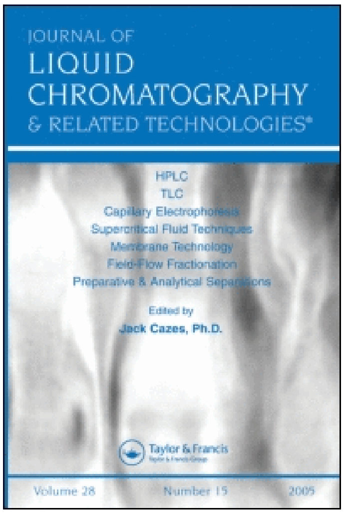

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PROTEIN BAND DISPERSION IN AXIAL AND RADIAL FLOW CHROMATOGRAPHY

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ABSTRACT

Radial flow (RF) column configurations have been developed for larger scale production liquid chromatography, with the primary purpose of increasing throughput rates and decreasing trans-bed pressure drops in comparison to conventional axial flow (AF) columns. The RF columns have been quite successful in attaining these two objectives. In this work, we investigated the nature of protein band dispersion in both axial and radial flow chromatography column configurations, utilizing S-200 Sephacryl gel filtration media with bovine serum albumin as the applied protein. The effects of input feed flow rate as well as input feed albumin concentration on the nature of protein band dispersion were studied. Protein band dispersion was quantified by examining the shape of the eluting protein peak and measuring peak height-to-width (HTW) ratio's for the various flow rates and feed concentrations used in the study. Our results indicate that protein dispersion was larger in RF columns as indicated by smaller peak HTW ratio's than those obtained for AF columns, operated at the same flow rates and feed protein concentrations. We also found that moderately increasing the flow rate increased peak HTW ratio's and resulted in sharper peaks for both AF and RF columns.

INTRODUCTION

Radial flow (RF) columns were first developed to handle large gas flow rates through packed catalyst beds with minimal pressure losses across the bed

(1). Since their development, the fluid mechanics (2) and kinetics (3) of RF systems, as well as their extension to reverse osmosis (4) and cell culture (5) systems have been studied.

Columns for RF chromatography were soon developed (6) and their applications demonstrated that reduced trans-bed pressure drops as well as increased throughputs could be achieved. RF columns have been commercially available for some time (6,7), and they have been utilized for the purification of plasminogen (8) and other biologicals (9). In our laboratories we have documented decreased process times and trans-bed pressure drops and increased throughputs (10) and studied their application to affinity systems and shown that both RF and AF systems perform equivalently (11).

Schematics of the RF and AF columns are depicted in Figure 1a and 1b, respectively. As can be seen from the figure, the cross sectional area normal to the flow direction will either be increasing (as in the case of centrifugal [CF] flow) or decreasing (as in the case of centripetal [CP] flow). Thus, the linear velocity in CF flow in RF columns is decelerating while that in CP flow is accelerating. In an AF column, on the other hand, the cross-sectional area normal to the flow direction is constant, resulting in a flow with constant velocity. The implications of this is that mass transfer coefficients and radial dispersion cannot be assumed constant in the flow direction (12). Thus, protein band spreading will differ for the RF and AF columns.

In this study, we investigated the nature of protein band dispersion in both AF and RF columns. We utilized columns of the same total volume (50 mls) and similar bed height (AF: 2.9 cm; RF 3.0 cm) packed with Sephacryl S-

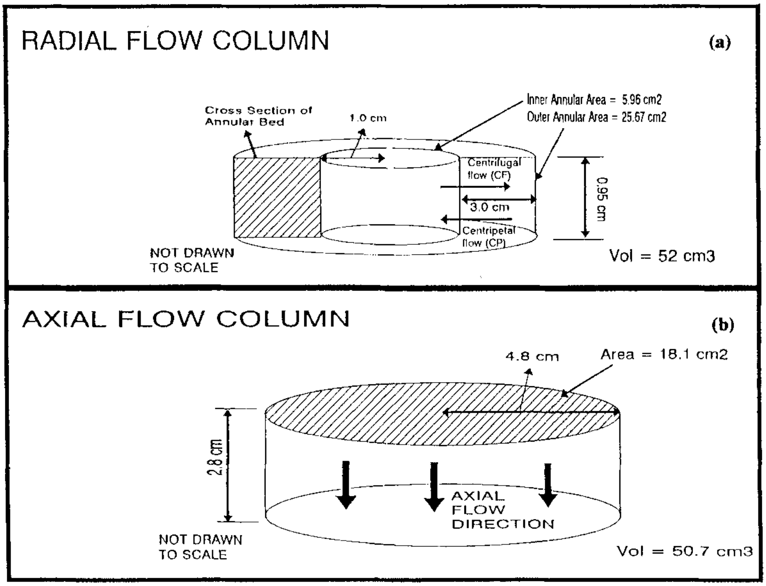

Figure 1: Schematic of radial flow (1a) and axial flow (1b) column flow configurations, indicating dimensions of experimental study columns.

200 gel filtration media. We utilize bovine serum albumin (BSA) to investigate the shape of the eluting peak from both columns packed with S-200 resin. Protein band dispersion was quantified by measuring peak height to width (htw) ratio's, and the effects of feed flow rate and inlet protein concentration on band spreading were also investigated.

MATERIALS AND METHODS

Columns

The axial flow column was purchased from Kontes Glass (Vineland, NJ). It had a diameter of 4.8 cm and was fitted with top and bottom flow adapters.

The resin was packed into the column by gravity settling and the height of the column was adjusted to yield a bed height of 2.8 cm, which resulted in a column of volume 50 cm^3. The area of the bed normal to flow was 18.1 cm^2.

The radial flow column was purchased from Sepragen (San Leandro, CA). The inner annulus had a radius of 1.0 cm and the outer annulus was 4.3 cm in radius, and the annular bed thickness (height) was 3.0 cm. The column was 0.95 cm high, resulting in a bed volume of 52 cm^3. The cross sectional area normal to flow at the inner annulus was 5.96 cm^2 and that at the outer annulus was 25.67 cm^2. Detailed diagrams of both columns are shown in Figure 1.

Resin

Sephacryl S-200 gel filtration resin was obtained from Sigma Chemical (St. Louis, MO).

Proteins and Chemicals

Bovine serum albumin (BSA) and blue dextran were obtained from Sigma. All chemical used were reagent grade and obtained from Sigma as well.

Experimental Protocol

The columns were packed with the resin according to the manufacturer's instructions. After packing, the resin beds were equilibrated with 5 column volumes (CV) of 0.3M NaCl, 0.02M NaH_2PO_4, 0.02% NaN_3 at pH 7.0. A 1-ml solution of protein at a concentration of 1.0 mg BSA/ml was injected in-line to the column and the pump (Masterflex Digital Unified Drive, Cole Parmer, Chicago, IL) was switched on at a flow rate of 2 ml/min. The column effluent was monitored by a UV detector set at 280 nm (Gilson 112 UV/VIS, Gilson Medical Electronics, Middleton, WI), and the output from the detector was recorded on a strip chart recorder (BD40, Kipp and Zonen, Delft, Holland).

The protocol was repeated using protein concentrations of 2.5,5.0 and 7.5 mg/ml at the same flow rate of 2 ml/min. These experiments were then repeated again at a fixed protein feed concentration for flow rates of 5, 10 and 15 ml/min.

Data on the peak height of the eluting protein band was collected and the peak width at half height was also measured. Peak height-to-width ratio's were then calculated and the data is presented to show the effect of feed protein concentration and feed flow rate on peak htw ratio for both AF and RF column configurations.

RESULTS AND DISCUSSION

In an RF column, the linear velocity with which the mobile phase moves through the packed bed is not constant. Figure 2 shows the radial velocity profile for four different flow rates that were utilized in this study, for the centrifugal flow situation. These plots were obtained by dividing the flow rate by the area, which is a function of radial distance. As the figure demonstrates,

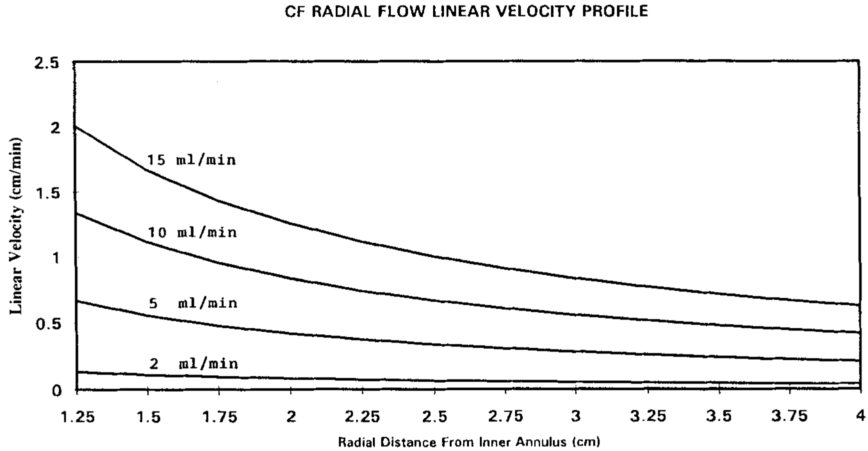

Figure 2: Radial velocity profile for centrifugal flow in an RF column.

larger flow rates result in larger differentials between the inlet velocity and the velocity at the outlet of the radial packed bed. In the case of the AF column, the cross sectional area normal to flow is constant and thus the linear velocity through the axial bed will be constant.

In the analysis of chromatography systems, the ratio of peak height to width provides information about the extent of protein band spreading that occurs as the protein flows through the chromatographic bed. When protein is introduced into a chromatography bed, it begins its passage as a tight plug that is subjected to radial and axial dispersion as it moves through the column. As it emerges from the column, a UV detector at the column outlet can monitor the spread of the protein band. Large peak HTW ratio's imply that the protein has remained in a relatively tight band and much dispersion has not occurred. As peak HTW ratio's decrease, the implication is that the protein band disperses more in the direction of flow resulting in shorter and broader peaks, or peaks with a low peak HTW ratio.

In Figure 3a, the peak HTW ratio is plotted as a function of increasing inlet protein concentration for both AF and RF columns at two flow rates, 2 and 10 ml/min, respectively. Figure 3b shows similar data for the same columns for flow rates of 5 and 15 ml/min. As we can see from the figure, as the concentrations increase, the peak HTW ratio also increases for all four flow rates. However, as both Figures 3a and 3b demonstrate, the rate of increase for the AF columns is greater than that for the RF columns. Additionally, the peaks that were obtained from the AF column were sharper and less dispersed, in general.

In Figure 4a, the peak HTW ratio is plotted as a function of increasing flow rate for feed inlet protein concentrations of 2.5 and 7.5 mg.ml. Once again, the rate of increase of the peak HTW ratio is larger for the AF columns than for the RF columns. In all four cases plotted, the HTW ratio increases linearly for flow rates increasing from 1 to 9 ml/min. After that point, the rate of increase decreases. Figure 4b shows the HTW ratio for increasing flow rates for both the AF and RF system, in this case for protein concentrations of 1 and 5 mg/ml. Again, the data demonstrates that the increase in HTW ratio for AF is more rapid that for the RF columns.

Both the RF and AF columns were tested for integrity and flow uniformity by injecting a plug of Blue Dextran into the columns and observing the movement and dispersion of the plug. Our observations showed that the

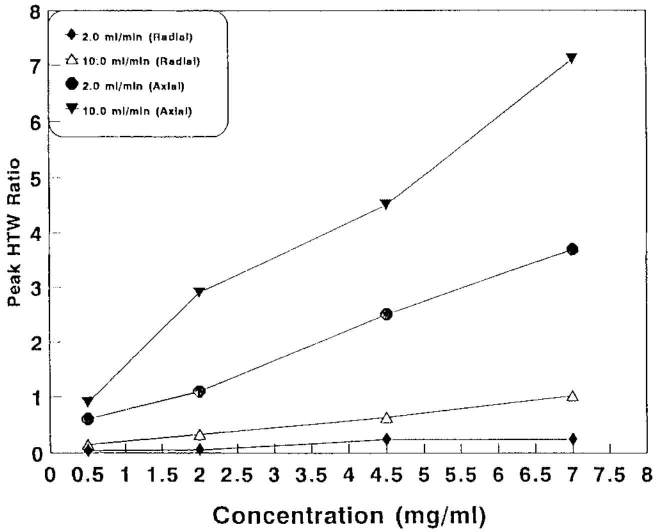

Figure 3a: Effect of concentration on peak HTW ratio for axial and radial flow.

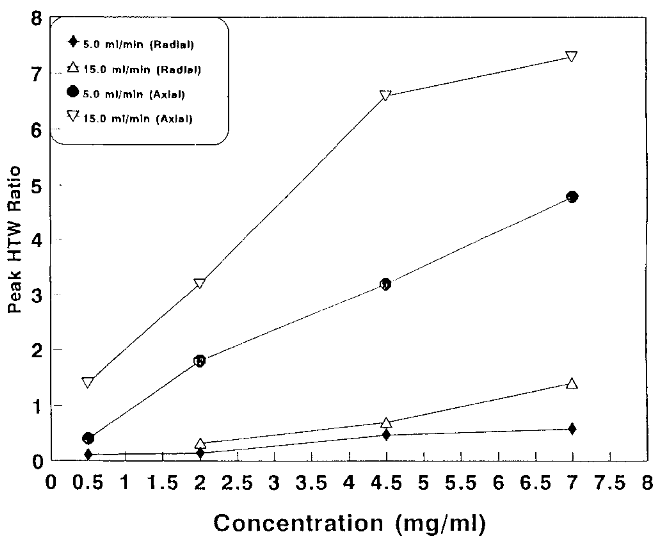

Figure 3b: Effect of concentration on peak HTW ratio for axial and radial flow

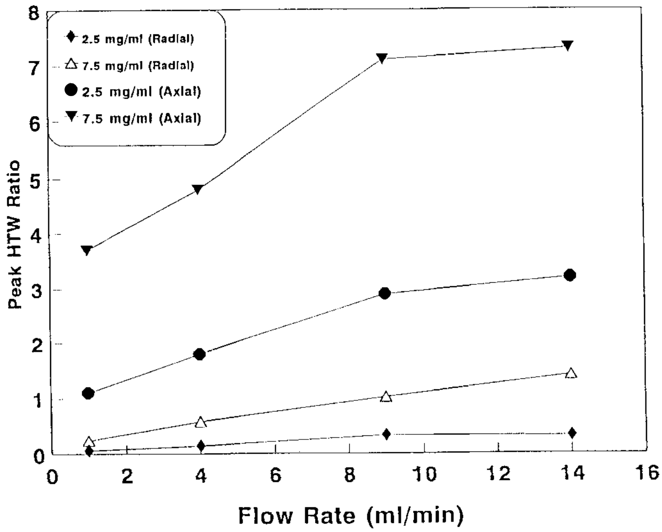

Figure 4a: Effect of flow rate on peak HTW ratio for axial and radial flow.

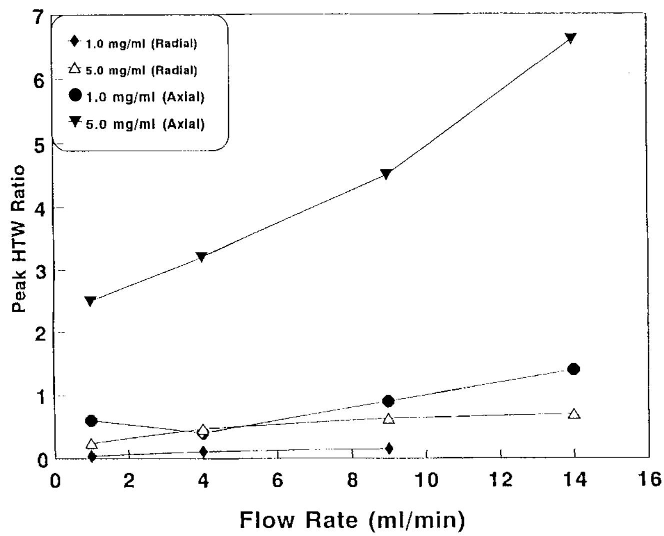

Figure 4b: Effect of flow rate on peak HTW ratio for axial and radial flow.

blue dextran moved uniformly through both the RF and AF columns. In this way, we ruled out the possibility of bed deformities or irregularities affecting the results.

Based on these data, it is clear that peaks will be flatter and broader for RF columns, confirming the theoretical determinations of Gu *et al* (12) that radial dispersion and mass transfer cannot be assumed constant for RF columns due to the accelerating and decelerating flows. Our data also shows that peaks become sharper in both AF and RF columns when the flow rates are increased. The results of these studies suggest that careful trial experiments need to be conducted to ascertain the effect of flow and protein loading on band dispersion before implementing an RF column at production scale.

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