Korean J. of Chem. Eng., **13**(5), 466-472 (1996)

COMPARISON OF AXIAL AND RADIAL FLOW CHROMATOGRAPHY ON PROTEIN SEPARATION SPEED AND RESOLUTION

Yun-Ha Kim* and Eun Kyu Lee†

Department of Chemical Engineering, Hanyang University, Ansan 425-791, Korea
(Received 10 April 1996 • accepted 12 July 1996)

Abstract – Fluid dynamic behavior, particularly the relationship between pressure drop and liquid flowrate, inside an axial and a radial flow chromatographic column packed with compressible porous media was theoretically analyzed using the modified Kozeny-Carman equation. The results were compared with experimental observations obtained using compressible DEAE-agarose as a model medium. At the 2-9 psi pressure drop range studied, theoretical derivation accounting for the 'gel compression' effect predicted simple Langmuirian-type response of volumetric flowrate to changes in pressure drop. On the other hand, the experimental result was more or less sigmoidal. At the same pressure drop, radial column yielded 2-3 times higher flowrates than those of axial column both theoretically and experimentally. Using r-HBsAg crude extract, protein resolution effects between the two types of columns at various flowrates were compared side-by-side. Though general chromatographic behaviors were very similar, the axial column was somewhat superior in terms of r-HBsAg recovery yield and specificity. However, the number of theoretical plates analysis indicated the protein resolution effects were comparable.

Key words: Chromatography, Radial Column, Protein Purification, Aspect Ratio

INTRODUCTION

Development of large-scale and economical protein purification processes is a key to successful manufacturing of biotechnology products. Compared to other bioseparation processes, chromatographic separation can achieve a relatively high purification factor and thus is widely used for various purposes in secondary and/or final purification steps. Continuous research is ongoing for new chromatographic media development, improvement of existing processes, and effective scale-up techniques [Cramer and Jayaraman, 1993]. Direct scale-up of analytical results to manufacturing scale chromatography, however, is ineffective and often results in significant loss in recovery yield and product purity. To circumvent this problem, various approaches have been taken. They include: to improve biospecificity of packing media for higher resolution effect, to decrease the media size to 3 to 5 microns for higher separation capacity, and to provide relatively large 'throughpores' as well as smaller 'diffusive pores' in the media for improved resolution and higher capacity [Afeyan et al., 1991].

It is frequently said that performance of a chromatographic process can be judged by three mutually incompatible parameters, i.e., resolution, capacity, and throughput or speed. While the aforementioned approaches are focused mainly on improved resolution and capacity, separation speed is also a critical parameter particularly in manufacturing scale. In such processes as ion exchange and affinity chromatographies where the interactions between solutes and a medium are relatively quick, higher speed can result in a shortened cycle time and thus improve the process economics.

Traditional chromatographic column has a long cylindrical shape with narrow bore. In large scale, this type of configuration often suffers a sudden drop in liquid flowrate by gel compression phenomenon from increased back pressure. Lowering the feedrate increases the residence time which in turn brings out other undesirable problems such as non-specific bindings, etc. Recently developed radial flow chromatographic column is designed to induce the liquid flow path in a radial direction. Compared to the cylindrical, axial-flow column, this new design could significantly increase the cross-sectional area of the liquid flow and therefore allow reduced packing bed height. Higher feedrate can be realized by reduced gel compression particularly in soft gels [Saxena and Weil, 1987]. Several studies have been reported for successful separation of various proteins such as trypsin [Lee et al., 1990], anti-melanoma IgG_2A antibody, riboflavin [Saxena et al., 1987], and egg white protein [Saxena et al., 1989].

In this study, to provide theoretical explanation for the higher speed in a radial column, a fluid dynamics analysis was performed focusing on the relationship between liquid flowrate and pressure drop. The results were compared with experimental data obtained using compressible medium as a packing material. Also, ion exchange chromatography experiments were performed at the identical condition to directly compare the protein resolution performances between the two types of the columns.

THEORETICAL CONSIDERATIONS ON THE RELATIONSHIP BETWEEN FLOWRATE AND PRESSURE DROP

Fig. 1 depicts the top and side views of axial and radial

†Corresponding author
*Present address: Hyundai Petrochemicals, Inc., New Projects Team, Seosan, Korea

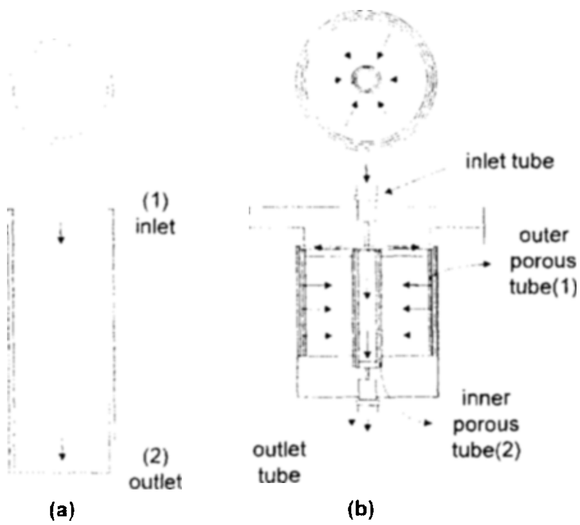

Fig. 1. Top and side views of (a) axial and (b) radial columns.

columns. In the axial column [Fig. 1(a)] the liquid is pumped to the top, flows through a packed media across a constant cross-sectional area with associated pressure drop, and then elutes out of the bottom. In the radial column [Fig. 1(b)] the feed is pumped to the 'inlet tube' in the top center, distributed radially through the 'flow distributor', flows radially from the 'outer porous tube' through the 'inner porous tube' to a hollow center tube, and then elutes out of the bottom. Here, the cross-sectional area is seen to change as the liquid travels from outside to inside radially.

The Bernoulli equation which describes the conservation of mechanical energy of fluid flow including friction force can be written as follows:

$$\frac{P_1}{\rho}+\frac{v_1^2}{2g_c}+\frac{g}{g_c}z_1=\frac{P_2}{\rho}+\frac{v_2^2}{2g_c}+\frac{g}{g_c}z_2+F \tag{1}$$

Here, P, ρ, v, g, g_c, z, and F are fluid pressure, fluid density, linear velocity of a fluid, gravitational force, conversion factor, height, and friction force, respectively. In the axial column considering the same cross-sectional area at the inlet and outlet and neglecting the gravitational term, Eq. (1) can be reduced to:

$$\frac{\Delta P}{\rho}=-F \tag{2}$$

In the radial column, neglecting the gravitational term in Eq. (1) gives:

$$\frac{\Delta P}{\rho}+\frac{\Delta v^2}{2g_c}=-F \tag{3}$$

For incompressible fluid flow through incompressible porous media, the friction factor, f_{pm}, can be described as follows by the Ergun correlation [Ergun, 1952].

$$f_{pm}=\frac{150(1-\varepsilon)}{Re_{pm}}+1.75 \tag{4}$$

$$Re_{pm}=\frac{D_P\,v_S\cdot\rho}{\mu(1-\varepsilon)} \tag{5}$$

Here, ε and Re_{pm} are the porosity of packed media and the Reynolds number for porous flow as defined in Eq. (5), respectively. And, the friction factor of incompressible fluid flowing through a column packed with incompressible media can be written as follows [McCabe and Smith, 1985]:

$$f_{pm}=\frac{-\Delta P\,D_P\,\varepsilon^3 g_c}{\rho L v_S^2(1-\varepsilon)} \tag{6}$$

where D_p, v_s, and μ are the media particle diameter, superficial velocity of a fluid, and fluid viscosity, respectively. The relationship between f_{pm} and F is then expressed as:

$$f_{pm}=F\cdot\frac{D_P}{L}\cdot\frac{\varepsilon^3}{(1-\varepsilon)}\cdot\frac{1}{v_S^2} \tag{7}$$

where L denotes the packed bed height in the flow direction. Combining Eqs. (5), (6), and (7), we can derive the following equation describing the friction force as a function of fluid velocity or volumetric flowrate:

$$F=1.75\cdot\frac{v_S^2}{D_P}\cdot\frac{(1-\varepsilon)L}{\varepsilon^3}+150\cdot\frac{v_S\mu(1-\varepsilon)^2L}{D_P^2\varepsilon^3\rho} \tag{8}$$

For the laminar flow, the first term in the right-hand side of the Eq. (8) can be neglected, and the following Kozeny-Carman equation can be derived:

$$-\Delta P=150\frac{L\mu(1-\varepsilon)^2}{D_P^2\varepsilon^3 g_c}\cdot v_s \tag{9}$$

Eq. (9) may be used to predict the pressure drop versus volumetric flowrate relationship for a given system of an axial column.

In the case of laminar flow through an radial column, the same relationship can be obtained from Eqs. (3) and (8) neglecting the right-hand side of the Eq. (8).

$$-\Delta P=\rho\left[\frac{1}{2g_c}v_S^2A_{LM}^2\left(\frac{1}{A_2^2}-\frac{1}{A_1^2}\right)+\frac{150}{g_c}\frac{L\mu(1-\varepsilon)^2}{D_P^2\varepsilon^3\rho}\cdot v_s\right] \tag{10}$$

Here, the linear velocities at the entrance and the exit, v_1 and v_2, are expressed as the average superficial velocity, v_s, applying the mass conservation law, i.e., $v_1A_1=v_2A_2=v_sA_{LM}$, where A_1 and A_2 are the cross-section areas at the entrance and the exit, and A_{LM} is the log mean area. Also, L denotes the distance between the outer and inner tubes and thus corresponds to the bed height in an axial column.

Since the Kozeny-Carman equation is for incompressible media only, some modifications are needed to apply the Eqs. (9) and (10) directly to a column packed with compressible media, of which the porosity and the bed height tend to decrease with an increased pressure drop. In this study, the decreases of the bed height and the porosity as a function of pressure drop were experimentally determined for a particular media (DEAE resin; see METHODS AND MATERIALS), and the results were inputted into Eqs. (9) and (10). Table 1 shows the numerical values and their units used in the calculations.

METHODS AND MATERIALS

The axial column used had a 100 ml packing volume with a

Table 1. Physical properties and dimensions used in Eq. (5) through (10)

Δp	: pressure drop [N/m^2]
ρ	: fluid density [1 g/cm^3]
L	: bed height [cm]
v_s	: superficial velocity [cm/sec]
μ	: fluid viscosity [0.01 g/cm/sec]
ε	: porosity [-]
g_c	: conversion factor [9.80665 $kg \cdot m/kg_f \cdot sec^2$]
A_{LM}	: log mean of cross-sectional area [cm^2]
A_1	: cross-sectional area of inlet [cm^2]
A_2	: cross-sectional area of outlet [cm^2]
Dp	: particle diameter of DEAE medium [10^{-4} cm]

2.54 cm ID and 19.74 cm bed height. The radial column used was SuperFlo 100® (Sepragen Corp., San Leandro, CA, USA). The outer diameter, inner diameter, and the axial height of the column were 8.56, 1.56, and 1.8 cm, respectively, and the packing volume was also 100 ml. Toyopearl-650® (Tosoh Corp., Tokyo, Japan), which is made of DEAE-agarose and has 100 micron average diameter, was used as a packing material. The column porosity was measured as follows; first, the column was packed at a constant pressure selected between 2 to 9 psi, and after each packing the excess liquid was first drained out before applying high-pressure nitrogen gas to purge out the residual, interstitial and intrastitial liquid. The porosity was determined by taking a ratio of the collected liquid volume to the apparent packed volume.

Recombinant hepatitis B surface antigen (r-HBsAg) crude extract used for chromatographic experiments was donated by Korean Green Cross Corp (Seoul, Korea). Axial and radial column were used side-by-side under the same hydraulic condition in order to compare their performances in an ion exchange step. The chromatography procedure was as follows; 100 ml of the crude extract was loaded into the column, washed with 50 mM TrisHCl buffer (pH=8.0), and then the same buffers containing 0.2 and 0.5 M NaCl were fed on a step gradient to elute the proteins. Three different volumetric flowrates, i.e., 35, 70, and 140 ml/min, were used for the washing and the elution. For each washing and elution the flowrate was maintained constant by using a self-compensating peristaltic pump (Digi-Staltic®, model 7526-00, MasterFlex Inc., Barlington, IL, USA). The total pressure drop of the columns was monitored by a differential pressure transmitter, and the protein content of the eluent was detected by UV absorbance at 280 nm (Econo UV monitor, model EM-1, Bio-Rad Lab, Hercules, CA, USA). Total protein was assayed by Bradford and Lowry methods, and the r-HBsAg was assayed by Sandwich ELISA method.

RESULTS AND DISCUSSION

1. Effect of Feed Pressure on Volumetric Flowrate

As the pressure is increased, compressible media such as DEAE-agarose are subject to gel compression that causes bed height and porosity to reduce. Fig. 2 shows the experimental data on the effect of pressure on bed height and porosity. As the feed pressure is increased from 2 to 9 psi, the DEAE bed height was reduced by about 10% from 21.2 to 19.1 cm and the porosity was decreased by about 17% from 0.403 to 0.334. These changes were well correlated as linear (regression coefficients for the bed height and the porosity were 0.9909 and 0.9923, respectively), and these correlations were inputted into Eqs. (9) and (10) to calculate superficial velocity and thus volumetric flowrate at each pressure.

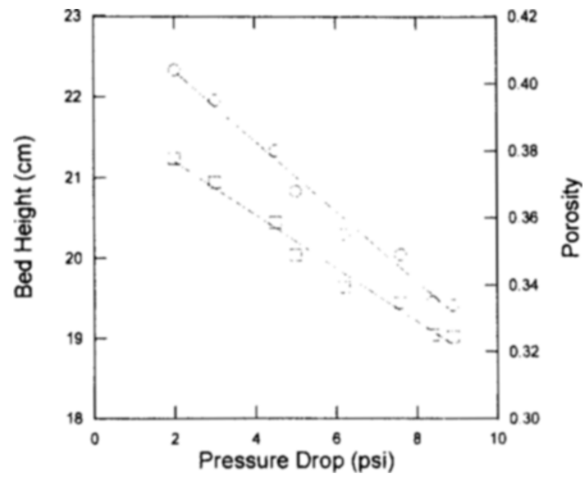

Fig. 2. Effect of pressure drop on bed height (□) and porosity (○).
(Porosity= −0.0140△P+0.424, Bed height= −0.3308△P+21.9)

Fig. 3 shows the calculated and experimental results on the total pressure drop and the corresponding volumetric flowrate. For the axial column, the theoretical prediction by Eq. (9) showed that due to the gel compression effect the rate of increase in the flowrate was somewhat slowed at a higher pressure range. The experimental data was more like a sigmoidal curve of which the reason was unclear. Below about 5 psi the theoretical prediction overestimated the experimental data, and the opposite was observed above about 6 psi. In the 5 to 8 psi range, however, they agreed quite well. In the case of the radial column, the theoretical estimate, which was obtained by adding the pressure drop calculated from Eq. (10) and 'empty' pressure drop, agreed very well with the experimental data. The 'empty' pressure drop was the pressure drop developed when the column was empty, and was due to 20 micron polyethylene filters installed at the inlet and the outlet. At the same pressure drop particularly at about 4 psi and higher, it was seen that the radial column flowrate were 2 to 3 times higher than that of the axial column. This was in good agreement with the experimental result obtained from 100 ml axial and radial columns packed with Sepharose CL-4B [Saxena et al., 1987]. Therefore, it was confirmed by the theoretical derivation that the main advantages of the radial column design, i.e., enlarged cross-sectional area and shortened bed height, allow higher flowrate due to reduced back pressure and gel compression.

2. Effect of Aspect Ratio on Pressure Drop vs. Flowrate Relationship

Aspect ratio (AR) is often used to describe a geometrical configuration of a chromatographic column, and is defined as:

$$AR = L/D \tag{11}$$

where L and D are bed height and diameter of an axial column.

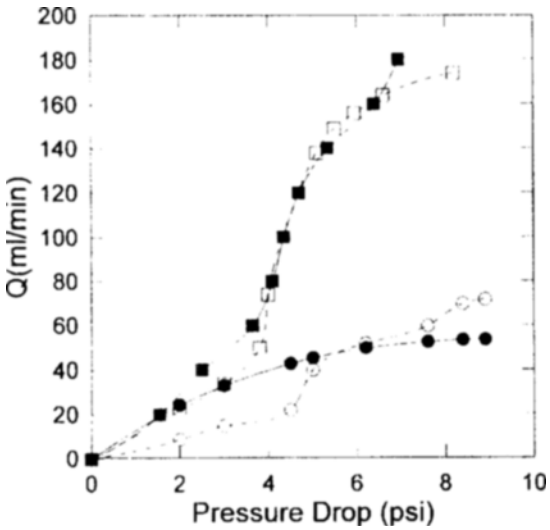

Fig. 3. Relationship between total pressure drop and volumetric flow rate.
○ Axial (Experimental), ● Axial (Theoreical), ■ Radial (Theoretical), + Experimental (Empty), □ Radial (Experimental)

This aspect ratio cannot be directly applied to a radial column because of the different configuration. To compare the two types of columns on a common basis, a new aspect ratio (AR') was defined as follows:

$$AR' = \frac{L^2}{\frac{\pi}{4} D^2} \text{ (for axial column)} \tag{12}$$

$$AR' = \frac{L^2}{\left(\frac{l_0 + l_i}{2}\right) z} \text{ (for radial column)} \tag{13}$$

In Eq. (13), L is $(d_o - d_i)/2$ (d_o and d_i are the outer and inner diameters) and thus corresponds to the bed height of an axial column. And, l_o and l_i are the perimeters of outside (column inlet) and inside (outlet), and z denotes axial, not radial, height of a radial column. Therefore, the denominators in Eqs. (12) and (13) mean the cross-sectional areas in the liquid flow direction. This new aspect ratio can be applied to columns of different geometries, and enables us to compare the relationship between pressure drop and flowrate on a common basis. Table 2 exemplifies the dimensions of axial and radial columns with various aspect ratios.

Table 2. Dimensions of axial and radial column with various aspect ratios (Bed volume is set constant at 100 ml)

Axial column			Radial column		
Aspect ratio	L (bed height)	D (diameter)	Aspect ratio	L $(d_o - d_i)/2$	z (axial height)
0.11	2.24 cm	7.54 cm	0.03	1.42 cm	7.55 cm
1.65	5.48 cm	4.82 cm	0.11	2.24 cm	3.73 cm
10.0	10.0 cm	3.57 cm	0.43	3.5 cm	1.8 cm
28.3	14.1 cm	3.0 cm	0.89	4.47 cm	1.18 cm
76.9	19.7 cm	2.54 cm	1.65	5.48 cm	0.83 cm

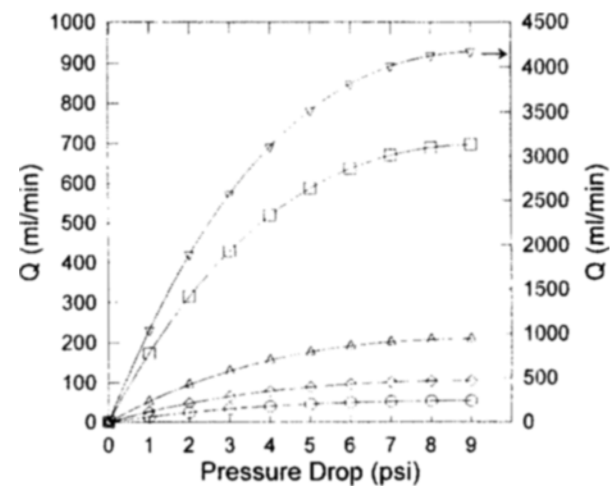

Fig. 4. Flow rate vs. pressure drop in axial columns with various aspect ratios.
○: AR'=76.9, ◇: AR'=28.3, △: AR'=10.0, □: AR'=1.65, ▽: AR'=0.11

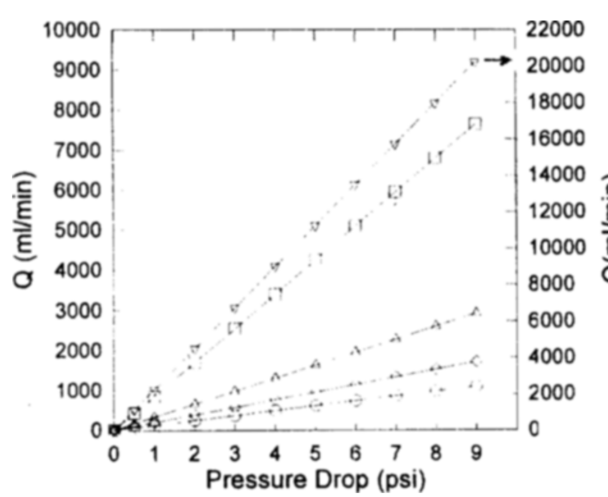

Fig. 5. Flow rate vs. pressure drop in radial columns with various aspect ratios.
○: AR'=1.65, ◇: AR'=0.89, △: AR'=0.43, □: AR'=0.11, ▽: AR'=0.03.

Fig. 4 and 5 show theoretical correlations of pressure drop and volumetric flowrate in axial and radial columns, respectively, with various aspect ratios. As expected, volumetric flowrate increased as the pressure drop was increased, and under the same pressure drop the flowrate increased as AR' was decreased. At AR'=1.65 both flowrates were comparable to each other, but the deviation became larger as AR' is decreased. For an axial column, there are limitations in designing a column with low AR'. For example, AR'=0.43 is rather a standard design in a radial column (d_o=8.56 cm, d_i=1.56 cm, z=1.8 cm), whereas in an axial column it means a quite stumpy shape (L=3.5 cm, D=6.1 cm) which may be susceptible to other potential problems such as channeling, uneven flow distribution, and so forth.

Fig. 6 and 7 show the effect of aspect ratio on volumetric flowrate at various pressure drops. As can be seen in Fig. 6,

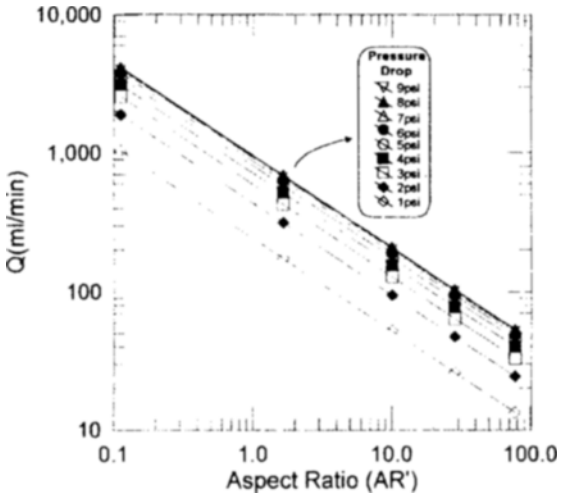

Fig. 6. Flow rate vs. aspect ratio in axial columns at various pressure drops.

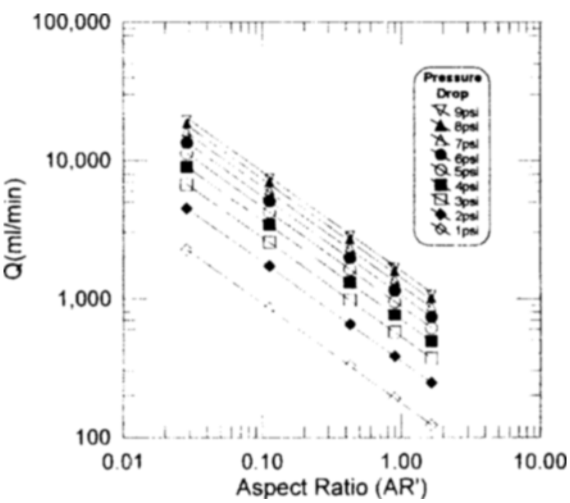

Fig. 7. Flow rate vs. aspect ratio in radial columns at various pressure drops.

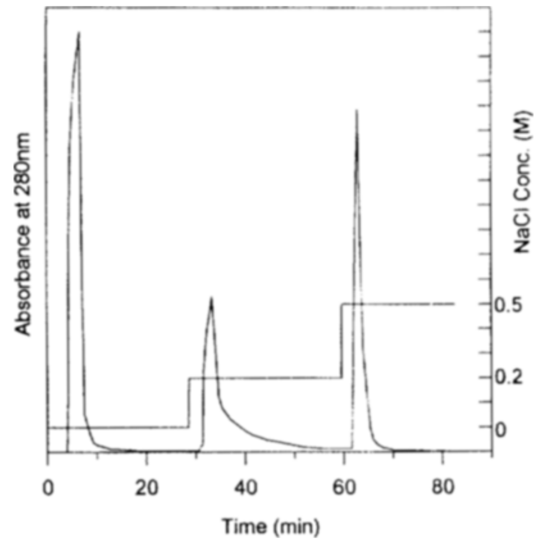

Fig. 8. Chromatogram of axial flow column at 35 ml/min flow rate.

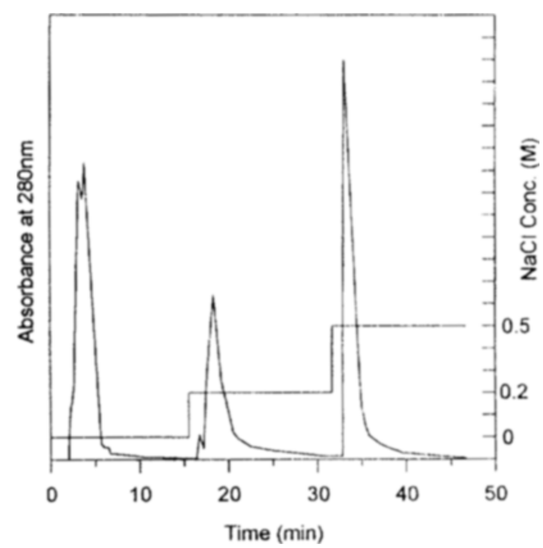

Fig. 9. Chromatogram of axial flow column at 70 ml/min flow rate.

the flowrate decreased as AR' increased, and the slopes were the same. At the same pressure drop there was a logarithmically linear relationship between AR' and the flowrate, that is:

$$\ln Q = a \ln AR' + b \tag{14}$$

Fig. 7 indicates the same correlation applies to radial columns. The slopes were nearly the same between the axial and radial columns; −0.666 and −0.718, respectively. This result can be used to estimate the flowrate in different shapes of columns.

3. Performance Comparison of r-HBsAg Purification

To the axial and radial columns, 100 ml of the r-HBsAg crude extract was loaded and eluted stepwise with buffers containing 0, 0.2, and 0.5 M NaCl at various flowrates. The r-HBsAg was eluted upon 0.2 M NaCl elution. Fig. 8 and 9 are the chromatograms from the axial column at 35 and 70 ml/min, respectively, and Fig. 10, 11, and 12 are those from the radial column at 35, 70, and 140 ml/min, respectively. For the axial column 140 ml/min was unobtainable because too high a pump pressure was required. Not much difference was observed among the chromatograms. Band broadening phenomenon at the increased flow rates was also unnoticeable.

Table 3 summarizes the recovery yield and specificity of the washing eluate and salt eluate pool under each experimental condition. Specificity was defined as a mass ratio of r-HBsAg to total proteins. The data in Table 3 indicate that there was little evidence of loss in either recovery yield or specificity with increased flowrate. However, it was clear the loss of r-HBsAg in the wash eluate increased as the flowrate increased. It could be explained as more unadsorbed r-HBsAg were washed off at a higher flowrate. Also, it appeared that at the same flowrate higher yield and specificity were resulted from the axial column. To quantitate this deviation, the number of theoretical plates was calculated at each condition.

Number of theoretical plates, N, is often used as a meas-

Table 3. Recovery yield and specificity of r-HBsAg in column eluate fractions

Fraction	Axial				Radial					
	@ 35 ml/min		@ 70 ml/min		@ 35 ml/min		@ 70 ml/min		@ 140 ml/min	
	Yield (%)	Specificity (μg/mg)	Yield (%)	Specificity (μg/mg)	Yield (%)	Specificity (μg/mg)	Yield (%)	Specificity (μg/mg)	Yield (%)	Specificity (μg/mg)
Wash	3.7	*	5.3	*	4.5	*	10.6	*	15.2	*
Salt eluate	58.2	197	62.9	242	42.7	134	55.6	180	50.5	169

* The amount of total proteins detected was insignificant

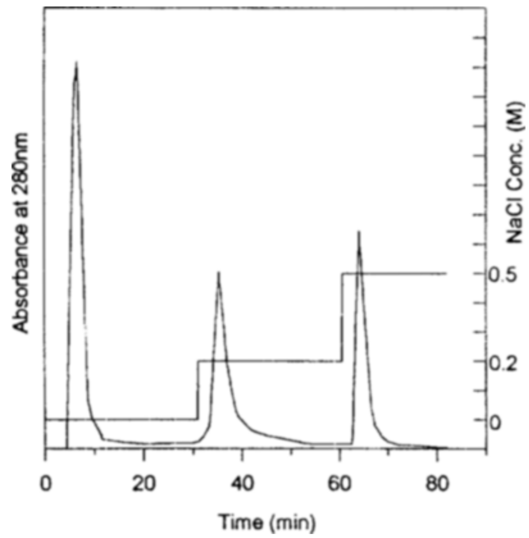

Fig. 10. Chromatogram of radial flow column at 35 ml/min flow rate.

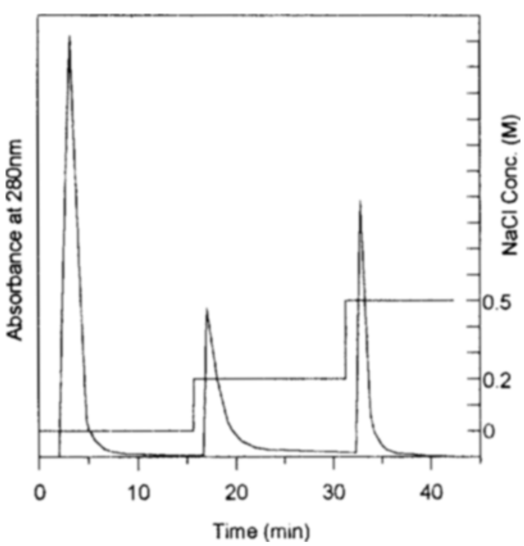

Fig. 11. Chromatogram of radial flow column at 70 ml/min flow rate.

urement parameter for chromatographic performance, and can be expressed as [Mant and Hodges, 1991]:

$$N = 16\left(\frac{t_R}{t_W}\right)^2 \tag{15}$$

where t_R is the time or distance from time zero (or time when a

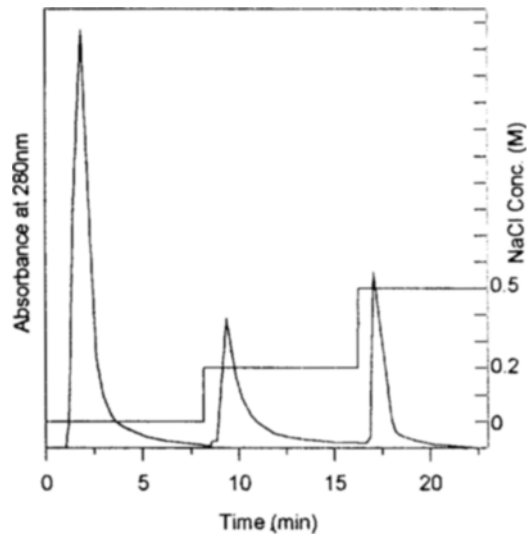

Fig. 12. Chromatogram of radial flow column at 140 ml/min flow rate.

Table 4. Number of theoretical plates analysis on NaCl eluate pools

Column/Flowrate	0.2 M	0.5 M
Axial/ 35 ml/min	31.9	46.3
70 ml/min	20.4	29.6
Radial/35 ml/min	24.6	41.6
70 ml/min	25.9	36.1
140 ml/min	19.0	26.7

step change is made) to the center of an eluted peak, and t_w is the peak width at the baseline. The N values determined are summarized in Table 4. As the flowrate increased, the N values were seen to decrease in both axial and radial columns. At 35 ml/min flowrate, the N value from the axial column was somewhat higher than that of the radial column, but at 70 ml/min the opposite was observed. Considering this mixed result and the errors inherent in the graphical determination procedures for the N value, it could be concluded that there was little difference in the resolution performance between the two columns.

CONCLUSIONS

Fluid dynamic behaviors between a traditional, axial chromatographic column and a more recently developed radial column were analyzed and compared, using Ergun correlation and Kozeny-Carman equations modified for incompressible fluid flow through compressible porous media. The theoretically derived results, which focused on the relationship between fluid pressure drop and volumetric flowrate, were in good agreement with the experimental observations. The response of flowrate to the increase in pressure from 2 to 9 psi was nonlinear in the axial column but linear in the radial column, which suggested the effect of back pressure was more pronounced in axial column. Under an identical pressure drop condition, both theoretical and experimental results suggested that two to three times higher volumetric flowrate could be obtained from a radial column. To compare columns with different geometries, a new aspect ratio was defined as a square of bed height divided by cross-sectional area of fluid flow. Logarithmically linear relationship existed between the new aspect ratio and the flowrate. Protein resolution experiments using r-HBsAg crude extract suggested that slightly higher recovery yield and specificity were obtained from the axial column. However, the number of theoretical plates analysis indicated there was little difference in the protein purification performance between the two columns.

ACKNOWLEDGEMENTS

The authors wish to thank Korean Green Cross Corp. for the donation of r-HBsAg crude extract and particularly Messrs K. N. Park and S. J. Ahn in the Bioprocessing Team for the ELISA and total protein assays.

REFERENCES

Afeyan, N. B., Fulton, S. P. and Regnier, F. E., "Perfusion Chromatography Material for Proteins and Peptides", *J. Chromatography,* **544**, 267 (1991).

Cramer, S. M. and Jayaraman, G., "Preparative Chromatography in Biotechnology", *Current Optinion in Biotechnology,* **4**, 217 (1993).

Ergun, S., "Fluid Flow through Packed Columns", *Chem. Eng. Progr.,* **48**, 89 (1952).

Lee, W. C., Tsai, G. J. and Tsao, G. T., "Protein Purification: from Molecular Mechanisms to Large-Scale Processes", Ladisch et al. ed., 104 (ACS Symposium Series, 427) (1990).

Mant, C. T. and Hodges, R. S., "High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation", CRC Press, Boca Raton, FL, USA (1991).

McCabe, W. L. and Smith, J. C., "Unit Operations of Chemical Engineering", 4th ed., McGraw-Hill, New York, NY, USA (1985).

Saxena, V. and Weil, A. E., "Novel Radial Flow Columns for Scaling Up Chromatographic Separations", *Biochromatography,* **2**(2), 90 (1987).

Saxena, V., Subramanian, K., Saxena, S. and Dunn, M., "Radial Flow Columns for Protein Separation", *Biopharm.,* **2**(3), 34 (1989).

Saxena, V., Weil, A. E., Kawahata, R. T., McGregor, W. C. and Chandler, M., "Applications of Radial Flow Columns for Fast Affinity Chromatography", *American Laboratory News,* **19**, 112 (1987).