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Evaluation of radial chromatography versus axial chromatography, practical approach☆

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Abstract

Developments in packing and packing port design of radial columns in recent years have resulted in a claimed significant increase in performance of this process chromatography technology. In this first study, the main chromatographic parameters as efficiency, capacity factor, asymmetry and resolution were evaluated in a unique one-to-one comparison between a 120 ml bed-volume and 6 cm bed length radial chromatography mini-process column against a 50 mm diameter, 6 cm bed height and 120 ml bed-volume axial chromatography column. Radial chromatography showed an increase in efficiency by 31% in the number of plates per meter while the equilibration could be reduced by 0.4–0.5 column volumes. The asymmetry factor for bovine serum albumin in radial chromatography showed a reduction of 20% while the reduction of the asymmetry factor of the smaller protein ovotransferrin decreased even by 46% in comparison to the performance of the comparative axial chromatography column. Therefore in radial chromatography resolution improved up to 20%. The retention volume was similar in both cases. For radial chromatography, the decrease in "width at half height" at Height Equivalent of Theoretical Plates (HETP) measurements was 40% while the decrease of the over-all width of the peak was 27%. For adsorbed/desorbed proteins, the elution peak showed similar results: "width at half height" decreased to 45% while the over-all width of the peak decreased by 28%. The concentration of the non-retained protein in the flow-through (lysozyme), increased by 35% while the concentration of the eluted fraction (serum albumin bovine), increased with 40% in the radial chromatography columns. The better results obtained with the radial column were probably the consequence of the geometrical design of this device (larger inlet surface area and small outlet surface area which concentrate the eluted fraction).

Keywords: Radial chromatography; Axial chromatography

1. Introduction

Since the late 1950s, liquid chromatography has progressed with the successive use of new supports [1–23]. This evolution was performed on the basis of classical chromatography so called by Rhee et al. [24] axial flow chromatography, widely applied in the separation of biomolecules since.

Late 1940s, radial chromatography technology (Fig. 1) was introduced with the work of Hopf [25], in which the concept and method was adopted to separate liquids by centrifugal forces. This method was improved for adsorption chromatography by Heftmann et al. [26].

Radial chromatography columns use two concentric cylindrical porous frits that hold the chromatographic media between them. Buffer and sample flow from the outer surface to the inner surface, across the radius of the column, which represents the effective bed height.

After proposing a separation scheme for analytical purposes using gas radial chromatography and a work on compressed beds [27–30], Rice and co-workers [31–34] solved the distribution of liquid in the column bed by a new column design. The introduction of radial chromatography to the commercial market in the mid 1980s [35,36] has opened the path for this technology in the separation of biological products by ion exchange or affinity chromatography [37–45]. Mathematical models and theoretical studies [46–49] have described the consequences of the

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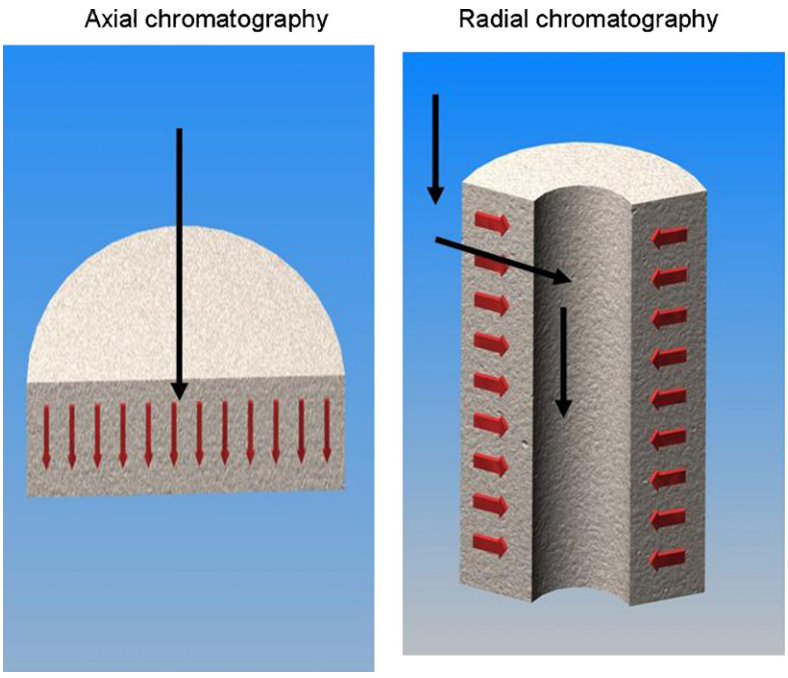

Fig. 1. Comparison of liquid flow through the axial and radial columns.

application of the radial geometry and that the cross-sectional area perpendicular to the flow direction is very large while the flow path is relatively short. These two factors help to reduce pressure drop in the bed and allow a much higher flow rate. In the radial flow mode, several authors [38–39,41,42] reported that the flow rate of soft media is improved compared to what is observed in an axial chromatography. Other theoretical studies [48,49] indicate that the design of a column with larger diameter and shorter bed height is advantageous for elevated separation efficiency and establishment of a robust separation method. Today three main radial flow chromatography technologies can be distinguished: membrane chromatography [50–62], preparative monolithic chromatography [63–67] and packed bed chromatography [68–71]. Some authors describe radial chromatography as intermediate and alternative step in scale up [69].

Before, several authors have compared axial with radial chromatography [68,70–71], however, apart from comparing the aimed geometry differences, they have either compared two columns with different bed volumes [70] or alternatively, same bed volume but different bed height [68]. Thus, apart from the geometrical differences between the two column types, at least one other chromatographically significant parameter was mixed in these experiments.

In this paper, the main chromatographic parameters: efficiency, capacity factor, asymmetry and resolution are evaluated in a unique one-to-one comparison between a 120 ml bed-volume 6 cm bed length radial chromatography mini-process column, against a 50 mm diameter and 6 cm bed height and 120 ml bed-volume axial chromatography column (Fig. 2). The radial mini-process chromatography column used in these experiments is a segment unit, specifically designed for linear scale-up and/or scale-down experiments in process chromatography (Fig. 3A and B).

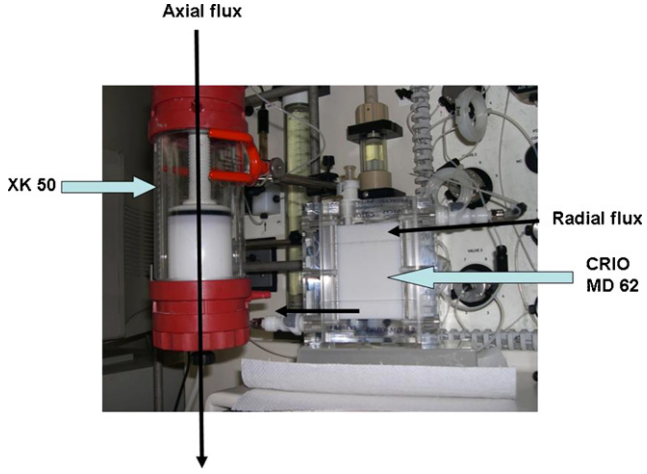

Fig. 2. Axial column (XK 50/20) and radial column (CRIO-MD 62) presented on the Biopilot workstation (GE Healthcare Life Sciences).

2. Experimental

2.1. Chemicals

All salts were HPLC grade and the buffers were filtered through a 0.22 μm membrane filter Minisart from Sartorius (Palaiseau, France).

All reagents and standard proteins were of analytical grade and purchased from Sigma–Aldrich (St Quentin, France).

Anion exchange chromatographic media Cellufine A 500 (DEAE) was kindly provided by CHISSO Inc. (Tokyo, Japan).

Axial column XK 50/20 was purchased from GE Healthcare Life Sciences (Saclay, France). Radial column CRIO-MD 62 was kindly provided by PROXCYS Downstream Biosystems b.v. (Emmen, The Netherlands).

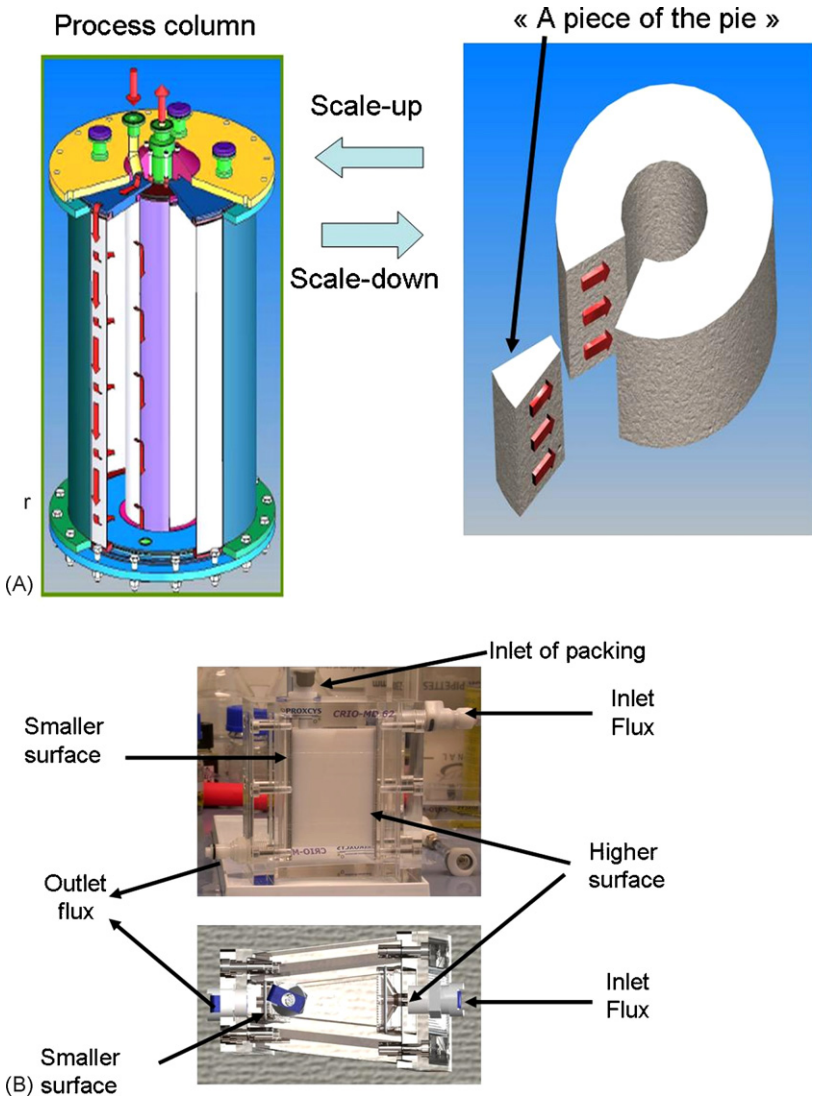

Fig. 3. (A) Radial flow technology: Process Development Column (CRIO) and mini Process Column (CRIO-MD). (B) CRIO-MD 62: side view and upper view.

2.2. Instruments

All chromatographic experiments were conducted using Biopilot and AKTA explorer 100 chromatographic systems controlled by the Unicorn Data system from GE Healthcare Life Sciences (Saclay, France). For recovery studies, we used a SAFAS UV mc spectrophotometer (SAFAS, Monaco).

2.3. Preparation of the samples

The protein standards (lysozyme, bovine serum albumin and ovotransferrin) used in these studies were prepared at different concentrations in equilibration buffer and filtered through a 0.22 μm membrane.

2.4. Chromatographic procedures

All experiments were done at ambient temperature. The equilibration buffer used was 50 mM Tris–HCl pH 9 and the elution buffer, 1 M NaCl, 50 mM Tris–HCl pH 9.

2.4.1. Packing procedure

Columns were packed with the chromatographic media Cellufine A500 (53–125 μm beads size).

2.4.1.1. XK 50/20 column. Slurry was prepared with elution buffer in a ratio of 75% settled gel to 25% buffer and was de-gassed. The column was filled through the outlet with a few centimetres of binding buffer and was closed. The slurry was poured into the column in one continuous motion. The remainder of the column was filled with buffer and the top mounted and connected to a pump. The bottom outlet of the column was opened and the pump set at 50 ml/min (153 cm/h). The packing flow rate was maintained during 3 bed volumes after a constant bed height was reached (6 cm).

2.4.1.2. CRIO-MD 62 column. Slurry was prepared with elution buffer in a ratio of 75% settled gel to 25% buffer and was de-gassed. The upper inlet (the packing port) of CRIO-MD 62 was used to fill the column with peristaltic pump, the upper

outlet from higher surface was used to eliminate the buffer and the bottom outlet was closed.

The slurry was introduced in the radial column at 50 ml/min (average linear velocity 135 cm/h), until the pressure increased up to 0.1 MPa. Bed length was 6 cm.

2.4.2. Measurement of HETP

Measurement of Height Equivalent of Theoretical Plates (HETP) was performed with acetone (injection of 2.5 ml of acetone 2% (v/v) in H_2O) at 50 ml/min.

2.4.3. Equilibration studies

The equilibration studies were performed with two modes, the first mode was by using alternately the different solutions and the second mode was during the different runs of the following studies.

2.4.4. Measurement of chromatographic parameters of protein peaks

Standard proteins (bovine serum albumin and ovotransferrin) were used as sample. The equilibration buffer used was 50 mM Tris–HCl pH 9 and elution buffer was 1 M NaCl, 50 mM Tris–HCl pH 9. The elution was performed with a 20 column volume linear salt gradient for the calculation of resolution, retention, capacity factor and asymmetry and by a step salt gradient for the measurement of the peak-width, half height peak-width and for the calculation of the protein concentration in the peaks.

3. Results and discussion

The assessment of linear velocity in radial column requires some explanation. Unlike the axial columns, the geometry of the radial chromatography column leads to an increase in linear velocity from inlet to outlet due to the smaller outlet surface area (Fig. 4A). Due to geometrical design of the column, the gel-volume distribution in the column is not linear along the radius and about 50% gel-volume is found at the inlet of the radial column where the linear velocity is slightly lower (beneficial for binding kinetics), followed by about 30% of the gel-volume where the linear velocity is slightly higher. The major increase in linear velocity is found in the last 20% of the gel-volume in the column (mostly not used for product binding). The crossing point between axial and radial linear velocity is at 70% gel-volume distribution (Fig. 4B), for safety we use the point where 55% of gel-volume distribution was present when the gel is used to its full capacity (80–90%) (Fig. 4B). Linear position in the column was expressed as per cent of the gel-volume along the column position and at each position correspond a relative surface area. This point corresponds to 0.85 times the inlet surface area. Therefore, the reference surface area for the calculation of an "average" linear velocity is not, as usually, in the middle of the column but at about 1/3 from the inlet.

The inlet surface area of the radial column is 25.8 cm^2 and therefore the calculated reference surface area for the average linear velocity in the CRIO-MD 62 is 21.93 cm^2 while for the axial column XK 50/20 it is 19.625 cm^2, which is a similar surface area between the two columns.

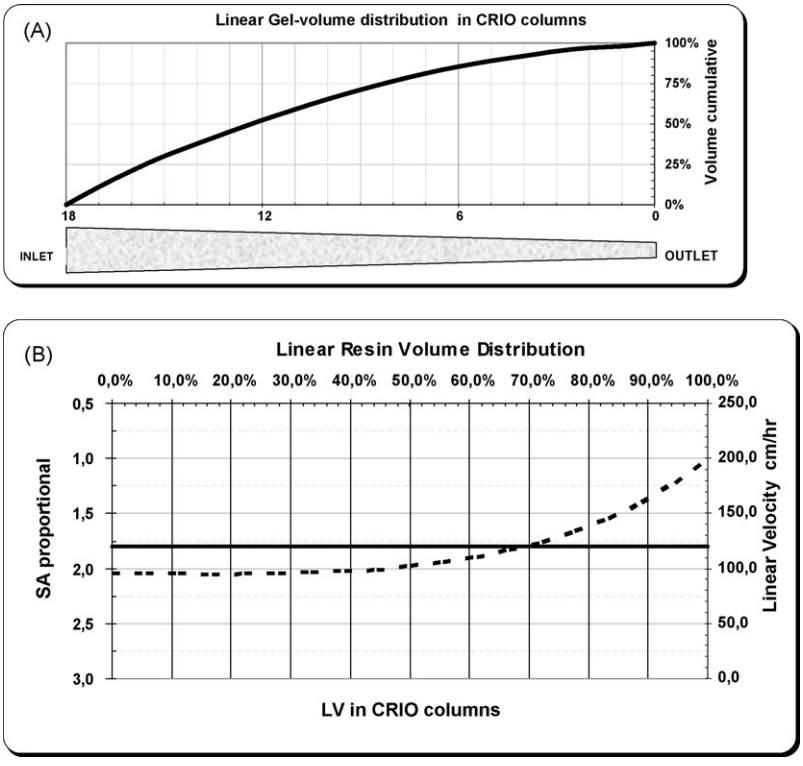

Fig. 4. (A) Linear gel-volume distribution in radial CRIO columns from inlet to outlet. (B) Linear velocity in radial CRIO columns with axial column (solid line is the average linear velocity and dot line is the real linear velocity along the radial column).

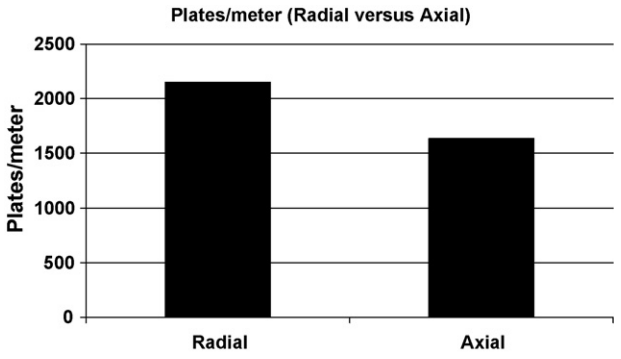

Fig. 5. Comparison of the number of plates per meter of radial chromatography versus axial chromatography.

3.1. Packing evaluation

The quality of column packing can be determined by running a low molecular mass non-interacting solute such as acetone and calculating the number of plates per meter of the packed bed. The higher this number, the more efficient the column is. Since the plate number is proportional to the column length, column quality can also be expressed in terms of height of a theoretical plate (HETP). Thus, for a "good" column, the value of HETP is small.

The comparison of the quality of the packing between the two columns (radial and axial) was done by measurement of the efficiency of the packed bed. The results (Fig. 5) show that radial chromatography resulted in an increase in the number of plates per meter by 31%. Thus, the HETP decreased (31%) in radial chromatography, indicating a better efficiency.

The quality of the peak shape can be expressed by the asymmetry factor, which should be close to unity (i.e. 0.9–1.1).

The acetone peak in the radial chromatography showed a better asymmetry value (1.1) compared to the axial chromatography (1.83), the peak from the radial column was more narrow, symmetrical and regular.

This can be explained by a more homogeneous packed bed and the symmetrical application of the product (typical to radial flow) resulting in less distortion of the plug-flow.

3.2. Equilibration

One important point for a chromatographic run is the quality and duration of the equilibration step before the start of a run. Therefore we have also studied the equilibration of the two columns. Firstly the equilibration steps to eliminate the storage solution (20% ethanol in deionised water) and reverse (equilibration buffer to storage solution). Secondly the equilibration steps to eliminate the elution buffer and reverse.

The results expressed in Fig. 6 show that the radial chromatography allowed a reduction in the length of equilibration with 0.4–0.5 column volumes. This decrease in equilibration step duration at the start and at the end of the run allowed a reduction of the total length of the run by 1 column volume. This accounts for a decrease in buffer consumption, which is considered to be an important point at process scale.

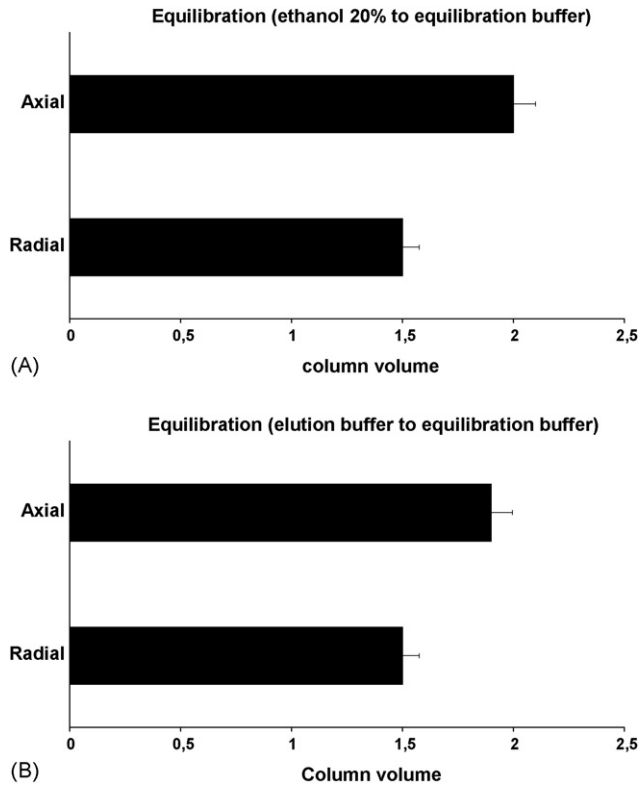

Fig. 6. Equilibration experiments: (A) Equilibration of column chromatography to eliminate storage solution (ethanol 20% to equilibration buffer). (B) Equilibration of column after regeneration to eliminate elution buffer (elution buffer to equilibration buffer).

3.3. Proteins elution by linear gradient

3.3.1. Resolution and asymmetry

Resolution, which is the separation factor, must be at a minimal value of 1.5. Figs. 7 and 8 show that resolution is acceptable for both column types. Nevertheless in radial chromatography the resolution was superior by 20% resulting from the decrease in asymmetry of both peaks in the elution (Fig. 9). The asymmetry for the bovine serum albumin peak in radial chromatography was reduced by 20% compared to axial chromatography. We measured a 46% reduction in the asymmetry factor of the ovotransferrin peak in radial chromatography when compared to axial chromatography. The combined decrease in width of both peaks resulted in the observed increase in resolution.

3.3.2. Retention volume and capacity factor (retention factor)

The retention volume was quite similar in both column types (Fig. 10). This was also expressed by a similar capacity factor for serum albumin bovine and lysozyme from both the radial and axial column (Fig. 11). Apart from the geometric differences between the axial and radial column the length of the columns was identical (bed height of both columns was 6 cm) as was the gel-volume (bed volume of both columns was 120 ml); therefore the proteins eluted the same way and had the same retention volume.

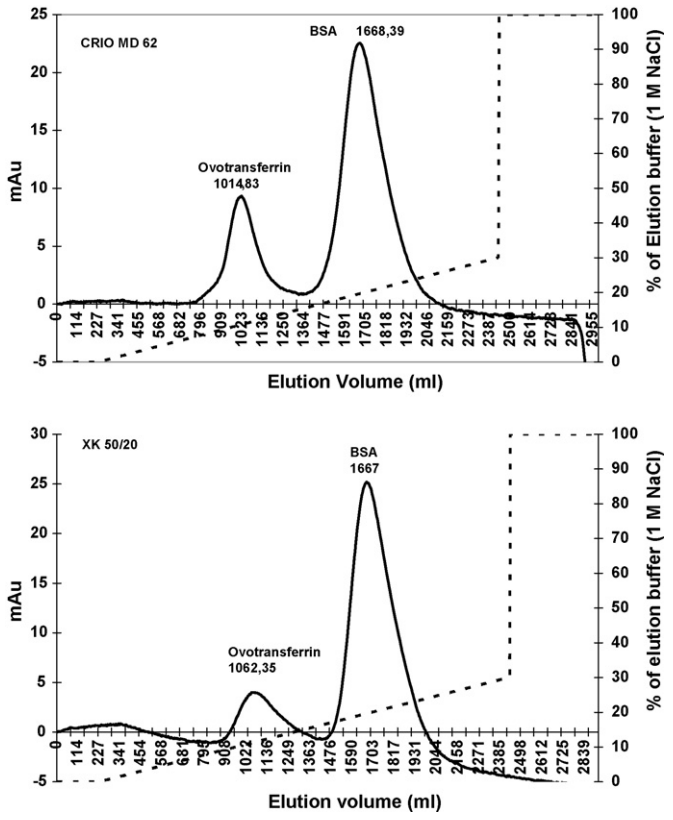

Fig. 7. Separation of protein mixture with the radial column (CRIO MD 62) and with the axial chromatography (XK 50/20); Chromatographic media: DEAE Cellufine A 500 (120 ml); Sample: 5 ml of protein mixture (ovotransferrin and bovine serum albumin); Equilibration buffer: 50 mM Tris-HCl pH 9; Elution buffer: 1 M NaCl, 50 mM Tris-HCl pH 9; Detection at 280 nm; Flow-rate: 50 ml/min.

3.4. Elution by step gradient

Using step gradient elution mode in the preparative chromatography, we have analyzed the width and the width at half height of the peaks of flow-through (lysozyme) and eluted peak of the adsorbed protein (serum albumin bovine). The absorbance at 280 nm by the protein was measured with a UV spectrophotometer. The protein concentration was thus calculated in function of the molar extinction coefficient of the protein.

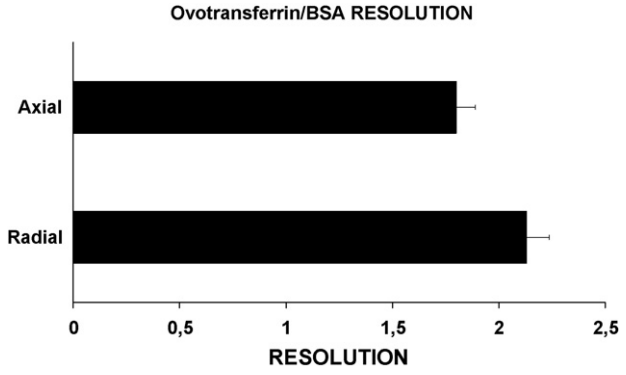

Fig. 8. Axial and radial chromatographic resolution comparison.

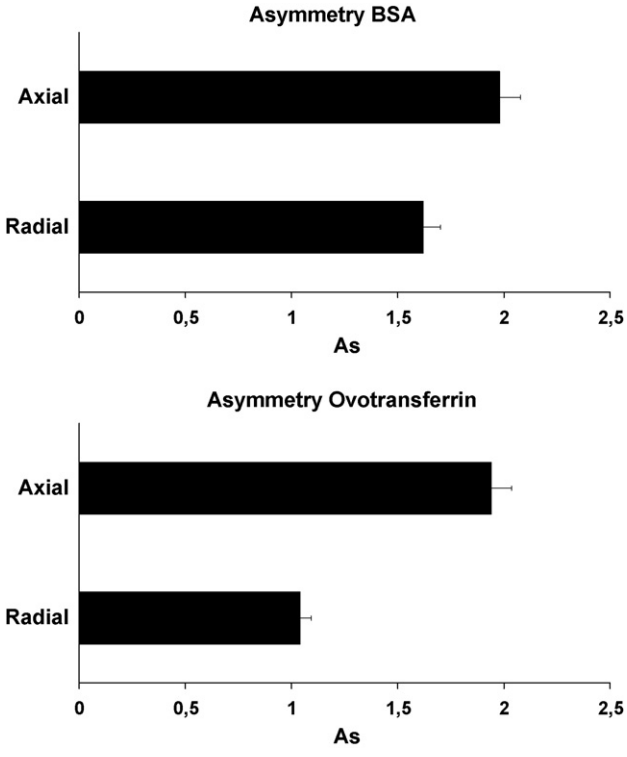

Fig. 9. Comparison of asymmetry factor of eluted peaks (ovotransferrin and bovine serum albumin) of axial chromatography versus radial chromatography.

The results expressed in Figs. 12 and 13 show that the radial chromatography gave the best results, width at half height of the flow-through product decreased by 40% and the peak-width decreased by 27%. For the adsorbed protein, we observed the same effect: width at half height decreased to 45% and the peak-width decreased to 28%. Due to a decrease in width of the peaks, the height of the peaks increased and these results were confirmed by the quantification of the protein concentration in the peaks (Figs. 12 and 13). Lysozyme, present in the flow-through was also more concentrated (35%) in the radial chromatography as compared to axial chromatography. The final concentration of the eluted fraction (serum albumin bovine) increased by 40% with the radial chromatography.

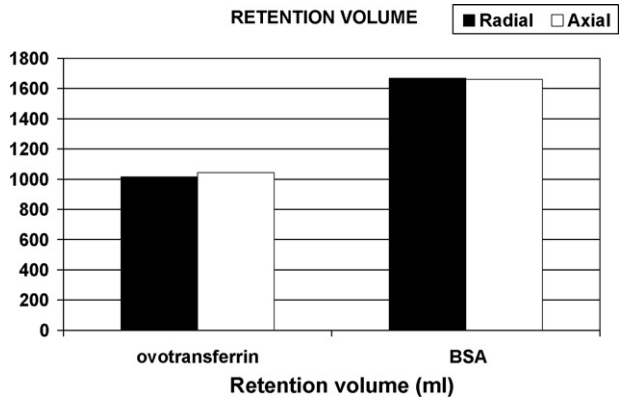

Fig. 10. Comparison of retention volume of eluted peaks (ovotransferrin and bovine serum albumin) of axial chromatography versus radial chromatography.

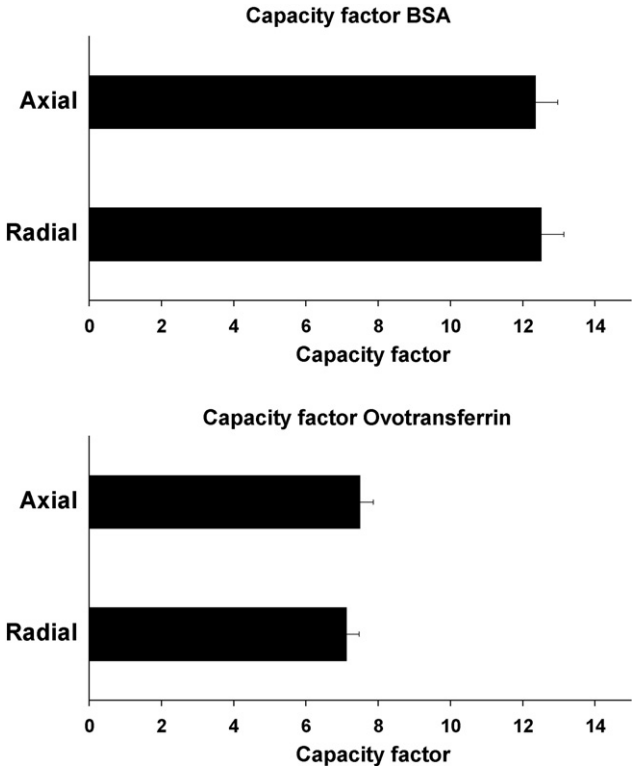

Fig. 11. Comparison of capacity factor of eluted peaks (ovotransferrin and bovine serum albumin) of axial chromatography versus radial chromatography.

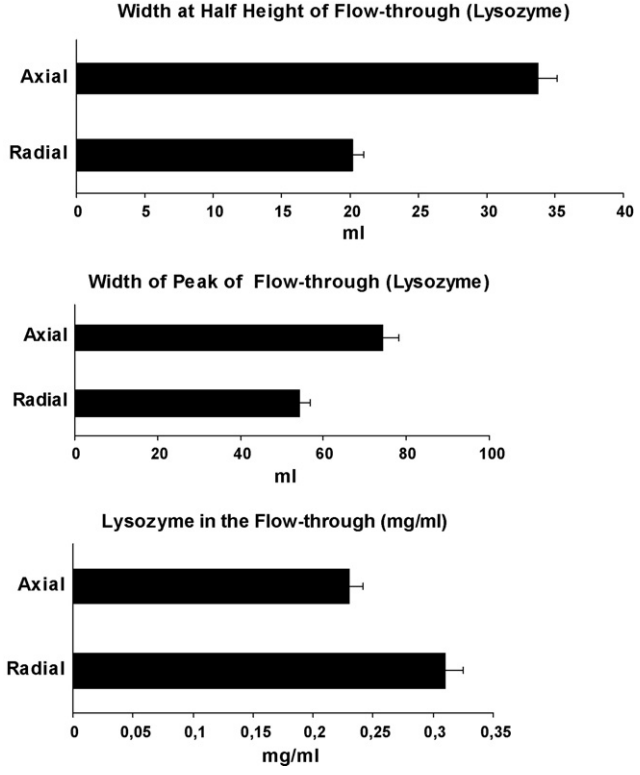

Fig. 12. Comparison of width at half height, width of flow-through peak and concentration of non-retained peak (lysozyme) of axial versus radial chromatography.

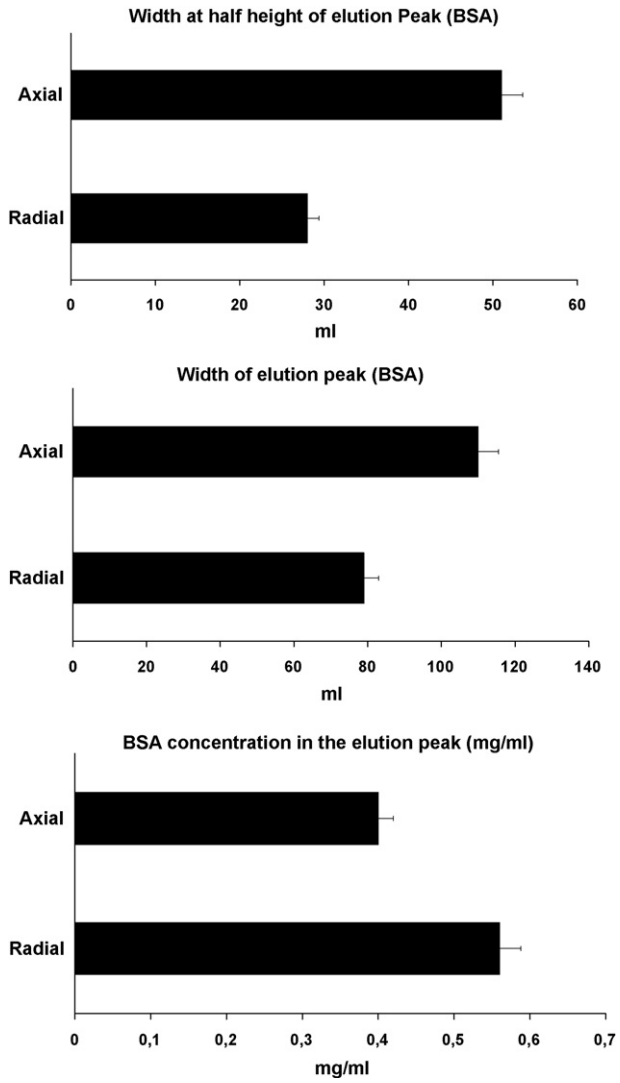

Fig. 13. Comparison of width at half height, width of flow-through peak and concentration of eluted protein (bovine serum albumin) of axial versus radial chromatography.

These results confirm the importance of the trapezoidal geometry of the radial column, which presents a larger surface at the inlet of the column allowing spreading the protein on a larger surface. This avoids a possible overloading of protein, which occurs in a smaller surface and could lead to aggregation or non specific interaction. This phenomenon could also concern possible contaminants in the feed stream. Therefore, it is expected that cleaning in place steps could be reduced or performed under milder conditions increasing the lifetime of the resin. During elution, the decrease of the surface area from the inlet to the outlet leads to a concentration of protein. In fact, a large quantity of protein is desorbed simultaneously and pushed towards a lowest surface. Therefore the protein was more concentrated.

4. Conclusion

During this study, a number of advantages were observed in the application of radial chromatography, in part explained by

the higher efficiency of the packed bed and by the superior peak symmetry. Each of these contributes to a reduction in cost, time and sometimes quality of product. The sum of these benefits, however, will make a considerable difference in process economics since buffer cost are a major cost factor in large-scale processing.

In comparing the radial flow mini-process column to the axial counterpart, we found a reduction of equilibration- and regeneration-buffer demand at the start and end of the chromatographic run that allowed reduction of this step into a single column volume. The second observation was a decrease of the flow-through volume (30%), reducing the time of this wash step to remove unbound material and therefore reducing the total run time and again buffer consumption.

Additionally, the eluted (product) peaks were reduced in volume (30%) during step gradient processing, this accounts for further reduction of time of processing. The better results obtained with the radial column were probably the consequence of the geometrical design of this device (larger inlet surface area and small outlet surface area which concentrate the eluted fraction).

In process scale chromatography, productivity is expressed as "Amount purified product per bed volume and per unit of time", since the use of radial chromatography results in a significant total run time reduction at increased product recovery. This technology offers a higher productivity in downstream chromatographic processing. Finally worth mentioning about the application of radial chromatography, is the elevated concentration of the eluted product that reduces the volumes further downstream during concentration steps or conditioning steps after the chromatographic run, therefore application of radial flow will improve total process economics.

Acknowledgements

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