ELSEVIER

Journal of Chromatography A, 760 (1997) 259–263

JOURNAL OF CHROMATOGRAPHY A

Short communication

Improved preparative electrochromatography column design

Kenneth D. Cole*, Heriberto Cabezas, Jr.[1]

Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Received 16 April 1996; revised 15 August 1996; accepted 10 September 1996

Abstract

Improved chromatography column fittings were developed for the efficient and reliable application of an electric field to a preparative chromatography column (a process termed electrochromatography). The improved fittings contained electrodes in close proximity to the column packing media and allowed uniform electric fields to be applied. Membranes in the fittings prevented mixing of the electrode and the column eluent buffers. The membranes prevented gases and electrolytic products generated in the electrode chamber from entering the column eluent buffer. An electrode buffer solution was pumped through the electrode chamber to a large external container. The circulation of buffer through the electrode chamber removed the gases and electrolytic products and ensured a uniform electric field by helping to maintain a constant buffer composition. The membranes prevented macromolecules being separated on the column from coming in contact with the electrodes.

Keywords: Electrochromatography; Preparative chromatography; Column design; Electrodes; Membranes; Instrumentation

1. Introduction

Application of an electric field to a chromatography system, the technique known as electrochromatography (EC), is a useful method to separate charged solutes. The availability of EC equipment that is easy to use and reliable would increase the use and applications of EC. The goal of this study was to develop an efficient and reliable EC apparatus that could be used on existing chromatography equipment.

The apparatus for early work in EC or electrophoresis in packed beds has been reviewed by Bloemendal [1], Kunkel and Trautman [2] and Porath [3]. The early columns were either U-shaped tubes or specially constructed glass columns in which the electrodes were isolated from the column. After a period of electrophoresis the separated sample zones were eluted from the separation media. The construction of a commercially manufactured electrophoresis column was described [3]. This column was constructed so that the separation region containing the chromatography medium was the annular space between two polymeric cylinders to allow cooling. The bottom electrode was isolated from the buffer coming out of the bottom of the separation system by a cylindrical membrane (gel impregnated porous plastic) coaxial with the end of the column. Nerenberg and Pogojeff [4] described an electrochromatography column that utilized a

*Corresponding author. Address: NIST, Building 222, Room A353, Gaithersburg, MD 20899, USA.

[1] Present Address: Environmental Protection Agency, 26 W. Martin Luther King Dr., Mail Code 443, Cincinnati, OH 45268, USA.

0021-9673/97/$17.00 Published by Elsevier Science B.V.
PII S0021-9673(96)00774-1

specialized end fitting, incorporating a gel to isolate the electrodes from the chromatography column buffer. Rudge et al. [5] used chromatography column fittings containing platinum electrodes, but the electrodes were not isolated from the chromatography column buffer. Cole and Cabezas [6] described an electrochromatography column that had membranes located in side-arms physically separated from the chromatography medium using dialysis membranes.

2. Experimental

2.1. Materials

Nylon chromatography fittings (No. 5837), porous polyethylene bed support disks (100 μm pores), O-rings and water-jacketed glass columns were obtained from Ace Glass (Vineland, NJ, USA). Water-jacketed columns were also made in-house by a glass blower using Ace Glass fittings. Nylon machine screws, thumb nuts, miniature tubing fittings and O-rings were from Small Parts (Miami, FL, USA). Dialysis membrane (Spectrapore 1, 6000–8000 molecular mass cutoff) was from Spectrum Medical Industries (Los Angeles, CA, USA). Sephadex G-25 fine (20 to 80 μm dry bead diameter), Sephadex G-75 (40 to 120 μm dry bead diameter), myoglobin (horse heart) and β-lactoglobulin (bovine) were obtained from Sigma (St. Louis, MO, USA).

2.2. Construction of the fittings

The electrochromatography column fittings were designed to apply an axial electric field to a packed bed in a chromatography column (Fig. 1). The commercial nylon fittings were modified to provide an isolated electrophoresis chamber just below the bed support position (Fig. 2a). Two sizes of fittings were constructed as shown in Fig. 2. A circular groove (the electrode chamber) was machined concentric to the outlet/inlet center hole on the surface below the bed support position (see Fig. 2a and b). In the bottom of the groove, four holes (two for the electrode and two for buffer ports) were drilled through the fittings parallel to the center line and spaced 90° apart. The central outlet/inlet holes were

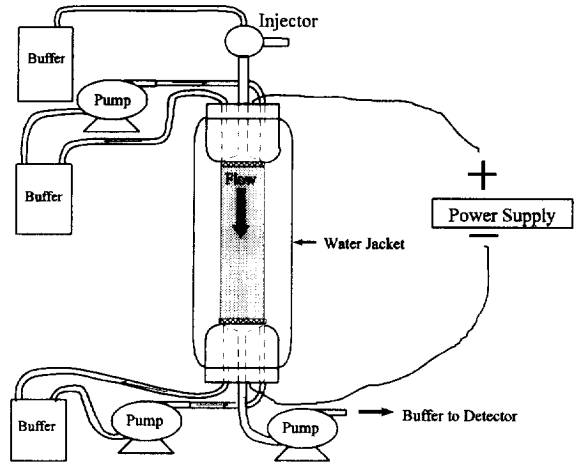

Fig. 1. Diagram of the electrochromatographic system.

drilled to 6.4 mm (1/4 in.) and counter sunk at 82°. The countersink was stopped short of breaking into the machined groove.

The electrodes were made by twisting two lengths (approximately 150 mm each) of platinum wire (0.4 mm diameter) together at the ends and forming a central circular region that will fit the diameter of the electrode chamber groove. The twisted ends were inserted in the two electrode holes in the groove and the central loop was pushed to fit into the groove. The twisted ends of the wires were sealed into the holes using silicone rubber adhesive (RTV 108, GE Silicones, Waterford, NY, USA), delivered using a large bore needle and syringe. Care was taken not to block the groove or coat the platinum wires exposed in the groove that forms the electrode chamber. One end of the protruding platinum wire was soldered to a female banana plug and heat shrink tubing added to insulate the wire. The other end of the wire was sealed with heat shrink tubing and left as a spare.

The other two open holes in the electrode chamber groove comprise the electrode chamber inlet and outlet. The external sides of the inlet and outlet holes were drilled and tapped to accept 2.4 mm (3/32 in.) threaded hose barbs. Because of the smaller diameter of the 15 mm fitting, 90° elbow fittings with inside diameter of 1.6 mm (1/16 in.) were fitted to exit on the side of the tubing below the thumb nut. The fittings were sealed in place with silicone rubber adhesive.

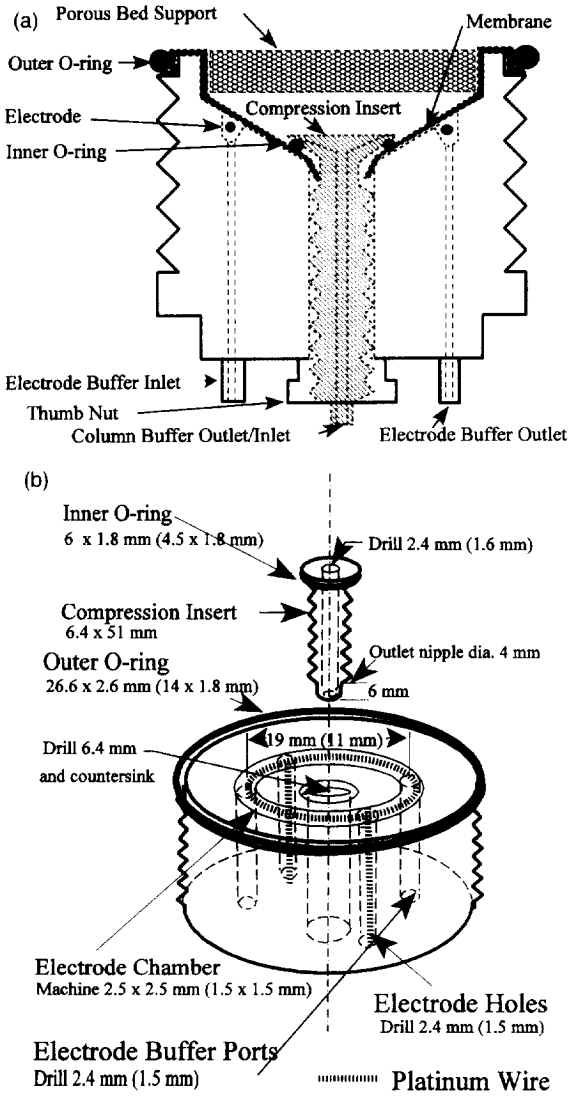

Fig. 2. (a) Cross-sectional and (b) 3-dimensional views of the electrochromatography column fitting. The porous bed support and membrane have been omitted for clarity in b. The dimensions shown are for the 25 mm fitting and dimensions for the 15 mm fitting are in parentheses.

The central compression insert was made from a 50.8×6.4 mm (2×1/4 in.) nylon machine screw. A hole was drilled through the central axis of the screw to provide for a new inlet or outlet. The lower portion of the threaded shaft was ground down to provide a nipple for tubing attachment. The diameters of the screw heads used in the 15 mm fittings were reduced to 9.5 mm. The upper surface of the screw head was tapered to provide a draining surface that approximates the original contour of the fitting. This was accomplished using a small rotary grinder to remove the nylon in the center of the screw head. The countersunk surface of the screw was polished to remove any ridges. A small O-ring was placed below the head of the central compression insert (Fig. 2a and b).

2.3. Assembly of the column fittings

A hole was punched in wetted dialysis membrane using a 6.4 mm diameter cork borer. The central compression insert with its central O-ring in place was inserted into the hole in the membrane and into the column fitting. The thumb nut was tightened to compress the inner O-ring against the membrane and the body of the column fitting. Care was taken to keep the membrane wet and free of wrinkles during compression. A wetted bed support (porous polyethylene) was pressed into position, sealing the membrane to the perimeter of the column fitting lip. The thickness of the porous bed supports used in the 15 mm columns was reduced to a thickness of approximately 1.3 mm by dividing the disk in half equatorially using a razor blade. In latter experiments the bed supports for the 15 mm fittings were used as supplied. The porous bed supports for the 25 mm fittings were 3 mm thick and were used as supplied. The outer O-ring was slipped over the wet membrane and onto the outer lip of the fitting. The dialysis membrane was trimmed so that only a small portion protruded beyond the outer O-ring. The assembled fitting was then carefully screwed into the column until the O-ring was compressed against the glass shoulder of the column sealing the membrane against the fitting. The electrode buffer recirculation pump was started and operated for approximately 10 min to observe for leaks. If the membrane was not properly sealed, buffer began to drip out of the inlet/outlet ports.

2.4. Operation of the column

The water-jacketed columns, thermostated at 25°C, had an inner diameter of 15 or 25 mm and a length of either 102 or 300 mm (between bed supports). A

Table 1
Number of theoretical plates (N) obtained from 10.2×1.5 cm column packed with Sephadex G-25, fine using either EC fittings or conventional fittings

Fitting type	β-Lactoglobulin (N±S.D.)	Glycine (N±S.D.)
EC	241±9	457±10
Conventional	241±22	477±31

Values are means of 3 determinations±S.D. (the standard deviation).

peristaltic pump placed at the outlet of the column was used to maintain a constant flow-rate of column eluent buffer. The eluent was monitored at either 230 or 280 nm by a UV detector. The electrode buffers were circulated to external buffer containers (25°C) by means of peristaltic pumps. Column inlet and outlet fittings were maintained as an independent loops (1 l of buffer) and circulated with a flow-rate of 15 ml/min. The column eluent and electrode buffers were 3.9 mmol/l tris(hydroxy)-aminomethane, 47 mmol/l glycine, 0.25 mmol/l ethylenediaminetetraacetic acid, pH 8.2.

3. Results and discussion

The influence of the column fitting on chromatographic performance of the column was tested. We packed a 10.2×1.5 cm column with Sephadex G-25 and compared the results between the EC fittings and conventional fittings. Table 1 indicates the number of theoretical plates obtained for β-lactoglobulin (excluded from the gel) and glycine (totally included in the gel) on a column packed with Sephadex G-25 fine using the EC fitting or conventional fittings. Fig. 3 shows the peak shapes obtained with both types of fittings. The two types of fittings yielded nearly identical results. These results indicate that the modifications in the EC fittings did not degrade the normal chromatographic performance of the system.

Fig. 4 shows a typical experiment done with an EC column (30×1.5 cm column packed with Sephadex G-75) and the EC column fittings. β-Lactoglobulin and myoglobin did not separate under these conditions. When a negative electric field (negative electrode at the column inlet) was applied to the column, β-lactoglobulin, with an electrophoretic mobility approximately 4.5-fold greater than myoglobin [7], was retained on the column much longer than myoglobin. Increasing the field allowed an almost complete separation of the two proteins (Fig. 4).

The EC column fittings described in this work offer significant advantages for the separation of charged macromolecules. The isolated electrode

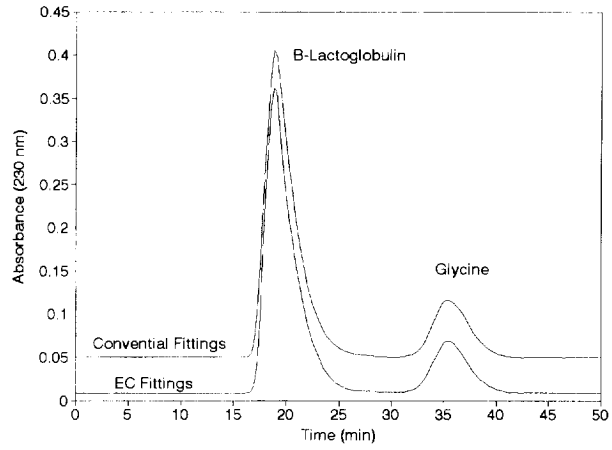

Fig. 3. Peak shapes of β-lactoglobulin and glycine obtained using the modified (EC) or unmodified (conventional) fittings. The column (10.2×1.5 cm) was packed with Sephadex G-25 and maintained at 25°C. The sample was 0.25 ml of a solution of β-lactoglobulin (1 mg/ml) and glycine (470 mmol/l). The buffer (1.0 ml/min) used was 3.9 mmol/l tris(hydroxy)aminomethane, 47 mmol/l glycine, 0.25 mmol/l ethylenediaminetetraacetic acid, pH 8.2.

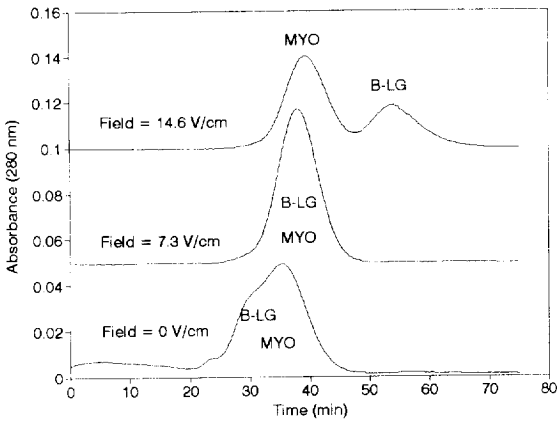

Fig. 4. Electrochromatographic separation of bovine β-lactoglobulin (B-LG) and horse heart myoglobin (MYO). The column (30×1.5 cm packed with Sephadex G-75) was injected with 0.5 mg of each protein. The flow-rate was 1.0 ml/min maintained at 25°C. Electric field strengths (E) were calculated by the formula, $E=I/Ak$, where I is the current, A is the cross-sectional area of the column and k is the conductivity of the buffer (174 μS/cm at 25°C). Buffer was the same as described in Fig. 3.

chambers are in close proximity to the packed bed providing a uniform field with most of the power applied axially across the chromatography medium. The electrode chambers use membranes that allow the passage of the electric field but prevent macromolecules from contacting the electrode surfaces. Continuous application of the electric field during sample loading and elution is possible because of this isolation feature. We have used dialysis membranes to isolate the electrode but any suitable membrane such as ultrafiltration, reverse-osmosis, or ion-exchange could be used.

The electrode buffer flow helps maintain a constant buffer composition in the electrode chamber, resulting in a uniform electric field. The electrode buffer flow also serves to remove any heat generated in the electrode chamber. The large volume of the external containers used for the electrode buffers allows the use of buffers that do not have high buffering capacity. The external containers allow the buffers to be maintained at a constant temperature and checked for any changes in pH or conductivity. The circulation rate of the electrode chambers can also be set independently of the column flow-rate.

Other chromatography fittings of similar design can be adapted for EC with minor modifications. These EC fittings gave similar performance to unmodified fittings and allow the adaptation of existing chromatography systems for EC separations. The ease of use and reliable performance of these columns should encourage the use of EC for the preparative separation of a wide variety of compounds.

4. Note

Certain commercial equipment, instruments or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by NIST, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

References

[1] H. Bloemendal, J. Chromatogr., 3 (1960) 1.
[2] H.G. Kunkel and R. Trautman, in M. Bier (Editor), Electrophoresis—Theory, Methods and Applications, Academic Press, San Diego, CA, 1959, Ch. 6, p. 225.
[3] J. Porath, J. Sci. Tools, 11 (1964) 21.
[4] S.T. Nerenberg and B.A. Pogojeff, Am. J. Clin. Path., 51 (1969) 728.
[5] S.R. Rudge, S.K. Basak and M.R. Ladisch, AIChE J., 39 (1993) 797.
[6] K.D. Cole and H. Cabezas Jr., Appl. Biochem. Biotech., 54 (1995) 159.
[7] K.D. Cole, P. Todd, K. Srinivasan and B.K. Dutta, J. Chromatogr. A, 707 (1995) 77.