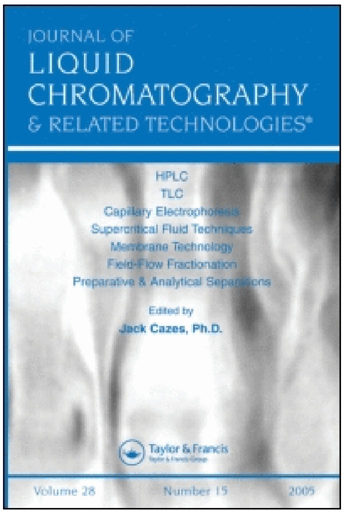

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Practical Approach to the Creation of a Concentration Gradient in a Stationary Phase Retained in a Rotating Coiled Column

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Abstract: Countercurrent chromatography (CCC) works with a liquid stationary phase. It is proposed to create a concentration gradient in the stationary phase during the chromatographic run. The gradient concentration is initiated putting a known volume of neat stationary phase at the head of the CCC column and a known volume of dithizone (a test die) concentrated stationary phase at the end of the CCC column. Water-organic solvent biphasic liquid systems were tested. The aqueous phase is the mobile phase. Hexane, decane, chloroform, methylisobutyl ketone, tetrachloromethane, or ethyl acetate were used as the dithizone containing stationary phase. It was found that the concentration gradient created inside the column is dependent on the operational hydrodynamic parameters and physicochemical properties of two-phase liquid systems used. The water-organic solvent liquid systems were divided

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into two types: 1. “Nimble” (easily mixed) systems that readily form concentration gradients, which can be completely leveled with time; 2. “Reluctant” (difficult to mix) systems that do not form a gradient.

Keywords: Please supply

INTRODUCTION

Countercurrent chromatography (CCC) is currently attracting great interest from investigators working on the separation and preconcentration of organic and inorganic substances. CCC is based on the retention of either phase (stationary) of a two-phase liquid system in a rotating coiled column (RCC) under the action of centrifugal forces, while the other liquid phase (mobile) is being continuously pumped through.[1]

The stationary phase volume retained in a column is characterised by the S_f -factor (ratio of the stationary phase volume, V_s, to the total column volume V_c). The value of S_f is dependent on the parameters of the planetary design centrifuge (rotation and revolution radii, tube diameter), on the operation conditions (rotation and revolution speeds, flow rate, and direction of pumping of the mobile phase), and on the physicochemical properties of the two-phase liquid system used. It should be mentioned that the influence of planetary centrifuge parameters and operation conditions on the stationary phase retention are well studied for simple two-phase liquid systems consisting of water and one or two organic solvents.[1–11] The stationary phase retention is known to increase with increasing the rotation speed and with decreasing the mobile phase flow rate. In the last 10 years, the influence of physico-chemical properties of two-phase liquid systems on the stationary phase retention is under study by different authors.[2,12–20] Parameters such as polarity of solvents, density, and viscosity differences of two phases, settling time of liquid system, and capillary wavelength on the interfacial surface have been considered.

The chromatographic behaviour of inorganic compounds, as well as organic compounds is dependent on the properties of the system used, partition coefficients of substances to be separated, and parameters of the planetary centrifuge operation such as rotation and revolution speeds, direction and speed of the mobile phase pumping, internal diameter of the column, sample volume. However, the systems for inorganic separations are very different from those for organic ones,[16,17,21,22] as in most cases they contain a complexing (extracting) reagent (ligand) in the organic phase and mineral salts and/or acids in the aqueous phase.

Gradient elution (e.g., pH gradient) is often used to improve the effectiveness of the separation of substances with close distribution ratios. This approach can be successfully employed, both in HPLC and CCC.[1,22] Variation in values of partition coefficients is achieved in gradient or step elution mode by changing the mobile phase (eluent) composition. It is known that the partition coefficients in liquid-liquid extraction and in partition chromatography may depend strongly on the extracting reagent concentration in the stationary phase. Therefore, the creation of a concentration gradient particulary in the stationary phase seems to be very attractive, especially for CCC as a support-free liquid-liquid partition chromatography. The CCC columns have a high preparative capacity provided by a high ratio (up to 0.9) of the stationary phase volume to the total column volume. In CCC, the availability of the stationary phase for separated components is determined by diffusion processes in liquids and efficiency of phase mixing (governed by design parameters of columns, operational hydrodynamic conditions, and physicochemical properties of a two-phase liquid system). That is why the modeling and numerical description of liquid behavior in rotating coiled columns is of special importance. It should be emphasized that despite a number of publications dealing with the simulation of stationary phase retention in RCC,[23–26] gradient systems have still not been analyzed.

The aim of the present work is to investigate the hydrodynamic mixing process in RCC as a function of different factors: column rotation speed (ω), mobile phase pumping rate (F), and composition of a two-phase liquid system. For the first time, the creation of a concentration gradient and the formation of separate areas in the stationary phase (organic or aqueous) are considered.

EXPERIMENTAL

Apparatus

Investigations were made on a planetary centrifuge of J-type (Ito's classification[1]) with a vertical column drum fabricated in the Institute of Analytical Instrumentation (St. Petersburg). The column of the device rotates around its axis and revolves at the same angular velocity around the central axis of the device. The planetary centrifuge model had the following design parameters: revolution radius $R = 100\,\text{mm}$, rotation radius $r = 36\,\text{mm}$. The column was made of a PTFE tube with an inner diameter of 1.5 mm and a wall thickness 0.75 mm. The total inner capacity of the column, V_c, was 14.5 mL. The rotation speed was varied from 600 to 900 rpm while the mobile phase flow rate F was varied to 0.5 to 2.0 mL/min.

A glass capillary viscometer was used for measurements of viscosities of the solutions. A tensiometer (Lauda, Germany) with a thermostatic cell was used for measurements of interfacial tension of the systems under investigation by the ring method. The wetting angles between the column material (PTFE) and liquids were measured directly in the column tube by the method of capillary lifting.

Reagents

All solvents (n-hexane, n-decane, chloroform, methylisobutyl ketone, tetrachloromethane, ethyl acetate) and reagents (dithizone, hydrochloric acid, ammonium chloride) were analytical grade chemicals.

Procedure

The following liquid-liquid systems were investigated: organic solvent–ammonium chloride aqueous solution (0.02 mol/L); dithizone (0.01–0.02 mol/L) in an organic solvent–ammonium chloride aqueous solution (0.02 mol/L). n-Hexane, n-decane, chloroform, methylisobutyl ketone, tetrachloromethane, ethyl acetate were used as stationary phase organic solvents. The aqueous phase was always the mobile phase. Some physicochemical characteristics of systems under study are given in Table 1. It has been preliminary estimated that all the liquid systems are characterized by the retention factor, S_f, greater than 0.54 in the ranges of rotation speed 600–900 rpm and flow rate 0.5–2.0 mL/min. In all the experiments the fixed volume of the stationary phase equal to 7.2 mL (corresponding to the retention factor $S_f \sim 0.5$) was used in order to prevent an occasional elution of the stationary phase. The stationary phase was introduced into the motionless column as two areas (3.6 mL each) separated by 4.0 mL of the mobile phase. The first area (zone) of an organic solvent contained dithizone, the second one contained no dye. The "closed" column was rotated during 2 min without flow, after which the flow of the mobile phase was switched on. After a certain time (10–60 min) the rotation was stopped and the content on the column was removed with the flow rate 1.0 mL/min. Fractions of 2 mL were collected. The dye concentration was determined by spectrophotometry ($\lambda = 400$ nm).

RESULTS AND DISCUSSION

Creation of a Concentration Gradient in the Stationary Phase

The curves for the dithizone distribution between two initially separated areas of the upper stationary phase (n-hexane) are shown in Figure 1 (the stationary phase areas were initially separated by 4 mL of aqueous mobile phase moving H → T). As can be seen, varying contact times of stationary and mobile phases and operational hydrodynamic parameters results in different patterns of dithizone concentration in the collected fractions. If the column rotation speed and flow rate are low, a stable concentration gradient can be created and maintained for a relatively long time. On the other hand, if the column rotation speed and flow rate are high (900 rpm and 1.5 mL/min,

Table 1. Some physicochemical characteristics of the water (0.02 M ammonium chloride)-organic solvent liquid systems under study

Organic solvent	$\Delta\rho$, g/cm^3 density	$\Delta\eta$, $\times 10^{-3}$ Pa*s viscosity	γ, $\times 10^{-3}$ N/m Interf. tension	Wetting angles on PTFE, (θ, degree)		Work of adhesion between a liquid and PTFE, (W_A, $\times 10^{-3}$ N/m)		Selective wetting, ($\sigma_{12}\theta_{12}$, $\times 10^{-3}$ N/m)
				Aq. phase	Org. phase	Aq. phase	Org. phase	
n-Hexane	−0.32	0.58	17.2	104 ± 5	43 ± 3	37.4	38.2	−28.4 ± 1.6
Ethyl acetate	−0.10	0.45	13.4	91 ± 3	52 ± 2	31.3	37.4	−11.4 ± 0.8
Methylisobutyl ketone	−0.19	0.37	10.1	92 ± 3	56 ± 2	32.3	38.9	−13.0 ± 0.3
n-Decane	−0.27	0.07	39.8	109 ± 5	48 ± 2	37.8	38.6	−33.8 ± 0.3
Chloroform	+0.48	0.34	19	119 ± 1	54 ± 3	34.2	39.1	−30.1 ± 1.8
Tetrachloro methane	+0.58	0.02	22	124 ± 1	40 ± 1	25.5	43.0	−46.5 ± 7.4

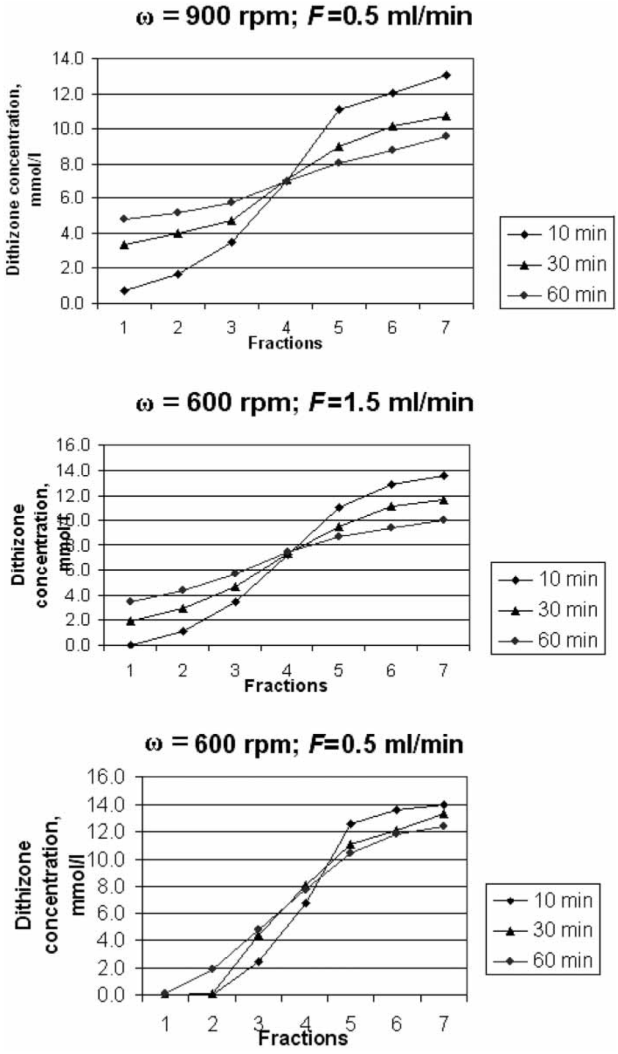

Figure 1. Dithizone distribution between two initially separated areas of the upper stationary phase (*n*-hexane) as a function of contact time at different rotation speeds and flow rates in the H → T direction.

respectively) the two initially separated stationary phase areas can be completely mixed within one hour. The liquid systems on the basis of n-decane, methylisobutyl ketone, ethyl acetate (all these solvents are lighter than water, see Table 1) have similar hydrodynamic behavior characterized by an uneven distribution of the stationary phase inside the column. The separated areas begin to get mixed within the first minutes of the experiment.

The behavior of the liquid systems on the basis of chloroform or tetrachloromethane (these solvents are heavier that water, aqueous mobile phase moving T → H) enables the stable gradient concentration in the stationary phase to be achieved even if the column rotation speed is high (Figure 2). The results are practically insensitive to the experimental flow rate and rotation speed conditions. This phenomenon may be explained by a considerable contribution from the work of adhesion forces to the retention process. The strong adhesion forces of the organic phase to the column material can provide an even distribution of the stationary phase along the whole column.

According to the data obtained, for organic solvents under study, the effectiveness of the mixing of two initially separated areas increase in the following rank order: lower phase solvents: tetrachloromethane, chloroform, then upper phase solvents in the order: *n*-decane, methylisobutyl ketone, ethyl acetate, *n*-hexane. This sequence and the values of wetting angles (Table 1) is somewhat correlated. It is possible to assume that wetting phenomena play an important role in the mixing of the stationary phase in the rotating column.

Hence, the water-organic solvent liquid systems can be divided into two types depending on their behavior in the coiled column at different hydrodynamic parameters: 1. "Nimble," easily mixed systems, readily form concentration gradients, which can be completely leveled in time. 2. "Reluctant," difficult-to-mix systems do not form a gradient; originally insulated areas of the stationary phase are practically not mixed in the column with time.

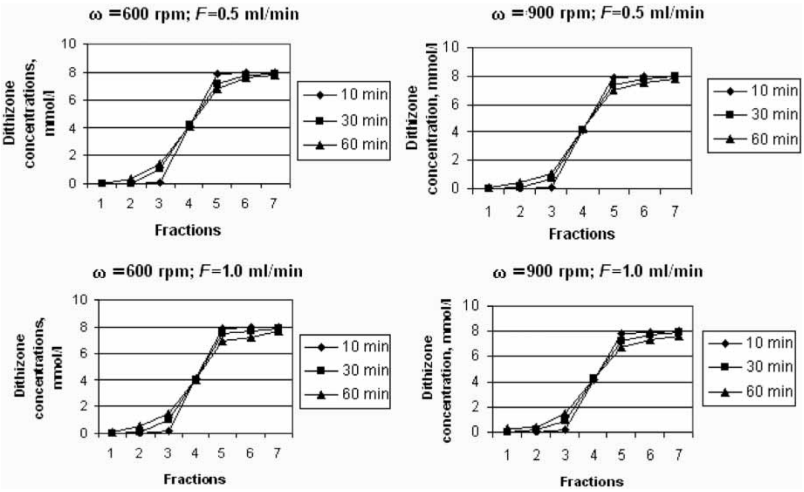

Figure 2. Dithizone distribution between two initially separated areas of the lower stationary phase (chloroform) as a function of contact time at different rotation speeds and flow rates in the T → H direction.

In general, the results obtained give the necessary prerequisites for a new approach to the control of stationary phase composition inside columns during a chromatographic run, and show the possibility of making a "chemical reactor" on the basis of one rotating coiled column with insulated areas of stationary phases containing different extracting reagents.

Work on the numerical simulation of the mixing process at the interface between different liquid phases under "stop-flow" and "continuous-flow" conditions are currently in progress.

CONCLUSIONS

The present work gives the necessary prerequisites for a new approach to the control of stationary phase composition inside columns during the chromatographic run, and shows the principal possibility of making a "chemical reactor" on the basis of one rotating coiled column with insulated areas of stationary phases containing different extracting reagents.

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