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GRADIENT ELUTION IN HOLLOW-FIBRE FLOW FIELD-FLOW FRACTIONATION

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SUMMARY

The scope of the technically simple hollow-fibre version fo field-flow fractionation has been extended to gradient elution. An arrangement of two liquid chromatographic pumps and a gradient controller permits sample introduction, relaxation and elution under gradient conditions to be conveniently performed. The retention times experimentally found can be theoretically explained. The technique was applied to model separations of polystyrene latex beads and plasmids.

INTRODUCTION

In a previous paper[1] we presented a novel version of flow field-flow fractionation (FFF) in which the classical parallel-plate channel is exchanged for a circular hollow fibre. This configuration appears to have several advantages, such as better mechanical properties and easier connection to liquid chromatographic equipment. Also, the flow-rates can be more precisely controlled. This, in addition to providing higher accuracy, also permits the application of gradient elution techniques.

In gradient elution techniques, the temperature or solvent composition is continuously varied in order to extend the number of components that can be separated within a given time. In the various FFF techniques, the external force field is varied to obtain the corresponding effect[2]. In sedimentation FFF, the gravity field, *i.e.*, the centrifuge speed is varied during elution. This is now a standard technique[3,4]. In thermal FFF the temperature difference between the plates is varied[5,6], while the parameter varied in flow FFF is the lateral flow-rate. This has been described for the parallel-plate system by Wahlund *et al.*[7].

In this paper we present a simple method for creating gradients in the hollow-fibre flow FFF system. The hollow-fibre unit is connected to a commercially available liquid chromatographic system, designed for gradient operation. The computerized, independent control of pump speeds facilitates the application of gradient techniques to this type of FFF.

EXPERIMENTAL

Materials

The hollow-fibre flow FFF system was previously described in detail[1]. Briefly, it

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is based on the FPLC™ range of liquid chromatographic equipment (Pharmacia LKB, Uppsala, Sweden). The hollow fibre, either a Model H10-P100-20 or H5-P100-43 with inner diameters of 500 and 1100 μm, respectively (Amicon, Danvers, MA, U.S.A.) was encapsulated in an empty, modified column tube (Model C 16, Pharmacia), see Fig. 1. The sample was injected with an injection valve (internal volume 5 μl, Model 7410; Rheodyne, Cotati, CA, U.S.A.), equipped with a pneumatic control unit.

The detector was a fixed wavelength (254 nm) UV detector (UV-1, Pharmacia). The axial and the radial liquid flows were created by two syringe pumps (Model P-500, Pharmacia), connected to the fibre. The pumps were further connected to a computerized control unit (LCC-500, Pharmacia), which was programmed to control the pumps independently as well as the injection valve.

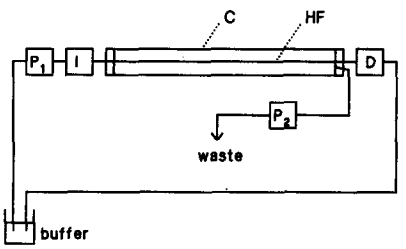
The gradient elution technique in the hollow-fibre system was tested with polystyrene latex beads, 0.09, 0.30 and 0.80 μm in diameter (Sigma, St. Louis, MO, U.S.A.) and the plasmid PUC-8 from the Molecular Biology Division, Pharmacia.

A buffer solution of 0.01 *M* Tris–HCl containing 1 m*M* EDTA, 100 m*M* sodium chloride, 0.04% sodium azide and 0.1% Triton X-100 at pH 7.0 served both as diluent for the latex beads and as a carrier in the separation. The same buffer, without Triton X-100, was used for the PUC-8.

Methods

In Fig. 2 the pumping sequence for sample introduction, relaxation, restoration and gradient elution is shown. The principle is the same as that described in the previous paper[1], except that the cross-flow, F_2, was now decreased during Phase IV.

Briefly, the sample was introduced into the fibre (Phase I) with the axial flow, F_1 (the flow pumped by pump P_1) at a constant rate (typically 30 or 80 μl/min). During Phase II (the relaxation period), F_1 was decreased to about 20 μl/min. During this time, the sample was allowed to migrate to its equilibrium distance from the fibre wall and was axially compressed. In Phase III, F_1 was increased to the flow-rate used in elution. The radial flow, F_2, which was controlled by pump P_2, was kept constant during Phases I–III and decreased successively during Phase IV. Thus, components with a wide range of particle sizes can be separated with optimum resolution. It is possible to use an initial radial flow that is considerably higher than under isocratic conditions, and this speeds up the relaxation process.



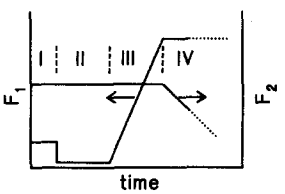


Fig. 1. Experimental set-up, including pumps P_1 and P_2, injection valve I, photometric detector D, porous hollow fibre HF and column tube C. For details, see the text.

Fig. 2. Pumping sequence for the pumps P_1 (creating the axial flow, F_1) and P_2 (creating the radial flow, F_2). The phases of the operation are: sample introduction (I), relaxation (II), restoration (III) to gradient elution condition (IV).

THEORETICAL

The general theory for hollow-fibre flow FFF was developed previously[1] for isocratic conditions and is here modified for gradients. The retention is governed by the Péclet number, Pé, which is defined as:

$$\text{Pé} = \frac{u_r(R) \cdot R}{D} \tag{1}$$

In eqn. 1, $u_r(R)$ is the radial flow-velocity component at the fibre wall, *i.e.*, at the distance R from the centre of the fibre, R being the radius of the fibre and D the diffusion coefficient of the sample particles to be separated. Assuming (a) that $u_r(R)$ is constant along the fibre and with time, and (b) that Pé > 50, the retention time, t_R, is given by (eqn. 14 in ref. 1)

$$t_R = \frac{1}{R_f} \cdot \frac{L}{\bar{u}_z} = \frac{\text{Pé}}{4} \cdot \frac{L}{\bar{u}_z} \tag{2}$$

where L is the length of the fibre and R_f is the retention ratio, *i.e.*, the velocity of the particles in question relative to $\bar{u}_z$ which is the axial flow velocity, averaged over the fibre cross-section.

The retention volume under gradient conditions is found as the solution of the following integral equation[2]

$$L = \int_0^{t_R} R_f \cdot \bar{u}_z \cdot \mathrm{d}t = \int_0^{t_R} \frac{4}{\text{Pé}} \cdot \bar{u}_z \cdot \mathrm{d}t \tag{3}$$

where $u_r(R)$ and, thus, Pé vary with time. A simple, linear gradient with a constant, initial period is expressed by:

$$u_r(R) = u_0 \qquad 0 < t < t_1 \tag{4}$$

$$u_r(R) = u_0 + b(t - t_1) \qquad t > t_1$$

With this, an explicit expression for t_R is readily obtained for the case where $t_R > t_1$

$$t_R = \frac{u_0}{b} \exp\left[\frac{\left(L - \frac{t_1 \cdot 4 \cdot \bar{u}_z}{\text{Pé}(0)}\right)\text{R} \cdot \text{b}}{4 \cdot \text{D} \cdot \bar{u}_z}\right] - 1 + t_1 \tag{5}$$

where Pé(0) is the initial Péclet number (at $t < t_1$). The case where $t_R < t_1$ is equivalent to isocratic (non-programmed) conditions and consequently trivial.

From eqn. 5, the expected retention times can be estimated. The use of eqn. 2 instead of more elaborate equations[1], which remove the restrictions necessary for eqn. 2, leads to an underestimation of the retention time in the isocratic mode. Thus,

also eqn. 5 may be expected to underestimate t_R. For more accurate calculations, numerical calculations are necessary.

RESULTS AND DISCUSSION

To evaluate the potential of gradient elution, polystyrene latex beads with different particle diameters (0.09 and 0.30 μm) were separated. Linear gradients from 40 to 0 μl/min in 15, 30 and 45 min and from 70 to 0 μl/min in 30 and 45 min were used. Baseline separation was obtained in all cases. In Fig. 3 two examples are shown.

Retention times, calculated with eqn. 5, agree fairly well with experimentally obtained values. The agreement is best (all differences < 1 min) if the value used for the fibre radius, R, is 300 μm in the cases where $u_0 = 40$ μl/min and 330 μm when $u_0 = 70$ μl/min. The nominal radius of the fibre used was 250 μm. Also, in the previous work[1] it was necessary to use a fibre radius larger than the nominal one in the calculations. Consequently, it seems as if the fibre expanded due to the pressure difference between the inside and the outside, which is created by the radial flow pump (P_2). This assumption was further substantiated by the observation that the dead (hold up) time also increased with the radial flow[1].

A time-optimized separation of three types of latex beads (0.09, 0.30 and 0.80 μm) with a three-segment gradient is shown in Fig. 4. The total time needed for elution was 45 min.

A separation of these components under isocratic conditions would require approximately 160 min (calculated as the sum of the relaxation time and the retention time of the largest particles with the lowest practical flow-rate of pump P_2).

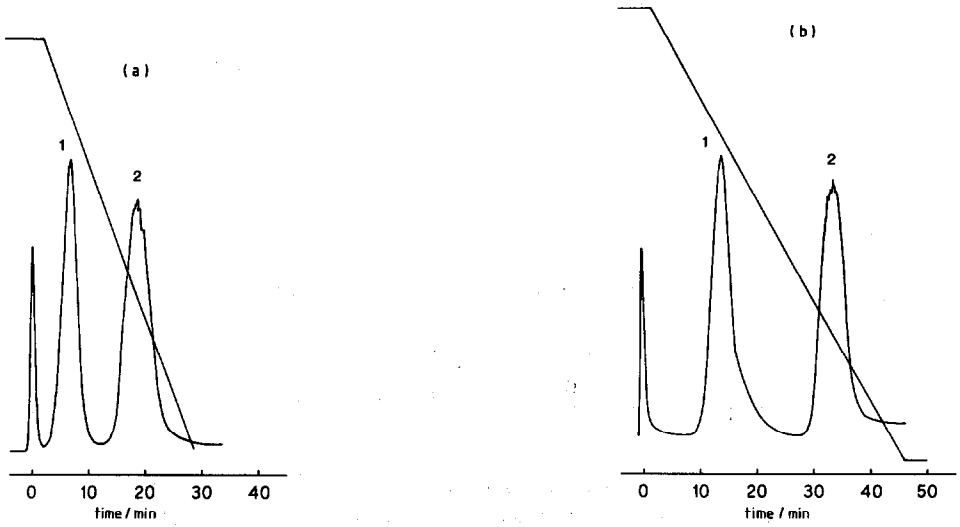


Fig. 3. Separation of polystyrene latex beads, 0.09 μm (peak 1) and 0.30 μm (peak 2) in diameter. Fibre radius: 250 μm. $F_1 = 200$ μl/min. (a) F_2 = gradient from 40 to 0 μl/min in 30 min; (b) F_2 = gradient from 70 to 0 μl/min in 45 min.

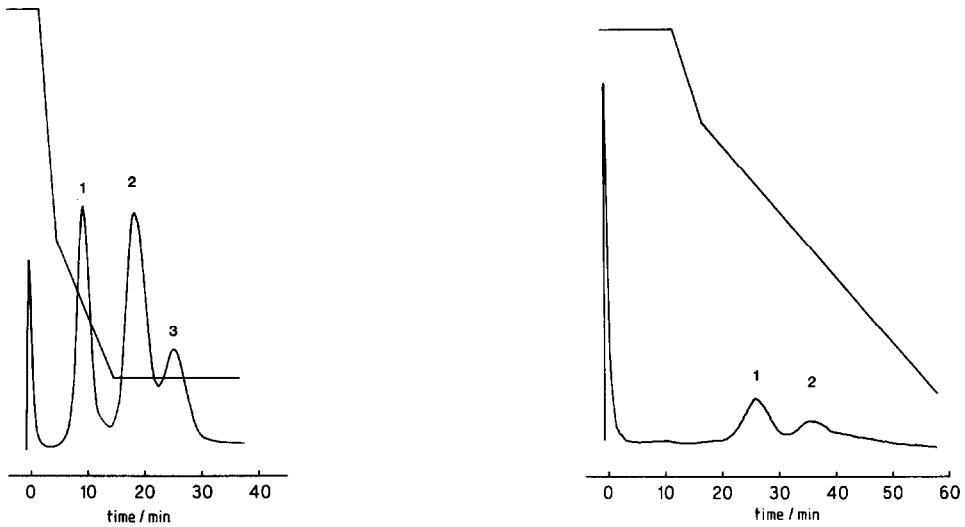


Fig. 4. Separation of polystyrene latex beads, 0.09, 0.30 and 0.80 μm in diameter (peaks 1–3). Fibre radius: 250 μm. F_1 = 200 μl/min; F_2 = 70 μl/min (initially), decreased as shown to the final value of 14 μl/min.

Fig. 5. Separation of the monomer (peak 1) and dimer (peak 2) of the plasmid PUC-8. Fibre radius: 550 μm. F_1 = 500 μl/min; F_2 = 100 μl/min (initially), decreased as shown to the final value of 20 μl/min.

As an application of the system described, we optimized a gradient separation of the monomer and dimer of the plasmid PUC-8, as shown in Fig. 5. Baseline separation was obtained in *ca.* 45 min. A similar separation of the plasmid PBR-325 was published by Kirkland and Yau[8], who used the technique of time-delayed exponential-sedimentation FFF. Using the technically much simpler hollow-fibre flow FFF technique, comparable results are obtained.

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REFERENCES

1 J. Å. Jönsson and A. Carlshaf, *Anal. Chem.*, 61 (1989) 11.
2 J. C. Giddings and K. D. Caldwell, *Anal. Chem.*, 56 (1984) 2093.
3 J. J. Kirkland, S. W. Rementer and W. W. Yau, *Anal. Chem.*, 53 (1981) 1730.
4 J. C. Giddings, P. S. Williams and R. Beckett, *Anal. Chem.*, 59 (1987) 28.
5 J. C. Giddings, L. K. Smith and M. N. Myers, *Anal. Chem.*, 48 (1976) 1587.
6 J. J. Kirkland, S. W. Rementer and W. W. Yau, *Anal. Chem.*m 60 (1988) 610.
7 K.-G. Wahlund, H. Winegarner, K. D. Caldwell and J. C. Giddings, *Anal. Chem.*, 58 (1986) 573.
8 J. J. Kirkland and W. W. Yau, *Science (Washington, D.C.)*, 218 (1982) 121.