

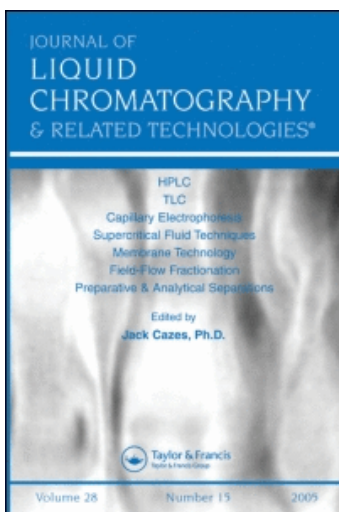
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A NEW APPROACH TO DESCRIBING THE REGULARITIES OF STATIONARY PHASE RETENTION IN COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Three-dimensional surfaces, based on a large set of experimental data, have been built to investigate and describe the interrelations between different physico-chemical properties of two-phase liquid systems, the operating conditions of the planetary centrifuge, and the stationary phase retention. The wide range of alteration of interfacial tension, density, and viscosity differences between two phases has been provided by changing the composition of liquid systems containing an organic solvent, an extracting reagent, water and a mineral salt, the organic solvents, and extracting reagents of different nature being considered. A great body of data presents a possibility of estimating the contributions from all the enumerated physico-chemical properties of liquid systems to the stationary phase retention.

It has been shown that the density difference between two phases ("the moving force" of the retention process) plays a dominant role. The range of density difference from 0.20 to 0.45 g/cm³ has been found to be favorable for stationary phase retention. It has been demonstrated that low values of interfacial tension and viscosity difference (less than 3 dyn/cm and 0.3 cP, correspondingly) have adverse effects on the stationary phase retention. The regularities evaluated enable the optimal operating conditions needed for the satisfactory retention of a certain liquid system to be chosen and offer additional insights into the hydrodynamic behavior of two immiscible liquids in a rotating coiled column.

INTRODUCTION

Countercurrent chromatography (CCC), a support-free partition chromatography, is a very promising technique for the separation and preconcentration of organic and inorganic substances. CCC is based on the retention of one phase (stationary) of a two-phase liquid system in a rotating column under the action of centrifugal forces, while the other liquid phase (mobile) is being continuously pumped through.¹ An important distinguishing feature of CCC as a chromatographic method is the absence of an adsorptive matrix for retaining the stationary phase. This feature determines the main advantages of the method, such as the absence of solute loss due to interaction with the sorbent matrix, a variety of two-phase liquid systems which may be used, easy change from one partition system to another, the possibility to change the volume of the sample solution from 0.1 to 1000 mL or more. It should be noted that the problem of column packing is also eliminated in CCC and the stationary phase is relatively inexpensive.

A few devices providing retention of the stationary phase in the field of mass forces, in the absence of a solid support, have been suggested. Among the various possible designs, the planetary centrifuge retains the liquid stationary phase effectively and enables the fastest and most efficient separation to be achieved. A column (or a column unit) of a certain configuration rotates around its axis and simultaneously revolves around the central axis of the device with the aid of a planetary gear.

An important factor that determines the separation efficiency, peak resolution, and preparation capacity for both organic and inorganic compounds is the S_f value, which is the ratio of the volume of the stationary liquid phase retained in a column (V_s) to the total column volume (V_c). In some cases, S_f ratio in CCC amounts up to 0.9; hence, this ratio can be much higher than that for the stationary solid phase used in HPLC.

For about 25 years, CCC has been successfully applied to the separation of various substances. However, regularities and the mechanism of the stationary phase retention in a rotating coil have not been adequately studied, though the investigations in this area are numerous. The influence of planetary centrifuge parameters and operation conditions on the stationary phase retention are well described for some simple two-phase liquid systems consisting of water and one or two organic solvents.² The stationary phase retention is known to increase with increasing the rotation speed and with decreasing the mobile phase flow rate.

The influence of physico-chemical properties of two-phase liquid systems on the stationary phase retention was also studied.³⁻⁷ Parameters such as polarity of solvents, density and viscosity differences of two phases, settling time of liquid system, and length of the capillary wave on the interfacial surface have been considered. In particular, it has been reported that the value of S_f increases linearly with an increase of the density difference between two phases ($\Delta\rho$).^{3,4}

According to the physico-chemical properties, liquid systems can be divided into three groups (Ito's classification¹): hydrophobic (high values of interfacial tension γ , low values of viscosities η), hydrophilic (low values of γ , high values of η), and intermediate systems, which behave in a rotating coil like hydro-phobic systems if β (ratio of rotation and revolution radii) is higher than 0.3 and behave like hydrophilic systems if $\beta < 0.3$.

Ito's classification is frequently used for choosing some operational conditions (directions and speeds of the column rotation and the mobile phase pumping) needed for the satisfactory retention of the stationary phase. For hydrophobic liquid systems, a hypothesis of the mechanism of stationary phase retention in a rotating coiled column has been proposed.⁸

In a previous publication⁹ we tried to correlate the composition and physico-chemical properties of some two-phase liquid systems used for CCC inorganic separations¹⁰ with their S_f factors. Complex liquid systems containing an organic solvent (n-decane or chloroform), an extracting reagent (di-2-ethylhexylphosphoric acid, D2EHPA), water, and a mineral salt (ammonium sulfate) have been considered.

It has been shown that the extractant additions in the organic phase and the salt additions in the aqueous phase lead to a change of the physico-chemical properties (viscosity, density, interfacial tension) of the liquid system and affect the stationary organic phase retention.

The results obtained may be important for both inorganic and organic applications of CCC because similar liquid systems, on the basis of one or sev-

eral organic solvents and aqueous mineral salt solutions, can be used for organic separations.

We have earlier studied the influence of each physico-chemical parameter (interfacial tension γ , density difference between two liquid phases $\Delta\rho$, or viscosity of the organic stationary phase η_{org}) on the stationary phase retention.¹¹ Two-phase liquid systems containing an organic solvent (n-decane), an extracting reagent (D2EHPA), water, a surfactant (sodium salt of dodecylbenzenesulphonic acid), and a mineral salt (ammonium sulphate) were chosen. Those systems provided the possibility of selectively alteration of certain parameters (γ , $\Delta\rho$ or η_{org}), all other conditions being the same.

The results obtained were in good agreement with the theoretical model proposed.⁸ However, the range of alteration of physico-chemical parameters was limited, and the number of data were not quite sufficient for their proper and full interpretation.

In the present work, we have investigated a rich variety of two-phase liquid systems on the basis of different organic solvents, extracting reagents, water, and mineral salts.

A great body of data has been used to construct three-dimensional surfaces that enable the interplay between several parameters to be conveniently demonstrated. In such a manner, physico-chemical properties of two-phase liquid systems, the operating conditions of the planetary centrifuge, and the stationary phase retention have been correlated.

EXPERIMENTAL

Apparatus

Investigations of the stationary phase retention were performed with a device consisting of a self-designed planetary centrifuge with a vertical one-layer coiled column drum (type J according to Ito's classification of planetary motion¹) and a peristaltic pump. The column 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 $R = 85$ mm, rotation radius $r = 35$ mm.

The column was made of a Teflon tube with an inner diameter of 1.5 mm and a wall thickness of 0.75 mm. The total inner capacity of the column V_c was

17 mL. The rotational speed was varied from 350 to 500 rpm. The mobile phase flow rate F was equal to 1.0 mL/min.

The densities were estimated simply by weighing a known volume of the liquid phase. A glass capillary viscometer was used for measurements of viscosities of the solutions.

A tensiometer (Lauda, Germany), with a thermostated cell, was used for measurements of interfacial tension of the systems under investigation by the ring method.

Reagents

All chemicals used were analytical grade reagents.

Procedure

Before the experiment on the stationary phase retention was begun, the components of the two phase liquid system were stirred and brought into equilibrium for mutual saturation of the phases; the two phases were separated shortly before use. The aqueous phase was used as the mobile phase and the organic as the stationary phase. First, the coil column, in the stationary mode, was filled with the organic phase. After that, while column rotation was started, the aqueous phase was fed into its inlet. The mass force field, which arose during rotation, made it possible to retain a constant volume of the stationary phase V_c while the mobile phase was continuously pumped through.

After equilibrium between the mobile and stationary phases had been established (about 30 min), both phases were removed from the column and their volumes were measured. The mobile phase pumping direction was from head to tail and from tail to head¹ for the upper and lower stationary phase retention, respectively.

The procedures for the measurements of physico-chemical parameters of the systems under investigation have been described in detail earlier.⁹

Construction of Three-Dimensional Surfaces

As had been expected, the obtained experimental data appeared to be almost completely unsorted, so it was necessary to rearrange them in order to build the three-dimensional surfaces. It is worth noting that the data had to be sorted for each surface separately, depending on which parameters were considered. As will be seen from the figures below, the stationary phase retention

S_f has always been taken as a function of two different parameters, one of which was singled out along the abscissa (X-axis) and another along the ordinate (Y-axis). In order to receive a quasi-regular two-dimensional grid in the argument space, the following procedure has been used.

First, the data were sorted towards the X-axis and then, for each value of X, the corresponding data were sorted towards the Y-axis. When the quasi-regular two-dimensional grid has been obtained, it is possible to build a three-dimensional surface using the known function values in the grid points. There are many different ways of function interpolation between the nodes. We used, perhaps, the most natural linear interpolation as can be seen in the figures.

RESULTS AND DISCUSSION

Choosing the Compositions of Two-Phase Liquid Systems

As we have mentioned above, a rich variety of two-phase liquid systems, on the basis of different organic solvents, extracting reagents, water, and mineral salts was under investigation. n-Hexane, n-decane, chloroform, carbon tetrachloride, and methylisobutylketone were used as organic solvents, offering different physico-chemical properties, the density (ρ) and viscosity (η) being varied from 0.68 to 1.59 g/cm³ and from 0.36 to 0.98 cP, respectively.

The interfacial tension γ for the organic solvent-water systems are in the range of 9-45 dyn/cm. The composition and, consequently, the physico-chemical properties of the organic phase were modified by adding the following extracting reagents of different nature: di-2-ethylhexylphosphoric acid, tributylphosphate (TBP), and trioctylamine (TOA).

Ammonium sulfate, ammonium chloride, and ammonium nitrate were used to modify the composition and properties of the aqueous phase. The concentrations of the extracting reagent in the organic phase and of the mineral salt in the aqueous phase amounted up to 30 and 15%, respectively. To extend the range of alteration of physico-chemical parameters the 1-butanol-water system was also investigated.

In general, more than 60 two-phase liquid systems were under study. Their physico-chemical properties vary in the following ranges. The density difference between two phases ($\Delta\rho$): from 0.14 to 0.59 g/cm³; the viscosity difference between two phases ($\Delta\eta$): from 0 to 0.9 cP; the interfacial tension (γ): from 1.5 to 45 dyn/cm.

According to Ito's classification,¹ all these systems are hydrophobic or intermediate. There are precisely two types of liquid systems, which are used for the major inorganic and organic separations by CCC.

Hence, the study on the regularities of their hydrodynamic behavior in a rotating coiled column is important with respect to the theory and practice. It should be noted that, under the given experimental conditions ($\beta > 0.3$) intermediate liquid systems behave like hydrophobic ones.

Effect of the Density Difference Between Two Liquid Phases

The density difference of two liquid phases is known^{1,8} to be the main “moving force” of the process of stationary phase retention in CCC. The interrelation between $\Delta\rho$, the rotation speed (ω), and the stationary phase retention (S_f) for systems under investigation is presented in Fig. 1. As can be seen, if the rotation speed is low (350 rpm), the $\Delta\rho$ -dependence of S_f is complicated. In this case, the influence of the density difference may be accompanied by the effect of other physico-chemical parameters. If the rotational speed increases up to 450-500 rpm, the surface becomes relatively smooth. Hence, in this area, the effect of the density difference can be predominant.

In the $\Delta\rho$ -range from 0.14 to 0.20 g/cm³ the stationary phase retention increases drastically, and with increasing the $\Delta\rho$ up to 0.40 g/cm³ the smooth increase in S_f is observed. If the value of $\Delta\rho$ is higher than 0.40 g/cm³, the stationary phase retention begins to decrease. This phenomenon can be explained by the high density of the stationary phase (1.5 g/cm³ or more) in systems on the basis of chloroform and carbon tetrachloride; these are, namely, liquid systems which provide high density differences between two phases.

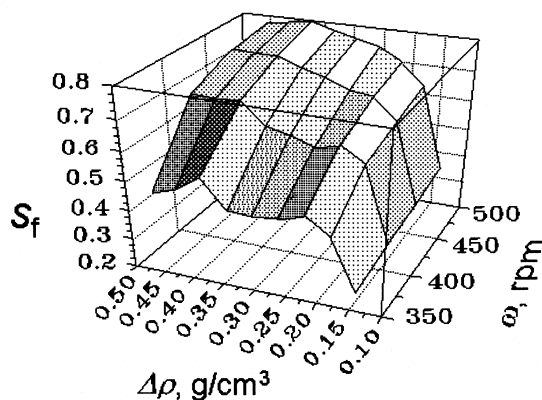


Figure 1. Interrelation between density difference of two phases, rotation speed of the device, and the stationary phase retention.

Generally, the retention of the stationary phase of a higher density can require a higher ω providing a sufficiently strong force field.⁹

Thus, under the given conditions the $\Delta\rho$ -range from 0.20 to 0.45 g/cm³ and the rotation speed equal to 450 rpm can be optimal for the stationary phase retention and CCC separations. The higher rotation speed (500 rpm) also provides high values of S_f . However, the high rotation speed can lead to the formation of films and difficultly separated emulsions on the interfacial surface. These phenomena adversely affect the process of chromatographic separation, especially its kinetics. It is of particular importance for the separation of inorganic species in systems containing complexing reagents.¹² So far, the lowest rotation speed providing stable retention of the appropriate volume of the stationary phase in the column has been considered as optimal.

It is interesting to note that single-layer and multi-layer columns with similar design parameters require different rotational speeds to attain the same retention factor.^{9,11} In the case of the multi-layer column, a higher rotation speed (700-750 rpm, instead of 450 rpm) is needed. This phenomenon has not been fundamentally investigated. From the practical point of view, if a large column volume is not necessary for certain separations, it is worthwhile to use a single-layer column.

Effect of the Viscosity Difference Between Two Liquid Phases

The interrelation between $\Delta\eta$, rotation speed, and the stationary phase retention for systems under study is presented in Fig. 2. If the rotation speed is

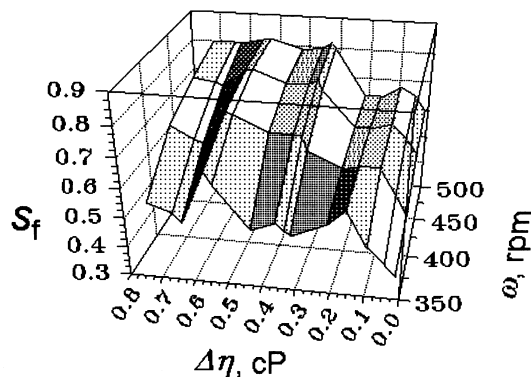


Figure 2. Interrelation between viscosity difference of two phases, rotation speed of the device, and the stationary phase retention.

low (350-400 rpm), it is difficult to estimate the influence of the density difference on the stationary phase retention. The observed waves on the surface may appear due to the contribution of other physico-chemical parameters. If the rotation speed increases up to 450-500 rpm, S_f has a tendency to increase with increasing $\Delta\eta$, the stationary phase being well retained in the column ($S_f = 0.7-0.8$) in the range of the viscosity difference from 0.3 to 0.8 cP. Hence, like the density difference, the viscosity difference between two phases might be the “moving force” of the retention process.

The observed trends are consistent with the data obtained for three liquid systems offering different $\Delta\eta$, the interfacial tension and density difference remaining practically the same.¹¹ Thus, the regularity evaluated should be taken into consideration when liquid systems and optimal operation conditions are needed to be chosen.

Effect of the Interfacial Tension

The interrelation between γ , rotation speed, and the stationary phase retention for systems under investigation is shown in Fig. 3. The observed surface is rather complicated. At first glance, it seems to be difficult to isolate the contribution of the interfacial tension to the retention process. However, the tendencies observed are not contradictory to the γ -dependencies of S_f obtained for systems with constant values of density and viscosity differences.¹¹ It is seen that, for all the values of rotation speed, S_f sharply increases with increasing the

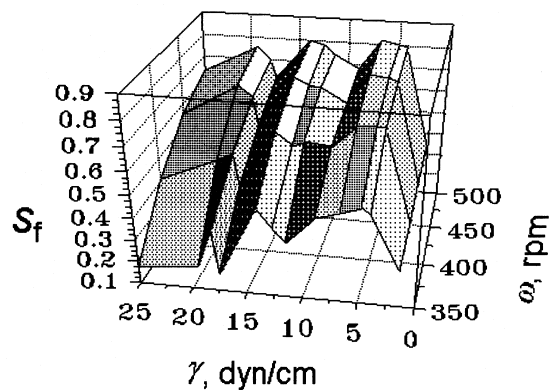


Figure 3. Interrelation between interfacial tension, rotation speed of the device, and the stationary phase retention.

interfacial tension from 1.5 to 3 dyne/cm. Then, the dependence becomes wave-like. These fluctuations can occur, due to the influence of other physico-chemical parameters. If the interfacial tension is higher than 20 dyn/cm, S_f begins to decrease. The moderate values of the interfacial tension may be most favorable for the retention of the stationary phase. The S_f values are in the range from 0.6 to 0.8, when γ varies from 3 to 20 dyne/cm, the rotation speed being equal to 450-500 rpm.

It should be noted that, if the interfacial tension is low (less than 3 dyne/cm), this physico-chemical parameter may be predominant. In this case, it has an adverse effect on the stationary phase retention.

Effect of the Capillary Wavelength

The dynamics of the macroscopic behavior of the interface between two immiscible liquids in a rotating coiled column has been reported to be governed by the evolution of waves.⁷ The occurrence of capillary instabilities and its signature were identified with the length of the capillary wave on the interfacial surface (λ_{cap} , cm). The following expression was proposed for estimating this value:

$$\lambda_{\text{cap}} = 2\pi [\gamma / (\Delta\rho \times 981 \times 100)]^{1/2} \quad (1)$$

The values of the capillary wavelength were utilized for classification purposes and applied to various solvent systems.⁷ This parameter is of great interest for our study because it allows us to combine into one quantity two physico-chemical parameters: the density difference and the interfacial tension. The interrelation between λ_{cap} , rotation speed, and the stationary phase retention for systems under investigation is shown in Fig. 4. It is seen that the dependence is undulated. Nevertheless, if the rotation speed amounts up to 450-500 rpm, the general tendency becomes evident. The higher the capillary wavelength, the lower is the value of S_f . Thus, the short capillary waves on the interface surface may favor stationary phase retention.

We have also tried to correlate the capillary wavelength (integrating $\Delta\rho$ and γ), the viscosity difference of two phases, and the stationary phase retention (Fig. 5). All the investigated physico-chemical parameters and the value of S_f have been integrated using one three-dimensional surface.

The correlation obtained is of theoretical interest. Apparently, the fluctuations observed on the dependencies in Figs. 2 and 4 result in a more complicated three-dimensional surface. However, it is difficult to draw some far-reaching conclusions from the resulting dependence because, in this case, the stationary phase retention varies within a narrow range. The matter is that, for

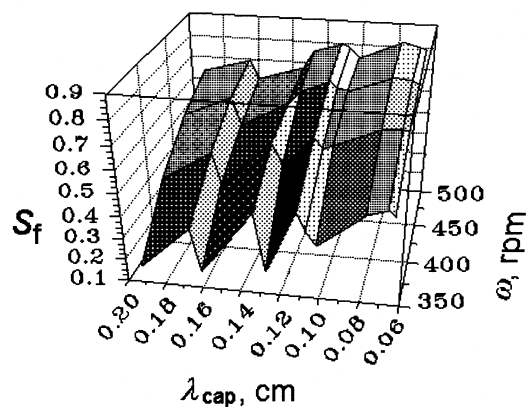


Figure 4. Interrelation between length of the capillary wave on the interfacial surface, rotation speed of the device, and the stationary phase retention.

the construction of a three-dimensional surface, a more or less regular two-dimensional grid is needed. We did not have enough data on the capillary wavelengths and viscosity differences to build a greater quasi-regular two-dimensional grid in the argument plane. Hence, the experimental investigation should be continued to estimate the general guideline for choosing the physico-chemical parameters favoring the stationary phase retention.

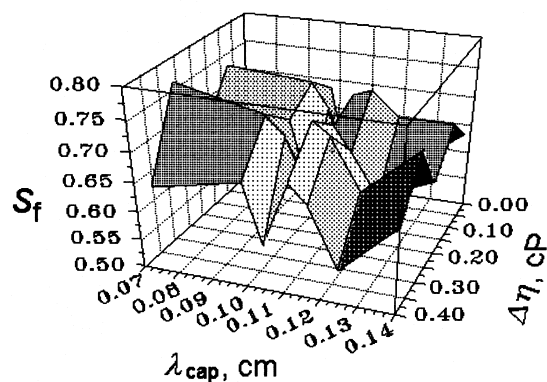


Figure 5. Interplay between length of the capillary wave on the interfacial surface, viscosity difference of two phases, and the stationary phase retention. $\omega = 450$ rpm.

CONCLUSIONS

A large set of experimental data has been theoretically handled. The criteria have been evaluated for choosing the appropriate combination of physico-chemical parameters of liquid systems and operation conditions of the planetary centrifuge needed for the satisfactory retention of the stationary phase in a single-layer rotating coiled column. In general, the following ranges of parameters under investigation can be optimal: $\Delta\rho$ from 0.20 to 0.45 g/cm³; $\Delta\eta$ from 0.3 to 0.8 cP; $\gamma > 3$ dyn/cm; $\omega = 450$ rpm.

It has also been shown that the higher the length of the capillary wave on the interfacial surface (capillary wavelength), the lower is the value of retention factor S_r . It should be noted that if a multi-layer coiled column with similar design parameters is used, a higher rotation speed (700-750 rpm) will be required. Therefore, if a large column volume is not necessary for certain separations, it is often rational to use a single-layer column.

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