ELSEVIER

Journal of Chromatography A, 890 (2000) 53–59

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Scale-down of continuous protein purification by annular chromatography
Design parameters for the smallest unit

Andrea Uretschläger, Alois Jungbauer*

Institute of Applied Microbiology, University of Agricultural Sciences, Vienna, Austria

Abstract

Theoretically, in fixed-bed chromatography an infinite separation time or volume is available. In annular chromatography the separation time is limited by the time required for a single rotation. In a chromatograph with a smaller annulus than the theoretical one, the equilibration zone will overtake the feed zone. The required separation time is determined by a number of steps such as equilibration, loading, washing, elution, regeneration and the retention of the solute. A mathematical model has been developed to estimate the minimum radius of an annular chromatograph. The minimal geometry of an annular chromatograph was calculated for a model system immunoglobulin and bovine serum albumin.

Keywords: Annular chromatography; Mathematical modelling; Instrumentation; Proteins; Albumin; Immunoglobulins

1. Introduction

Annular chromatography (AC), simulated moving bed (SMB) and true moving bed (TMB) are the most frequently used continuous chromatographic separation methods. The advantages of the AC over SMB and TMB are the application of gradient elution, and the separation of multi-component mixtures [1]. Annular chromatography has been used for a big variety of separation problems ranging from small molecules to biopolymer separations [2–5]. The original concept of the annular chromatograph, as proposed by Martin [6] and realized by Fox et al. [7–9], was further developed at the Oak Ridge National Laboratory to operate the system under a certain pressure [2,10,11]. The pressurized continuous annular chromatography (P-CAC) system is designed as a closed system. Two concentric cylinders form an annulus into which the chromatography medium is packed. Feed and eluent are introduced in a continuous way at the top of the bed. The entire bed is slowly rotated while the feed material is introduced from a stationary entry and the eluent uniformly everywhere else around the annulus. The separation of the feed into single components is caused by the rotation of the sorbent. The separated components appear as helical bands each of which has a characteristic, stationary exit point. Three factors have an effect on the exit point: (a) eluent velocity, (b) rotation rate of the annulus, and (c) the distribution coefficient.

In contrast to the discontinuous fixed bed chromatography, in annular chromatography a hypothetically infinite separation time is not available. A sepa-

*Corresponding author. Tel.: +43-1-36006-6226; fax: +43-1-36006-1249.

E-mail address: jungbaue@hp01.boku.ac.at (A. Jungbauer).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00395-2

ration cycle usually consists of several steps such as equilibration, loading, washing, elution, and regeneration. These steps must be completed within a time span equivalent to one rotation. The separation time is dependent on axial dispersion, the number of separation steps, the isotherms of the solutions, the height of the bed, and the radius [3,12]. Especially when complex solutions are processed, extensive regeneration must be performed to prevent fouling. Together with a large elution volume, often observed in size-exclusion chromatography, the required separation time may exceed the time equivalent to a single rotation. Respectively, for continuous separation processes, special emphasis has to be put on scale down. We developed a model, based on an analytical solution described by Carta et al. [13], for the calculation of the minimum radius which still enables a complete separation cycle. This model can be extended to non-linear systems and axial dispersion.

The scale-down of the system is important to perform laboratory experiments in a reasonable scale. This implicates less material and therewith a reduction of the costs.

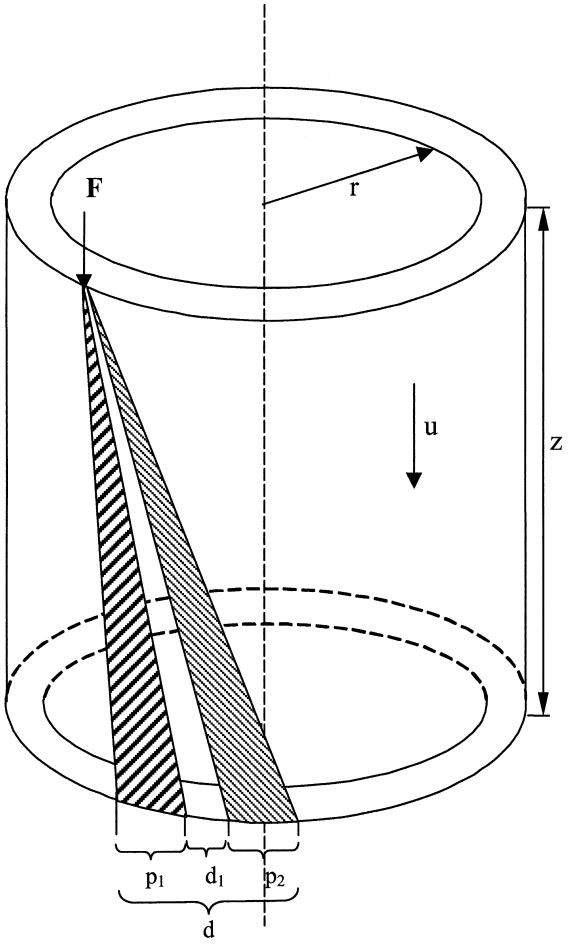

Fig. 1. Schematic drawing of solute train in annular chromatography. r is radius, z is column length, and u is the superficial velocity. The other notations are explained in the text.

2. Theory

Assume a sample consists of n components. After separation into single components, they appear at the bottom of the annulus with a certain outlet width (p_1, p_2, ..., p_n), see Fig. 1. The distances between the peak onset of the various components (d_i) are defined as:

$$d_i = (\theta_{i+1} - \theta_i) \cdot \frac{\pi r}{180} - \left(\frac{p_i + p_{i+1}}{2} \right) \quad (1)$$

with $i \in \{1,2, \ldots n-1\}$.

θ_i is the displacement of the peak maximum of a component from the sample inlet port and r is the minimal inner radius of the annulus. The subscript i denotes the number of components. $i=1$ is the least retarded component and $i=n$ the most retarded component. The average velocity (u) of a single component is defined as:

$$u_i = \frac{z}{t_i} \quad (2)$$

where t_i is the retention time and z the column length.

The sum of the outlet widths of the n components (p) is:

$$p = \sum_{i=1}^{n} p_i + \sum_{i=1}^{n-1} d_i \quad (3)$$

θ_i, as defined by Eq. (4),

$$\theta_i = \omega t \quad (4)$$

can also be dependent on the annular velocity (ω).

Combining Eqs. (1)–(4) yields:

$$p = \sum_{i=1}^{n} p_i + \sum_{i=1}^{n-1} \left[\left(\frac{z}{u_{i+1}} - \frac{z}{u_i} \right) \cdot \frac{\omega \pi r}{180} - \left(\frac{p_i + p_{i+1}}{2} \right) \right] \quad (5)$$

Regeneration and equilibration are applied steps, necessary in biochromatography. We have defined the outlet width for regeneration as g and for equilibration as e.

According to Fig. 2, the distance between the adjacent peak maximums (y_i) of the most retarded component and the regeneration buffer at the outlet are defined as:

$$y_i = \left(\frac{z}{u_g} - \frac{z}{u_n} \right) \cdot \frac{\omega \pi r}{180} + x_g \quad (6)$$

where x_i is the distance of two adjacent inlet ports at the top of the annulus. For an isocratic separation such as size-exclusion chromatography, only three inlet ports are required. The distance between the sample inlet port and the regeneration inlet port (x_g) is calculated according to Eq. (7):

$$x_g = \frac{p_n + g}{2} - \left(\frac{z}{u_g} - \frac{z}{u_n} \right) \cdot \frac{\omega \pi r}{180} \quad (7)$$

The distance between inlet port for regeneration and equilibration (x_e) is defined as:

$$x_e = \frac{e + g}{2} - \left(\frac{z}{u_a} - \frac{z}{u_g} \right) \cdot \frac{\omega \pi r}{180} \quad (8)$$

The sum of all distances at the outlet (b) is:

$$b = \sum_{i=1}^{n} p_i + \sum_{i=1}^{n-1} \left[\left(\frac{z}{u_{i+1}} - \frac{z}{u_i} \right) \cdot \frac{\omega \pi r}{180} - \left(\frac{p_i + p_{i+1}}{2} \right) \right] + g + e \quad (9)$$

b cannot exceed $2r\pi$. In order to calculate the minimal radius Eq. (9) is equated with $2r\pi$. After rearrangement the radius (r) required for a complete separation cycle is:

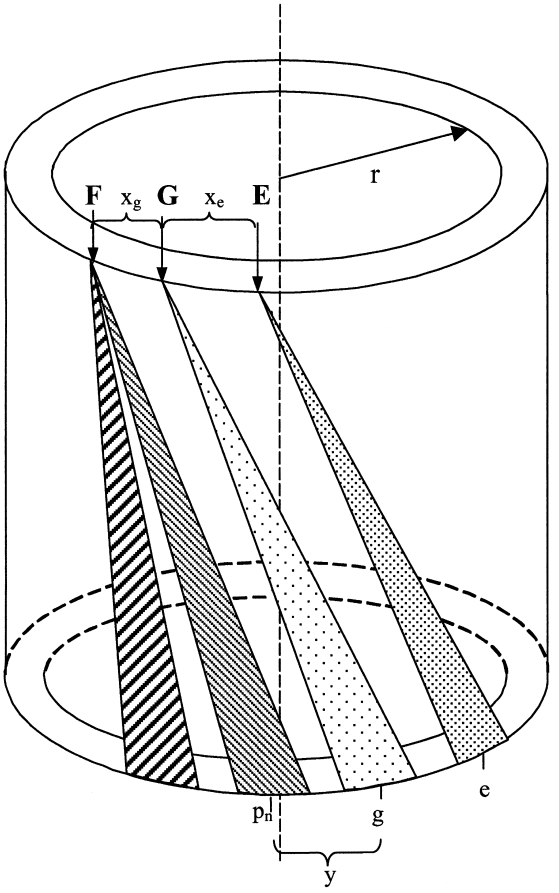

Fig. 2. Schematic drawing of the bands of feed components, regeneration medium and equilibration medium.

$$r_{\min} = \frac{g + e + \frac{p_1 + p_n}{2}}{2\pi - \frac{\omega \pi}{180} \cdot (t_{Rn} - t_{R1})} \quad (10)$$

Further it is possible to calculate the outlet width in respect to the mass transfer coefficients of the individual components. For simplicity we have neglected other peak dispersion phenomena. Thus, peak broadening is only caused by mass transfer resistances and σ^2 can be defined as:

$$\sigma^2_{\text{mass transfer}} = \frac{1}{k_0 a} \cdot \frac{u}{z} \quad (11)$$

where $k_0 a$ is the mass transfer with k_0 as the global mass transfer coefficient and a the effective interfa-

cial mass transfer area per unit volume. $(1/k_0a)(u/z)$ can be also related to the reciprocal of the number of transfer units (*NTU*). Furthermore the number of plates (*N*) is defined as:

$$N = \left(\frac{t_R}{\sigma}\right)^2 \tag{12}$$

With $NTU = 2N$ and the peak width of half maximal height (Δ):

$$\Delta = 2\sigma \cdot \sqrt{2 \ln 2} \tag{13}$$

the mass transfer coefficient can be expressed as:

$$k_0a = 16(\ln 2) \cdot \left(\frac{t_R}{\Delta}\right)^2 \cdot \frac{u}{z} \tag{14}$$

After some conversions, Eq. (14) can be inserted into Eq. (10) that leads to:

$$r_{min} = \frac{\sqrt{\frac{32 \cdot u}{z}} \cdot \left(\frac{t_{Rg}}{\sqrt{k_0a_g}} + \frac{t_{Re}}{\sqrt{k_0a_e}} + \frac{t_{Rp1}}{\sqrt{k_0a_{p1}}} + \frac{t_{Rpm}}{\sqrt{k_0a_{pm}}}\right)}{2\pi - \frac{\omega\pi}{180} \cdot (t_{Rn} - t_{R1})} \tag{15}$$

The exact transformations are shown in Appendix A.

For size-exclusion chromatography a relationship between molecular mass and time has been described. This can be inserted again in Eq. (15) providing an estimate for the minimal radius required for a given separation problem. Using this equation a minimal radius can be calculated without wasting separation space. The influence on radial dispersion is neglected in this model.

3. Experimental

3.1. Chromatography system

All chromatography experiments were performed with a chromatography-workstation (Prosys, BioSepra, Marlborough, MA, USA) consisting of four HPLC pumps, an injection loop, and three monitors. The pH, conductivity, and UV absorbance were continuously recorded. A data acquisition system was used for processing of chromatograms. Data were exported to a peak evaluation program (Peak Fit, Version 4.0, SPSS, Chicago, IL, USA). Peak width and retention time were determined by fitting data pairs with the Gaussian function.

3.2. Chromatography column and medium

As a chromatography column a HR10×28 (18 mm×10 mm) column from Amersham-Pharmacia (Uppsala, Sweden) was used. Size-exclusion gel Superdex 75 prep grade from Amersham-Pharmacia, pre-suspended in 20% ethanol, was packed into the column.

3.3. Buffers and running conditions

As a running buffer a 10 m*M* phosphate buffer pH 7.0 supplemented with 150 m*M* NaCl was used. The column was operated at 76.4 cm/h. After equilibration 25, 200, 500, 1000, 2500, and 5000 μl of bovine serum albumin (BSA) (1 mg/ml) and in a different experiment the same amounts of IgG (1 mg/ml) were injected. In the third experiment, 25, 200, and 1000 μl of 0.1 *M* NaOH were applied to the column. To obtain the mass transfer coefficient for an equilibration medium, 1000, 2500, and 5000 μl of 200 m*M* phosphate buffer pH 7.0 were injected to the column. Elution was made by the same buffer as described above.

3.4. Determination of mass transfer coefficients

Mass transfer coefficients k_0a and the retention times t_R were obtained from conventional batch experiments with fixed bed chromatography using Eq. (14) as described in the theory section. To compensate the influence of the loading volume, the peak variance (σ^2) was corrected by subtracting the variance of the excessive sample volume ($V^2_{Sample}/12$).

4. Results and discussion

In conventional chromatography in batch-wise operation mode, as well as in continuous chromatography, operated as annular chromatography, a certain column height is required to resolve a mixture of solutes. Additionally in the continuous operation

mode, the separation time is limited. To enlighten the problem of limited separation time in annular chromatography, separation of IgG from albumin was used as a model system emulating industrial separation conditions. The column must be continuously regenerated by 0.1 *M* NaOH, which is in the lower limit for efficient sanitization and regeneration of a column [14–16]. The high pH, necessary for an efficient regeneration procedure, must be followed by a step suited for lowering the pH. Application of a strong buffer, in our case a 200 m*M* phosphate buffer was selected for this purpose. The buffer inlet port was placed in the closest distance adjacent to the inlet port of NaOH buffer to minimize separation time. Equilibration is performed by the eluent buffer. Such an operation cycle must be completed within a single rotation. Especially the removal of residual NaOH consumed a big fraction of the available separation time. NaOH must be efficiently displaced. At least five different steps must be completed within the time span necessary for one rotation. In our model we assumed that the outlet zones consumed by each individual step are followed by the next zone without delay. The inlet ports were placed at optimal positions to fulfill this requirement.

For calculation of the minimum radius for the given separation examples, several parameters must be experimentally estimated. The mass transfer coefficient k_0a (Eq. 14) must be determined for each individual step. To simplify the complex situation, we assumed that in each single step only one component is interacting with the sorbent and that the individual components are not interacting.

Mass transfer coefficients were determined for all components applied to the chromatographic separation, as described in the Experimental section. The data are summarized in Table 1. The value for k_0a obtained for BSA as described by Bart et al. is 0.033 s^{-1} [5]. We observed a value of 0.75 s^{-1}. Differences can be explained by the different stationary phases.

Using the experimental data set, the minimum radius was calculated using Eq. (15). The radius is dependent on the single mass transfer coefficient, retention time and angular velocity. Mass transfer coefficients are given by the nature of the chromatographic sorbent and the physical properties of the mixture. The retention time is also dependent on the aforementioned properties, the column height, and velocity. Column height and velocity are dictated by the resolution, which is necessarily required to fulfill the separation task. The sole variable which can be used for optimization is the angular velocity.

In Fig. 3 the relationship between radius and the angular velocity of the annular chromatograph is shown for a particular separation. We selected the separation of IgG and BSA with subsequent regeneration by NaOH. For calculation of the minimum radius, the experimentally determined k_0a values (listed in Table 1) were inserted into Eq. (15). For NaOH and the 200 m*M* phosphate buffer a quite low k_0a value was observed. Neutralization of the buffer or NaOH occurred at the front of the pulse, reducing the zone-width. Neglecting this process would yield in a 5–10 times higher k_0a value. We are aware that we empirically lumped together a chemical reaction and a chromatographic partition into one parameter. The empirically determined k_0a reflects the practical situation. For more rigorous treatment, the reaction constants have to be incorporated. The most dominant influence are the retention time and the number of steps. As already depicted from Eq. (15), the number of components and the efficiency of the separation dictate the minimum radius.

Currently a commercial system with a 14 cm inner radius is available as smallest unit. For a simpler separation task, a smaller radius would be sufficient.

Table 1
Mass transfer coefficients of 200 m*M* phosphate buffer (equilibration medium), 0.1 *M* NaOH (regeneration medium), BSA (feed), and IgG (feed)

k_0a of compounds (s^{-1}) 200 mM Phosphate buffer	BSA (1 mg/ml)	IgG (1 mg/ml)	0.1 *M* NaOH
1.76	0.75±0.25	0.78±0.3	1.76

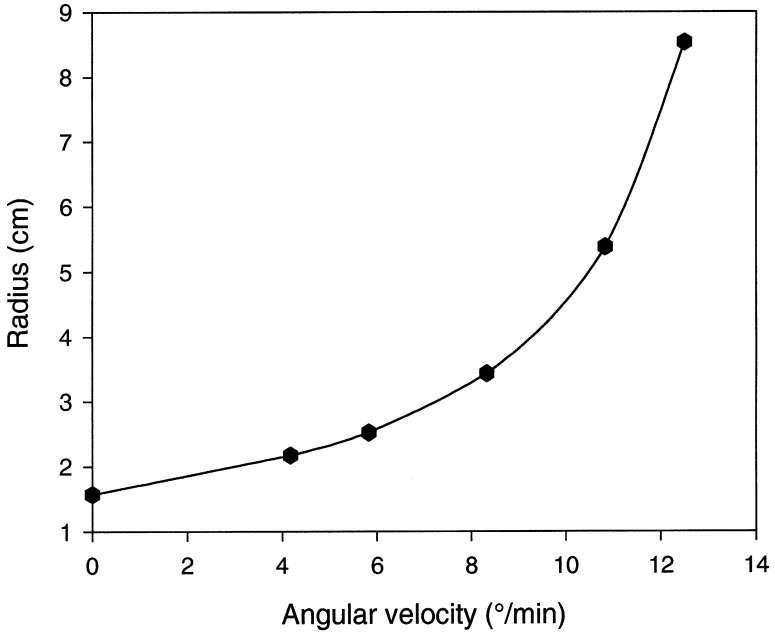

Fig. 3. Relationship between the angular velocity and the radius for separation of albumin and IgG with subsequent regeneration using NaOH.

Finally we conclude, that in continuous annular chromatography the limited separation must be obeyed, when a separation is scaled down.

5. Nomenclature

a	effective interfacial mass transfer area per unit volume, cm^2/cm^3
b	sum of all distances at the outlet, cm
d	distance between peak onsets of different components, cm
e	outlet width for equilibration, cm
g	outlet width for generation, cm
k_0	global mass-transfer coefficient, cm/s
NTU	number of transfer units
p_i	outlet width of component, cm
p	sum of p_i, cm
r	radius, cm
t	retention time, s
u	superficial velocity, cm/s
w_b	peak width at peak basis, s
x	distance of inlet ports, cm
y	distance between adjacent peak maximums, cm
z	column length, cm

Greek symbols

ω	rotation rate, degrees/h
σ^2	peak variance, s
θ	displacement from feed point, degrees
Δ	peak width at half peak height, s

Acknowledgements

This work has been made possible by a grant from the ‘Österreichischer Forschungs Förderung Fond (FFF) der gewerblichen Wirtschaft’, Projectnumber, BIOCAC (FFF Nr.3/12992) and by the company Prior Separation Technology, Götzis, Austria.

Appendix A

The mass transfer coefficient can be expressed as:

$$k_0 a = 16\,(\ln 2)\cdot\left(\frac{t_R}{\Delta}\right)^2\cdot\frac{u}{z} \tag{14A}$$

Rearranging for Δ yields into:

$$\Delta = \sqrt{16\ln 2}\cdot\frac{t_R}{\sqrt{k_0 a}}\cdot\sqrt{\frac{u}{z}} \tag{A.1}$$

For a Gaussian peak Δ can be expressed as:

$$\Delta = 2\sigma\cdot\sqrt{2\ln 2} \tag{13A}$$

and this equation is equated with the one described before.

$$\sqrt{16\ln 2}\cdot\frac{t_R}{\sqrt{k_0 a}}\cdot\sqrt{\frac{u}{z}} = 2\sigma\cdot\sqrt{2\ln 2} \tag{A.2}$$

We have defined g, e, p as the width corresponding to the peak width at the basis w_b:

$$w_b = 4\sigma \tag{A.3}$$

Eq. (A.2) is solved for σ:

$$4\sigma = \frac{2\sqrt{16\ln 2}}{\sqrt{2\ln 2}}\cdot\frac{t_R}{\sqrt{k_0 a}}\cdot\sqrt{\frac{u}{z}} \tag{A.4}$$

The required radius for a complete separation cycle is given by:

$$r_{\min} = \frac{g + e + \dfrac{p_1 + p_n}{2}}{2\pi - \dfrac{\omega\pi}{180}\cdot(t_{Rn} - t_{R1})} \tag{10A}$$

Inserting the basis width of each component (Eq. (A.4)) into Eq. (10A) yields Eq. (15A) describing the radius required for a complete cycle dependent on the $k_0 a$ of each individual component.

$$r_{\min} = \frac{\sqrt{\dfrac{32\cdot u}{z}}\cdot\left(\dfrac{t_{Rg}}{\sqrt{k_0 a_g}} + \dfrac{t_{Re}}{\sqrt{k_0 a_e}} + \dfrac{t_{Rp1}}{\sqrt{k_0 a_{p1}}} + \dfrac{t_{Rpm}}{\sqrt{k_0 a_{pm}}}\right)}{2\pi - \dfrac{\omega\pi}{180}\cdot(t_{Rn} - t_{R1})} \tag{15A}$$

References

[1] C. Heuer, H. Kniep, T. Falk, A. Seidel-Morgenstern, Chem. Ing. Tech. 69 (1997) 1535.
[2] J.M. Begovich, C.H. Byers, W.G. Sisson, Sep. Sci. Tech. 18 (1983) 1167.
[3] G. Carta, G.F. Bloomingburg, J.S. Bauer, C.H. Byers, Ind. Eng. Chem. Res. 30 (1991) 1061.
[4] H.J. Bart, R.C. Messenböck, C.H. Byers, A. Prior, J. Wolfgang, Chem. Eng. Proces. 35 (1996) 459.
[5] H.J. Bart, K. Reissner, A. Prior, J. Wolfgang, C.H. Byers, J. Chromatogr. A 763 (1997) 49.
[6] A.V.P. Martin, Faraday Soc. 7 (1949) 332.
[7] J.B. Fox, R.C. Calhoun, W.J. Eglinton, J. Chromatogr. 43 (1969) 48.
[8] J.B. Fox, J. Chromatogr. 43 (1969) 55.
[9] J.B. Fox, R.A. Nicholas, J. Chromatogr. 43 (1969) 61.
[10] C.D. Scott, R.D. Spence, W.G. Sisson, J. Chromatogr. 126 (1976) 381.
[11] C.D. Scott, W.G. Sisson, J.M. Begovich, C.H. Byers, Prep. Chromatogr. 1 (2) (1989) 139.
[12] G. Carta, G.F. Bloomingburg, Chem. Eng. J. 55 (1994) B19.
[13] C.H. Byers, W.G. Sisson, J.P. De Carli II, G. Carta, Biotechnol. Prog. 6 (1990) 13.
[14] A. Jungbauer, H.P. Lettner, BioPharm 5 (1994) 46.
[15] A. Jungbauer, H.P. Lettner, L. Guerrier, E. Boschetti, BioPharm 7 (1994) 37.
[16] G. Sofer, L. Hagel, Handbook of Process Chromatography: A Guide to Optimization, Scale Up, and Validation, Academic Press, San Diego, 1997.