

Comparison of adsorption properties of Florisil and silica in high-performance liquid chromatography

II. Retention behaviour of bi- and trifunctional model solutes

Monika Waksmundzka-Hajnos

Department of Inorganic and Analytical Chemistry, Medical Academy, Staszica 6 20-081 Lublin (Poland)

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ABSTRACT

The retention of several bi- and trifunctional model solutes using different adsorbents, Florisil and silica, and different binary eluent systems containing isopropanol, dioxane or tetrahydrofuran as modifiers in *n*-heptane was investigated by high-performance liquid chromatography. The retention parameters are presented as $\log k' - \log k'$ relationships obtained for solutes in different chromatographic systems. The selectivity of the systems investigated and the influence of modifiers on the adsorptive properties of the adsorbents are discussed.

INTRODUCTION

In previous papers, the special properties of Florisil in comparison with silica were demonstrated using various model solutes such as aromatic hydrocarbons, phenols, anilines and nitroarenes [1] and aromatic acids and aldehydes [2] using continuous thin-layer chromatography (TLC) (quasi-column conditions). In a recent paper [3], the results obtained for monofunctional aromatic model solutes in high-performance liquid chromatography (HPLC) were reported. Although some analogies between adsorption on Florisil and silica for derivatives of anilines and phenols (solutes of class AB according to the Pimentel–McClellan classification [4]) were found, a better capability for the separation of isomers on Florisil was observed.

The investigations revealed distinct differences in the acidity of adsorption centres and their distribution on Florisil and silica surfaces. Quinoline bases (class B) were more strongly adsorbed and better separated on Florisil in all eluent systems investigated [3].

The influence of modifiers on the adsorptive properties of Florisil was also examined. It was found that dioxane modified both Florisil and silica surfaces [3,5,6] in such a way that solutes interacted with the ether oxygens of the solvent film on the surface (co-adsorption effect) or displaced the solvent molecules from the adsorbent surface (displacement effect). On the other hand, the molecules of tetrahydrofuran deactivate and shield the surface of Florisil. For this reason, separation on Florisil in the latter system is poorer [3].

It was considered of interest to examine these effects using compounds with more complex molecular structures. In this work, the retention behaviour of several bi- and trifunctional aromatic com-

Correspondence to Dr M. Waksmundzka-Hajnos, Department of Inorganic and Analytical Chemistry, Medical Academy, Staszica 6, 20-081, Lublin, Poland.

pounds was examined by HPLC in chromatographic systems using Florisil as an adsorbent and isopropanol, dioxane or tetrahydrofuran in *n*-heptane as mobile phases. The chromatographic data were compared with those obtained earlier by HPLC on silica [7,8].

The analysis of steric and mesomeric effects of solutes also permits a better recognition of the distribution and chemical properties of active centres on the Florisil surface.

EXPERIMENTAL

The chromatographic experiments with Florisil were performed at $19 \pm 1^\circ\text{C}$ using a Type 302 liquid chromatograph (Institute of Physical Chemistry, Polish Academy of Sciences), equipped with a syringe pump (at a flow-rate of 1.2 ml/min) and a UV detector (254 nm). Single solutes dissolved in the eluent were injected with the help of a 5- μl injection valve.

HPLC-grade 10- μm Florisil was prepared in the Institute of Inorganic Chemistry (Gliwice, Poland)

Florisil (Fluka, Buchs, Switzerland) for TLC was milled in a Model 100 AFG counterflow-fluid mill produced by Alpine (Augsburg, Germany). Particle segregation of ground Florisil was carried out in a Model 100 MZR pneumatic separator (Alpine). The column (150 \times 4 mm I.D.) was packed with 10- μm Florisil [specific surface area (BET) 340 m²/g, total porosity 46.2%] by the slurry method. The experimental results represent the averages of three runs. As mobile phases *n*-heptane solutions of isopropanol (for HPLC, E. Merck, Darmstadt, Germany), 1,4-dioxane (for HPLC, Romil Chemicals, Shephed, UK) or tetrahydrofuran (for chromatography, redistilled, International Enzymes, Windsor, UK) were used. For further details, see ref. 3.

The chromatographic experiments with silica were performed using a Perkin-Elmer (Norwalk, CT, USA) Model 1210 liquid chromatograph with dual syringe pumps and UV detector. The column (125 \times 4 mm I.D.) was packed with 10- μm LiChrosorb Si 60 (E. Merck). For further details, see refs. 7 and 8.

TABLE I
COMPOUNDS INVESTIGATED

Solute	Abbreviation	Polar groups type
1,3-Dihydroxynaphthalene	13HN	AB-AB
1,3-Dihydroxy-5-methylbenzene (orcinol)	13H5MB	AB-AB
1,4-Dihydroxybenzene (hydroquinone)	14HB	AB-AB
4-Aminophenol	4AP	AB-AB
3-Aminophenol	3AP	AB-AB
4-Hydroxybenzaldehyde	4HBal	AB-B
2,6-Dichloro-4-nitroaniline	26C4NtAn	AB-B
2-Chloro-4-nitroaniline	2C4NtAn	AB-B
4-Chloro-3-nitroaniline	4C3NtAn	AB-B
4-Nitroaniline	4NtAn	AB-B
3-Nitroaniline	3NtAn	AB-B
2-Nitroaniline	2NtAn	AB-B
2,4-Dinitroaniline	24NtAn	AB-B-B
4-Nitrophenol	4NtP	AB-B
5-Aminoacridine	5AAcr	AB-B
1,2-Dinitrobenzene	12NtB	B-B
1,4-Dinitrobenzene	14NtB	B-B
4-Nitrobenzaldehyde	4NtBal	B-B
6-Nitroquinoline	6NtQ	B-B
2-Chloro-3-nitropyridine	2C3NtPy	B-B
2-Chloro-5-nitropyridine	2C5NtPy	B-B

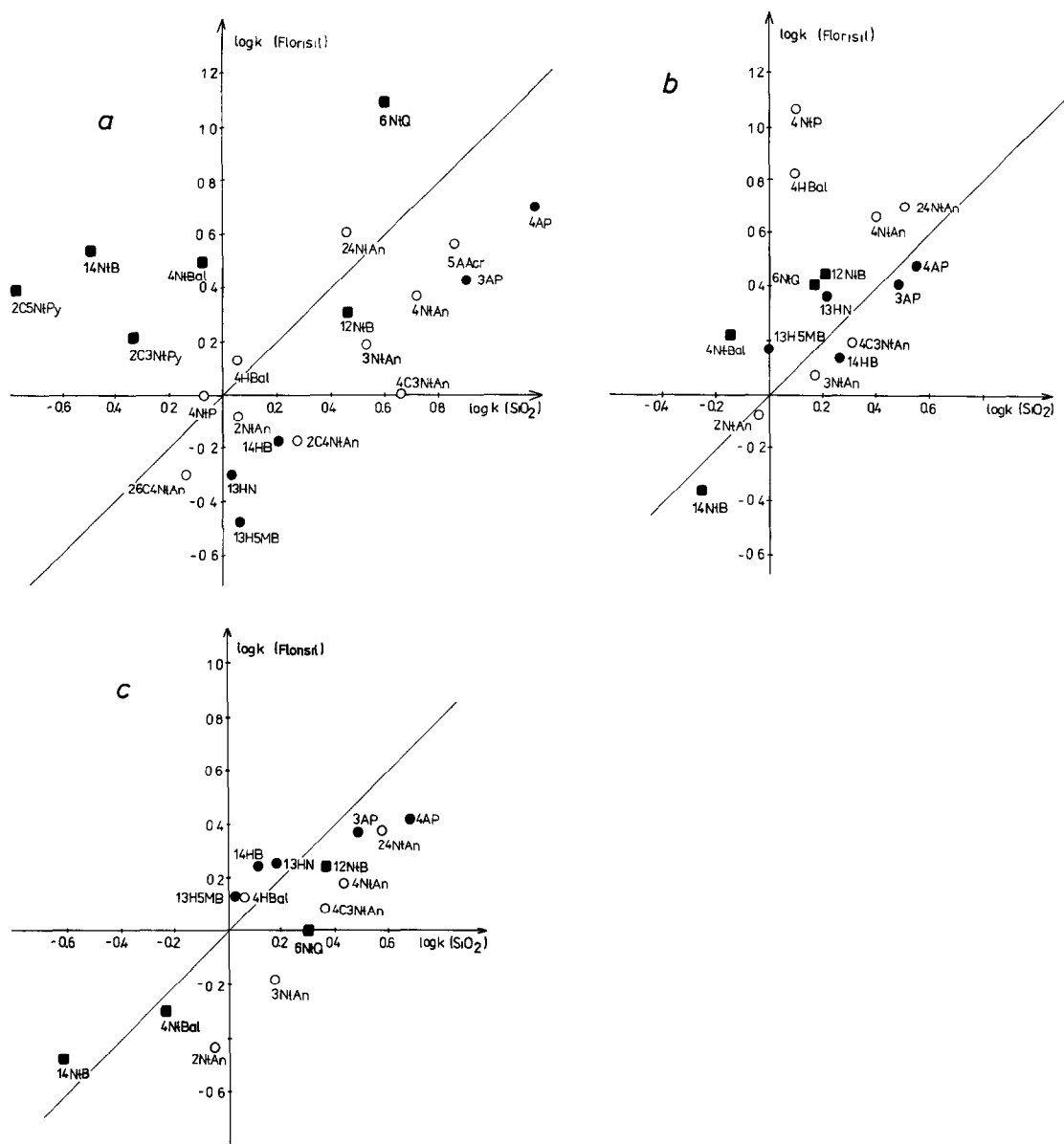


Fig 1 Correlation between $\log k'$ values of bi- and trifunctional aromatic compounds on Florisil and silica. Solutes: ■ = compounds with B-B groups, ○ = compounds with AB-B groups, ● = compounds with AB-AB groups. For abbreviations of solutes, see Table I. Mobile phases: (a) isopropanol-*n*-heptane [30/70 (v/v) for Florisil and 12.5/87.5 (v/v) for silica], (b) dioxane-*n*-heptane [50/50 (v/v) for Florisil and 40/60 (v/v) for silica], (c) tetrahydrofuran-*n*-heptane [40/60 (v/v) for both adsorbents].

RESULTS AND DISCUSSION

The experimental results obtained for the bi- and trifunctional model solutes listed in Table I are given in Fig 1 as the relationships of $\log k'$ values

(k' = capacity factor) of the solutes adsorbed on Florisil against $\log k'$ values for the same solutes adsorbed on silica in different eluent systems.

Fig 1a shows $\log k'$ (Florisil) vs $\log k'$ (silica) relationships in the isopropanol-*n*-heptane system

Molecular interactions in the isopropanol system are complex owing to its linear autoassociation [9,10], which leads to an apparent decrease in their solvent strength especially with respect to solutes possessing functional groups of class B. Such a difference in the solvation mechanism of the solutes belonging to class B or AB is a reason for the different retentions of compounds. Hydroxyl groups on the surface of either silica or Florisil interact competitively with isopropanol and solute molecules and adsorption of solutes can be considered as a result of displacement and solvation effects. Hence, it appears that solutes interact directly with active centres of adsorbents and differences in retention parameters depend largely on the adsorptive properties of the adsorbents. The analysis of chromatographic data and the structures of the solutes shows many dissimilarities in the type, distribution and properties of active centres of both adsorbents, as can be seen in Fig. 1a. The solutes with B–B groups (6-nitroquinoline, 4-nitrobenzaldehyde, 2-chloro-3-nitropyridine, 2-chloro-5-nitropyridine and 1,4-dinitrobenzene) are adsorbed more strongly on the active acidic centres of Florisil than on the silica surface. The solutes with AB–AB groups are adsorbed less strongly on Florisil than on the silica surface (e.g. 1,3-dihydroxy-5-methylbenzene, 1,3-dihydroxynaphthalene, 1,4-dihydroxybenzene, 3-aminophenol and 4-aminophenol), nevertheless, they are better separated on Florisil (see Fig. 1a). Bifunctional compounds with B- and AB-type groups (e.g., nitro and amino or chloro, nitro and amino substituents) are similarly adsorbed on both adsorbents. Although the nitro group has a relatively weak adsorption energy, the introduction of a second nitro group into the solute molecule usually increases the adsorption affinity (compare 2-nitroaniline and 2,4-dinitroaniline, Fig. 1a), especially of *ortho* and *para* derivatives owing to the ability to create resonance forms. The weaker adsorption of 2,6-dichloro-4-nitroaniline, 4-nitroaniline and 2-chloro-4-nitroaniline is presumably due to the steric shielding effects of the halogen substituents.

In the dioxane–heptane system the dispersion of correlation points is less pronounced (Fig. 1b). For example, solutes with B–B groups are adsorbed less strongly than in the isopropanol–*n*-heptane system. This indicates that the strongly acidic centres of the Florisil surface are blocked with dioxane molecules

[3], which consequently permits a co-adsorption effect comparable to that described for silica [5,6]. Nevertheless, compounds with B and AB groups, such as 4-nitrophenol, 4-hydroxybenzaldehyde, 4-nitroaniline and 2,4-dinitroaniline, are more strongly adsorbed on Florisil than on the silica surface. These compounds reveal a positive mesomeric effect (+*M*) of AB groups intensified by the negative mesomeric effect (–*M*) of the B groups (NO₂, CHO), so that the proton-donor effect of OH and NH₂ AB groups is intensified. This causes stronger adsorption on the Florisil surface covered with a bonded dioxane film (see Fig. 1b).

Fig. 1c presents the log *k'* (Florisil) vs log *k'* (silica) correlations in the system tetrahydrofuran–*n*-heptane. The spread of points in this system is less pronounced and the slope of the correlation line is less than 1, indicating that silica in this instance is generally a more selective adsorbent. Moreover, all compounds except 1,4-dihydroxybenzene, 1,3-dihydroxynaphthalene, 1,3-dihydroxy-5-methylbenzene and 4-hydroxybenzaldehyde are more strongly adsorbed on the silica surface than on the Florisil surface in this system, including compounds with electron-donor B–B groups (Fig. 1c). Similarly to dioxane molecules, tetrahydrofuran modifier molecules cover the Florisil surface, blocking the adsorption centres. The oxygen atoms in tetrahydrofuran molecules interact strongly with the acidic centres of the Florisil surface, forming a film that deactivates the adsorbent surface.

To summarize these results, the spread of points in the correlations indicates the accumulation of several effects such as the chemical character of polar groups and internal hydrogen bonding, *ortho* and mesomeric effects also have an influence on the adsorption energy of solutes. The interaction of solutes with adsorbent sites also depends on the distance between two polar substituents, adjusting better or worse to the arrangement of surface hydroxyl groups on the adsorbent (localization and delocalization effects) [11].

The correlation of log *k'* for two different solvents is very useful in the search for optimum systems for the separation of the investigated compounds (Fig. 2). It allows the selectivities of modifiers to be compared and the composition of the mobile phase to be chosen for rechromatography of complex fractions not separated in one system. For

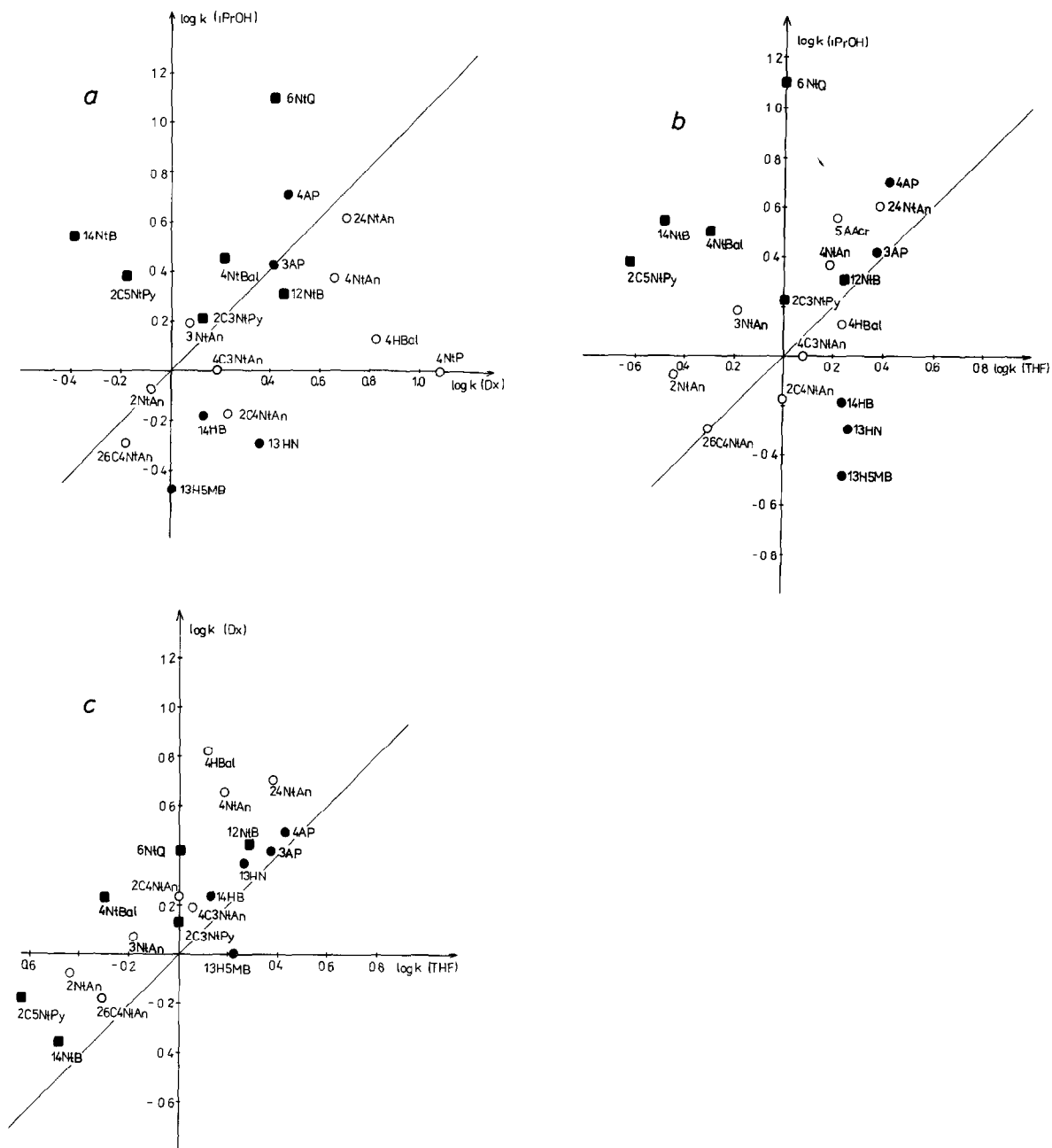


Fig 2 (a) Correlation of $\log k'$ values for isopropanol-*n*-heptane [30 70 (v/v)] against $\log k'$ values for dioxane-*n*-heptane [50 50 (v/v)] Adsorbent Florisil (b) Correlation of $\log k'$ values for isopropanol-*n*-heptane [30 70 (v/v)] against $\log k'$ values for tetrahydrofuran-*n*-heptane [40 60 (v/v)] Adsorbent Florisil (c) Correlation of $\log k'$ values for dioxane-*n*-heptane [50 50 (v/v)] against $\log k'$ values for tetrahydrofuran-*n*-heptane [40 60 (v/v)] Adsorbent Florisil Solutes as in Fig 1

example, 2,4-dinitroaniline and 4-nitroaniline, 4-chloro-3-nitroaniline and 2-chloro-4-nitroaniline, 4-aminophenol and 3-aminophenol and 2-nitroaniline and 2,6-dichloro-4-nitroaniline, which are not separated in the dioxane-*n*-heptane system, have a much greater resolution in the 2-propanol-*n*-heptane system (Fig 2a), on the other hand 2-chloro-3-nitropyridine and 2-chloro-5-nitropyridine, 1,4-dihydroxybenzene and 1,3-dihydroxynaphthalene and 1,4-dinitrobenzene and 1,2-dinitrobenzene, which are not separated in the isopropanol-*n*-heptane system, are better separated using the dioxane-*n*-heptane system (Fig 2a) Similarly, 1,4-dihydroxybenzene, 1,3-dihydroxynaphthalene and 1,3-dihydroxy-5-methylbenzene or 4-aminophenol and 3-aminophenol, 6-nitroquinoline and 2-chloro-3-nitropyridine eluted together with the tetrahydrofuran-*n*-heptane system but can be separated when 2-propanol is used as a modifier (Fig 2b) Some solutes from each group of compounds investigated have similar retentions in tetrahydrofuran-*n*-heptane Most of the investigated compounds are less strongly retained in the tetrahydrofuran-*n*-heptane system (Figs 2b and 2c) This verifies the hypothesis that the molecules of tetrahydrofuran form a film that deactivates the surface of Florisil, so that its specific properties decrease

Another method for comparing the investigated eluent systems is to plot the $\log k'$ "spectrum" obtained for a constant concentration of a modifier (comparable solvent strength) for bi- and trifunctional model solutes This diagram (Fig 3) gives a possibility of observing selectivity and sequence changes for the investigated solutes in three systems The influence of the structural effects on the retention of the chromatographed compounds can be elucidated from this diagram

Correlations of retention parameters (Figs 1 and 2) are very useful in planning separations of complex mixtures A hypothetical correlation diagram in two chromatographic systems is as follows

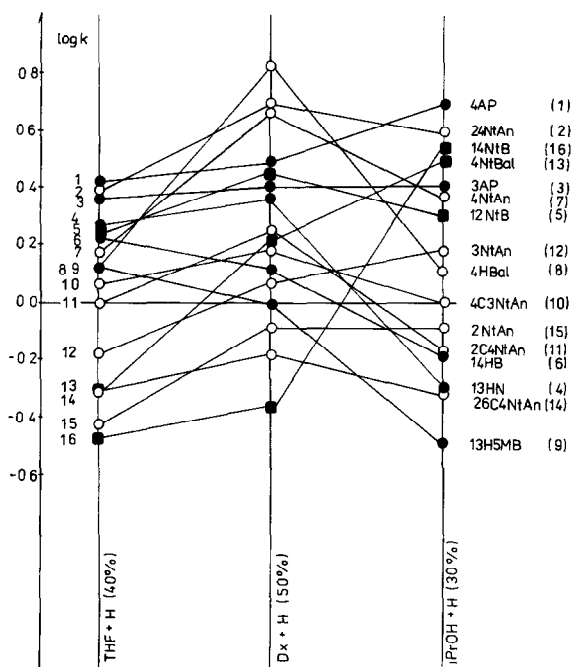
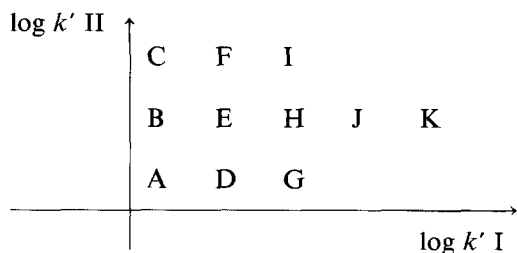


Fig 3 Graphical comparison of $\log k'$ values obtained for Florisil Mobile phases tetrahydrofuran-*n*-heptane [40 60 (v/v)], dioxane-*n*-heptane [50 50 (v/v)] and isopropanol-*n*-heptane [50 50 (v/v)]

The diagram indicates that in system I group separation is possible (ABC) + (DEF) + (GHI) + J + K In system II isolation of individual solutes from separated groups can be obtained

(ABC) \rightarrow A + B + C

(DEF) \rightarrow D + E + F

(GHI) \rightarrow G + H + I

This makes application of correlations for planning separations on the analytical and micropreparative scales possible

CONCLUSIONS

Differences in selectivity of most popular adsorbents (e.g., silica and Florisil) and eluent systems can be utilized in difficult separation problems with isomers or closely related compounds

The specific properties of Florisil depend on modifiers being used as polar components of binary solvents Dioxane molecules modify the surface of both Florisil and silica so that especially proton-donor solutes can interact with the oxygen atoms of the solvent film It changes the specific surface prop-

erties of adsorbents. The film of tetrahydrofuran shields and deactivates especially acidic centres on the Florisil surface

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