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# Preparative desalting of bovine serum albumin by continuous annular chromatography

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**Abstract**

Bovine serum albumin (BSA) was continuously desalted from alkaline salts (a mixture of sodium phosphate, sodium chloride and potassium chloride) using a size-exclusion gel as the stationary phase. An annular chromatograph was used to achieve a continuous mode of operation and therefore a reasonable throughput. Distribution and mass transfer coefficients of the substances as well as bed properties were obtained by batch chromatography. These separations were simulated mathematically applying an approximate linear chromatographic theory. It was shown experimentally and theoretically that the BSA and the salt solution could be recovered continuously in a purity higher than 98%. The influence of rotation rate on the resolution of the individual peaks was investigated.

*Keywords:* Desalting methods; Annular chromatography; Preparative chromatography; Proteins; Albumin

## 1. Introduction

Purification of biological material is an important part of any biochemical research. Among other separation processes, desalting is often an essential step during the isolation and purification of amino acids, peptides and proteins [1]. Gel chromatography is one of the effective methods for desalting proteins [2]. Assuming a proper size-exclusion gel, proteins, which are large molecular structures, cannot enter the pores of the resin and are therefore eluted quickly. Salts which are small molecules do enter the pores, are held by weak bonds and hence are eluted after the large molecules. Using the technique of size-exclusion chromatography, mixtures of small molecules which are retained by the gel cannot be separated and therefore are eluted in a common fraction. Using an ion retardation resin makes it possible to further separate the individual salts which were to be separated from the protein [3].

However, conventional chromatography where a small amount of a solution including the solutes (feed) is applied to the top of the bed and eluted with an adequate eluent, has some disadvantages. The operation is discontinuous and the species are diluted during the elution, a drawback of most chromatographic methods.

Many attempts have been made to increase the capacities of chromatographic devices either through repetitive or cyclic operation on large diameter fixed bed columns, or through continuous feeding and removal of mixtures and their components in a moving-bed system [4]. By using the simulated moving bed (SMB) technology for desalting proteins

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[2] it is necessary to use at least three stationary columns to get a complete separation. This work is focused on a rotating annular chromatograph (AC) which is a cross-flow system where the chromatographic bed moves perpendicularly to the direction of fluid motion within the bed. In an annular chromatograph the stationary phase is located in a single column resulting in an easier operating and process control than in the SMB. The AC in its present form was developed at the Oak Ridge National Laboratory. The concept has been successfully applied to a number of separations of potential commercial interest, including the separation of metals [5–8], the separation of sugars [9,10], the separation of amino acids [11] and recently the separation and cleaning of proteins [12]. The apparatus consist of two concentric cylinders forming an annulus into which the stationary phase is packed. The eluent and feed solutions are continuously fed into the top of the annular bed. While the eluent is uniformly fed to the entire circumference, the feed mixture is introduced in only one sector of the annular bed. The column assembly is slowly rotated while the feed nozzle remains stationary. The rotation effects the separated components to appear as helical bands each of which has a characteristic, stationary exit point. This exit point of each compound is dependent on two factors: (a) linear flow velocity of the eluent; (b) rotation rate of the annulus. As long as conditions remain constant, the retention time of each component and thus the angular displacement from the fixed feed entry will also remain constant. Hence the separation process is truly continuous.

The desalting of bovine serum albumin (BSA) dissolved in an alkaline solution with Toyopearl HW 40F as stationary phase was chosen as a model desalting system. The method described here may be applicable to other separation systems.

## 2. Experimental

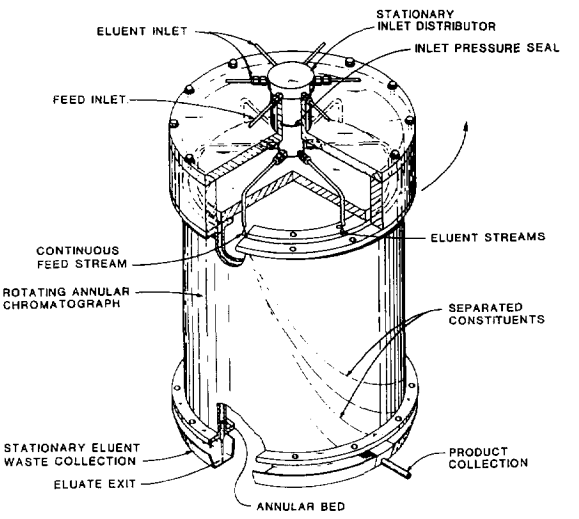
### 2.1. Fixed bed apparatus

Equilibrium and mass-transfer parameters were determined by batch-experiments in a conventional low-pressure liquid chromatography column (Pharmacia C10/20). A defined volume of the pure BSA and of the individual salt solution was injected onto the column. The main part of the apparatus was a 20 cm×1 cm I.D. glass column (Pharmacia) filled with Toyopearl TSK HW-40 F gel (TosoHaas). The gel had a particle size form 30–60 μm and a pore size of 50 Å. The column was equipped with a 1 ml sample loop from where the sample was injected by a polyether ether ketone (PEEK) six-port valve (Upchurch). Eluent was supplied by an Alltech HPLC pump (model 325). Piping and fittings were all made from PTFE. Blue Dextran, a high-molecular-mass saccharide ($M_r=2\cdot10^6$) was used as a tracer to determine the bed void fraction. Blue Dextran fractions were analyzed using a UV spectrophotometer (Hitachi U 1100) at 650 nm the peak maximum representing $\lambda_{max}$. The void fraction was determined as:

$$\varepsilon = \frac{V_0}{V_t} \quad (1)$$

### 2.2. AC apparatus

A schematic of the apparatus used for the continuous separation is shown in Fig. 1. The AC unit was constructed by IsoPro International (Knoxville, TN,




Schematic drawing of an AC

Fig. 1. Schematic drawing of an annular chromatograph.

Table 1
Characteristics of the AC

| | |
|---|---|
| Outer cylinder I.D. | 12.7 cm |
| Inner cylinder O.D. | 11.4 cm |
| Annulus | 0.65 cm |
| Cross-sectional area | 24.6 $cm^2$ |
| Pressure limit | 3 bar |
| Bed length | 36 cm |

USA) of clear Plexiglass, poly(vinyl chloride) (PVC) and stainless steel and is similar to the unit described by Bloomingburg [13]. Table 1 sums up the dimensions and the operating conditions of the laboratory-scale AC used during the experiments. The annular bed is located between two concentric cylinders. The outer cylinder is closed at the top by a PVC flange. The cylinder itself is made of clear Plexiglass to make visual observation during the experiment easier. The inner cylinder is constructed of solid PVC and is shorter than the outer one leaving a head space at the top which allows the eluent to distribute evenly over the entire annulus. A stationary stainless steel header with two PTFE O-ring seals is inserted through the top flange for the introduction of feed and eluent streams. At the bottom of the unit the two cylinders are attached to a second PVC flange. This flange contains 90 1/8 in. exit holes (1 in. = 2.54 cm), each fitted with a porous PTFE plug and a short section of PVC tubing. The exit holes are evenly distributed at 4° intervals along the annulus. To sample the product as a function of angular position, flexible capillary tubing is attached to one of the chromatograph's exit tubes and to a fraction collector (Model AB, LKB, Sweden). The concentration of BSA was determined spectrophotometrically (Hitachi U-1100) at a wavelength of 280 nm. An electric conductivity meter (WTW TetraCon 96) was used to measure the concentration of salts. A digital speed drive system with feedback control and the appropriate gear reducers allowed the AC to be rotated over a wide range of rates (10–600°/h).

Table 2
Base conditions for the AC experiment

| | |
|---|---|
| Eluent flow-rate | 9.3 ml/min |
| Feed flow-rate | 0.3 ml/min |
| Rotation rate | 50°/h<br>100°/h<br>150°/h |
| Feed concentration | 10 g/l BSA<br>10 g/l $Na_2HPO_4$<br>10 g/l NaCl<br>10 g/l KCl |

The AC experiments were performed under the conditions shown in Table 2. The influence of the rotation rate on the resolution of the peaks was studied by varying the rotation rate of the annular bed. The resolution, $R$, is defined as

$$R = \frac{2(\theta_2 - \theta_1)}{W_1 + W_2} \quad (2)$$

where $\theta_1$ and $\theta_2$ represent the angular displacements (from the feed point) of the maximum concentrations of constituents 1 and 2, and $W$ is the constituent bandwidth. Measurements were made at least 1–2 h after start to ensure steady state conditions, although it is likely that steady conditions prevailed almost from the beginning of the experiments.

The size-exclusion gel (TSK HW-40 F) was slurry packed in the annular unit. A 4 cm deep layer of glass beads (100–200 μm in diameter) was packed on top of the gel. The mixture of BSA and salts were eluted by a physiological buffer. The buffer composition, per 1000 ml, includes 1.15 g $Na_2HPO_4 \cdot 2H_2O$, 8 g NaCl, 0.2 g KCl, and 0.2 g $KH_2PO_4$.

The buffer was introduced to the column by a Milton Roy (LMI) positive displacement pump. A pulse dampener was inserted between the pump and the column to reduce pulsation. Feed was delivered to the stationary inlet port by an Alltech HPLC pump (model 325). The tip of the stationary plastic feed nozzle was located in the middle of the glass bead layer. While the AC unit rotates, the glass beads flow around the feed nozzle without significantly disturbing the gel bed below and preventing convective mixing of feed and eluent in the head space of the AC.

### 2.3. Materials

BSA and the salts used in the study were of analytical grade purchased from Fluka and not further purified. The concentration of any salt species and of BSA in the feed solution during the experi-

ments was 10 g/l from each and the feed flow-rate was 0.5 ml/min. Blue Dextran (Fluka) in a concentration of 0.3 g/l was used as a tracer with its peak maximum representing $\lambda_{max}$. Toyopearl TSK HW-40 F gel (1 l) used as the stationary phase was purchased from TosoHaas.

## 3. Theory

The modeling of an AC has been extensively investigated and since it has been reported elsewhere [14–16], we shall only outline the part used in the current studies. It has been shown that the one-dimensional continuity equation is analogous to the two-dimensional by making the following change of variable:

$$\theta = \omega \cdot t \tag{3}$$

As described elsewhere [16] the AC continuity equation neglecting axial dispersion is:

$$\omega\varepsilon \cdot \frac{\delta C}{\delta\theta} + \omega(1-\varepsilon)\cdot\frac{\delta q}{\delta\theta} + u \cdot \frac{\delta c}{\delta z} = 0 \tag{4}$$

Using a film model, as proposed by Howard [9] the fluid particle mass transfer may be written as:

$$\omega(1-\varepsilon)\cdot\frac{\delta q}{\delta\theta} = k_0 a(c - c^*) \tag{5}$$

Assuming linear isotherms (can be made for there is no interaction of the BAS with the stationary phase and the salts are used only in a small concentration) the concentration of a species in the solid and the liquid phase can be related by a linear distribution coefficient, $K$.

$$q = Kc \tag{6}$$

The mass transport parameters $K$ and $k_0a$ could be calculated out of the response to the pulse injection experiments by:

$$K = \frac{\hat{t}_{max} u}{(1-\varepsilon)z} \tag{7}$$

and

$$k_0 a = 16 \ln(2)\cdot\left(\frac{\hat{t}_{max}}{\Delta}\right)^2 \cdot \frac{u}{z}\cdot\frac{1}{K} \tag{8}$$

Carta [17] developed an exact analytical solution for the general case of finite-width, periodic-feed applications while retaining the assumption of linear equilibrium and negligible axial dispersion. Carta's solution, originally describing the behavior of a fixed bed, can be transformed with the use of Eq. (3) to given the solution applicable to geometry and operation of the AC. The analytical solution is presented in Eq. (9):

$$\frac{c(z,\theta)}{c_F} = \frac{\theta_F}{\theta_F+\theta_E} + \frac{2}{\pi}\sum_{j=1}^{\infty}\left\{\begin{array}{l} \frac{1}{j}\exp\left[-\frac{j^2 k_0 a z}{(j^2+r^2)u}\right]\cdot\sin\left[\frac{j\pi\theta_F}{\theta_F+\theta_E}\right]\cdot \\ \cdot\cos\left[\frac{j\pi\theta_F}{\theta_F+\theta_E}+\frac{2j\pi\theta}{\theta_F+\theta_E}-\frac{2j\pi z\omega\varepsilon}{u(\theta_F+\theta_E)}-\frac{jrk_0 az}{u(j^2+r^2)}\right] \end{array}\right\} \tag{9}$$

with

$$r = \frac{k_0 a(\theta_F+\theta_E)}{2\pi(1-\varepsilon)K\omega} \tag{10}$$

Assuming no interactions between species, and conditions of the linear assumptions, Eq. (9) can then be applied to each component in the feed mixture independently to compute concentration profiles for the individual components. A simple computer pro-

Table 3
Distribution and transport parameters

| Species | Distribution coefficient | Mass-transfer coefficient |
|---|---|---|
| BSA | 0.10 | 0.033 |
| $Na_2HPO_4$ | 0.43 | 0.098 |
| NaCl | 0.67 | 0.105 |
| KCl | 0.68 | 0.144 |

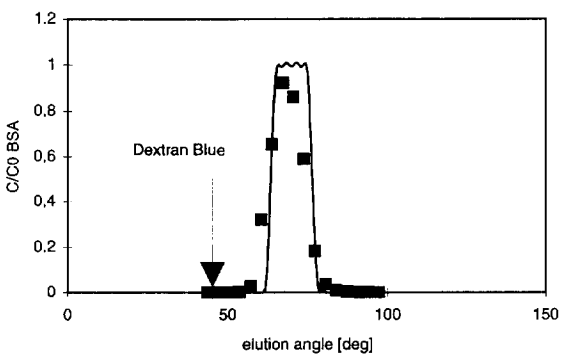



Fig. 2. Comparison of experimental and calculated BSA elution.

gram has been written to handle the series computations leading to the concentration profiles.

## 4. Results and discussion

The distribution and mass-transfer parameters for the BSA and the three salts determined as mean values are listed in Table 3. Feed concentrations were typically 10 g/l of BSA and each salt. The values are estimated from pulse injections of the individual salts and BSA onto the stationary column. The retention time of each component was used to calculate $K$, the distribution coefficient, and the mass transfer coefficient, $k_0a$, was computed using the distribution coefficient and the width of the peak using the standard method for linear chromatograms described by Howard et al. [18]. Injecting Blue Dextran onto the column, and observing its retention time, allowed computation of the bed void fraction,

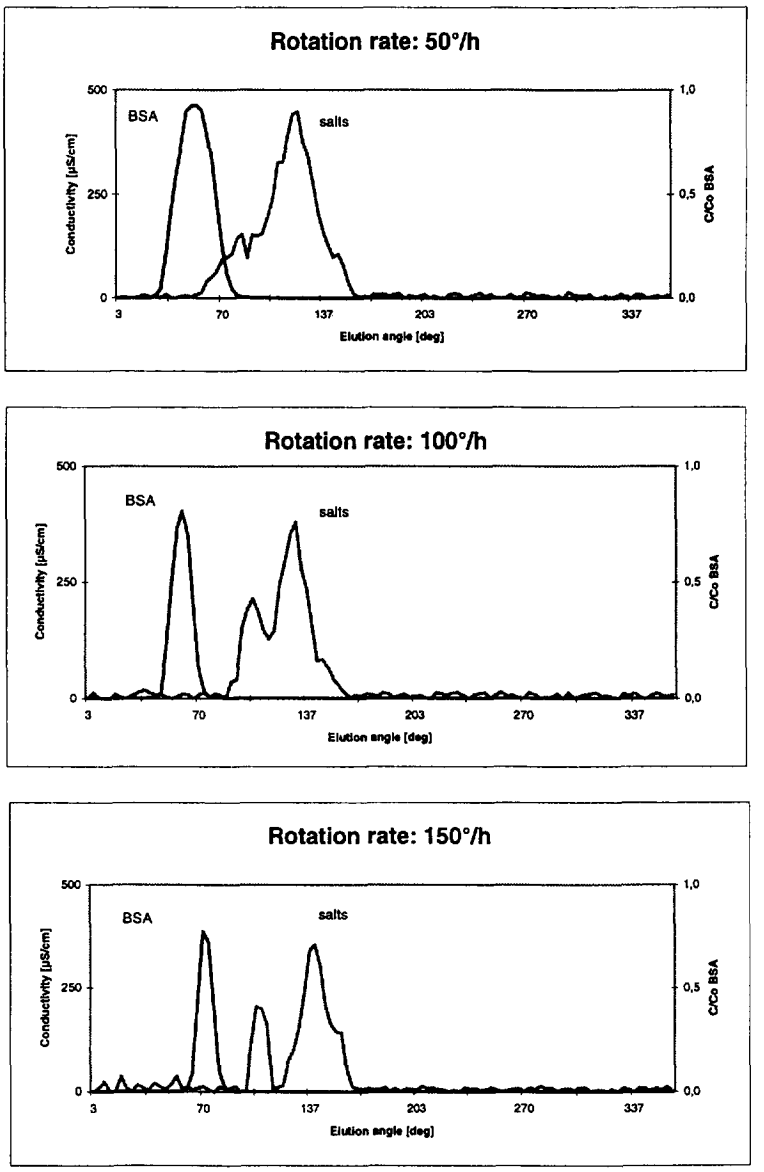



Fig. 3. Effect of the rotation rate on the desalting.

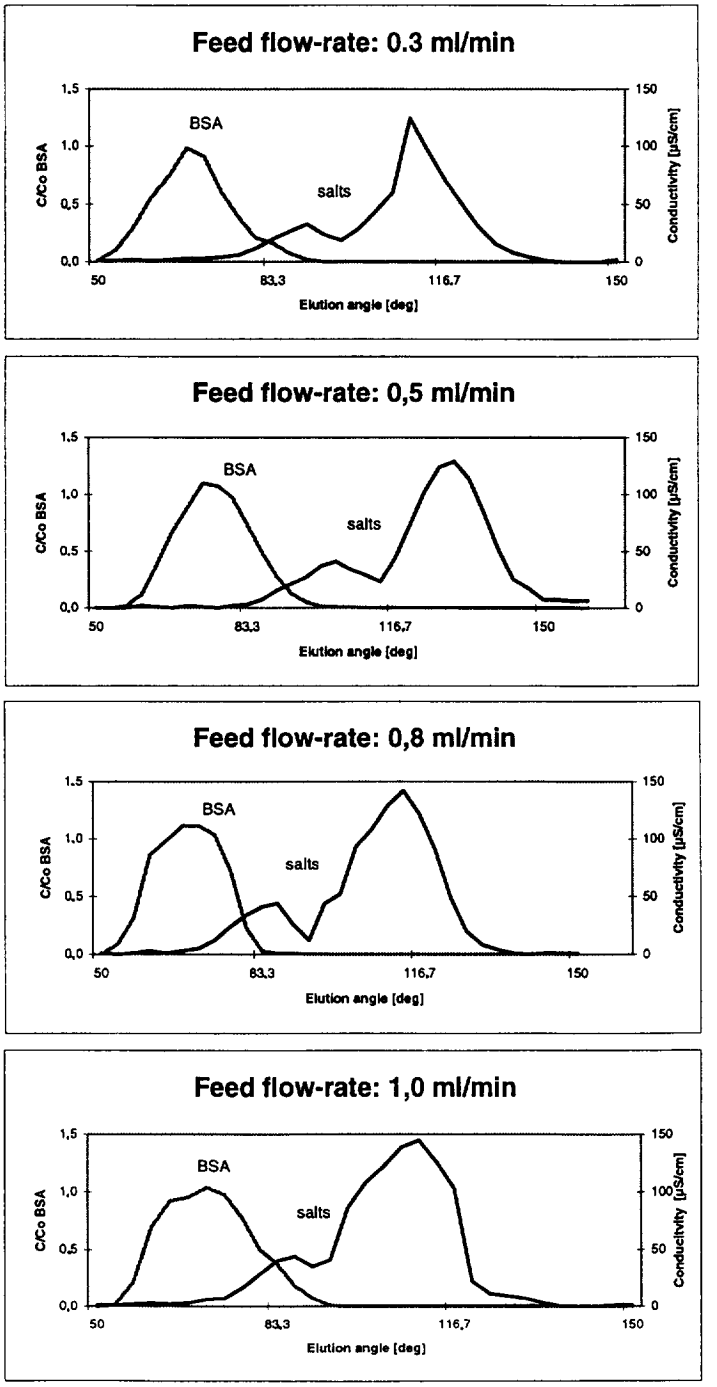



Fig. 4. Effect of the feed flow-rate on the desalting.

$\varepsilon$. It was found to be 0.37, which is normal for closely packed spheres with a narrow size range. The calculated values quoted in Table 3 were subsequently used for the theoretical prediction of the continuous experiments, based on the Carta solution [17].

Fig. 2 shows the comparison of an experimental and calculated BSA peak resulted from the elution in the AC with a rotation rate of 100°/h. The theoretical values can be calculated using Eq. (9). It is obvious that the agreement between theory and experiment is within expectation for the experimental system used here.

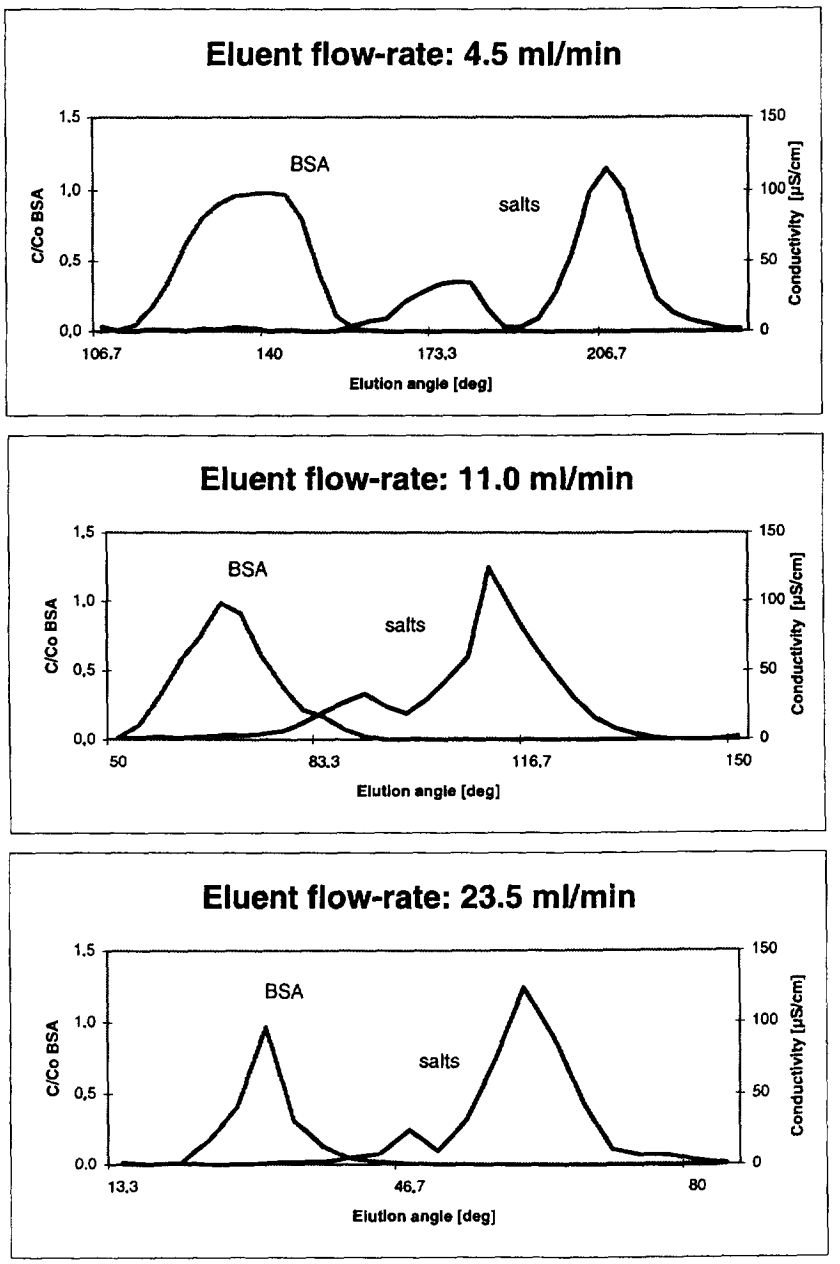



Fig. 5. Effect of the eluent flow-rate on the desalting.

The effect of rotation rate was studied in a series of experiments at the basic conditions listed in Table 2. Fig. 3 shows the effect of the rotation rate on the separation. As expected the separation decreases with decreasing the rotation rate. A good agreement between the theory and the experimental values is obtained. This result is similar to that found by Begovich et al. [15]. We do not know why the peak area of BSA is decreased by increasing the rotation rate.

The effect of the feed flow-rate on the resolution of the individual bands was studied in a series of experiments. The analysed peaks are shown in Fig. 4. It can be seen that increasing the feed rate decreases the resolution. This is in analogy with the mathematical theory.

The elution rate was varied between 4.5 ml/min and 23.5 ml/min. The experimental values in Fig. 5 shown that increasing the elution rate results in narrower peaks, as it can be understood from theory.

Nevertheless, the investigated range of the superficial velocity has no influence on the resolution, which indicates that the separation between large and small molecules using a size-exclusion gel is very simple.

In this study the continuous desalting of BSA using an AC was presented. The practicability of desalting is easily and completely. Because the separation is easy to accomplish the throughput can be increased by increasing the feed flow-rate significantly. Other effects on the separation, like the eluent flow-rate and the feed concentration, were only attempted in batch column until this series of experiments and will be transferred to the AC later.

## 5. Symbols

| | |
|---|---|
| $c$ | liquid phase solute concentration [g/l] |
| $c^*$ | equilibrium solute concentration [g/l] |
| $c_F$ | feed solute concentration [g/l] |
| $K$ | distribution coefficient dimensionless |
| $k_0a$ | global mass-transfer parameters [$s^{-1}$] |
| $q$ | solid-phase average solute concentration [mmol/g gel] |
| $t_{max}$ | reduced retention time [s] |
| $\hat{t}_{max}$ | retention time of Blue Dextran [s] |
| $u$ | linear superficial velocity [cm/s] |
| $W$ | bandwidth [deg] |
| $z$ | bed length [cm] |
| $\varepsilon$ | bed void fraction |
| $\theta$ | displacement from feed point [deg] |
| $\omega$ | rotation rate [deg/h] |
| $\Delta$ | peak width at half concentration [deg] |

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