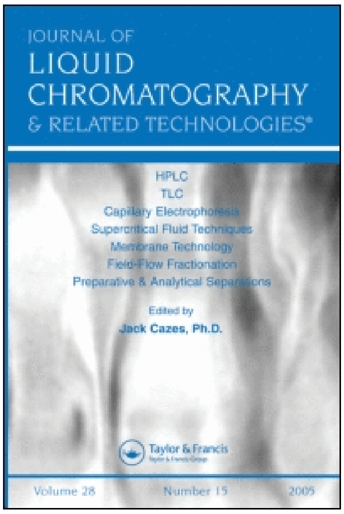

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RETENTION OF STATIONARY PHASE AND PARTITION EFFICIENCY OF MULTILAYER HELICAL COLUMN ROTATED AROUND ITS HORIZONTAL AXIS

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RETENTION OF STATIONARY PHASE AND PARTITION EFFICIENCY OF MULTILAYER HELICAL COLUMN ROTATED AROUND ITS HORIZONTAL AXIS

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ABSTRACT

Using a multilayer helical separation column coaxially mounted on a horizontal rotary shaft, retention of the stationary phase and partition efficiency were studied at various rotation speeds ranging from 20 to 800 rpm. A binary two-phase solvent system composed of chloroform/water was used to elute the lower organic phase through the head of the rotating column. The results indicated that best retention of the stationary phase was obtained at a low rotation speed of 75 rpm yielding the highest peak resolution between caffeine and theophylline. Because of its simplicity and low rotation speed, the system has a potential to be scaled-up for an industrial-scale separation simply by increasing the capacity of the helical column.

INTRODUCTION

It has been reported that high retention of the stationary phase, that is necessary to yield a good peak resolution, is attained in a helical column slowly rotating around its horizontal axis.[1-3] In these previous studies the hydrodynamic distribution of a two-phase solvent system was studied mainly using an end-closed helical column.

In the present study, we conducted a series of experiments to measure the retention of the stationary phase under continuous elution of the mobile phase through a multilayer helical column rotated at various rates ranging from 20 to 800 rpm. The partition efficiency of the system is measured by the peak resolution of caffeine and theophylline at three selected rotation speeds.

EXPERIMENTAL

Apparatus

The present study was performed using a simple apparatus equipped with a horizontal rotary shaft which coaxially holds a multilayer helical separation column. The separation column was prepared by winding a 97m length of 5.5mm ID PTFE (polytetrafluoroethylene) tubing onto a 15cm OD holder hub making 6 layers (26 loops per layer) with a total capacity of 2,300 mL. Each end of the rotary shaft was equipped with a rotary seal to permit continuous elution of the mobile phase through a rotating column. The rotation speed of the column was regulated from 0-900 rpm with a speed controller.

Reagents

Chloroform was purchased from Shanghai Chemical factory, Shanghai, China, and caffeine and theophylline from Sigma Chemical Co., St Louis, MO, USA.

Preparation of the Two-Phase Solvent System and Sample Solution

About equal volumes of chloroform and distilled water were thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated shortly before use. The sample solution was prepared by dissolving caffeine (1g) and theophylline (200 mg) in 50 mL of the lower organic phase.

Determination of Retention Percentage of Stationary Phase

The retention of the stationary phase was determined at various rotation speeds with the following procedure: The column was entirely filled with the aqueous stationary phase (water phase). Then the organic mobile phase (chloroform phase) was pumped into the head end of the column at a desired flow rate while the column was rotated at a given rate. After the mobile phase front emerged and the two solvent phases had established the hydrodynamic equilibrium state, the volume of the stationary phase displaced from the column was measured to compute the % retention relative to the total column capacity.

Determination of Partition Efficiency

The column efficiency was evaluated by separation of caffeine and theophylline using a binary solvent system composed of chloroform and water at three different rotation speeds of 75, 300, and 800 rpm. In each separation, the column was first partially filled with 1,650 mL of the organic mobile phase followed by introduction of 650mL of the aqueous stationary phase. Then the apparatus was rotated at a give rate while the organic mobile phase was pumped into the column at a desired flow-rate. After 3 hours of elution, the sample solution was injected into the column. The effluent from the outlet of the column was collected with a fraction collector and each fraction was analyzed with a UV detector (Model UV-2401PC, Shimadzu Corporation, Kyoto, Japan) to draw a chromatogram.

RESULTS AND DISCUSSION

Effect of Rotation Speed on Stationary Phase Retention

Figure 1 shows % retention of the stationary phase measured at various flow-rates of the mobile phase. The retention curve revealed a peculiar shape which represents a sequence of four different stages of hydrodynamic equilibrium between the two phases, which had been observed in an enclosed rotating helical column.[2,3] The first 3 stages are ruled by an Archimedean screw force of unit gravity while the fourth stage is governed by the centrifugal force induced by column rotation.

Under 50 rpm of the rotation speed (first stage), the two phases establish a basic hydrodynamic equilibrium where each phase is evenly distributed from the head of the helical column while any excess of either phase occupies on the tail side. Under this basic hydrodynamic equilibrium, elution of either phase through the head of the column at a low flow rate permits retention of the other phase as the stationary phase. In the present situation, however, a relatively high flow rate of the mobile phase depleted the amount of the stationary phase

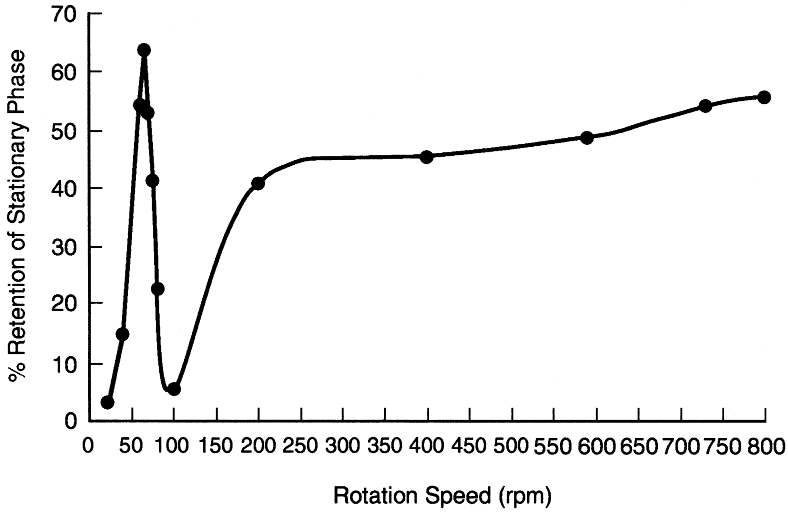

Figure 1. The effect of rotation speed on stationary phase retention of chloroform/water binary system. Experimental conditions: Column: 97m long, 5.5mm ID PTFE tube wound onto a 15cm OD holder hub with a total capacity of 2,300 mL capacity; mobile phase: chloroform phase; flow rate: 3 mL/min, elution mode: head to tail direction.

in the column resulting in poor retention. When the rotation speed is increased (second stage), the above hydrodynamic equilibrium is gradually altered in such a way that the water phase occupies more space at the head side, and at the critical rate of around 75 rpm, the head of the column is entirely occupied by the water phase.[3] Under this unilateral phase distribution, elution of the chloroform phase from the head through the tail produces excellent retention of the stationary phase even at a high flow rate, thus forming a peak in the diagram (Figure 1). When the rotation speed is further increased to 100 rpm (third stage), the above hydrodynamic equilibrium is suddenly reversed so that the chloroform phase tends to occupy more space on the head side. Consequently, the elution with the water phase from the head end results in a detrimental loss of the stationary phase from the column. As the rotation speed is further increased (fourth stage), this third stage of hydrodynamic equilibrium is gradually altered by an increased centrifugal force which distributes the two phases in such a way that the chloroform phase occupies the outer portion and the water phase the inner portion throughout the helical column. Under this hydrodynamic equilibrium, elution with either phase in either direction produces retention of the stationary phase at around 50% of the total column capacity as indicated in the diagram. In this fourth stage of the hydrodynamic distribution, the two phases form a strip of continuous interface along the helical path, the area of which is much smaller than that in the earlier stages where multiple droplets are created by an Archimedean screw effect. Consequently, the partition efficiency, which highly depends on the area of interface for mass transfer, would decrease as the rotational speed is increased. Overall results of the retention studies closely correlate with those of hydrodynamic studies using a chloroform/water binary system in an end-closed helical column.[3]

Here, it is important to note that the chloroform/water binary system used in the present study is a unique solvent pair, since all other solvent systems (including chloroform/acetic acid/water at a 2:2;1 volume ratio) distribute the

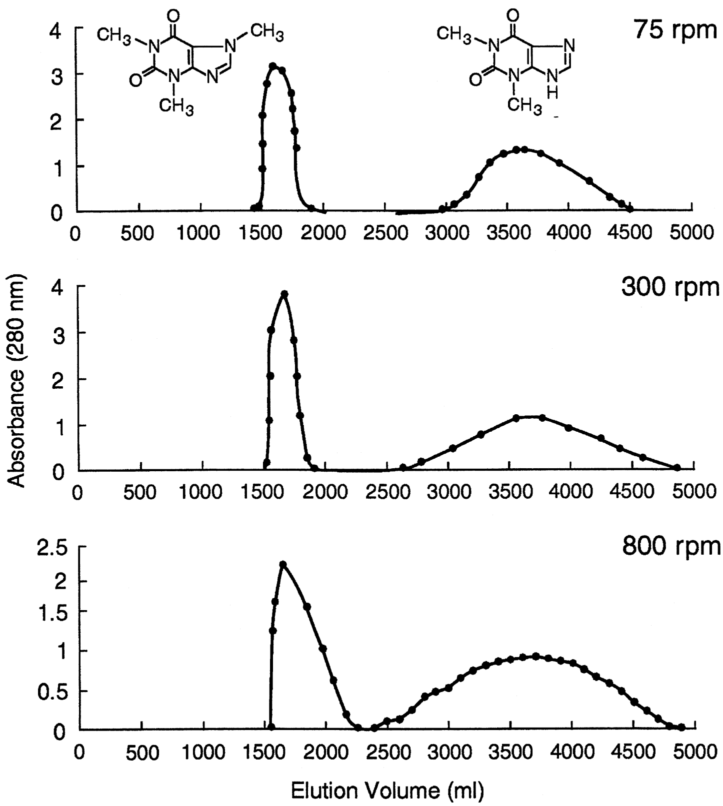

Figure 2. Separation of caffeine and theophylline by a multilayer helical column rotated at three different speeds. Experimental conditions: sample: caffeine (1 g) and theophylline (200 mg) in 50 mL of chloroform phase; stationary phase: 650 mL of chloroform phase; mobile phase: chloroform phase; flow rate: 4 mL/min; elution mode: head to tail direction.

heavier phase toward the head at the second hydrodynamic equilibrium stage so that they form completely inverted phase distribution curves.[3] This exceptional behavior of chloroform/water binary system may be due to a large difference in density between the two phases (0.5 g/mL) associated with its high interfacial tension. The mechanism involved in the two-phase distribution in a rotating helical tube is discussed in detail elsewhere.[4] Accordingly, for the application of other two-phase solvent systems the separation should be performed by either eluting the lighter phase from the head toward the tail or the heavier phase in the opposite direction at the second stage of hydrodynamic equilibrium.

Effect of Rotation Speed on Partition Efficiency

The partition efficiency of the present system was assessed by separation of caffeine and theophylline at three different rotation speeds of 75 rpm (second stage), 300 rpm (early fourth stage), and 800 rpm (late fourth stage). Figure 2 shows three chromatograms obtained from a mixture of caffeine (1 g) and theophylline (200 mg) at three different rotation speeds as indicated in the diagram. The results clearly indicate that the rotation at 75 rpm produced the best peak resolution. As discussed earlier, higher rotation speed produced broad peaks mainly due to a lack of effective interface area for mass transfer.

The present studies indicate that slow rotation of the helical column produces the best results in both the stationary phase retention and peak resolution measured by caffeine and theophylline as test samples. Because of its simplicity and slow motion of the column, the present device has a high potential of scaling-up for industrial separation by increasing the column capacity.

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