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Convective Transport in Chromatographic Media

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New liquid chromatographic media have been developed in the last decade containing large pores for convective mass transport and smaller diffusive pores to provide adsorption capacity. These permeable packings are at the heart of perfusion chromatography, patented in 1991, leading to better column efficiency and speed of separation with applications in the rapid analysis of biological macromolecules and preparative scale purification of proteins. This improved column efficiency is based on the concept of augmented diffusivity by convection, available from parallel development in the chemical reaction engineering area.

The analysis of column performance requires an extended van Deemter equation for the HETP (height equivalent to a theoretical plate) as a function of the superficial velocity. A methodology for obtaining basic data for design of perfusive chromatography is suggested involving elution chromatography of proteins in nonretained and frontal chromatography experiments.

Permeable Chromatographic Media

Packing materials in bead form can be grouped into four classes: homogeneous cross-linked polysaccharides (e.g. agarose), macroporous polymers based on synthetic polymers (e.g. POROS particles used in perfusive chromatography with large pores of 600–800 nm and diffusive pores of 50–100 nm), tentacular adsorbents allowing faster interaction between proteins to be separated and functional groups and materials based on the concept of 'soft gel in a rigid shell' combining the good capacity of soft gels with the rigidity of composite materials.

In conventional packings, solutes are carried to sites on the particle surface by bulk convective flow of the mobile phase through the column and then diffuse to binding surfaces inside the particle. The intraparticle diffusion process can be quite slow for large molecules (proteins, peptides). In order to maximize capacity and resolution, interaction with as many sites as possible is required; however, this will be more difficult at high flow rates and therefore trade-offs between speed, resolution and capacity are needed. In permeable, flow-through particles used in perfusive chromatography the flow rate can be increased one order of magnitude compared with conventional packings because of the short diffusion path length inside the microspheres.

New materials aim to achieve higher sorption capacity and better sorption kinetics. Some developments consider new geometries: fibres, membranes or discs (cellulose, polymethacrylate) or continuous beds (rods, monoliths). In continuous rods of acrylamide-acrylate polymers, flow pores are of 3–4 μm diameter and in methacrylate-styrene polymers large pores of 0.5–2 μm and even 20 μm are obtained with microspheres of less than 0.5 μm. Similar developments on continuous silica rods are taking place. In continuous bed technology, channels are of 3–5 μm with microspheres of 0.5–1 μm, whilst in bead form, packings are typically of 3–50 μm with pore size of 0.1 μm. Also superagarose beads of 300–500 μm have been prepared with superpores of 30 μm as well as 3 mm thick membranes. **Table 1** reports examples of convective chromatographic media.

The Concept of Augmented Diffusivity by Convection

The design of chromatographic media aims to eliminate or reduce the mass transfer resistance inside particles by coating a nonporous support with active species, decreasing the particle size or increasing par-

Table 1 Examples of convective chromatographic media

Base material	*Trade name*	*Process*	*Producer*
Polystyrene	PL4000	HPLC	Polymer Laboratories, UK
Polystyrene	POROS	HPLC	PE Biosystems, USA
Agarose	Sepharose	Fast flow	Pharmacia, Sweden
Silica gel	Daisogel SP2705	HPLC	Daiso Co, Japan
Polymeric	TSK-PW	SEC	Toso-Haas, USA
Hydroxyapatite	Macro-Prep	Preparative chromatography	Bio-Rad, USA
Methacrylate copolymer	Macro-Prep	HPLC	Bio-Rad, USA
Alumina	Ceraflo	Membrane	Norton, UK
Cellulose	MemSep	MCLC	Millipore, USA
Polymer matrix	CIM	CBT IEX	BIA Separation, Slovenia JM Science, USA
Polymer matrix	UNO	CBT IEX	Bio-Rad, USA

CIM, Convective interaction media; MCLC, membrane convective liquid chromatography; CBT, continuous bed technology; IEX, ion exchange; SEC, size exclusion chromatography; HPLC, high performance liquid chromatography.

ticle permeability as in convective chromatographic media (**Figure 1**). The use of flow-through particles (**Figure 2**) has been increasing recently in relation to protein separation by HPLC with perfusive chromatography. Intraparticle forced convection is a mass transport mechanism which, in addition to diffusive transport, cannot be neglected in large pore materials. The key concept to be retained is the augmented diffusivity by convection, which explains why the efficiency of adsorptive processes is improved with convective chromatographic media.

The effect of intraparticle convective flow due to a total pressure gradient was quantified in 1982 by a group measuring effective diffusivities by chromatographic techniques. The analysis of experimental results was first made with a conventional model which included an apparent diffusion (lumping diffusion and convection) of tracer inside pores. The apparent diffusivity was found to increase with the superficial velocity. From the equivalence of the conventional model and a detailed model which allows for the separate contribution of diffusive and convective flow (**Figure 3**) the apparent diffusivity or augmented diffusivity by convection, $\tilde{D}_e$ was calculated for an inert tracer as a function of the true effective diffusivity D_e and the intraparticle mass Peclet number λ:

$$\tilde{D}_e = D_e \frac{1}{f(\lambda)} \qquad [1]$$

where:

$$f(\lambda) = \frac{3}{\lambda}\left(\frac{1}{\tanh\lambda} - \frac{1}{\lambda}\right)$$

The intraparticle Peclet number, λ, is the ratio between the time constant for pore diffusion, τ_d, and the time constant for intraparticle convection, τ_c. For slab geometry, $\tau_d = \varepsilon_p \ell^2 / D_e$ and $\tau_c = \varepsilon_p \ell / v_0$ so $\lambda = v_0 \ell / D_e$ where ℓ is the half-thickness of the slab particle and v_0 is the intraparticle convective velocity inside large pores; for sphere geometry with particle radius R_p, the diffusion time constant is $\tau_d = \varepsilon_p R_p^2 / D_e = R_p^2 / D_p$ and $\lambda = v_0 R_p / 3D_e$.

The enhancement of diffusivity by intraparticle convection is $1/f(\lambda) = \tilde{D}_e / D_e$ and so the apparent diffusion time constant $\tilde{\tau}_d = \tau_d f(\lambda)$. **Figure 4** shows the enhancement factor $\tilde{D}_e / D_e$ as a function of the intraparticle Peclet number λ. At low bed superficial velocities, u_0, the convective velocity inside pores, v_0, is also small; therefore $f(\lambda) = 1$ and $\tilde{D}_e = D_e$ (diffusion-controlled case); at high superficial velocities u_0, and therefore high v_0 and high λ,

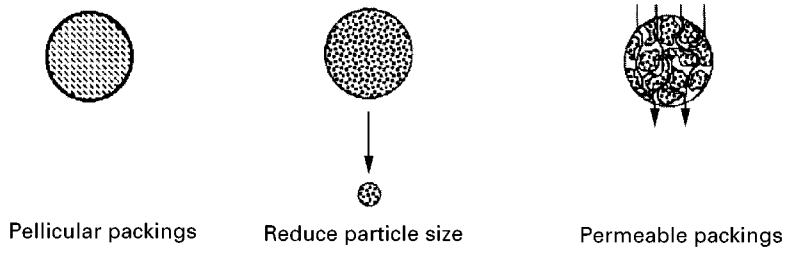

Figure 1 Strategies for eliminating or reducing intraparticle mass transfer resistances. (Reprinted from Rodrigues AE (1997) with permission from Elsevier Science.)

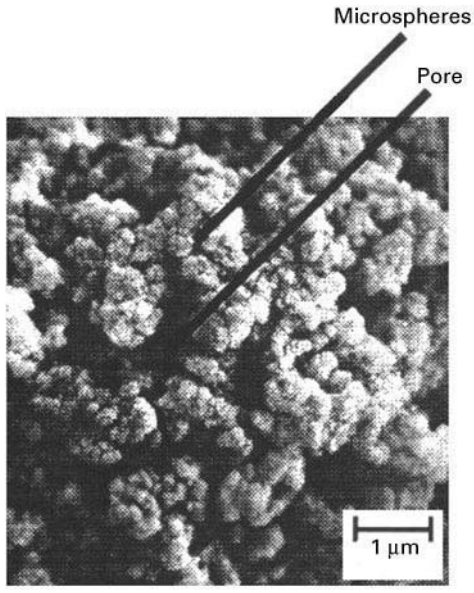

Figure 2 An example of permeable or flow-through chromatographic media with large pores and gel microspheres.

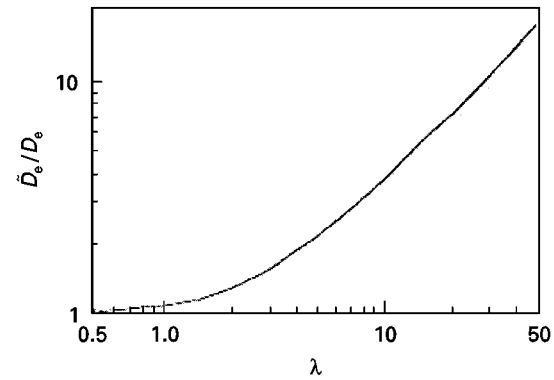

Figure 4 Enhancement factor $\tilde{D}_e/D_e$ versus intraparticle Peclet number λ. (Reprinted from Rodrigues AE (1997) with permission from Elsevier Science.)

$f(\lambda) = 3/\lambda$; the augmented diffusivity is $\tilde{D}_e = v_0\ell/3$ for slab geometry and $\tilde{D}_e = v_0 R_p/9$ for spheres (convection-controlled case), which depends only on the particle permeability, fluid viscosity and pressure drop across the particle.

Column Performance Using Convective Chromatographic Media

Conventional Packings

The column performance can be assessed in terms of HETP as a function of bed superficial velocity following the classic van Deemter analysis. The van Deemter equation for linearly retained species in conventional packings of sphere geometry is:

$$\mathrm{HETP} = A + \frac{B}{u_0} + \frac{2}{15}\frac{\varepsilon_p(1-\varepsilon_b)b^2}{[\varepsilon_b + \varepsilon_p(1-\varepsilon_b)b]^2}\tau_d u_0 \qquad [2]$$

where ε_p is the intraparticle porosity, ε_b is the bed porosity and $b = 1 + \{(1-\varepsilon_p)/\varepsilon_p\}K$ is the adsorption equilibrium parameter for a linear isotherm with slope K. In a condensed form the van Deemter equation is:

$$\mathrm{HETP} = A + \frac{B}{u_0} + Cu_0$$

with:

$$C = \frac{2}{15}\frac{\varepsilon_p(1-\varepsilon_b)b^2}{[\varepsilon_b + \varepsilon_p(1-\varepsilon_b)b]^2}\tau_d$$

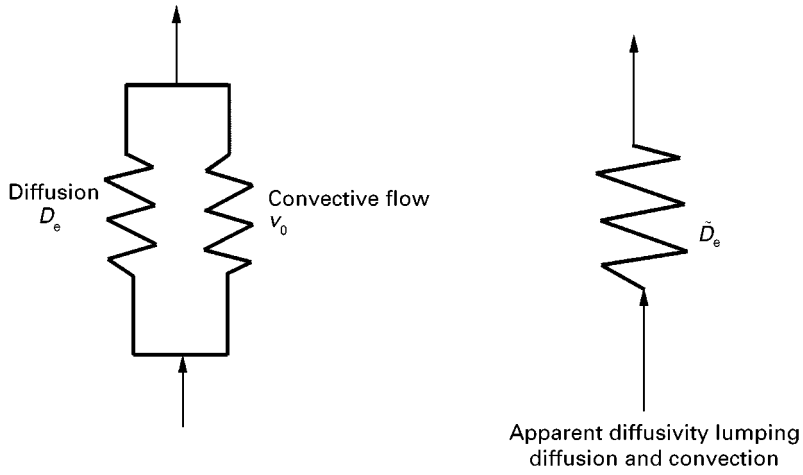

Figure 3 Analogues of mass transport mechanisms in chromatographic media – model equivalence.

For protein separation the B term is negligible and the plot HETP versus u_0 in HPLC is a straight line in most of the domain when conventional supports are used. Moreover, the HETP increases with the square of the particle size.

Permeable Packings

For convective chromatographic media, since $\tilde{\tau}_d = \tau_d f(\lambda)$, the extended van Deemter equation (Rodrigues equation) is:

$$\text{HETP} = A + \frac{B}{u_0} + Cf(\lambda)u_0 \qquad [3]$$

In the above equation one can notice that the last term pertaining to intraparticle mass transfer is reduced for permeable particles since $f(\lambda) < 1$. The van Deemter equation for conventional packings and Rodrigues equation for large pore supports are shown in **Figure 5**. At low velocities both equations lead to similar results. However, at high superficial velocities the last term in the Rodrigues equation becomes a constant since the intraparticle convective velocity v_0 is proportional to the superficial velocity u_0. The HETP reaches a plateau which does not depend on the value of solute diffusivity but only on particle permeability and pressure gradient (convection-controlled limit). The column performance with permeable adsorbents is improved since HETP is reduced when compared with conventional packings and the speed of separation can be increased without losing column efficiency.

Bidisperse Chromatographic Media

In perfusive chromatography, packings contain both convective large pores and smaller diffusive pores. Carta and Rodrigues provided an equation for the column performance measured by HETP:

$$\text{HETP} = A + \frac{B}{u_0} + C\left\{f(\lambda) + \frac{b-1}{b^2\delta}\right\}u_0 \qquad [4]$$

where $\delta = (D_c/r_c^2)/(D_p^2/R_p^2)$ is the ratio of time constants for diffusion in macropores and in microspheres with radius r_c. **Figure 6** shows the effect of δ on the HETP versus u_0 plot when the fraction of flow permeating the packing is 1%. A criterion to determine when microparticle diffusion is the limiting step is given by:

$$\frac{b-1}{b^2\delta} \gg f(\lambda)$$

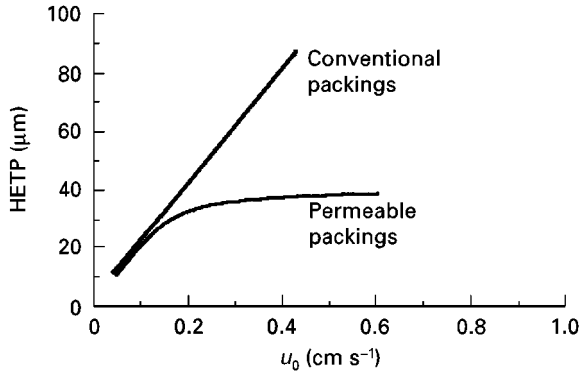

Figure 5 HETP versus superficial velocity u_0 (van Deemter equation for conventional packings and Rodrigues equation for large pore packings). (Reprinted from Rodrigues AE (1997) with permission from Elsevier Science.)

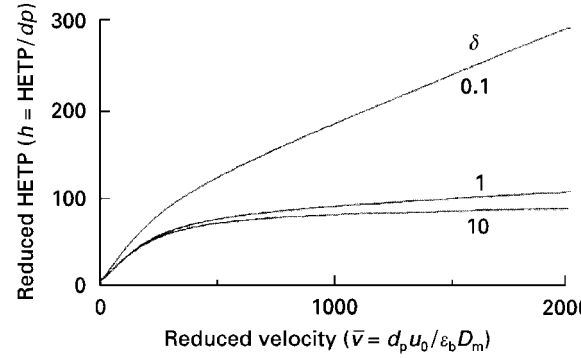

Figure 6 Influence of the ratio of time constants for diffusion in macropores and gel microspheres δ on the HETP versus u_0 plot for bidisperse permeable packings. (Reprinted from Carta G and Rodrigues AE (1993) with permission from Elsevier Science.)

Extension to Include Adsorption/Desorption Kinetics

In protein separation by high performance liquid chromatography (HPLC) with flow-through particles, the effect of convective flow inside pores (say, 1% of the total flow through the bed) is sufficiently important to enhance the low diffusion coefficient for proteins ($\approx 5 \times 10^{-7}\,\text{cm}^2\,\text{s}^{-1}$) and intraparticle Peclet numbers λ of 30 are easily obtained. When the kinetics of adsorption/desorption is considered with rate $r_{ads} = k_a c_i' - k_d q_i'$ where c_i' and q_i' are the species concentrations in the fluid phase inside pores and in the adsorbed phase, respectively and k_a and k_d are the kinetic constants for adsorption and desorption, the extended van Deemter equation becomes:

$$\text{HETP} = A + \frac{B}{u_0} + C\left\{f(\lambda) + \frac{5}{Bi_m} + \frac{3(b-1)}{b^2\phi_d^2}\right\}u_0 \qquad [5]$$

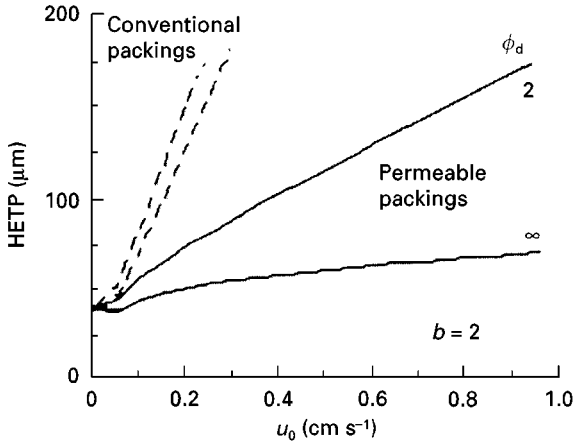

Figure 7 Effect of adsorption/desorption kinetics on HETP versus superficial velocity u_0. (Reprinted from Rodrigues AE *et al.* (1992) Influence of adsorption/desorption kinetics on the performance of chromatographic processes using large-pore supports. *Chemical Engineering Science* 47: 4405–4413, with permission from Elsevier Science.)

Parameters are the Biot number $Bi_m = k_f R_p / D_e$, accounting for film mass transfer and the Thiele modulus $\phi_d = \sqrt{k_d \tau_d}$. **Figure 7** shows the effect of ϕ_d on the HETP versus u_0 plot. The performance of permeable packings is still better than that of conventional materials; intraparticle convention enhances pore diffusivity but has no effect on the mechanism of adsorption/desorption. Adsorption/desorption kinetics have to be taken into account in process such as affinity chromatography.

Methodology for Design

Estimating Intraparticle Peclet Number λ

The importance of convective flow in permeable particles can be assessed by intraparticle Peclet number λ. The convective velocity v_0 inside pores can be calculated from the equality of pressure drops across the particle Δ_p/d_p and across the bed $\Delta P/L$; in laminar flow both for the bulk fluid phase and pore fluid as in HPLC we obtain:

$$v_0 = \frac{B_p}{B_b} u_0$$

where B_b and B_p are bed and particle permeability, respectively. The fraction of flow rate entering the column which goes through the macropores by convection is:

$$(1 - \varepsilon_b)\left(\frac{B_p}{B_b}\right)$$

Bed Permeability

The measurement of the bed pressure drop, ΔP, versus u_0 allows the calculation of bed permeability. In laminar flow, the pressure drop ΔP across a bed of length L packed with particles d_p is given by Darcy's law:

$$\frac{\Delta P}{L} = \frac{\eta u_0}{B_b}$$

where:

$$B_b = \frac{\varepsilon_b^3 d_p^2}{150(1 - \varepsilon_b)^2}$$

Bed permeabilities B_b are obtained from the slope of the plot $\Delta P/L$ versus u_0 and then the bed porosity ε_b can be calculated. As a typical example for a POROS Q/M (PE Biosystems, USA) column 4.6 mm i.d. × 100 mm long with a bed volume of 1.7 mL filled with 20 μm particles, the bed permeability is $B_b = 2.35 \times 10^{-9}$ cm^2 s^{-1} and $\varepsilon_b = 0.34$.

Elution Chromatography with Nonretained Proteins

Elution chromatography experiments under nonretained conditions ($b = 1$) allow the understanding of mass transport inside particles. Flow rates up to 10 mL min^{-1} corresponding to superficial velocities of 1 cm s^{-1} were used. The diffusivities of proteins myoglobin, ovalbumin and bovine serum albumin (BSA) in aqueous solution at 25°C are 16.1×10^{-7}, 6.4×10^{-7} and 1×10^{-7} cm^2 s^{-1}, respectively.

The HETP is calculated from the experimental chromatographic peaks by:

$$\text{HETP} = \frac{\sigma^2 L}{\mu_1^2}$$

where σ^2 is the peak variance, μ_1 is the first moment of the peak, μ_2 is the second moment and L is the column length. **Figure 8** shows the experimentally measured reduced HETP $h = \text{HETP}/d_p$ as a function of the bed superficial velocity.

The efficiency of chromatographic columns can be characterized by the HETP; for columns packed with permeable packings, eqn [3] applies. The A term accounts for eddy dispersion effects and becomes a constant at high superficial velocities, $A = 2d_p$; $B = 2D_m$ and so the term B/u_0 can be neglected in the case of proteins. The simplified equation for HETP with permeable packings is $\text{HETP} \cong A + Cf(\lambda)u_0$. In the low velocity region

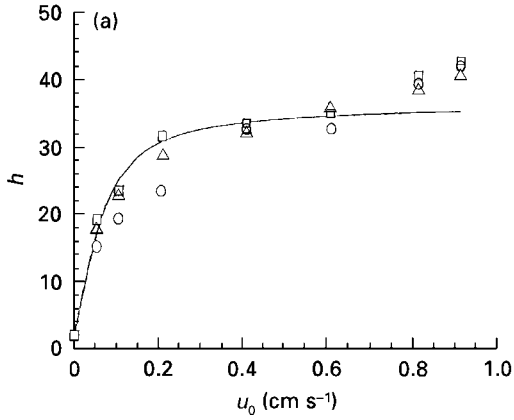

Figure 8 HETP versus superficial velocity u_0 for elution chromatography of proteins under unretained conditions in a POROS *Q/M* column. Solvents were TRIS-HCl 50 mmol L^{-1}, pH 8.6, mixed with NaCl 0.5 mol L^{-1} at 22°C. Circles, myoglobin; squares, ovalbumin; triangles, bovine serum albumin; continuous line, Rodrigues' equation. (Reprinted from Rodrigues AE *et al.* (1996) Protein separation by liquid chromatography using POROS *Q/M* particles. *Chemical Engineering Journal* 61: 191–201, with permission from Elsevier Science.)

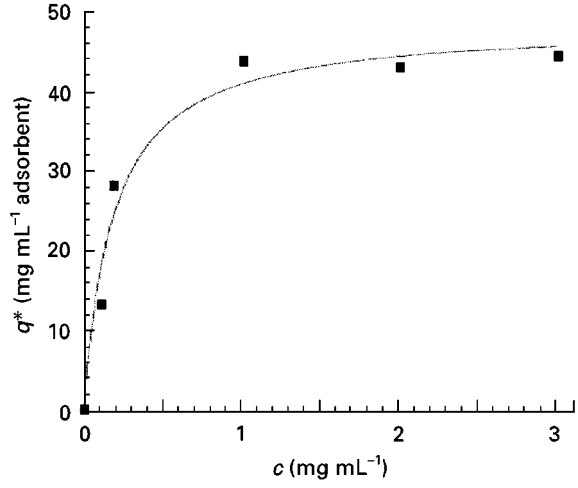

Figure 9 Adsorption equilibrium isotherm for bovine serum albumin on POROS *Q/M* packing. (Reprinted from Rodrigues AE *et al.* (1996) Protein separation by liquid chromatography using POROS *Q/M* particles. *Chemical Engineering Journal* 61: 191–201, with permission from Elsevier Science.)

where pore diffusion is the controlling mechanism of mass transfer, the slope of HETP versus u_0 is

$$C = \frac{1}{30}\frac{v}{(1+v)^2}\frac{\varepsilon_p}{\varepsilon_b}\frac{d_p^2}{D_e}$$

At high flow rates a plateau is reached with:

$$H_{plateau} = A + \frac{3}{5}\frac{v}{(1+v)^2}\frac{\varepsilon_p}{\varepsilon_b}\frac{B_b}{B_p}d_p$$

where:

$$v = \frac{(1-\varepsilon_b)}{\varepsilon_b}\varepsilon_p$$

When the particle structure characterized by the intraparticle porosity, ε_p, is known, the initial slope and the plateau values provide measured values of D_e and B_p.

The straight line at low flow rates crosses the plateau at critical point where

$$u_{0,c} = \frac{18D_e}{d_p}\frac{B_b}{B_p}$$

For POROS *Q/M*, $\varepsilon_p = 0.5$ and $B_p = 1.5 \times 10^{-11}$ cm^2 for an experimental h plateau of 36 (reduced HETP) and $D_e = 7 \times 10^{-8}$ cm^2 s^{-1}.

Frontal Chromatography Experiments

In frontal chromatography experiments a solution of protein with concentration c_0 is continuously passed through the column under retained conditions. The breakthrough curve is measured from which the amount of protein retained in the adsorbent, q_0^* is calculated by mass balance leading to a point on the adsorption equilibrium isotherm. The adsorption equilibrium isotherm of BSA in POROS *Q/M* is shown in **Figure 9** and follows the Langmuir equation. Breakthrough curves with BSA at feed concentration of 2 mg mL^{-1} and various flow rates merge together when the outlet concentration is normalized by the feed concentration and the time is reduced by the stoichiometric time, as shown in **Figure 10**. Moreover, breakthrough curves are very sharp, indicating that the useful dynamic capacity approaches the total column capacity.

Future Developments

Convective chromatographic media have found applications in the preparative chromatography of peptides and proteins. The use of these materials in preparative and industrial scale simulated moving bed (SMB) technology for chromatographic processes will eventually occur in view of the trade-off between pressure drop and packing efficiency; the simplified design of the SMB unit with simple linear driving force models to describe intraparticle mass transfer is straightforward, using an apparent mass transfer coefficient $\tilde{k} = k/f(\lambda)$.

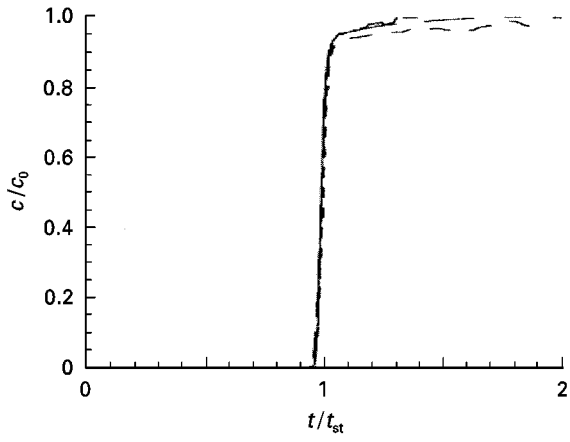

Figure 10 Normalized breakthrough curves for BSA 2 mg mL^{-1}, c/c_0 versus t/t_{st}, on POROS *Q/M* column at various flowrates. Continuous line, 2 mL min^{-1}; dashed line, 5 mL min^{-1}; dotted line, 7 mL min^{-1}. (Reprinted from Rodrigues AE *et al.* (1996) Protein separation by liquid chromatography using POROS *Q/M* particles. *Chemical Engineering Journal* 61: 191–201, with permission from Elsevier Science.)

Continuous bed technology is a promising area which allows convective flow in wider channels and at the same time smaller diffusion limitations in microspheres since they have a very small diameter.

See also: **II/Chromatography:** Size Exclusion Chromatography of Polymers. **Chromatography: Liquid:** Mechanisms: Size Exclusion Chromatography. **III/Peptides and Proteins:** Liquid Chromatography.

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Correlation Chromatography

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Principles

Correlation chromatography (CC, multiplex chromatography, multiple input chromatography) belongs to the family of multiplex methods and is essentially statistical in nature. It is a typical example of an integrated product of chemometric principles and an analytical technique. A schematic set-up of a CC system is shown in **Figure 1**.

In conventional chromatography the sample is injected over a short time, and the response of the chromatographic system – the chromatogram – can be considered as an impulse response. In CC the input