. Ruthven, Principles of Adsorption and Adsorption Processes, Wiley, New York, 1984.
[130] P.C. Wankat, Large Scale Adsorption and Chromatography, CRC Press, Boca Raton, FL, 1986.
[131] G. Zhong, G. Guiochon, Adv. Chromatogr. 39 (1998) 351.
[132] D.B. Broughton, C.G. Gerhold, US Patent, 2,985,589 (May 23, 1961).
[133] D.B. Broughton, Sep. Sci. Technol. 19 (1985) 723.
[134] G. Storti, M. Masi, S. Carra, M. Mazzotti, M. Morbidelli, Chem. Eng. Sci. 44 (1989) 1329.
[135] G. Storti, M. Mazotti, M. Morbidelli, S. Carra, AIChE J. 39 (1993) 471.
[136] M. Mazzotti, G. Storti, M. Morbidelli, AIChE J. 40 (1994) 1825.
[137] G. Storti, R. Baciocchi, M. Mazzotti, M. Morbidelli, Ind. Eng. Chem. Res. 34 (1995) 288.
[138] M. Mazzotti, G. Storti, M. Morbidelli, AIChE J. 42 (1996) 2784.
[139] M. Mazzotti, G. Storti, M. Morbidelli, AIChE J. 43 (1997) 64.
[140] G. Zhong, G. Guiochon, Chem. Eng. Sci. 51 (1996) 4307.
[141] T. Yun, G. Zhong, G. Guiochon, AIChE J. 43 (1997) 935.
[142] G. Storti, M. Masi, S. Carra, M. Mazzotti, M. Morbidelli, Chem. Eng. Sci. 44 (1989) 1329.
[143] M. Mazzotti, G. Storti, M. Morbidelli, AIChE J. 40 (1994) 1825.
[144] F. Charton, R.M. Nicoud, J. Chromatogr. A 702 (1995) 97.
[145] G. Zhong, G. Guiochon, Chem. Eng. Sci. 52 (1997) 4403.
[146] S. Khattabi, D.E. Cherrak, K. Mihlbachler, G. Guiochon, J. Chromatogr. A 893 (2000) 307.
[147] G. Guiochon, J. Chromatogr. A 908 (2001) 1.
[148] F. Helfferich, G. Klein, Multicomponent Chromatography. A Theory of Interference, Marcel Dekker, New York, 1970.
[149] D.E. Cherrak, J. Lee, G. Guiochon (in preparation).