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Continuous Electrochromatography Using a Rotating Annular System

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Abstract

A rotating cylinder containing an annulus of adsorbent can be operated as a continuous chromatograph if the feed point and the product takeoff points are stationary with respect to the annulus. A system of this type will develop helical bands of the separated solutes with radial symmetry. It has been shown that such a system can operate with enhanced throughput by extending the annular area to more than 90% of the total cross-sectional area. The larger operating area also makes it possible to consider a multidimensional separation where an external force can be applied radially to the chromatograph.

When an electric field is applied across the annulus, both chromatography and electrophoresis can be carried out simultaneously on a continuous basis. This capability gives the potential for continuous separation of a variety of substances such as complex biochemical mixtures. The concept has been demonstrated for the separation of hemoglobin and other large-molecular-weight substances.

INTRODUCTION

Over 30 years ago, Martin proposed a concept for a continuous chromatograph that included an annular bed of adsorbent moving with respect to a feed stream and eluate collection points (*1*). Later, this concept was considered on a theoretical basis by Giddings (*2*). Other workers evaluated various versions of the concept (*3, 4*); however, the most successful demonstration of continuous chromatography was made

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with a rotating cylinder containing an active annulus of adsorbent used with stationary feed and eluent streams as well as stationary eluate collection ports. The most significant advance has involved the introduction of feed and eluent through appropriate seals that permit pressurized operation, thus affording greater productivity and higher resolution (*5–7*). The separated constituents appear as discrete helical bands that start at the feed point and terminate at different angular positions at the column effluent (see Fig. 1).

Systems of this type have been scaled up successfully, and several potential applications have been demonstrated (*7–9*). Various studies showed that a very wide annulus (>90% of the free area) could be used with radial symmetry and, thus, without loss of resolution. This operational mode resulted in an even higher throughput, providing adequate attention was given to feed introduction (*10*).

In a different approach to separations, Vermeulen et al. investigated a concept for continuous-flow electrophoresis in which the feed was introduced as a constant stream through an annular ring positioned above a stationary, cylindrical column that had electrodes both at the center and at the outer circumference (*11*). As the feed constituents flowed down through the column, those with electrophoretic mobility progressed radially toward one of the electrodes and exited as annular bands that were displaced from the path of the original feed stream. This system was demonstrated for the separation of mixtures of dyes and amino acids.

Somewhat similarly, if a radial electrical field is imposed across the rotating annulus, both of the above effects can be utilized, thus providing a continuous multidimensional separation that could properly be described as continuous electrochromatography.

PRINCIPLE OF OPERATION

The continuous, annular electrochromatograph has many similarities in construction and operation to the previously developed continuous chromatograph. However, the imposition of an electrical field adds some complications.

Description of System

The system is composed of an annular column of adsorbent that slowly rotates past a stationary feed point and the eluate takeoff points (see Fig. 2). The annulus is relatively wide with respect to the feed stream that is

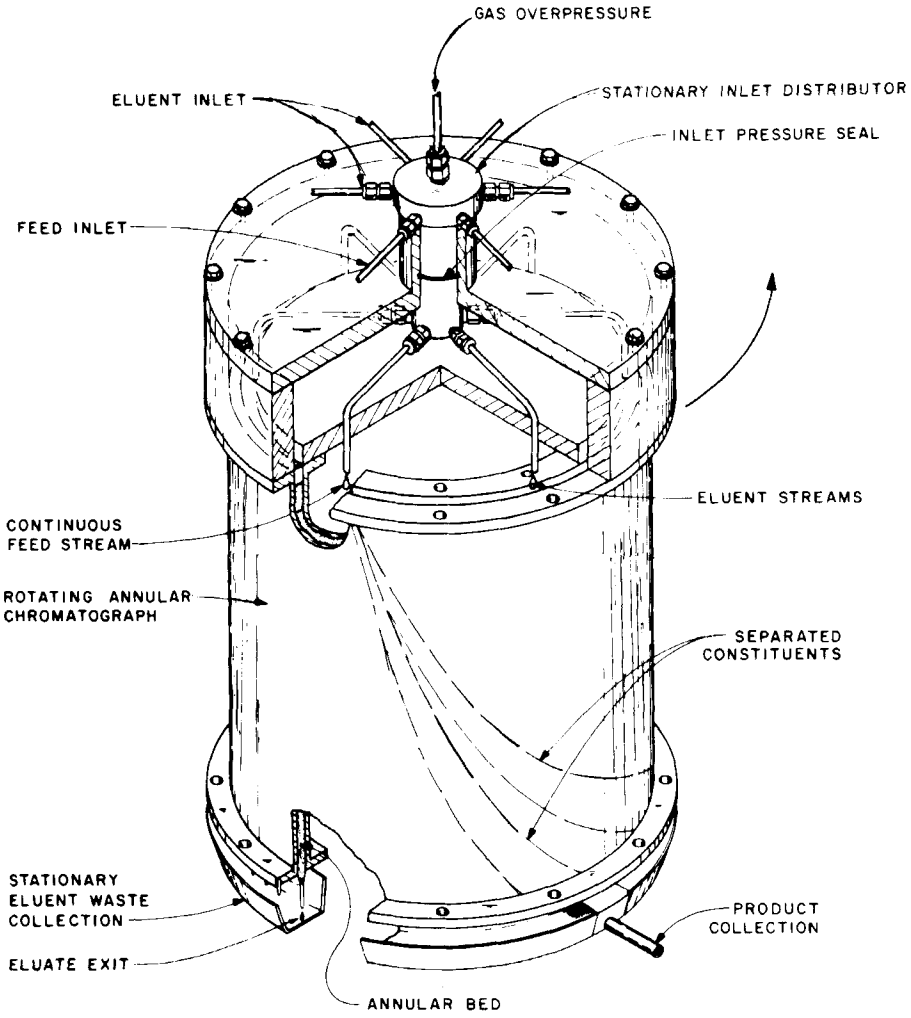

FIG. 1. Pressurized, continuous annular chromatograph (reproduced from Ref. 5).

introduced through a nozzel nominally located in the middle of the annulus. Typically, the two electrodes are placed within the annular column immediately next to the two inside walls. Alternatively, they can be separated from the adsorbent by porous membranes. In the latter case the resulting electrode annuli would serve to separate any resulting off-

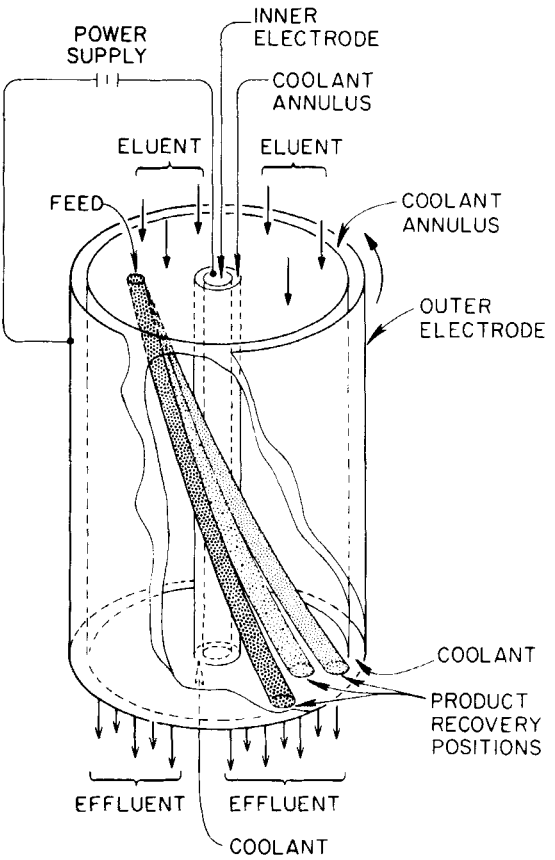

FIG. 2. Possible configuration for a continuous annular electrochromatograph.

gas from the adsorption bed and a small electrode buffer stream could be used to sweep out the off-gas and provide column cooling.

As in the case of the continuous, annular chromatograph (5), the eluent is introduced by a single nozzle to the headspace above the top of the adsorbent bed and the feed is introduced below the top surface of the adsorbent bed to prevent mixing with the eluent. If gradient elution is required, then multiple eluent introduction nozzles must be used with the eluent level maintained just at the top of the adsorbent bed. An inert sweep gas is introduced into the top headspace to remove potentially explosive electrode gases and to provide system overpressure. All entrance and exit streams and electrode connectors at the top of the

column progress through a central stationary shaft with a pressure seal, thus allowing the annulus to be rotated under pressurized operation.

Exit ports, each of which includes a small porous filter at the entrance and terminates with a small drip tube, are arranged in radial rows around the bottom of the annulus. Each product is collected by placing a stationary channel at the angular and radial position expected for elution.

Theoretical Considerations

Continuous annular electrochromatography represnts a "two-dimensional" separation in which each of the separated products will exit from the system at a radial and angular position that depends on the geometry and dynamics of the system as well as the sorption and electrophoretic properties of each of the solutes. In general, the chromatographic component will contribute to the angular position while the electrophoretic component affects the radial position.

Using a simple, theoretical plate concept, it has been previously shown that the angular displacement of the exiting solute band, $\bar{\theta}$, can be represented by (*5*) (Fig. 3):

$$\bar{\theta} = (L\omega/u_f)[\varepsilon + (1 - \varepsilon)K] \quad (1)$$

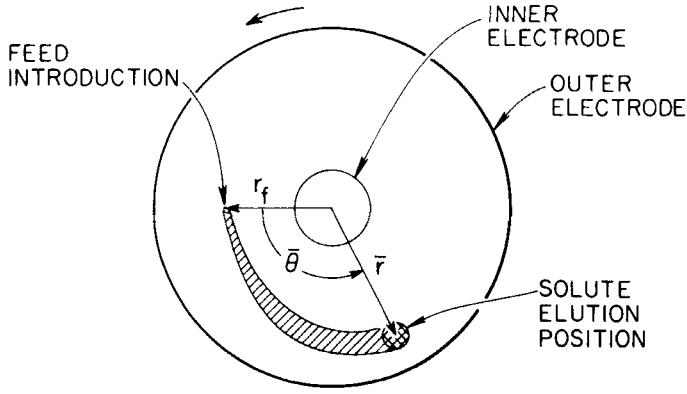

FIG. 3. Top view of electrochromatograph, indicating the positions of an eluted solute.

where $\bar{\theta}$ = angular displacement of the maximum solute concentration exiting the annulus, rad
L = vertical distance from feed point to exit, cm
ω = angular velocity or rotational speed, rad/s
u_f = superficial eluent velocity through the column, cm/s
ε = void fraction in the chromatographic column, cm^3 fluid phase/cm^3 total volume

$$K = \text{distribution coefficient, } \frac{\text{mol solute/cm}^3\text{ (sorbent phase)}}{\text{mol solute/cm}^3\text{ (fluid phase)}}$$

This represents the separation component due to the sorption phenomena coupled with the geometry and dynamics of the annular column.

The electric field results in a radial deflection of the solute, depending on its electrophoretic mobility in the nonsorbed state. Radial mobility will be limited during the time the solute is sorbed in or on the stationary phase; however, since this will also be true for migration in the vertical direction, the effects will cancel out and we can simply consider migration rates in the adsorption bed as a whole. Assuming that the electrophoretic properties are constant throughout the column, the apparent radial distance of component movement can be expressed as (Fig. 3):

$$\bar{r} - r_f = L \tan \alpha \tag{2}$$

where $\bar{r}$ = radial position of the exit of the maximum solute concentration, cm
r_f = radial position of the feed point, cm
L = column length, cm
α = angle of radial deflection of the solute, rad

But the angular deflection is also dependent on the relative axial and radial movements:

$$\tan \alpha = v_e/v_a \tag{3}$$

where v_e = electrophoretic mobility in the sorbent bed, cm/s
v_a = axial velocity of component through the sorbent bed, cm/s

The axial velocity of the component through the column can be

described as the length of the column divided by the length of time for the component to traverse the column:

$$v_a = L\omega/\bar{\theta} \tag{4}$$

Combining these relationships, the radial eluate point should occur at

$$r = r_f + (v_e\bar{\theta}/\omega) \tag{5}$$

or

$$r = r_f + (v_eL/u_f)[\varepsilon + (1 - \varepsilon)K] \tag{5a}$$

Thus, each component should exit the column at a specific position, $\bar{\theta}$ and $\bar{r}$, that is totally dependent on its sorption and electrophoretic properties.

For a stationary annulus, the following equation can be used:

$$\bar{r} = r_f + (v_e/t) \tag{6}$$

where t = residence time of solute in the column, s.

MATERIALS AND EQUIPMENT

Materials

Preliminary tests were made with two macromolecular solutes. Bovine Hemoglobin (Type I, Sigma Chemical Company), with an approximate molecular weight of 60,000 daltons (*12*), is known to be separated into several fractions by electrophoresis (*13*) and thus was used to demonstrate the concept of continuous separation by electrophoresis. Blue Dextran 2000 (Pharmacia Biotechnology), with a molecular weight of 2,000,000 daltons (*14*), was used to determine column void fraction, and, in conjunction with the hemoglobin, it was also used to demonstrate separation by gel permeation chromatography. Both solutes were used in a 0.05 *M* phosphate buffer at a nominal pH of 8.0.

Each packed bed was composed of either noninteracting glass beads (0.17 to 0.18 mm diameter B. Braun Melsungen) or polyacrylamide gel beads (Bio-Gel P-150, 50-100 mesh, Bio-Rad Laboratories).

Experimental System

Tests were carried out with a 8.8-cm-ID column that had a 30-cm active height and an annulus of approximately 3.8 cm (see Fig. 4 and Ref. 5). The column, which was externally jacketed by an aqueous ice bath for cooling, contained an inner platinum-mesh electrode wrapped around an inert plastic sleeve and an outer nickel-mesh electrode immediately adjacent to the inside of the outer wall. The electrodes were in direct contact with the adsorbent material.

The annular column was slowly rotated by a variable-speed motor in the range of 0.15 to 1.5 rph. There were provisions for the introduction of a continuous feed stream through a stationary 1.6-mm-diameter stainless steel nozzle that was suspended 0.5 cm below the surface of the adsorbent bed and an eluent buffer stream that was introduced into the headspace of the column. Both of these streams entered through a pressurized seal. The electrodes were connected to an external dc power supply, and the eluate exited through a series of concentric, filtered nozzles arranged in four circumferential rows with equal radial spacing. An inert sweep gas was also introduced into the headspace at the top of the column, and it exited through a pressure letdown valve.

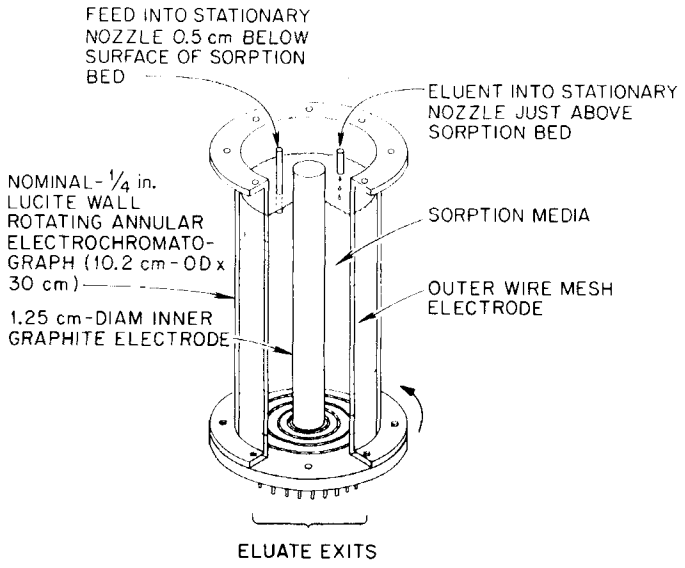

FIG. 4. Simplified view of experimental apparatus for electrochromatography.

Operation

The experimental system was operated by first initiating the eluent flow until the top of the adsorbent bed was covered with eluent. Then the sweep gas was started, and system pressure was adjusted by a pressure regulator on the off-gas. If the electrodes are isolated from the adsorbent bed, the electrode sweep and cooling solutions must also be initiated. Thereafter, the electrical field was initiated and the feed stream was started. The product collection channels could then be put in place.

Analytical Procedure

A Beckman DB UV-Visible Spectrophotometer was used to measure the solute concentrations of discrete samples collected from individual eluate nozzles. The hemoglobin concentration was measured at 410 nm, and Blue Dextran 2000 concentration was measured at 610 nm.

EXPERIMENTAL RESULTS

Several preliminary tests were performed in which hemoglobin and/or Blue Dextran were introduced as the feed into the annular column containing either adsorbing or nonadsorbing packed beds. The concept of continuous electrochromatography was demonstrated with these two solutes.

Nonadsorbing Bed

Annular beds of packed glass beads were used to demonstrate continuous electrophoresis in the absence of adsorption. Both Blue Dextran and hemoglobin could be eluted in a relatively narrow band when there was no electrical field imposed on the system either as a stationary column or as a rotating cylinder containing an annulus of packed solids. For example, hemoglobin at the concentration of 2 g/L was introduced as the feed stream at the rate of 0.25 mL/min while the eluent buffer was introduced at the rate of 43 mL/min at ambient temperature and pressure. The solute residence time of approximately 18 min indicated that the bed had a void fraction of 0.44.

With no applied electrical field, 97% of the hemoglobin exited at a single port, suggesting that there was very little hydrodynamic dispersion

within the bed (Table 1). As the electrical field was imposed, radial migration of the hemoglobin fractions increased. The apparent electrophoretic velocity of the most rapidly moving hemoglobin fraction, as determined by Eq. (6), was 7.4×10^{-4} cm/s at 5 V.

Blue Dextran 2000 was not significantly affected by the electrical field; therefore, with this relatively short residence time, it tended to exit the column as a narrow stream. Since the glass beads represented a noninteracting packed bed, rotation of the annulus did not affect the dispersion of either the hemoglobin or the Blue Dextran streams. Blue Dextran could not be separated from hemoglobin in these tests since there was no chromatographic component.

An external ice bath and the high eluent flow rate were adequate to control the bed temperature to <35°C even at voltages up to 40 V, where the maximum current flow was approximately 1 A. Extensive off-gasing was observed at the electrodes with the higher voltages, but the gas exited the annulus adjacent to the electrodes, thereby limiting the effects on the migrating solutes.

Adsorbing Bed

Bio-Gel P-150 was used for a series of tests in which the molecular sieving action could be used to chromatographically separate macro-

TABLE 1
Elution of Hemoglobin in an 8.8-cm-diameter Electrochromatograph

ω (rad/s$^{\omega} \times 10^4$)	$\bar{\theta}$ (rad)	ΔE (V)	Fraction of hemoglobin eluted at radial position (cm)					
			(−)	1.7	2.5	3.3	4.0	(+)
Glass Beads (residence time, ~18 min)								
0	0	0		0.01	0.01	0.97	0.01	
0	0	5		0.03	0.10	0.86	0.01	
0	0	20		0.16	0.31	0.51	0.02	
Bio-gel P-150 (residence time, ~100 min)								
0	0	0		0.02	0.08	0.77	0.13	
0	0	10		0.39	0.24	0.29	0.08	
3.1	1.8	10		0.44	0.16	0.29	0.11	

molecules by size. This material has a molecular exclusion limit of 150,000 daltons (*15*); therefore, the hemoglobin fractions should penetrate the gel matrix and be retarded in its exit, but the Blue Dextran should be much too large to interact with the gel beads.

The elution time of Blue Dextran indicated that the void fraction of the packed bed was 0.40; however, the smaller beads tended to cause a higher flow resistance so that typically the eluent buffer flow rate was only 8.0 mL/min. The feed rate was again 0.25 mL/min, with a hemoglobin concentration of 1 g/L and/or a Blue Dextran concentration of 1 g/L. The longer residence time (typically 100 min for hemoglobin) resulted in a somewhat greater dispersion of the feed stream; however, in the absence of an electric field, 77% of the original material was still exiting at a single nozzle (Table 1).

Although an external ice bath was used to cool the system, only a modest electrical potential could be used without excessive bed temperatures when the eluent flow was less than 10 mL/min. (The bed temperature was not allowed to exceed 35°C.) But, even a potential of 10 V resulted in appreciable migration of the hemoglobin components, with a clear indication of two different fractions. This separation was also maintained when the annulus was rotating. The electrophoretic velocity of the more mobile hemoglobin fraction was determined by Eq. (5) to be 2.8×10^{-4} cm/s at 10 V, a value less than half that measured in the bed of glass beads at a lower voltage. Obviously, the bed of gel beads presented greater resistance to electrophoretic mobility.

When both Blue Dextran and hemoglobin were introduced into the feed stream, there was almost complete angular separation between these two solutes (continuous chromatography) as well as radial separation of the hemoglobin components (continuous electrophoresis) (Fig. 5). Thus, the concept of continuous electrochromatography has been demonstrated on an experimental basis.

Operational Problems

Although the experimental apparatus described here was adequate for demonstrating the concept of continuous electrochromatography, some operational problems need to be addressed before such systems can be utilized for routine applications. Since no attempt was made to isolate the electrodes from the sorption media, gas formation at the electrodes caused some disruption in the vicinity of the electrodes. The electrodes could be isolated from the separation media via separate electrode channels by using a permeable membrane similar to the design tested by

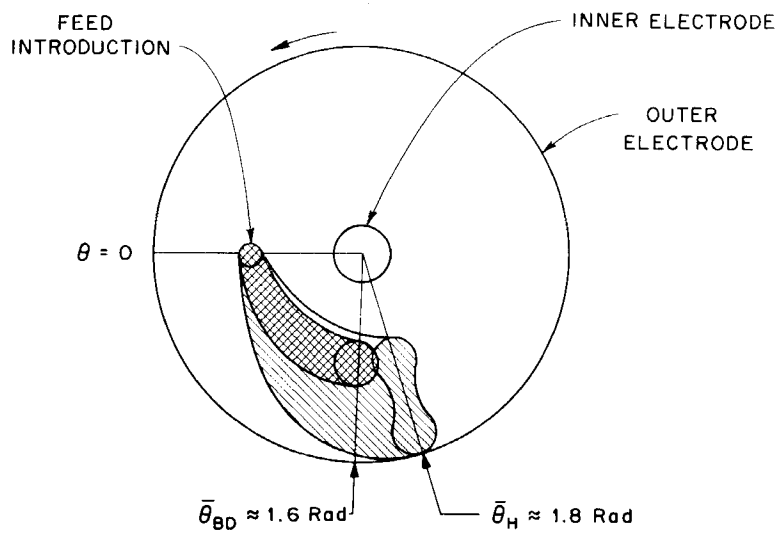

FIG. 5. Top view of the 8.8-cm-diameter electrochromatograph during separation of Blue Dextran and hemoglobin.

Vermeulen et al. (*11*). Separate electrode buffer streams (Fig. 2) could also be used to sweep away the electrode gases.

Excessive temperature buildup in the sorption bed could be controlled by using buffer streams that flow through the electrode chambers and act as coolants. This would allow the use of higher voltages, which would effect more rapid radial separations.

CONCLUSIONS

The results of this study show that simultaneous electrophoresis and chromatography can be carried out continuously in a slowly rotating annulus containing a sorption bed. A simplified theoretical model has been developed for such a separation based on the "theoretical plate" approach. Such a concept may be useful in separating mixtures of macromolecules; however, additional work on system design will be required to control electrode off-gases and bed heating adequately.

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