Convection-Enhanced Mass Transfer in Aggregated Beads for Gel Chromatography

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Convection-enhanced mass transfer in aggregated κ-carrageenan beads was assessed using experimental and mathematical modeling studies. Aggregated beads, prepared from subsidiary beads in a fluidized bed, were employed for gel chromatography of proteins. The efficiency of a chromatography column was measured by the plate height. The plate height of the aggregated beads was much lower than that of conventional carrageenan beads of the same size. This was attributed to intraparticle convection, which occurred even at relatively low flow rates due to the large intraparticle channels resulting from the low degree of aggregation. A general equation for predicting the reduced plate height was modified by using an apparent diffusivity in order to incorporate intraparticle convection. The modified equation was in good agreement with experimental data. The protein productivity of an aggregated bead column (mg/h/mL column) was estimated to be six times higher than that of a conventional column.

Introduction

The development of high performance liquid chromatography (HPLC) has provided a rapid technique for protein separation. Typical separation times for HPLC can range from one minute to two hours (Farnan et al., 1997; Sukla et al., 1998; Majors, 1977; Kalghatgi and Horvath, 1987, 1988; Liao and Hjerten, 1988; Hjerten and Liao, 1988). The fastest processing speed, for example, was reported to be only 15 s for an analytical column packed with 2 μm silica particles (Kalghatgi and Horvath, 1987) and 20 μm macroporous beads (Fulton et al., 1991). Higher speed separation in HPLC was originally achieved by using smaller particles as a column packing. This reduced the mass-transfer resistance in porous beads, increased the surface area for mass transfer, and, therefore, increased the total mass-transfer rate. However, the smaller particles employed as column packing created greater pressure drops and required the use of rigid particles such as silica beads. Various hydrogel beads (such as dextran, agarose, and cellulose) were excluded from HPLC applications due to their low mechanical strength (Janson and Hedman, 1982). Interestingly, these soft gel beads are still widely employed for chromatographic separation, especially for large-scale preparative chromatography, because of their superior chemical stability, versatile chemical derivatization, and easy handling during setup (Knight, 1989; Jungbauer, 1993).

A solution to the dilemma of faster intraparticle mass transfer at the cost of a higher-pressure drop is the concept of intraparticle convection enhanced mass transfer. This can occur, for example, in macroporous beads or in porous polymer rods (Vogt and Freitag, 1998). A typical pore structure of macroporous beads consists of two sizes of pores, small ones allowing for molecular diffusion and large ones allowing for through-pore convection (Afeyan et al., 1990a, 1991; Lloyd and Warner, 1990; Lloyd, 1991; Frey et al., 1993). The intraparticle convection greatly improved the mass-transfer rates

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in porous beads, especially at high flow rates (Rodrigues et al., 1982; Rodrigues and Fereira, 1988; Farnan et al., 1997). This technique opened up opportunities for designers to manipulate the pore structure of porous beads to increase intraparticle mass transfer without sacrificing particle size. It has been shown, for example, that the efficiency of 20 μm rigid macroporous beads, made from poly (styrene-divinyl benzene) (Afeyan et al., 1990a), was higher in terms of shorter separation time than that of conventional HPLC with 5–10 μm particles. With the former, the processing time was reduced by a factor of ten and the protein productivity (mg/h/mL column) increased by a factor of nine.

Macroporous beads can be prepared by emulsion (Nilsson et al., 1986; Lim et al., 1992) or aggregation methods (Frey et al., 1993). With the former method, pore forming agents, such as toluene or sodium carbonate, are mixed with the polymer solution before the gel beads are formed. Macropores are created after removing the pore forming agent. To produce a well connected macroporous system, a high loading of the pore forming agent, usually 20–25 vol. %, is required (Bawa et al., 1985; Zentner et al., 1985; Baker, 1987). However, the primary disadvantage of a high loading of pore forming agent is the great reduction in mechanical strength of the gel bead. For the aggregation method, sub-particles are aggregated to produce larger ones. The large pores then consist of the interstices among the sub-particles. In this case, the original mechanical strength of the sub-gel beads can be preserved.

It has been recognized since the early 1980s that intraparticle convection (fluid flow) can be utilized to enhance mass transport in a porous system (Nir and Pismen, 1977; Cogan et al., 1982; Rodrigues et al., 1982, 1984; Cresswell, 1985). This means that mass transport in porous particles may no longer be limited by slow molecular diffusion. A variety of studies have recently been performed to gain a better understanding of fluid flow through porous media (Bergendahl and Grasso, 1999; Kulkarni et al., 1998; Fredd and Fogler, 1998; Din and Michaelides, 1998; Tessier et al., 1997). Thompson and Fogler (1997), for example, solved fundamental fluid mechanics equations at the pore scale and then translated the results to macroscopic behavior using a network approach. The results showed excellent fits with experimental permeabilities.

In a porous particle surrounded by fluid, solutes in the fluid, carried by convection, can directly enter or escape from the porous system. Convective mass transfer coupled with molecular diffusion can greatly reduce the mass-transfer resistance in porous beads and increase the mass-transfer rate. This technique has been increasingly employed in catalyst reaction (Nir and Pismen, 1977; Cogan et al., 1982; Rodrigues et al., 1982, 1984, 1991; Rodrigues and Ferreira, 1988; Cresswell, 1985), cell immobilization (Nilsson et al., 1986; Stephanopoulos and Tsiveriotis, 1989; Prince et al., 1991; Lim et al., 1992) and chromatography (Fulton et al., 1991, Afeyan et al., 1990a,b, 1992; Lloyd and Warner, 1990; Lloyd, 1991; Frey et al., 1993; Farnan et al., 1997) to improve the processing efficiency by either a shorter processing time or a higher protein productivity. Higher productivity is defined here as processed solute mass per unit time per unit column volume.

There are basically two requirements for intraparticle convection to take place: a large pore size and a driving force (that is, pressure drop). For example, to increase mass-transfer rates, the pore size should be larger than 4,000 Å before effective intraparticle convection occurs in a column packing with 8 μm particles (Lloyd and Warner, 1990; Lloyd, 1991). In a study with 20 μm macroporous poly (styrene-divinyl benzene) beads, the large pores ranged from 6,000 Å to 8,000 Å (Afeyan et al., 1990b). Intraparticle convection is also greatly affected by eluent flow rate. This has usually been represented by a change in the apparent diffusivity or chromatographic plate height with eluent flow rate. In chromatography, the separation measure in a column is defined by the plate height. The apparent diffusivity of porous beads increased dramatically with an increase in flow rate when the Reynolds number was greater than ten (Rodrigues et al., 1982; Rodrigues and Ferreira, 1988). Below this value, the apparent diffusivity was shown to be a constant. For chromatographic applications, the plate height showed less dependence on flow rate when the reduced velocity, a dimensionless parameter, was over 100 (Li et al., 1995).

In the current study, intraparticle convection in polysaccharide beads was investigated because such beads have the advantage of chemical stability, ease of handling, and versatility in applications. Specifically, carrageenan beads, prepared by using a toluene emulsion, were aggregated in a fluidized bed. The stability of the beads was further enhanced by steam treatment and chemical cross-linking. The aggregated beads were assessed for their performance in gel filtration of a protein mixture at varying flow rates and compared to conventional nonaggregated carrageenan beads. It was expected that at high flow rates, an increase in intraparticle convection would reduce the masstransfer resistance in macroporous beads, as indicated by an increase in the apparent diffusivity in aggregated beads. Alternatively, the mass-transfer resistance could be expressed as an equivalent plate height. In this case, the Knox equation (Knox, 1977) typically used to predict the plate height was modified by using the apparent diffusivity in order to incorporate the contribution of intraparticle convective mass transfer to the plate height. The modified equation was fitted to the experimental data from a chromatographic column packed with carrageenan beads of a low degree of aggregation.

Chromatographic Plate Height

The separation power of a chromatographic column is measured by the plate height H. The lower the plate height, the greater the plate number for a given column, and the better the separation power. For easy comparison of various column packings, the dimensionless reduced plate height h is derived by dividing the plate height with the bead diameter d_p. The plate height contributions from several dispersion processes in the rate theory may be added linearly (van Deemter et al., 1956; Knox, 1977; Giddings et al., 1977; Horvath and Lin, 1978)

$$h = h_{\text{disp}} + h_{\text{ext}} + h_{\text{int}} + h_{\text{kin}} \tag{1}$$

The first term in the equation h_{disp} is the reduced plate height contribution due to flow anisotropy, axial diffusion, and packing quality. At high flow rates, it can be simplified to (Knox, 1977; Horvath and Lin, 1978)

$$h_{\text{disp}} = 2\frac{\lambda}{\omega}\nu^{1/3} = A'\nu^{1/3} \tag{2}$$

where λ is a factor related to the eddy diffusivity, ω a column packing parameter, A' a constant for a specific column, and ν is the reduced velocity. This last quantity is related to the interstitial fluid velocity u, the particle diameter d_p, and the effective diffusivity in the stationary phase D_e, as follows

$$\nu = u d_p / D_e \tag{3}$$

Two contributions to the reduction in plate height generated by the mass-transfer resistance in the mobile and stationary phases are h_{ext} and h_{int}, respectively. Compared with mass transfer in the mobile phase, mass transfer in the stationary phase is usually limited due to slow molecular diffusion at very high flow rates (van Deemter et al., 1956; Giddings et al., 1977; Horvath and Lin, 1978). In fact, the contribution from mass transfer in the mobile phase is normally smaller than the other terms (Horvath and Lin, 1978) so that h_{ext} can be ignored. The height associated with the stationary phase (Knox, 1977), meanwhile, is given by

$$h_{\text{int}} = \frac{q k' D_m}{(1 + k')^2 D_e} \nu = C' \nu \tag{4}$$

where q is the configuration factor for particle shape, k' is the ratio of solute mass per unit column volume in the stationary phase to the mass per unit column volume in the mobile phase, D_m and D_e are the diffusivities in the mobile phase and the stationary phase, respectively, and ν is the reduced velocity defined earlier. The parameter C' is therefore a function of diffusivity in the mobile phase and stationary phase.

The last term in Eq. 1, h_{kin}, is the adsorption kinetic contribution which is considered negligible in gel chromatography and will not be discussed here (Horvath and Lin, 1978). Combining Eqs. 1–4 gives the following expression (Knox equation) for the total reduced plate height

$$h = A' \nu^{1/3} + C' \nu \tag{5}$$

Convective Mass Transfer in Aggregated Beads

At low Reynolds numbers ($Re < 1$), the bulk motion of fluid through a column of packed particles is governed by Darcy's law (Giddings, 1965; Nir and Pismen, 1977), which states that the flux of fluid per unit cross section is proportional to the pressure drop per unit column height. For a column packed with gigaporous particles, which are aggregates of a set of subsidiary nonporous particles (Frey et al., 1993), the fluid velocity for each type of particle can be evaluated from the correlations which combine Darcy's law with the so-called Kozeny-Carmen relationship (Janson and Hedman, 1982)

$$u = K \frac{d_p^2 \Delta p}{\mu L}$$

$$u' = K' \frac{d_p'^2 \Delta p}{\mu L} \tag{6}$$

where K and K' are the permeabilities for the aggregated bead within the bed and within the bead between the subsidiary particles, respectively, d_p and d_p' are the diameters of aggregated beads and subsidiary beads, respectively, u and u' are the interstitial fluid velocity for aggregated beads and the intraparticle fluid velocity for subsidiary beads, respectively, $\Delta P/L$ is the pressure gradient in the column, and μ is the viscosity of the fluid. From Eq. 6, the relationship between the intraparticle fluid velocity and the interstitial fluid velocity can be expressed as

$$u' = \frac{K' d_p'^2}{K d_p^2} u \tag{7}$$

Mass transport in aggregated beads can be divided into two parts: molecular diffusion into aggregated beads and convective mass transfer into the aggregate combined with molecular diffusion into the subsidiary beads making up the aggregate. In the first case, solutes in the bulk solution diffuse into or out of the beads, driven by the concentration gradient between the beads and the bulk solution. The mass flux into the aggregated beads by diffusion can therefore be approximated by (Frey et al., 1993)

$$J_{\text{diffusion}} = 2 A_d D_e \frac{(C_m - C_s/K_a)}{d_p} \tag{8}$$

where the dimensionless factor A_d corrects for diffusional resistances in the aggregated beads and also takes into account the intraparticle void fraction, C_m is the concentration of solute on the bead surface in solution, C_s is the average concentration in the beads, and K_a is the partition coefficient. Frey et al. (1993) developed a similar relation for the case where diffusion is the sole transport mechanism inside the bead and the subsidiary particles are nonporous.

For intraparticle convective mass transport, solutes, which are carried by fluid, directly enter the aggregated beads and then diffuse into the subsidiary beads. The equation for convective mass flux into the beads is similar in form to the equation for convective flux across membranes with series resistances (Geankoplis, 1993) and is given by

$$J_{\text{convection}} = \frac{C_m - C_s/K_a}{\dfrac{1}{A_c u'} + \dfrac{d_p'}{2 A_s D_e}} \tag{9}$$

where A_c and A_s are dimensionless correction factors to account for the change in surface areas in a unit column volume for intraparticle convection and for diffusion into the subsidiary beads, respectively. On the right side of Eq. 9, the first term in the denominator is the mass-transfer resistance for intraparticle convection. It decreases with an increase in flow rate. The second term is the resistance generated by molecular diffusion in subsidiary beads. Equation 9 is similar to Eq. 8 with one additional resistance added to account for convection effects.

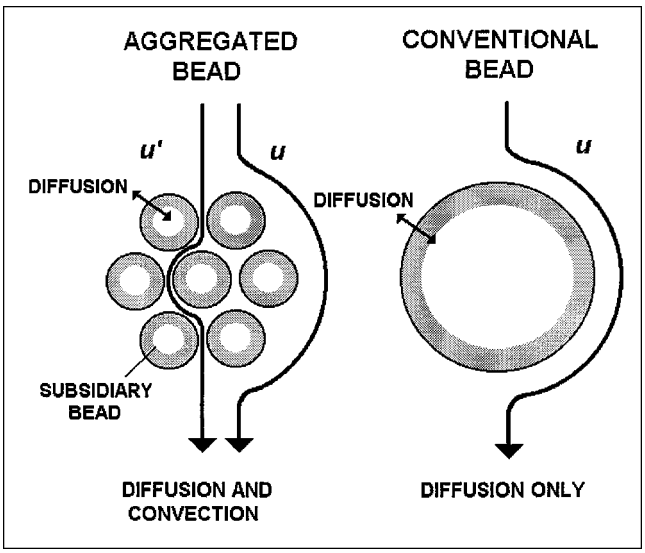

Figure 1. Beneficial effect of convective intraparticle fluid flow on solute diffusion inside an aggregated bead made from subsidiary beads.

Intraparticle convection increases the total mass transfer in the aggregated bead independently. Conventional beads show only interstitial fluid flow.

At high flow rates, solutes moving along a chromatographic column in a concentrated band contact each stationary bead for only a very short period of time. Solute diffuses into the surface region of the bead; however, the exterior contact time is short compared to the internal diffusion times to travel well into the bead interior. Most of the internal volume of the bead is therefore not affected by diffusion in such a short time. This reasoning was supported by Hines and Maddox's (1985) analysis on solute diffusion into a spherical particle. Thus, with high velocities, intraparticle convective mass transfer might dominate in most of the internal volume of the aggregated bead. If diffusion flux and convective flux are assumed to be two independent processes (Figure 1), then the total mass flux into the aggregated beads might be represented by a linear addition of the diffusion flux and the convective flux

$$J_{\text{total}} = J_{\text{diffusion}} + J_{\text{convection}} \tag{10}$$

Treating this total flux as a dispersive process, we have

$$J_{\text{total}} = 2\,A_d\,D_p(C_m - C_s/K_a)/d_p \tag{11}$$

where D_p is the apparent diffusivity in the aggregated beads.

With the substitution of Eqs. 3, 8, 9, and 10 into Eq. 11, the effective diffusivity can be written as

$$D_e = D_p\frac{1+a\nu}{1+b\nu} \tag{12}$$

where constants a and b are

$$a = \frac{A_c K' d_p'^3}{2\,A_s K d_p^3}$$

$$b = a + \frac{A_c K' d_p'^2}{2\,A_d K d_p^2} \tag{13}$$

The effective diffusivity D_e in Eq. 4 for conventional beads is now replaced by an apparent diffusivity D_p for the aggregated beads. The latter accounts for intraparticle convection in addition to diffusion. The contribution of intraparticle mass transfer to the plate height in Eq. 4 becomes

$$h_{\text{int}} = C'\nu\frac{1+a\nu}{1+b\nu} \tag{14}$$

Due to the presence of convection inside aggregated beads, the eddy diffusivity $E = \lambda u d_p'$ is generated by fluid flow around the subsidiary beads. Equation 2 can therefore be corrected to

$$h_{\text{disp}} = A'\frac{d_p'}{d_p}\nu^{1/3} \tag{15}$$

Substituting Eqs. 14 and 15 into Eq. 1 yields

$$h = A'\frac{d_p'}{d_p}\nu^{1/3} + C'\nu\frac{1+a\nu}{1+b\nu} \tag{16}$$

Equation 16 is a modified Knox equation which accounts for intraparticle convective mass transfer. It can be employed for predicting the reduced plate height of aggregated beads in a chromatographic separation.

Experimental Procedures

Materials

κ-Carrageenan, epichlorohydrin, surfactant Span 60, and a molecular weight marker standard kit, MW-GF-1000, were obtained from Sigma Chemical Co. (St. Louis, MO). Toluene, ethanol, and acetone were supplied by BDH Inc. (Toronto, Canada). All other chemicals were of analytical grade.

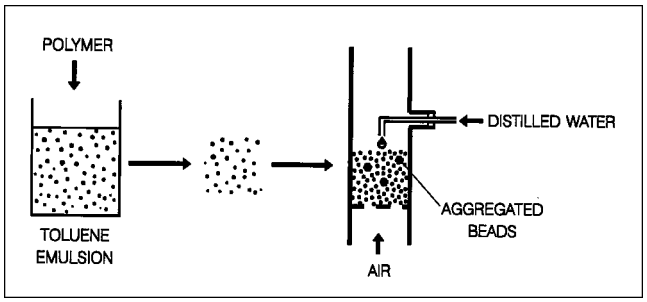

Figure 2. Procedure for preparation of aggregated carrageenan beads in a fluidized bed.

Preparation, cross-linking and desulfurization of aggregated carrageenan beads

The preparation of macroporous carrageenan beads is shown in Figure 2. In the first step, 100 mL κ-carrageenan solution, 4% (w/v), at 80°C was added to 250 mL toluene with 4% (w/v) Span 60. The mixture was stirred at 1,500 rpm with a 2 in. turbine propeller (Cole-Parmer Instrument Co., Anjou, Canada) for 2 min at 80°C to give an emulsion. The temperature of the emulsion was lowered to room temperature, 25°C, under continuous stirring to allow the gel beads to form. After the stirring was stopped, the beads were allowed to settle for 15–20 min. The toluene on top of the beads was removed by decantation. The collected beads were then washed with ethanol, followed by acetone, and sieved in acetone at a range 75–180 μm (sieve No. 200 and No. 80, Endecotts Ltd., London, U.K.). The beads, which were called subsidiary beads since they were used to make the aggregated beads, were finally dried in vacuum. Conventional sized beads, 180–500 μm, were prepared in the same way as subsidiary beads, but using different sieves (No. 80 and No. 35).

In the second step, dried carrageenan subsidiary beads were aggregated using 60 mL filtering funnels (Fisher Scientific, Toronto, Canada). Between 8 and 10 g of beads were placed inside the funnels and fluidized by air at a flow rate of 1,000–1,500 mL/min. Distilled water was sprayed into the top of the bed to allow for aggregation of the beads. To prevent bed quenching (in which the beads could not be fluidized due to too much liquid in the bed), a strong vibration (that is, mechanical shaking) was needed to activate the bed. After injecting the aggregating liquid for 10 min, the beads were dried in the fluidized bed for 20 min and then sieved in a range of 100–300 μm (sieve No. 140 and No. 50) to obtain the final dry aggregated beads.

The beads were then treated with steam at 120°C for 2 min to increase the aggregated bead strength by adhesion between beads. Environmental scanning electron microscopy (ESEM) micrographs were taken of the dried beads.

The aggregated beads were first placed in 0.3 M KCl solution for 2 h to allow for swelling. The swollen carrageenan beads, 200 mL, were thoroughly washed with 0.3 M KCl, followed by 2 M KOH. The beads were then suspended in 500 mL of 2 M KOH with 2.5 g sodium borohydride ($NaBH_4$) and 25 mL epichlorohydrin at 60°C for 1 h with adequate stirring. The aggregated beads were chemically cross-linked by epichlorohydrin. After the reaction, the cross-linked beads were filtered, washed with 0.3 M KCl, and resuspended in 1 L of 2 M KOH with 5 g sodium borohydride. The mixture was desulfated by autoclaving at 120°C for 20 min. In the final step, the cross-linked and desulfated carrageenan beads were thoroughly washed with 0.1 M KCl and sieved in a size range of 180–500 μm (No. 80 and No. 35). The final bead structure was examined visually under a microscope.

Gel filtration

A slurry of aggregated carrageenan beads (180–150 μm) in 0.1 M phosphate buffer with 2% KCl and 0.02% sodium azide at pH 7 was loaded onto a 1.6 cm (ID) glass column (LBK Instruments, Inc., MD). The beads were then compacted at a flow rate of 50–100 mL/h for 1 h. A phosphate buffer (0.01 M) containing 0.1 M KCl and 0.02% sodium azide at pH 7 was washed through the column for one day at a flow rate of 10 mL/h. Protein samples (molecular weight marker standard proteins from a MW-GF-1000 kit for gel chromatography) were dissolved in phosphate buffer and loaded onto the column from the top (0.4–2 mL). The eluent was collected with a fraction collector (LKB 2070 UltroRac II, Pharmacia Biotech Inc., Baie D'Urfé, Canada) in 0.5–2 mL samples and analyzed at 280 nm. Protein concentration in the eluent was determined by measuring the absorbance at 280 nm with a UV/Vis spectrophotometer (Philips PU8700 Pye Unicam Ltd., Cambridge, U.K.).

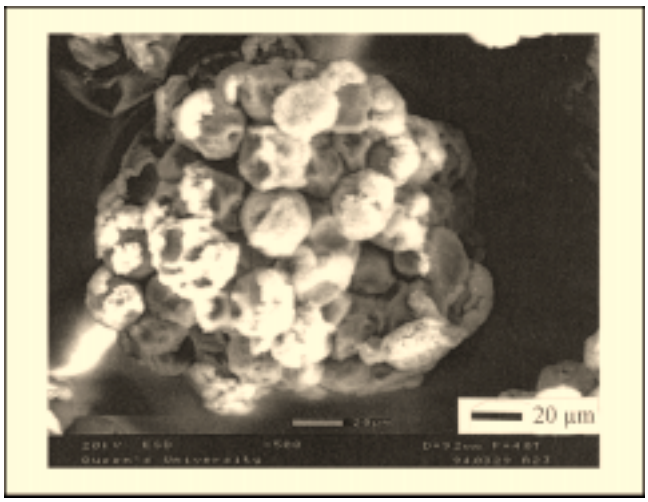

Figure 3. Macroporous carrageenan beads prepared from smaller subsidiary beads by aggregation in a fluidized bed.

Results and Discussion

Preparation of aggregated carrageenan beads

Large aggregated κ-carrageenan beads, 180–500 μ, were successfully prepared from smaller subsidiary beads, 75–180 μm diameter, using a fluidized bed (Figure 3). However, aggregated beads not exposed to steam treatment were unstable in 0.3 M KCl solution. After ten minutes, for example, all the beads disintegrated into smaller particles in the solution. In contrast, beads treated with steam for 2 min showed good stability in KCl solution (Figure 4) with only a few of the aggregated beads disintegrating. This may be associated with partial melting of the surface of the subsidiary beads due to the high temperature and their subsequent merging through adhesion at the contact points. When the beads returned to the ambient temperature, the contact between the subsidiary particles became much stronger. Nevertheless, the contact mechanical strength, determined visually, varied among aggregated beads; after cross-linking and desulfating, not more than 40% (v/v) of the aggregated beads could be recovered. Many aggregated beads with weak connections were broken down by extensive stirring.

Gel filtrations using aggregated beads

The flow rate as a function of column pressure drop was determined from a column (34×1.6 cm ID) packed with ag-

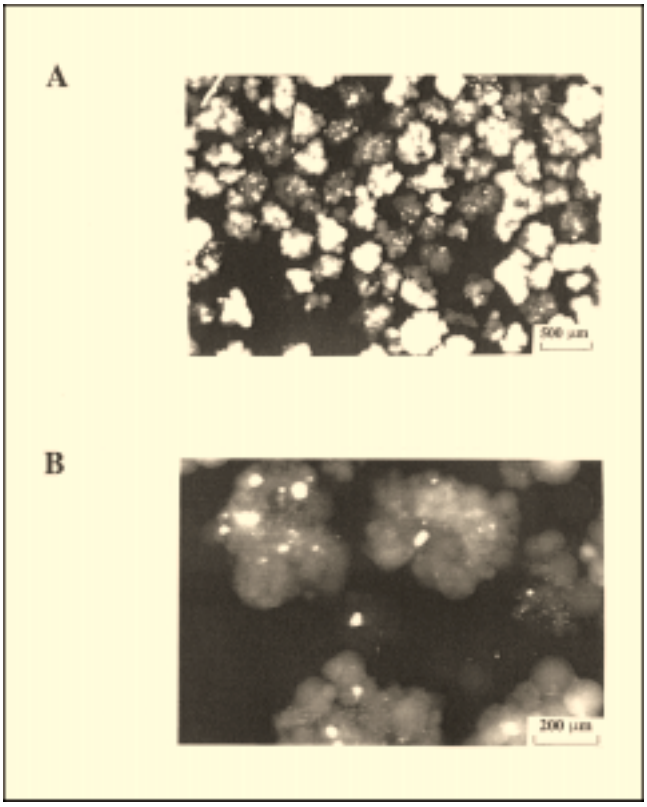

Figure 4. Aggregated carrageenan beads were stable in 0.1 M KCl solution.

A: 26× magnification, B: 88× magnification.

gregated carrageenan beads or conventional carrageenan beads. The flow rate was a linear function of pressure drop when the former was under 120 mL/h for an aggregated bead column, 190 mL/h for a conventional bead column, and under 46 mL/h for a subsidiary bead column (Figure 5). Above these flow rates, bed compression was observed. The increase in flow rate followed a nonlinear behavior apparently slowing down as the pressure drop was continuously raised. Particularly, for the small subsidiary beads (75–180 μm), the flow rate actually decreased slightly from 67 mL/h to 64 mL/h as the column pressure drop increased from 5.8 cm H_2O to 7.0 cm H_2O. After removal of the pressure, the height of the packed beds returned to its original position.

The flow resistance of aggregated beads, 180–500 μm, was apparently lower than that for subsidiary beads, 75–180 μm diameter. At a column pressure drop of 3.2 cm H_2O/cm column, for example, the flow rate obtained with a subsidiary bead column was 56 mL/h compared with 100 mL/h for the aggregated column. The reduction in resistance was mainly attributed to an increase in bead size due to the aggregation. However, compared with conventional beads of the same size (that is, 180–500 μm), aggregated beads showed a higher resistance to fluid flow; the flow rate was 150 mL/h at a column pressure of 1.1 m H_2O for a column filled with 180–500 μm conventional beads.

The molecular weight calibration curve of a column filled with aggregated beads changed slightly compared with that of conventional carrageenan beads (Figure 6). Generally, the

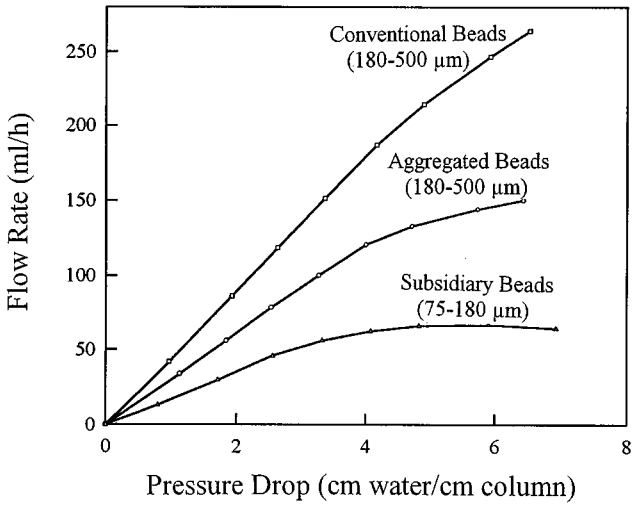

Figure 5. Flow rates as a function of pressure drop in a column (34×1.6 cm ID) filled with conventional beads, aggregated or subsidiary beads.

elution volume for thyroglobulin (M.W. 669,000), for example, went down from 1.49 to 1.46. In contrast, smaller molecules were eluted slightly faster from the aggregated beads than from the conventional beads. For example, the dimensionless elution volume went down from 2.20 to 1.98 for albumin (M.W. 66,000) and from 2.62 to 2.37 for carbonic anhydrase (M.W. 29,000). The decrease in the elution volume was caused by an increase in the void volume of the column due to aggregated packing. The void volume determined by the elution of the blue dextran was 0.36 for the aggregated column and 0.31 for the conventional column. These results were to be expected due to the channels inside aggregated beads.

Thyroglobulin and albumin were eluted from a column (32.5×1.6 cm ID) packed with aggregated beads (180–500 μm) at varying flow rates (Figure 7A). The results were compared with elution of two proteins from a column (34×1.6 cm ID) filled with conventional beads of the same size (Figure 7B). The two proteins were separated better with the aggregated column than with the conventional column. At a flow rate of 1.3 mL/h, for example, the resolution for protein separation was 1.57 for the aggregated column and 0.98 for the conventional column (Figure 8). As the flow rate increased to 4.5 mL/h, the rate of decrease in resolution began to slow down with the resolution dropping to 1.15 for the aggregated column and 0.63 for the conventional column. The separation of thyroglobulin and albumin was still distinguishable at a flow rate of 35 mL/h for the aggregated column with a resolution of 0.46. In contrast, at such flow rates, the two proteins were barely separated with the conventional column.

Compared to conventional carrageenan beads, aggregated carrageenan beads produced much lower reduced plate heights in gel chromatographic separation at various reduced velocities (Figure 9), particularly at high flow rates. The reduced plate height of the aggregated beads (180–500 μm), for example, was 55% lower at a reduced eluent velocity ν of 30, and 70% lower when ν equaled 600 for albumin. For thyroglobulin, it was 60% lower at a reduced velocity of 1,400.

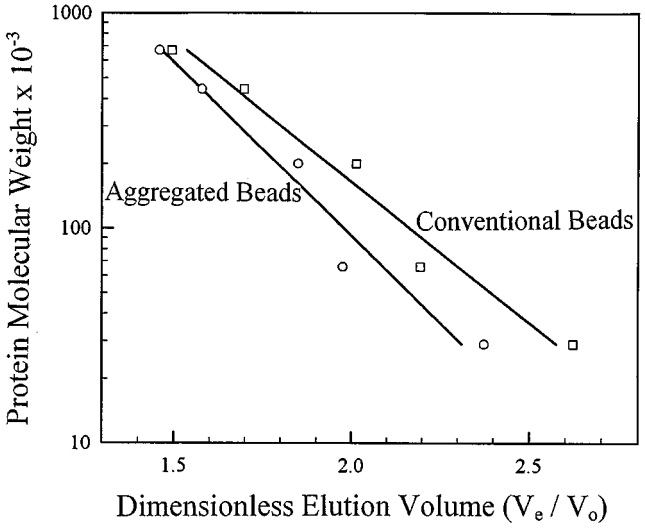

Figure 6. Molecular weight calibration curves for aggregated and conventional carrageenan beads respectively.

V_e is the protein elution volume and V_o is the void volume of column determined by blue dextran (MW 2,000,000).

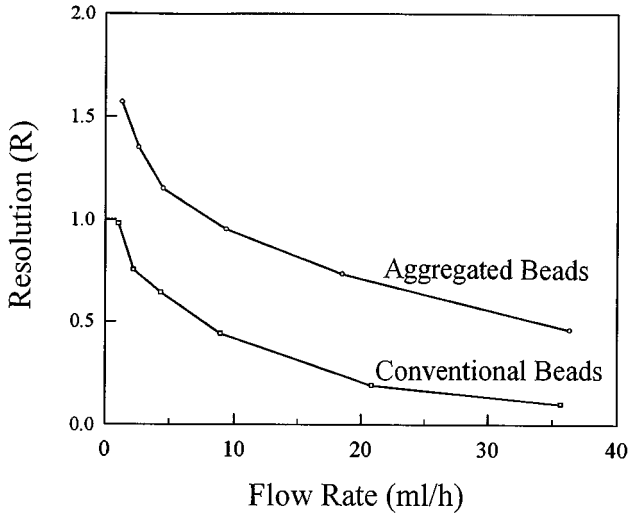

Figure 8. Resolution of an aggregated carrageenan column (32.5×1.6 cm ID) and a conventional column (34×1.6 cm ID) for separating thyroglobulin and albumin as a function of flow rate.

$R = 2(V_{e1} - V_{e2})/(W_1 + W_2)$.

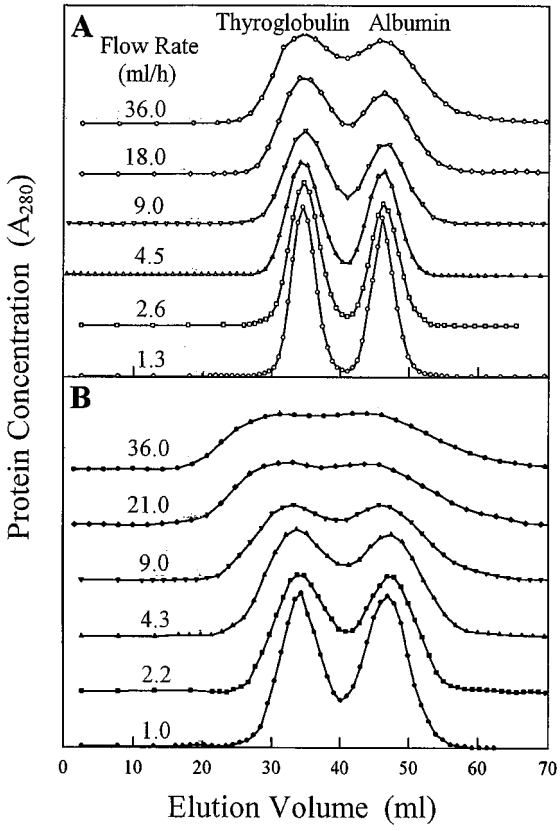

Figure 7. Elution of thyroglobulin and albumin from A: a column (32.5×1.6 cm ID) packed with aggregated carrageenan beads (180–500 μm); B: a column (34×1.6 cm ID) packed with conventional carrageenan beads (180–500 μm).

The lower reduced plate height indicated that intraparticle convection increased mass-transfer rates in the aggregated beads, even at reduced velocities as low as 30. This result differed from those of other researchers working with macroporous beads (Afeyan et al., 1990b; Lloyd and Warner, 1990; Frey et al., 1993; Li et al., 1995), who reported values for reduced velocities down to 100. The presence of intraparticle convection in aggregated carrageenan beads at low flow rates could be explained by the low degree of aggregation, which is defined as the ratio of aggregated bead diameter to subsidiary bead diameter. The degree of carrangeenan bead aggregation was only 2.7 in the present study, while the degree of aggregation of the polystyrene-divinyl benzene macroporous bead system (Frey et al., 1993; Farnan et al., 1997) was 10−50. For an aggregated bead, intraparticle channels become larger with a decrease in the degree of aggregation. Thus, in turn, provides a lower intraparticle resistance to fluid flow.

Comparison of theory with experimental results

The reduced plate height of conventional carrageenan beads was expressed as a function of the reduced velocity by Eq. 5. The calculated results (solid lines in Figure 9) were in good agreement with experimental data (open and filled circles) obtained from albumin and thyroglobulin. The constants A' and C' in Eq. 5 were 0.65 and 0.019 for albumin, and 0.80 and 0.028 for thyroglobulin, respectively (Table 1). The value of the constant A' was within the range from 0.5 to 3.0, as previously indicated by Knox (1977). The higher the A' value, the poorer the column packing quality. The constant C' usually varies widely from 0.02 to 0.30 (Knox, 1977; Giddings et al., 1977). Our value of C' for albumin was at the low end of the range observed by most researchers. The cal-

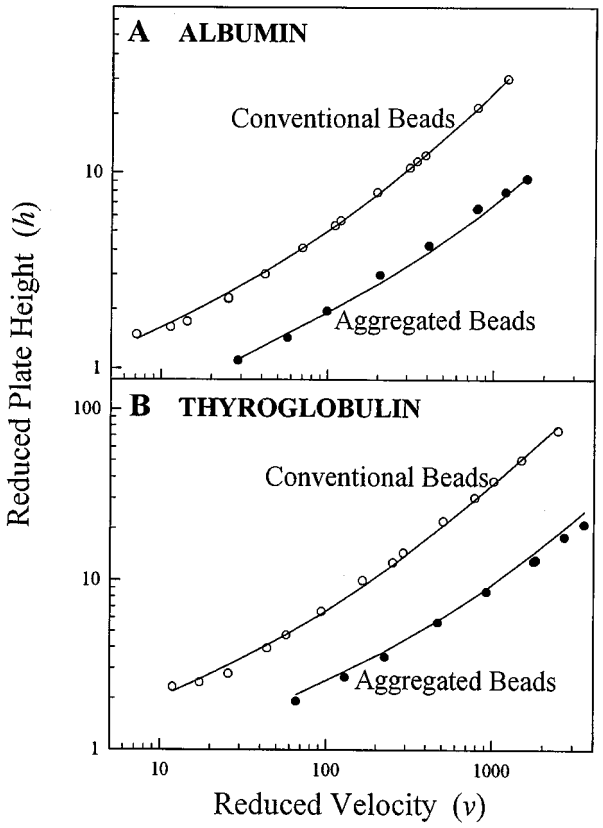

Figure 9. Reduced plate height as a function of the reduced velocity for A: albumin; B: thyroglobulin.

Solid lines are calculated by Eq. 5 for conventional beads and by Eq. 16 for aggregated beads. Circles are experimental results.

culated results showed that the simplified Knox equation provided a reliable prediction of reduced plated height for the conventional nonaggregated beads at high flow rates.

Equation 16 was modified from Eq. 5 by considering intraparticle convective mass transfer. The ratio $(1+a\nu)/(1+b\nu)$ in Eq. 16 represents the change in mass-transfer resistance caused by intraparticle convection in aggregated beads. Since the present carrageenan beads had a low degree of aggregation (2.67), intraparticle convection could easily be generated at low flow rates. Owing to this and also to allow for better curve fitting, the eddy diffusion term was modified by using the subsidiary particle size instead of the aggregated particle size. This was represented by d'_p/d_p in Eq. 16. The calculated results based on this equation agreed well with experimental observations obtained by running albumin and thyroglobulin through a column loaded with aggregated carrageenan beads (Figure 9). The two constants a and b in Eq. 16 were found to be 0.006 and 0.03, respectively. Apparently, an $\nu \to \infty$, the ratio of $(1+a\nu)/(1+b\nu)$ in Eq. 16 tends to a/b. This suggests that the contribution of intraparticle mass transfer in aggregated beads to the reduced plate height is reduced by a factor of five at high flow rates (that is, $a/b = 1/5$). In constrast, there was only a 38% reduction at low flow rates (such as $\nu = 30$). However, because Eq. 16 reflects axial diffusion, it does not apply at very low flow rates (that is, below $\nu = 30$).

Owing to the presence of intraparticle convection, intraparticle mass transfer was greatly improved. The reduced plate height of aggregated beads was much lower than that of conventional beads at the same reduced velocity. This indicated that, when the particle size of aggregated beads is the same as that of conventional beads, the plate height H of the former is lower than that of the latter. Aggregated beads should therefore provide better separation resolution (that is, more clearly defined peaks) than conventional beads in a column of the same height. Alternatively, if the resolution is to remain constant, an aggregated column can be operated at a higher flow rate than a conventional column. An aggregated column should thus be able to provide a higher productivity in terms of processed protein mass per unit time per unit column volume.

The productivity of an aggregated column was compared with a conventional column in separating thyroglobulin from albumin through a theoretical calculation based on experimental results and Eqs. 5 and 16 (Figure 10). The purity of thyroglobulin was arbitrarily set at 99.9% (w/w). The elution

Table 1. Constants in Knox Equation* for Albumin and Thyroglobulin Obtained by Curve Fitting Experimental Results Based on a Conventional Carrageenan Column

	Constants in Eq. 5	
Proteins	A'	C'
Albumin	0.65	0.019
Thyroglobulin	0.80	0.028

*Knox equation was simplified by removing the term B'/ν.

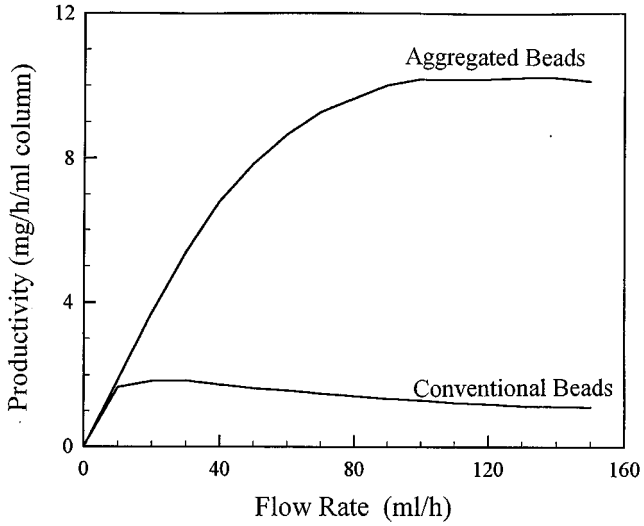

Figure 10. Thyroglobulin productivity of an aggregated bead column with a conventional column in gel chromatography as a function of flow rate.

Assuming column length, $L = 40$ cm, cross-section area $A_t = 1$ cm^2, particle diameter, $d_p = 200$ μm, sample concentration $C_0 = 25$ mg/mL for each thyroglobulin and albumin. The sample loading was 3% of column volume. The purity of the collected product was 99.9% (w/w).

profile of both proteins was assumed to have a normal distribution (Glueckauf, 1955). The column assumed length L was 40 cm, the column cross-sectional area A_t was 1 cm^2, the particle diameter d_p was 200 μm, the sample concentration C_o was 25 mg/mL for each thyroglobulin and albumin, and the sample loading volume was assumed to be 3% of the column volume (12 mL). The calculated results showed that both columns had almost the same productivity at low flow rates (<10 mL/h), so that almost all the thyroglobulin was recovered within the purity limit. However, with an increase in the eluent flow rate, the concentration of albumin increased quickly in the thyroglobulin eluent from a conventional column. This reduced the amount of recoverable thyroglobulin and decreased the productivity. The productivity of a conventional column reached the maximum, a 1.85 mg/h/mL column, at 30 mL/h flow rate, after which it slowly decreased to 1.1 mg/h/mL column at 150 mL/h. On the other hand, because the aggregated column provided a better separation between the two proteins, there was not as much overlap. The amount of recoverable thyroglobulin eluted from the aggregated column was greater than that of the conventional column and, as a consequence, the productivity of the aggregated column was therefore higher.

The productivity also increased with an increase in flow rate. However, when the flow rate was over 60 mL/h, the increase in productivity of the aggregate column began slowing down. At a flow rate of 135 mL/h, the maximum productivity of the aggregated column was a 10.2 mg/h/mL column. The maximum productivity of the aggregated column was about six times higher than that of a conventional column. The simulations suggest that intraparticle convection greatly improves mass-transfer rates in the aggregated beads, which results in an increase in the separation resolution and the processing speed or the productivity.

Conclusions

Aggregated carrageenan beads with a low degree of aggregation were successfully prepared with a fluidized-bed technique. The dimensionless elution volume of small proteins decreased as the total void volume increased in the aggregated beads. Intraparticle convection occurred in aggregated beads at reduced velocities as low as 30. This effectively decreased the reduced plate height of the aggregated beads in gel filtration. At high flow rates, a greater decrease in reduced plate height was observed with aggregated beads compared to conventional carrageenan beads of the same size. Mass transfer in aggregated beads was further improved by better intraparticle convection at high flow rates.

A general equation proposed by Knox (1977) (Eq. 5) was modified to incorporate intraparticle convective mass transfer for predicting the reduced plate height. A change in mass-transfer resistance with intraparticle convection in aggregated beads was represented by the ratio $(1+a\nu)/(1+b\nu)$ in the modified Knox equation (Eq. 16). The two constants a and b were found to be 0.006 and 0.03, respectively. The equation was in excellent agreement with experimental observations obtained from protein gel chromatography in a column packed with macroporous carrageenan beads with a low degree of aggregation. At high flow rates, the contribution of intraparticle convective mass transfer to the reduced plate height was greatly reduced. The modified equation was only applicable to beads with a low degree of aggregation at reduced flow velocities over 30, where intraparticle convection was present.

Intraparticle mass transfer may be greatly improved by intraparticle convection. This should result in higher separation resolution and/or a higher processing speed. Our preliminary investigation on the column performance in gel filtration suggests that an aggregated column could provide at least five times higher productivity than a conventional column. Future experiments should include a more rigorous uncertainty analysis.

Notation

C_o = sample concentration
C_s = average concentration of solute in the beads
d_p' = diameter of subsidiary bead
h_{kin} = reduced plate height contribution due to adsorption kinetics
$J_{\text{convection}}$ = intraparticle convective mass transport
$J_{\text{diffusion}}$ = mass flux into beads by diffusion
J_{total} = total mass flux
Δp = pressure drop in column
V_e1 = elution volume of protein 1
V_e2 = elution volume of protein 2
W_1 = peak width of protein 1
W_2 = peak width of protein 2

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