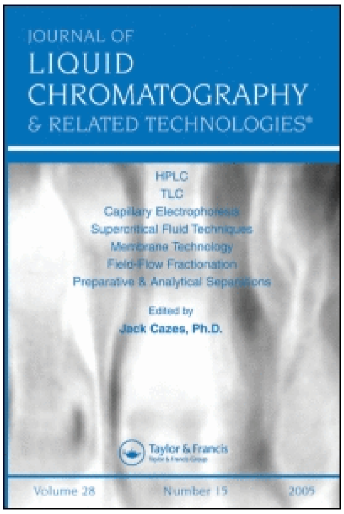

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STUDIES OF LIQUID ADSORPTION CHROMATOGRAPHY PROCESS WITH MIXED MOBILE PHASES

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ABSTRACT

Description of chromatographic retention mechanism in the systems with ternary mobile phase is very complex. Designation of the effect of individual mobile phase components on this process is difficult. The number of moles (or molecules) of less polar solvents of mobile phase referring to one mole (or molecule) of polar solvent must be taken into account. In this connection, a new method of experimental data presentation was proposed. The association effects and changes of surface phase composition were analysed. Experimental data are presented as relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$. The influence of a chromatographed substance on the retention process was also analysed. The relationships mentioned above enable the effect of individual solvent on the chromatographic process.

INTRODUCTION

The optimum conditions of mixture separation in liquid adsorption chromatography very often require application of mixed, multicomponent (e.g. ternary) mobile phases. Many authors investigated the influence of ternary mobile phase composition on separation conditions using normal and reversed phases.[1-5]

In such chromatographic systems, the mechanism of chromatographic retention process is very complex. Therefore, attempts to use relationships derived for systems with binary mobile phases for systems with ternary mobile phases were made.[6-7] Three component mobile phases were prepared as follows: In the binary mobile phase 1+2 (1, 2-polar and less polar solvent, respectively), the mixture of solvents 2 + 3 (3 non-polar solvent) was used instead of less polar solvent 2. The ratio of their molar fractions ($r = x_2/x_3$) was constant. In this way, ″binary″ mobile phases $x_1 + r$ were obtained (x_1, x_2, x_3 - molar fractions of mobile phase components in bulk phase). One can observe changes of chromatographic parameter values (k' or R_M) along with changes of mobile phase properties. Five mobile phases with different physicochemical properties were obtained: binary mobile phases 1 + 2, 1 + 3, and ternary mobile phases 1 + r ($r = 1/3$, $r = 1$ and $r = 3$). In the earlier papers,[8,9] experimental data were presented on the Gibbs triangle as isolines of R_M values of analysed substances. The analysis of these isolines was useful for investigation of process mechanism. The changes of R_M values of test substances with change of mobile phase composition, for constant values of x_1, were investigated (Figure 1A and 1B).

The properties of polar mobile phase component (solvent 1) influence, first of all, the mechanism of chromatographic retention with mixed mobile phase. Two remaining solvents (2 and 3) are less active, but their physicochemical properties are different. For that reason, the changes of their molar fractions ratio, r, may distinctly modify the mechanism of chromatographic process and its parameters.

We stated that molecule association of mobile phase components plays a very important role in liquid adsorption chromatography.[10-12] It is also connected with formation of structure and composition of surface phase in the adsorption process. The association effects exist, first of all, between molecules of solvent 1 (auto association 1-1 type) and between molecules of solvent 1 and test substance s (association s-1 type). If the component 2 of mobile phase is not quite inactive, the association of type 1-2 may exist, too. The properties of molecules decided their association, the concentration of mobile phases influence the degree of association. In the analysis of retention process mechanism the number of moles of components 2 and 3, referring to one mole of solvent 1 in the mixed mobile phase, must be taken into account. It is synonymous with the number of solvent 2 and 3 molecules referred to one molecule of solvent 1 (to one molecule of substance s, too).

For the compared systems with binary and ternary mobile phases, for identical values of x_1, ratios mentioned above are different $(x_1/x_2)_2$, $(x_1/x_3)_2 \neq (x_1/x_2)_3$, $(x_1/x_3)_3$ (the index outside the parenthesis shows number of mobile phase components) (Table 1). Therefore, comparison of the retention mechanism in such systems is very difficult. The situation is more complex when the association of molecules of mobile phase components is taken into consideration.

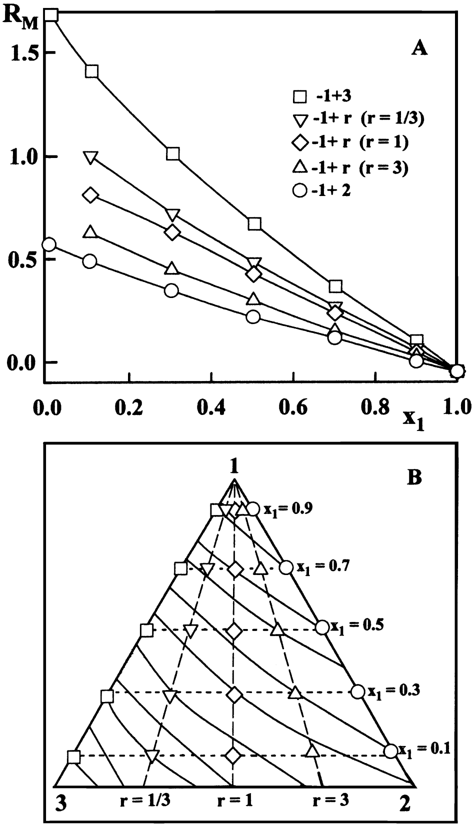

Figure 1. The relationships R_M vs. x_1 for mobile phases ethylene chloride (1) + toluene (2) + n-heptane (3), test substance – 2-nitroaniline; (A). Isolines of R_M values on Gibbs triangle for 2-nitroaniline in the system ethylene chloride (1) + toluene (2) + n-heptane (3); (B).

Table 1

Number of Molecules of the Mobile Phase Components 2 and 3 Referring to One Molecule of Component 1 in Binary and Ternary Mobile Phase

	1 + 2	1 + 2 + 3 r = 1/3	1 + 2 + 3 r = 1	1 + 2 + 3 r = 3
x_1	x_1 : x_2	x_1 : x_2 : x_3	x_1 : x_2 : x_3	x_1 : x_2 : x_3
0.1	1 : 9	1 : 2.25 : 6.75	1 : 4.5 : 4.5	1 : 6.75 : 2.25
0.3	1 : 2.3	1 : 0.58 : 1.75	1 : 1.17 : 1.17	1 : 1.75 : 0.58
0.5	1 : 1	1 : 0.25 : 0.75	1 : 0.5 : 0.5	1 : 0.75 : 0.25
0.7	1 : 0.43	1 : 0.11 : 0.32	1 : 0.21 : 0.21	1 : 0.32 : 0.11
0.9	1 : 0.11	1 : 0.03 : 0.08	1 : 0.06 : 0.06	1 : 0.08 : 0.03

The new manner of experimental data presentation was proposed to eliminate the mentioned difficulties. The results obtained in ternary mobile phases were presented as relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$ for constant values of $r = x_2/x_3$. These relationships enable one to evaluate the effect of solvent 2 and 3 on chromatographic process. It may be very interesting, if physicochemical properties of these solvents are different.

The aim of our investigations was to study whether the proposed new method of description of chromatographic data makes the retention mechanism more clear.

EXPERIMENTAL

The measurements were made using thin-layer adsorption chromatography. Chromatograms were developed on 20 x 10 cm plates (Silica gel 60H, Merck, Darmstadt, FRG) at 293K. In TLC experiments DS horizontal chambers (Chromdes, Lublin, Poland)[13,14] were used. These chambers permitted continuous development and application of the samples through a slit in the coverplate after equilibration of the liquid-solid system. Any gradient and solvent demixing effects could thus be eliminated, and the conditions were similar to those in column development.[15] Azobenzene and squalane were used as markers of the solvent flow-rate.[15,16]

The following binary mobile phases were used:

I. 1+2 (ethylene chloride + benzene) and 1+3 (ethylene chloride + cyclohexane).

II. 1+2 (acetone + toluene) and 1+3 (acetone + n-heptane).

The ternary mobile phases were developed:

I. 1+2+3 (ethylene chloride + benzene + cyclohexane).

II. 1+2+3 (acetone + toluene + n-heptane).

In the ternary mobile phases, the ratio of components 2 and 3 molar fractions (benzene and cyclohexane or toluene and n-heptane) $r = x_2/x_3$ was constant and equal to 3, 1, and 1/3.[8,9] All measurements were made for $x_1 = 0.1$, 0.3, 0.5, 0.7, 0.9, and 1.0 (molar fraction of ethylene chloride or acetone).

Test substances are presented in Table 2.

Table 2

Test Substances Used for the Chromatographic Measurements

	Mobile Phase	
Test Substance	**Cyclohexane + Benzene + Ethylene Chloride**	**n-Heptane + Toluene + Acetone**
Phenol	+	-
2,3-Dichlorophenol	-	+
2-Nitrophenol	+	+
4-Nitrophenol	+	+
Aniline	+	+
2-Nitroaniline	+	+
3-Nitroaniline	+	+
4-Nitroaniline	+	+
Carbazole	-	+
2-Toluidine	+	+
4-Toluidine	-	+
2-Nitro-r-toluidine	+	+
4-Nitro-2-toluidine	+	+
5-Nitro-2-toluidine	+	+
Fluorenone	+	+

RESULTS AND DISCUSSION

Figures 2A-5A show experimental data as isolines of R_M values on the Gibbs triangle, while Figures 2B-5B show relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$ for selected substances and mobile phases from I and II groups. Thick straight lines on the Gibbs triangles represent some constant values of mole fraction ratios $(x_1/x_2)_3$ and $(x_1/x_3)_3$ (these ratios are equal to 0.11, 0.5, 1.0, 1.5, 2.0, and 2.5). The points of intersection of these lines with sides of the triangle indicate concentrations of binary mobile phases (1+2 or 1+ 3). The points of intersection of mentioned lines with thin dashed lines (constant values of $r = x_2/x_3$) indicate composition of ternary mobile phases.

One can state that curves of relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$ differ from one another in both groups of mobile phases. For the relationships R_M vs. $(x_1/x_2)_3$, the shape of analysed curves varies insensibly. The curves are only displaced towards the ordinate axis - the values of R_M parameters are changed (Figure 2B and 4B).

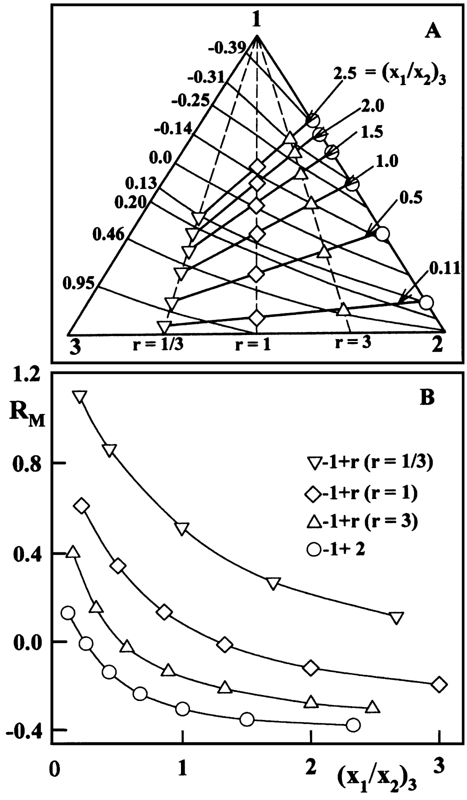

Figure 2. Isolines of R_M values on Gibbs triangle for 2-nitroaniline in the system acetone (1) + toluene (2) + n-heptane (3). Thick lines on Gibbs triangle represent constant values of $(x_1/x_2)_3$ ratio; (A). The relationships R_M vs. $(x_1/x_2)_3$ for the same system; (B).

The relationships R_M vs. $(x_1/x_3)_3$, look differently from R_M vs. $(x_1/x_2)_3$. The curves are insensibly displaced towards the R_M axis with change of mobile phase composition (change of ratio r). They lie near one another and intersect. The great influence of the kind of chromatographed substances on the mutual position of the obtained curves is also observed (Figure 3B and 5B).

Such shapes of analysed relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$, are closely connected with the mutual position of R_M isolines and of lines illustrating ratios $(x_1/x_2)_3$ or $(x_1/x_3)_3$ (Figures 2A - 5A). For R_M vs. $(x_1/x_2)_3$ relationships, the mentioned lines intersect R_M values of isolines almost at a right angle. For individual experimental points (points on $r = x_2/x_3$ line), for selected $(x_1/x_2)_3$

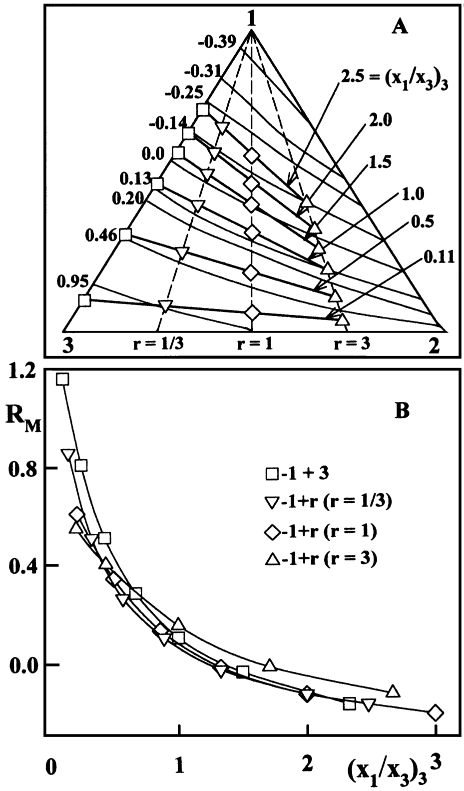

Figure 3. Isolines of R_M values on Gibbs triangle for 2-nitroaniline in the system acetone (1) + toluene (2) + n-heptane (3). Thick lines on Gibbs triangle represent constant values of $(x_1/x_3)_3$ ratio; (A). The relationships R_M vs. $(x_1/x_3)_3$ for the same system; (B).

ratio, the values of isolines are different (Figure 2A and 4A). Because of this, R_M vs. $(x_1/x_2)_3$ relationships are distant from one another (Figure 2B and 4B).

In the case of R_M vs. $(x_1/x_3)_3$ relationships, some lines of $(x_1/x_3)_3$ ratio are almost parallel to the isolines of R_M values (Figure 3A and 5A). Then, for a selected value of $(x_1/x_3)_3$ ratio, the values of isolines are the same, Figure 3A and 5A. In this connection, the relationships R_M vs. $(x_1/x_3)_3$ lie close to one another and intersect.

The mechanism of chromatographic process depends on mobile phase properties. Association effects in bulk phase determine the equilibrium con-

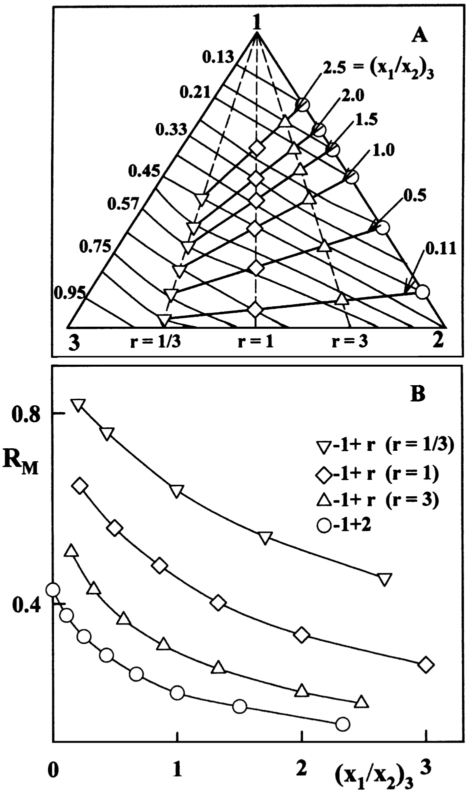

Figure 4. Isolines of R_M values on Gibbs triangle for 2-toluidine in the system acetone (1) + toluene (2) + n-heptane (3). Thick lines on Gibbs triangle represent constant values of $(x_1/x_2)_3$ ratio; (A). The relationships R_M vs. $(x_1/x_2)_3$ for the same system; (B).

stant of phase exchange reaction of molecule s and solvent 1. The composition of surface phase forming during the retention process in the adsorbent layer also influences the adsorption equilibrium constant.

If the mobile phase contains molecules of solvent 1 besides the molecules of solvent 2 (benzene or toluene), the molecules of this solvent exist in surface phase, too. Then, changes of composition of binary and ternary mobile phases have an effect on surface phase composition. When solvent 3 is practically inactive (n-heptane or cyclohexane), its addition to binary mobile phase 1+2 does not have any effect on surface phase composition. New types of molecular associates do not form, then, only the degree of existing complexes is mod-

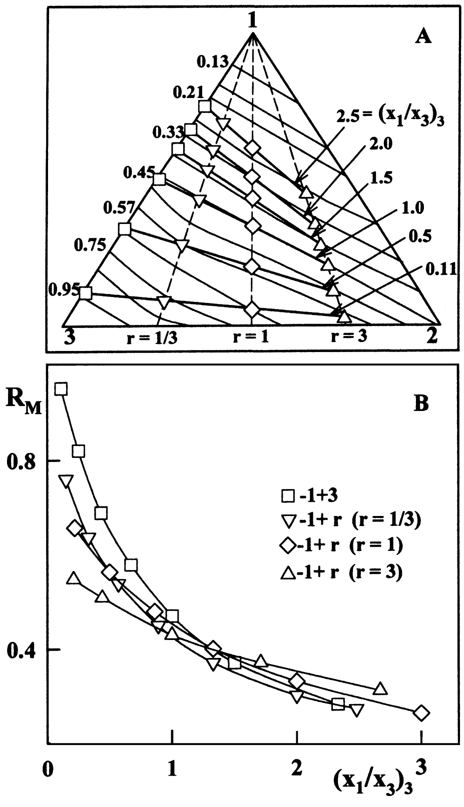

Figure 5. Isolines of R_M values on Gibbs triangle for 2-toluidine in the system acetone (1) + toluene (2) + n-heptane (3). Thick lines on Gibbs triangle represent constant values of $(x_1/x_3)_3$ ratio; (A). The relationships R_M vs. $(x_1/x_3)_3$ for the same system; (B).

ified. The change of properties of binary mobile 1+2 transformed into ternary mobile phase 1+2+3, with variable values of parameter r, causes inconsiderable changes of relationships R_M vs. $(x_1/x_2)_3$ shapes. They are only displaced towards R_M values of chromatographed test substance s.

In the case of binary mobile phase 1+3, the properties of solvent 1 molecule determines the shape of relationships R_M vs. $(x_1/x_3)_3$ (existence of associates s-1 and 1-1). The molecular interactions of type 1-3 may be neglected. For all ranges of solvent 1 concentration in bulk phase, only its molecules are present in the surface phase. Therefore, high at the beginning, the R_M values of test substance s (when in mobile phase which consists of only solvent 3 and for small x_1 values) quickly decrease with the increase of values $(x_1)_2$. Addition of solvent 2 to the mobile phase 1+3 creates a new type of associates (type 1-2). Then, the properties of mobile phase change significantly. The changes of association effects in this phase change the adsorption of the test substance. Addition of solvent 2 to solution 1+3 causes considerable change of surface phase composition, too. Its molecules exist in the surface phase (especially for smaller $(x_1)_3$ values). That is why the character of relationships R_M vs. $(x_1/x_3)_3$ is so complex.

CONCLUSIONS

Influence of association effects of mobile phase components on the retention process mechanism was noticed.

The significant difference of relationships R_M vs. $(x_1/x_2)_3$ and R_M vs. $(x_1/x_3)_3$ is visible.

The great influence of the kind of chromatographed substance on the shape of R_M vs. $(x_1/x_3)_3$ relationships is observed.

The new method of data presentation enables better investigation of the liquid adsorption chromatography mechanism.

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