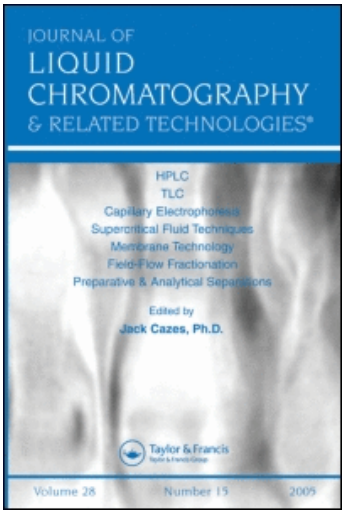

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On-Line Flow-Injection Detection System and Its Application to the Measurement of Ethanol Transfer and Osmosis Rate in Centrifugal Precipitation Chromatography

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On-Line Flow-Injection Detection System and Its Application to the Measurement of Ethanol Transfer and Osmosis Rate in Centrifugal Precipitation Chromatography

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ABSTRACT

An on-line flow-injection detection system is newly designed and successfully used in the basic research on determination of the mass transfer and osmosis rates of ethanol through the membrane in centrifugal precipitation chromatography. The system improves the linearity range of the calibration curve, which showed linearity coefficient of correlation of 0.998 between 10 and 80% ethanol solution.

Key Words: On-line flow-injection method; Centrifugal precipitation chromatography; Mass transfer; Osmosis; Ethanol; Seal-free continuous-flow centrifuge.

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INTRODUCTION

Centrifugal precipitation chromatography[1] is a novel support-free chromatographic technique recently developed for the separation and purification of various compounds ranging from small to macromolecules, including protein,[2] polycatechin,[3] polysaccharide,[4] dextran,[5] and DNA.[6] In centrifugal precipitation chromatography, the separation depends on the difference in solubility of samples in solvents containing a precipitant, e.g., ammonium sulfate for proteins,[2] ethanol for polysaccharide,[7] and cetyltrimethylammonium bromide for DNA[6] as a precipitating agent. The separation column consists of a pair of disks equipped with mutually mirror-imaged spiral grooves, and a sheet of dialysis membrane sandwiched between the disks to form two identical channels separated by the membrane. This disk assembly is mounted on a seal-free continuous-flow centrifuge developed in our laboratory.[8] In the course of our studies on the hydrodynamics and optimization of operating conditions for centrifugal precipitation chromatography, we found it necessary to measure the mass transfer and osmosis rates of the precipitant, e.g., ethanol, through the membrane. Refraction index is a robust and readily available detection system for such purposes, but it is exceeding non-linear measuring concentrated organic aqueous solutions.

In this paper, we report the design of a single six port valve that provides dilution of an aliquot of the concentrated solution with a fixed amount of water, allowing its measurement in a mode linear region. This flow-injection detection system is then applied to the measurement of mass transfer and osmosis rates of methanol, through the membrane that was required in our research on polysaccharide fractionation.

EXPERIMENTAL

Apparatus

The design of our new flow-injection detection system is schematically illustrated in Fig. 1. It consisted of a Waters 515 HPLC pump, a Model 7725 switching valve with a 5-μL loop, a PTFE tubing ($370 \times 0.85\,mm^2$ I.D.) as a mixer, a Waters 2410 refractive index detector set at 40°C (Waters, Milford, MA), and a CR501 Chromatopac integrator (Shimadzu Corporation, Kyoto, Japan). The system was assembled particularly for the on-line quantitative analysis of ethanol from the sample channel outlet of the centrifugal precipitation chromatograph. HPLC grade water was used as the mobile phase at a flow-rate of 0.7 mL/min. The diagram drawn in Fig. 1

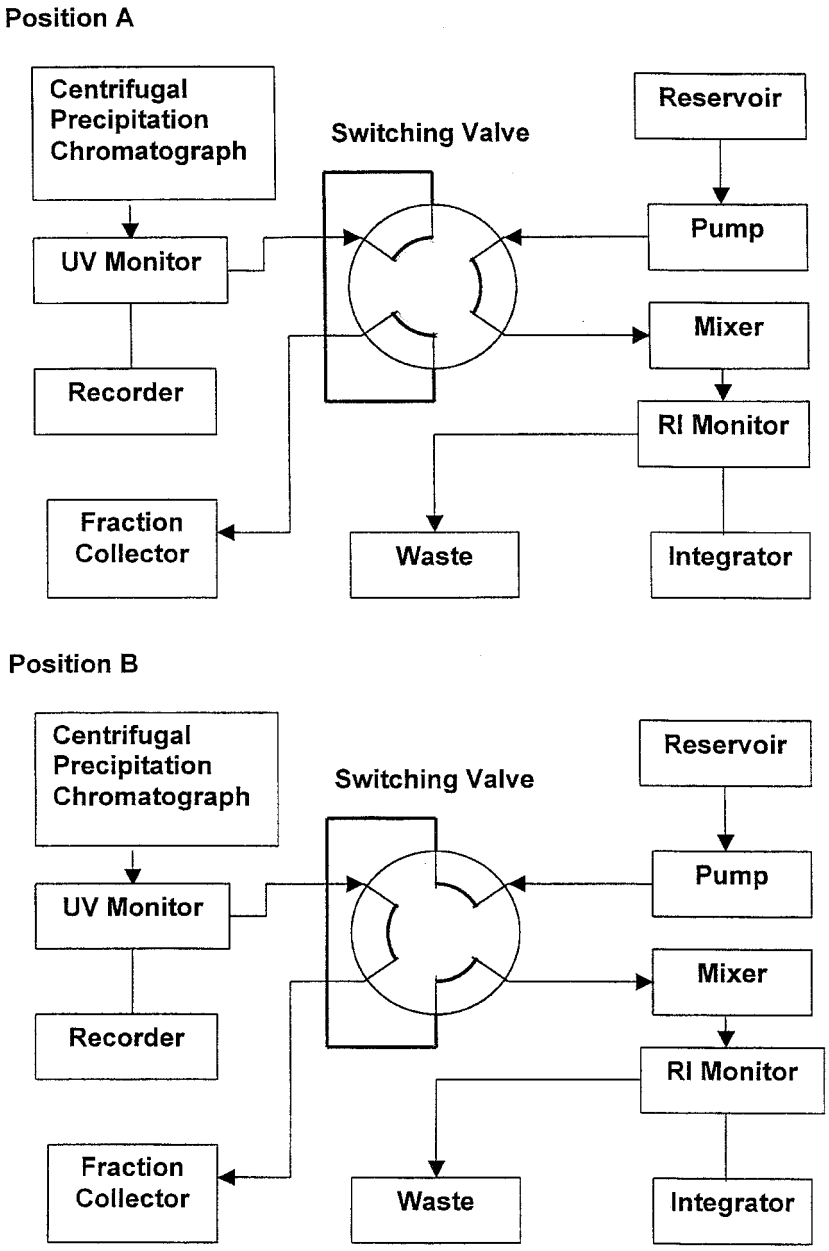

Figure 1. On-line flow-injection detection system.

indicates two positions of the injection valve, i.e., filling (A) and injecting (B). This on-line flow-injection system gives accurate values of the ethanol concentration directly from the flow line, without loss by evaporation.

The centrifugal precipitation chromatograph used in the present study consists of a compact seal-free centrifuge produced by Pharma-Tech research Corporation (Baltimore, MD). The separation column was newly designed and fabricated in the NIH machine shop. The column consists of a pair of disks (high-density polyethylene, 13.2 cm diameter and 1.5 cm thick), each with a rectangular spiral channel measuring 1.5 mm wide, 0.5 mm (for sample channel) and 2 mm (for ethanol channel) deep, and ca 2 m in length. A dialysis membrane (regenerated cellulose, molecular weight cut off: 6000–8000, Spectrum, Laguna Hills, CA) is sandwiched between these two disks to form two matched spiral channels partitioned by the membrane. The sample channel has a 2 mL capacity, and the ethanol channel has a 7.3 mL capacity. The design of the apparatus was described previously.[1,2,5]

Reagents

Ethanol (anhydrous) was purchased from the Warner–Graham Company (Cockeysville, MD) and HPLC grade water from Fisher Scientific Co. (Fair Lawn, NJ).

Determination of Ethanol Transfer and Osmosis Rates Through the Membrane

The mass-transfer and osmosis rates of ethanol through the membrane were measured according to the following procedure: the anhydrous ethanol was eluted through one channel at a fixed flow-rate of 1 mL/min, while water was eluted through the other channel at various flow-rates ranging from 0.1 to 1 mL/min. No sample was used in this study. After hydrodynamic equilibrium was reached, the relative ethanol concentration from the water channel was determined using the on-line flow-injections (1) the stationary column, by eluting ethanol through the lower channel; (2) the column rotated at 1000 rpm; and (3) the column rotated at 2000 rpm. The ethanol transfer rate through the membrane was illustrated by plotting the ethanol concentration in output of the water channel against input flow-rate in the water channel. The ethanol osmosis rate through the membrane is illustrated by plotting the relative water output rate (%) against input flow-rate in the water channel.

RESULTS AND DISCUSSION

For measuring the mass-transfer and osmosis rates of ethanol through the membrane of the centrifugal precipitation chromatograph, an on-line flow-injection detection system (Fig. 1) was designed using a Waters 2410 refractive index detector. In order to increase the linear range of the calibration curve of ethanol concentration, a 5-μL loop was used for controlling the sample volume of ethanol solution, and a short PTFE tubing ($370 \times 0.85\,mm^2$, I.D.) was used as a mixer. Using the present system, a set of 10 standard ethanol solutions (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, by volume) were analyzed in duplicate. The calibration curve for ethanol concentration was produced by plotting the peak height against the ethanol concentration. This system improves the linearity range of the calibration curve, which now shows an excellent linearity (*y*-intercept: −0.096; slope: 0.0804; correlation coefficient, *r*: 0.998) in a range of 10–80% concentration of ethanol. This calibration curve was used for the quantitative analysis of ethanol during our basic studies on the mass-transfer and osmosis rates of ethanol through the membrane of our centrifugal precipitation chromatograph.

A series of experiments was performed by varying the water input rate from 1 to 0.1 mL/min while the anhydrous ethanol was eluted through the ethanol channel at a fixed flow-rate of 1 mL/min. No sample was used in this study. As shown in Fig. 2(A), the ethanol concentration in the water output (ethanol transfer rate) increases as the water input flow-rate is decreased, and very slightly as the revolution speed is increased. The ethanol concentration in the water output reaches 82 and 88% at 0.1 mL/min of the water input rate at the revolution speed of 1000 and 2000 rpm, respectively.

The osmosis rate through the membrane is similarly illustrated in Fig. 2(B), where the relative water output rate (%) is plotted against the water input rate. Osmosis curves form an inverted shape of the ethanol transfer curves, i.e., the osmosis rate becomes enhanced by the centrifugal force, and at 2000 rpm the water output reduces to 23% at a 0.1 mL/min water input rate. When the water input is further reduced to 0.05 mL/min, no liquid is collected from the outlet of the channel.

The results of our studies clearly demonstrated the successful application of the newly designed on-line flow-injection system, for the basic research on ethanol transfer and osmosis rates through the membrane in centrifugal precipitation chromatography. This injection system gives accurate calibration values of the ethanol concentration without evaporation losses. The same technique should be suitable for the quantitative analysis of other precipitants, such as ammonium sulfate, at a very high concentration.

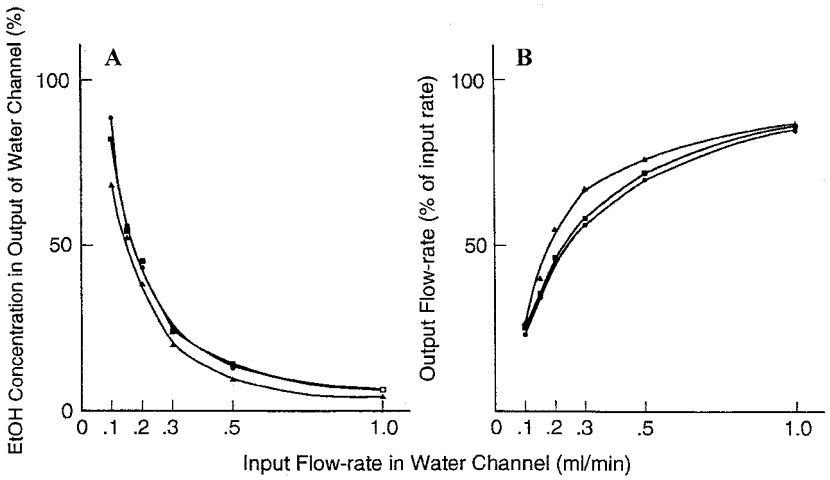

Figure 2. Ethanol transfer and osmosis rates through the dialysis membrane. (A) Ethanol transfer through the membrane measured at various water input rates. Experimental conditions: ethanol channel: ethanol at 1.0 mL/min; water channel: water at various flow rates from 0.1 to 1 mL/min; sample: not charged; revolution speed. 0 rpm (▲), 1000 rpm (■), and 2000 rpm (●).

The application of the present system may be extended to the CCC/MS junction, where a minute amount of eluate ($<0.1\,\mu g$) can be intermittently injected into a mass spectrometer through the conventional HPLC elution system.

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