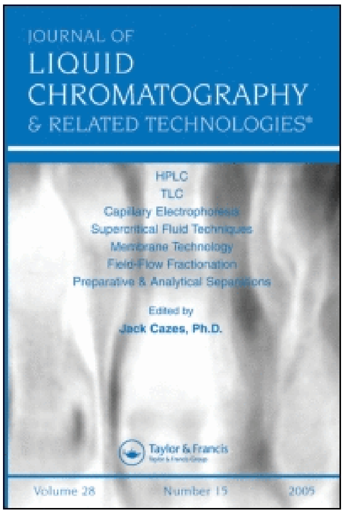

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STUDIES OF LIQUID-LIQUID CIRCULATION

EFFECT OF PHYSICOCHEMICAL PROPERTIES OF TWO-PHASE LIQUID SYSTEMS ON THE RETENTION OF STATIONARY PHASE IN A CCC COLUMN

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ABSTRACT

The effect of physicochemical properties of two-phase liquid systems (interfacial tension and differences in the densities and viscosities of the two phases) on the retention of the stationary phase in a rotating coil column was studied, taking as examples complex liquid systems containing an organic solvent (*n*-decane, *n*-hexane, chloroform, carbon tetrachloride and methylisobutylketone), an extracting reagent (di-2-ethylhexylphosphoric acid, tri-*n*-butyl phosphate, trioctyl amine), water, and a mineral salt (ammonium sulfate). Extracting reagent additions to the organic solvent and salt additions in the aqueous phase lead to changes in physicochemical properties of the liquid system and affect, strongly, the stationary organic phase retention. The influence of the rotational speed on the stationary phase retention for different systems was also investigated. Some recommendations for the selection of proper systems for CCC separations are formulated.

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INTRODUCTION

Countercurrent chromatography (CCC), as a technique for separation and preconcentration of organic and inorganic substances, is currently attracting a great deal of interest with investigators. A few devices providing the retention of a stationary phase in a coil column in a centrifugal force field in the absence of a solid support have been proposed. The most successful one that has gained acceptance is a coil planet centrifuge.[1]

The stationary phase volume retained in a column is characterized by the S_f factor (the ratio of the stationary phase volume V_s to the total column volume V_c). It is well known that the value of S_f depends on the parameters of the coil planet centrifuge (rotation and revolution radii, tube internal diameter), on the operating conditions (rotation and revolution speeds, flow rate, and direction of pumping of the mobile phase), and on the physicochemical properties of the two-phase system used.[1-19] Du et al.[18] have proposed a correlation between the retention of stationary phase and the square root of mobile phase flow for 12 different phase systems. Sutherland[19] has shown that there is a linear relationship between the square of the mobile phase linear velocity and flow. It should be mentioned, that the influence of coil planet centrifuge parameters and operating conditions on the stationary phase retention are rather well studied for simple two-phase liquid systems, consisting of water and one or two organic solvents. A number of papers, describing the influence of the physicochemical parameters of two-phase liquid systems (interfacial tension, polarity of solvents, difference in the densities and viscosities of the phases, time of phase separation, and the length of capillary waves at the phase boundary) on the retention of the stationary phase in a column, have been published.[1-5,12-17]

Recent investigations by Sutherland[20] show that there are simple relationships between retention, linear flow, and density difference in CCC. Our paper is aimed at establishing relationships between the retention behavior and both the density difference of some two-phase systems and the operating characteristics/ geometry of the coil planet centrifuge.

It is worth noting, that the systems for inorganic separations are very different from those for organic ones,[12-16] 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. The complexation processes are the main factors that determine the separation efficiency in these cases. Moreover, the addition of extracting reagents and mineral salts can strongly affect some physicochemical properties of liquid systems and, consequently, their hydrodynamic behavior and the S_f factor.

To solve a particular problem of preconcentration and separation of inorganic substances it is necessary to know how the system, consisting of different organic solvents and extracting reagents, behaves in a CCC column. This work is an attempt to correlate composition and some physicochemical properties of such two-phase systems with the S_f factor. Complex liquid systems containing an organic solvent (*n*-decane, *n*-hexane, chloroform, carbon tetrachloride, and methylisobutylketone), an extracting reagent (di-2-ethylhexylphosphoric acid, tri-*n*-butyl phosphate, trioctyl amine), water, and a mineral salt (ammonium sulfate) are considered.

EXPERIMENTAL

Apparatus

Studies on the stationary phase retention were made with a device consisting of a homemade coil planet centrifuge of J-type (Ito's classification[1]) with a vertical column drum and a peristaltic pump.[21] 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 capacity of the column, V_c, was 17 mL. The rotation speed was varied from 350 to 550 rpm (from 36.65 to 57.60 rad/sec). The mobile phase flow rate, F, was equal to 1.1 mL/min. The densities were estimated simply by weighing a known volume of the liquid phase.

Reagents

Di-2-ethylhexylphosphoric acid (D2EHPA, Sigma), tri-*n*-butyl phosphate (TBP, Aldrich), trioctyl amine (TOA, Aldrich) were used as extracting reagents. Chemically pure organic solvents were employed without additional treatment. The other chemicals were analytical grade reagents.

CCC Procedure

Before the experiment was begun, the components of the two phase liquid system were stirred for equilibration and 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 one as the stationary phase. This results in the lower phase being the mobile phase in all cases, apart from the chloroform and carbon tetrachloride phase systems, when the upper phase was the mobile phase. First, the stationary coil column was filled with the organic phase.

After starting column rotation, the aqueous phase was fed to its inlet. Heavy phase should be pumped into the head end while lighter phase should enter the tail end. The inertial force field, which arose during rotation, made it possible to retain a constant volume of the stationary phase V_s 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.

Measurements of Viscosity

Viscosities were measured using a glass capillary viscometer. The kinematic viscosity ν (in centistokes) was computed using the formula:

$$\nu = k\tau \tag{1}$$

where k is the constant of the capillary tube (k=0.00832) and τ is the observed time (in seconds). The dynamic viscosity η (in centipoise) is then obtained from the equation:

$$\eta = \nu\rho \tag{2}$$

where ρ is the phase density (in grams per cubic meter). All measurements were performed at 20 ± 1°C.

Measurements of Interfacial Tension

A tensiometer (Lauda, Germany) with a thermostated cell, was used for measurements of interfacial tension of the systems under investigation by the ring method.[13] When the interfacial tension of the systems under investigation was measured, the volumes of upper and lower phase were 20 and 25 mL, respectively. The liquid phases were presaturated and thermostated (25°C).

The interfacial tension γ was calculated with the use of the following expressions:

$$\gamma = K\gamma_{\mathrm{mes}} \tag{3}$$

$$\gamma_{\mathrm{mes}} = [m_{\mathrm{et}} H_{\mathrm{mes}} \times 10^3 \times 0.981] / [H_{\mathrm{et}} \times 4\pi \times 0.955] \tag{4}$$

$$K = 0.725 + [4.033 \times 10^{-4} \times \gamma_{\mathrm{mes}}/\Delta\rho + 0.0128]^{1/2} \tag{5}$$

where γ_{mes} - measured interfacial tension, dyn/cm; K - correction factor; H_{mes} - height of the peak obtained by a recorder when the interfacial tension is measured, cm; m_{et} - mass of a standard, g; H_{et} - height of the peak obtained using the same recorder when the standard is weighted, cm; $\Delta\rho$ - density difference between the two phases, g/cm^3 ($\Delta\rho > 0$).

RESULTS AND DISCUSSION

We used organic solvents and extracting reagents significantly differing in viscosity and density (Table 1). As we have shown before,[14,15] the properties of liquid systems can also be changed by varying the composition of an aqueous mobile phase. Solution of a mineral salt was used as the mobile phase.

Systems Based on Different Organic Solvents

For the saturated hydrocarbon - water systems, the retention factor of stationary phase in a column decreases with decreasing the value of the difference between the viscosities of the phases.[15] The greater the difference between the viscosities of the phases, the better an organic solvent of this type is retained in a column.

Systems based on chlorine-containing hydrocarbon derivatives such as chloroform and carbon tetrachloride have much higher densities than hexane and decane (Table 1). In the systems based on these solvents, the retention factor for the stationary phase in a column also decreases on going from chloroform to carbon tetrachloride.[15] The noted organic solvents are heavier than water, therefore, the stable retention of a stationary phase based on them is achieved at higher column rotation speeds, in comparison with systems based on saturated hydrocarbons.[15] The stationary phase was completely washed out of the column at a column rotational speed up to 450 rpm (47.12 rad/sec), whereas, the retention factor

Table 1. Physicochemical Parameters of Solvents and Extracting Reagents

Solvent (extracting reagent)	ρ, g/mL	η, cP
n-Hexane	0.65	0.29
n-Decane	0.72	0.92
Chloroform	1.48	0.62
Carbone tetrachloride	1.58	0.97
Methhylisobutylketone	0.80	0.80
D2EHPA	0.97	45.6
TBP	0.97	3.39
TOA	0.80	0.50

was 0.62 at ω = 500 rpm (52.36 rad/sec) in the carbon tetrachloride – water system. Note that, in comparison with systems based on saturated hydrocarbons, systems with chlorine-containing derivatives of hydrocarbons possess much lower interfacial tension.

The systems, on the basis of methylisobutylketone (MIBK), are well retained in the column though the values of the interfacial tension are not higher than 9 dyn/cm. The physicochemical parameters and retention factors of liquid systems described are presented in Table 2. As seen from the table, the retention of the stationary phase depends on the value of difference between the viscosities of the phases.

Sutherland[20] has suggested a method for the calculation of the dependence of the square of linear velocity of mobile phase (u_m^2) on the acceleration field ($g=R\omega^2$ expressed in m/sec^2), where R is distance in meters from the center of the main rotor to the planetary axis, ω – the angular speed of rotation expressed in rad/sec). Such dependences for the solvent systems described are presented in Fig. 1. As seen from Fig.1, in an interval of acceleration field, 16-22 m/sec,^{2} the values of the square of linear velocity of mobile phase increases with increasing of the difference between the viscosities of the phases. For the planet centrifuge used for our studies, the nature of the solvents does not practically influence the retention factor of the stationary phase if speed of rotation is more than 550 rpm (57.60 rad/sec).

Effect of Extracting Reagent

We used three extracting reagents (D2EHPA, TBP, TOA) with different viscosities and similar densities (Table 1). These extracting reagents have been often used in conventional solvent extraction processes and proven to be useful for CCC metal separations.[21]

Table 2. Physicochemical Parameters and Organic Phase S_f Retention Factors with Water Mobile Phase (1 mL/min, 450 rpm)

Organic Solvent	Δρ, g/cm^3	Δη, cP	γ, dyn/cm	S_f
n-Hexane	0.32	0.62	45	0.73
$CHCl_3$	0.48	0.34	19	0.70
MIBK	0.20	0.10	9	0.65
n-Decane	0.27	0.06	39	0.55
CCl_4	0.59	0.02	22	0 (0.62*)

*ω = 500 rpm.

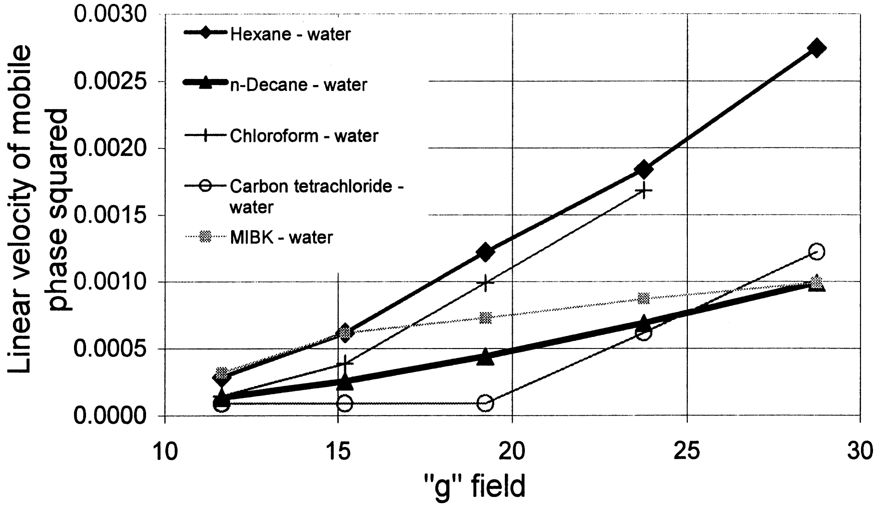

Figure 1. The variation of the square of linear velocity of the mobile phase versus the acceleration field (g=$R\omega^2$) for various systems solvent - water. F=1.0 mL/min.

A small addition (5%) of D2EHPA to the solvents under study (except MIBK), leads to a considerable increase in the S_f factor.[15] This influence is especially strong for carbon tetrachloride solution at a rotation speed of 450 rpm (47.12 rad/sec) (S_f increases from 0 to 0.7 on addition of the extractant). Among the physicochemical properties studied, only the difference between the phase viscosities rises significantly in this case. This parameter seems to be most important for the solvents that are heavier than water. The further increase in the column rotation speed does not lead to a change in the retention factor.

The physicochemical parameters and retention factors for the liquid systems described are presented in Table 3. As seen from the table, there is also a

Table 3. Physicochemical Parameters and Organic Phase (+5% D2HPA) S_f Retention Factors with Water Mobile Phase (1 mL/min, 450 rpm)

Organic Solvent	$\Delta\rho$, g/cm^3	$\Delta\eta$, cP	γ, dyn/cm	S_f
n-Hexane	0.20	0.53	23	0.80
$CHCl_3$	0.45	0.31	15	0.76
CCl_4	0.53	0.06	21	0.73
n-Decane	0.26	0.01	18	0.64
MIBK	0.19	0.05	7	0.60

relationship between the retention of the stationary phase (except MIBK) and the value of difference between the viscosities of the phases. The addition of D2EHPA to MIBK to a concentration of 5% does not change the retention factor, although, the difference between the phase viscosities significantly decreases (from 0.10 to 0.05 cP). The differences between the phase densities remain practically unchanged in this case. The value of the interfacial tension is equal to 7 dyn/cm. It is the lowest value of the interfacial tension among all the systems investigated.

The dependences of the square of linear velocity of mobile phase on the acceleration field for various solvent systems containing 5% D2EHPA are presented in Fig. 2. As seen from the figure, at least in a range of acceleration field 16-22 m/sec^2 the values of the square of linear velocity of mobile phase increase with increasing viscosity difference between the phases. The exception is the system on the basis of MIBK. A low value of the interfacial tension might be the reason for this exception.

With further increasing of the D2EHPA concentration in the organic phase (up to 50%), S_f as well as $\Delta\rho$ begin to decrease. In the case of *n*-decane as solvent, an increase in D2EHPA concentration leads to a considerable increase in the viscosity difference between the phases, and to a decrease in the interfacial ten-

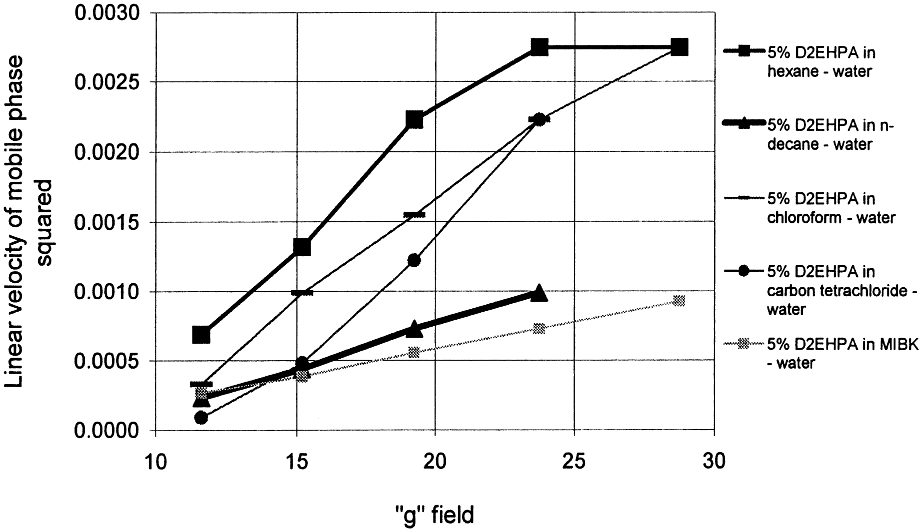

Figure 2. The variation of the square of linear velocity of the mobile phase versus the acceleration field (g=$R\omega^2$) for various systems 5% D2EHPA in solvent - water. F=1.0 mL/min.

Table 4. Physicochemical Parameters and Organic Stationary Phase (+% D2HPA) S_f Retention Values (Aqueous Mobile Phase, 1 mL/min, 350 rpm)

	Mobile Phase: H_2O (ρ =0.9995 g/ml)					
Stationary Phase	ρ g/mL	$\Delta\rho$, g/mL	S_f	η cP	$\Delta\eta$ cP	γ dyn/cm
n-Decane	0.73	0.27	0.18	0.92	0.06	39.8
5% D2EHPA	0.74	0.26	0.63	0.96	0.01	18
30% D2EHPA	0.78	0.22	0.47	1.61	0.64	14
50% D2EHPA	0.87	0.13	0.38	3.37	2.40	12

sion (Table 4). It should be noted, that the viscosity of the organic phase increases by more than 1 cP on going from one D2EHPA concentration to another (Table 4), and $\Delta\eta$ value should be determined modularly. At a D2EHPA concentration more than 5%, the value of S_f decreases linearly with a decrease in the density difference between the two phases. This dependence agrees with those reported by Berthod et al.[2,6,17] However, the addition of 5% D2EHPA into the solvent system led to a substantial change of the S_f value at a constant density difference (Table 4). In this case, the value of interfacial tension is probably the main factor responsible for this dramatic change in S_f.

TBP, as well as D2EHPA, is more viscous than *n*-decane. The addition of such a reagent in the *n*-decane - water system to a concentration of 5% results in a change in the viscosity difference, and leads to an increase in S_f values (S_f increases by 16% in case of D2EHPA addition and by 23% in case of TBP). The same addition of TOA having the viscosity less than 1 cP (as that of *n*-decane), does not lead to an increase in S_f values. For the *n*-decane – water system, the S_f value is 0.55, whereas for 5% TOA in the *n*-decane - water system, the S_f value is slightly lower (0.53).

This is also observed in the dependences of the square of linear velocity of mobile phase on the acceleration field for phase systems with different extracting reagents (Fig. 3). 5% TOA in *n*-decane has a slightly lower slope than *n*-decane alone, whereas the addition of 5% TBP and 5% D2EHPA shows a significant increase in S_f , with TBP having the greater effect. The variation of the retention factor with $1/g^2$, calculated according to Ref. [20] is shown in Fig. 4. The higher the stationary phase viscosity the better this phase is retained in the column (the viscosity of 5% TBP in *n*-decane is 1.044, that of 5% D2EHPA in *n*-decane is 0.962).

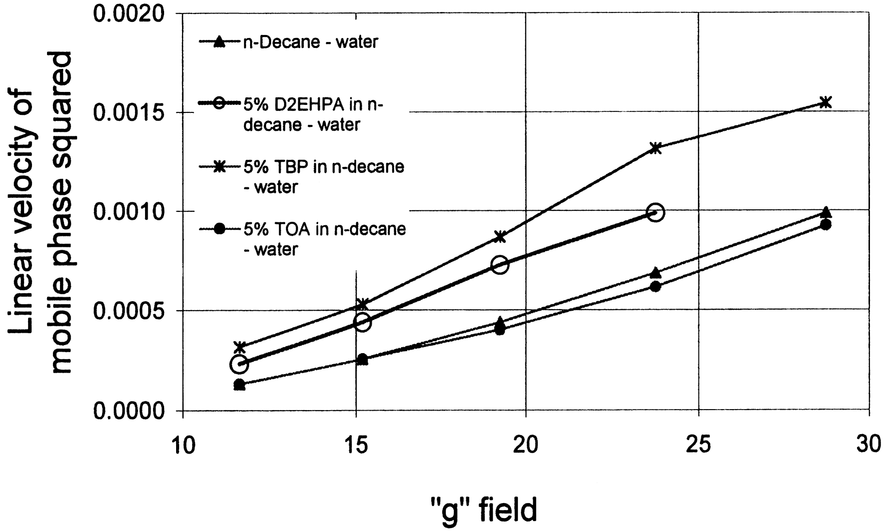

Figure 3. The variation of the square of linear velocity of the mobile phase versus the acceleration field (g= $R\omega^2$) for various systems based on *n*-decane. *F*=1.0 mL/min.

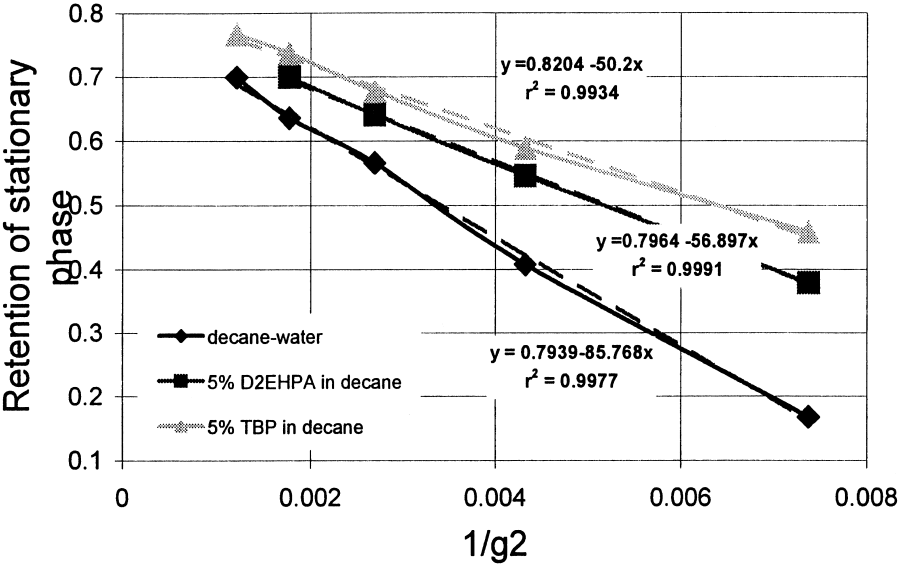

Figure 4. The variation of the retention factor of the stationary phase based on *n*-decane versus $1/g^2$. *F*=1.0 mL/min.

It should be noted, that the S_f value for more viscous D2EHPA is somewhat lower than for TBP. It can be explained by the interfacial tension influence. In the system *n*-decane – water, the interfacial tension value decreases after addition of 5% D2EHPA from 40 to 18 dyn/cm, whereas after addition of 5% TBP it changes to 14 dyn/cm. As we already mentioned in Ref. [15], an addition of an extracting reagent to a saturated hydrocarbon leads to a decrease in the interfacial tension. For systems under investigation, the interval 9-15 dyn/cm is most favourable for the retention of the stationary phase in the column. In the case of TBP, the values of interfacial tension are located in the most favourable range.

Effect of Mobile Phase Composition

Varying the aqueous phase composition can change the physicochemical properties of the systems. The effect of the mobile phase composition on the retention factor was examined using aqueous ammonium sulfate solutions of different concentration as an example. Liquid systems based on different organic solvents exhibited different behavior on changing the concentration of ammonium sulfate in the aqueous phase.[15]

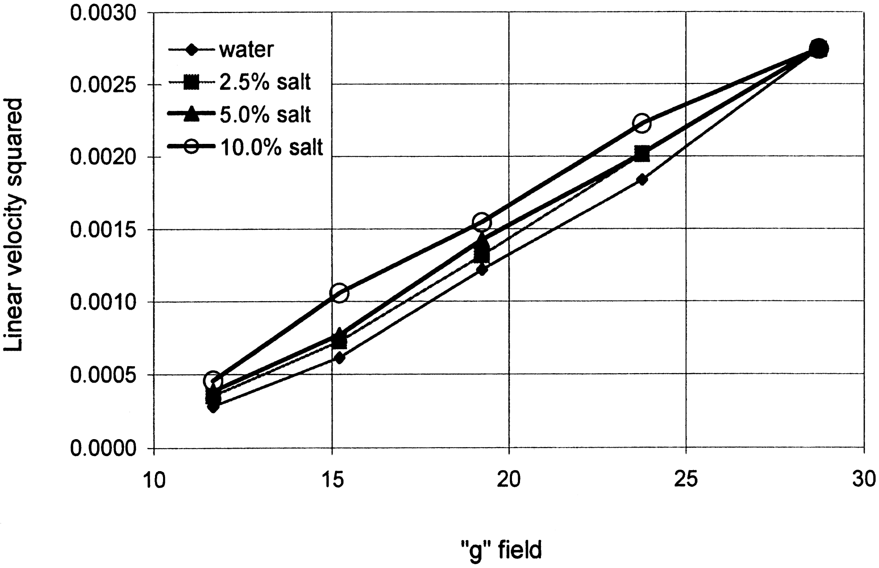

Figure 5. The variation of the square of linear velocity of the mobile phase versus the acceleration field (g= $R\omega^2$) for systems hexane – salt solution of various concentrations. F=1.0 mL/min.

The dependences of the square of linear velocity of mobile phase on the acceleration field for hexane - salt solutions of different concentration are presented in Fig. 5. The values of the square of linear velocity of mobile phase increase with increasing the concentration of the salt solution. The effect of salt concentration in the mobile phase on the retention of the stationary phase on the basis of *n*-decane containing different extracting reagents, is shown in Fig 6. The addition of some amount (2.5%) of ammonium sulfate to the mobile phase leads to an increase in the S_f value for the systems described.

The interfacial tension can also be an important factor responsible for the behavior of the stationary phases in the column. Addition of ammonium sulfate to the mobile phase up to 2.5%, leads to an increase in the value of S_f for the system based on D2EHPA by 32%, whereas the values of interfacial tension decrease to 11.4 dyn/cm. However, the value of interfacial tension in this case lies in the most favorable interval for the retention of the stationary phase in the column. For the systems on the basis of 5% TOA in *n*-decane, the retention is practically independent on the salt concentration (Fig. 6, curve *4*).

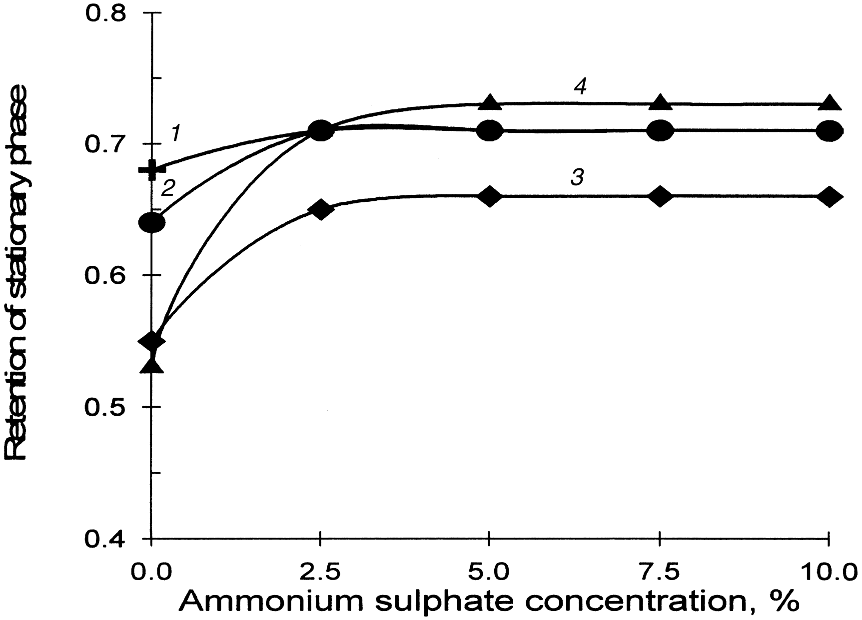

Figure 6. The influence of ammonium sulfate concentration in the aqueous phase on the retention factor for *n*-decane-based systems. ω =450 r/min, F=1.0 mL/min. Mobile phase: aqueous $(NH_4)_2SO_4$ solution. Stationary phase: *(1)* *n*-decane, *(2)* 5% D2EHPA in *n*-decane, *(3)* 5% TBP in *n*-decane, *(4)* 5% TOA in *n*-decane.

In the systems with TBP, the same addition of the salt leads to increasing the retention factor only by 3 %. In this case, the values of $\Delta\rho$ and $\Delta\eta$ are practically independent on the salt concentration. The values of interfacial tension change in the range 12-14 dyn/cm and remain in the most suitable interval for the retention of the stationary phase. It should be noted that all the described systems with *n*-decane as organic solvent, at the salt concentration 2.5% in the aqueous phase, have the same retention factor (Fig.6). This may be explained by practically equal values of interfacial tension (11.4-12.0 dyn/cm) for all the systems.

CONCLUSIONS

This study has shown that physicochemical properties of two-phase systems influence the retention factor of the stationary phase in the column. The addition of an extracting reagent to the organic solvent and salt additions in the aqueous phase, lead to changes in physicochemical properties of the liquid system and strongly affect the stationary organic phase retention. Our investigations confirm the conclusions made by Du and Sutherland[18,20] (a good correlation between the square of the linear velocity of mobile phase and retention factor of the stationary phase and the acceleration field). More work is required to obtain a clearer understanding of how the process works and to predict the behavior of two-phase systems in the column from the physicochemical parameters and the operating conditions of the centrifuge.

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