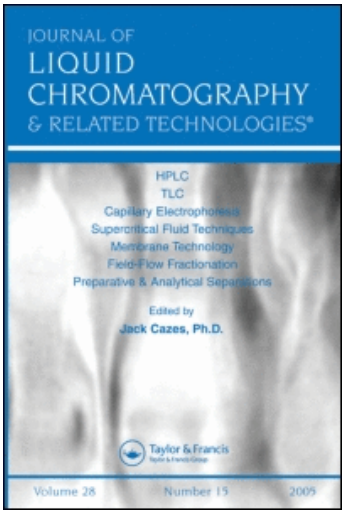

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Influence of Stationary Phase Properties on the Retention of Ketones and Alcohols in RP-HPLC Mode: A New Test for the Stationary Phase Characterization

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Abstract: Two types of octadecyl stationary phases, monomeric and polymeric, were characterized using a simple test based on aliphatic alcohol and ketone retentions. Chromatographic measurements in reversed phase systems (acetonitrile/water, methanol/water) permitted reporting changes of the separation selectivity. For the tested compounds, shape selectivity is different in systems with packings with different surface morphologies and depends also on the type of modifier used in the mobile phase. Separation selectivity was also investigated at three different temperatures: 20°C, 40°C, and 60°C. Standard enthalpy and entropy change calculations confirmed the heterogeneity of surface coverage density and conformational changes of chemically bonded silica gel ligands for various solvents. Presentation of differences between stationary phases, surface architecture, and the application of simple compounds and chromatographic systems for this purpose was the aim of the paper.

Keywords: Alcohols, Chemically bonded stationary phases for liquid chromatography, Enthalpy and entropy changes, Ketones, Selectivity, Surface characterization

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INTRODUCTION

Development of chemically bonded stationary phases for liquid chromatography and column preparation guarantee high precision, speed, and efficiency of analysis. Silica based stationary phases still are the most popular in liquid chromatography.[1]

The stationary phase might be represented as a uniform layer of chemically bonded organic ligands. Due to the organic groups grafted to the silica surface, the steric effects there is no possibility of blocking all superficial hydroxyl groups of the silica gel by molecules of organic modifiers and their homogenous distribution on the surface.[2,3,5–7] Residual silanols are strongly polar groups and weakly acidic and affect the retention of non-ionic polar compounds by hydrogen bonding interactions and the retention of ionic compounds, especially the basic ones, and by electrostatic interactions, which influence peak asymmetry and variations in the retention and selectivity.[8] The residual silanols can also react with the solvent molecules by the specific and non-specific interactions, which causes surface solvation and modifies its properties.[9] Very often analyte structure may change under the above conditions and also preferential interactions with the stationary phase. The bonded alkyls are more solvated and better ordered at a higher concentration of organic solvent in the mobile phase. In pure water or in mobile phases with low concentration of organic component, the bonded alkyl chains may collapse because of the pure surface wetting.[9,10]

The separation process in high performance liquid chromatography (HPLC) is based on specific and non-specific interactions between analyte $\Longleftrightarrow$ eluent $\Longleftrightarrow$ stationary phase. Which type of interactions and retention mechanism (adsorption, partition, size exclusion, ion-exchange) will predominate depends on the analyte structural properties, the mobile phase composition, and the properties of the chemically bonded phase.[1–3] The stationary phase selectivity, as well as chromatographic data acquisition depend on: (i) the chemical nature of bonded ligands, (ii) coverage density and homogeneity of the surface, (iii) the conformation of the chemically bonded film. Arrangement of chemically bonded film depends on ligands properties, mobile phase compositions, and temperature.[1,2,5–8]

For molecules with similar physicochemical properties, molecular shape and size can provide a basis for separation. Parameters affecting shape selectivity have been already studied, but still there are questions with uncertain answers.[9–14] Some experiments established that shape selectivity is enhanced by increased phase loading, longer chain length bonded phase ligands, reduced column temperature, increased organic modifier composition in the mobile phase, and the use polymeric stationary phases.[9,10,12,13]

Sander and Wise proposed "slot model" of retention based on effective contact area between analyte and the stationary phase as also the

mobile phase.[16] In this model the stationary phase is consisted of a number of slots into which solute molecules penetrate during retention. Planar solutes are penetrated and retained in preference to non-planar molecules. This is an explanation of bulky analytes faster eluting than planar ones.

Selection of a column plays an important role in optimization of separation conditions. Adsorbents structure and physicochemical character determination permitted predicting the pattern of the analyte's molecule behavior during the chromatographic process, and provided some information about its quality. Advanced physicochemical techniques, such as porosimetry, elemental analysis, ^{29}Si and ^{13}C CP/MAS NMR, FTIR, differential scanning calorimetry, and others can be applied for surface characterization.[1,15–17]

Spectroscopic techniques are proper for the characterization of packing, but problems arise when we have to evaluate a chromatographic column and choose the best one for laboratory practice. Spectroscopic techniques are not sufficient and require pure stationary phase not whole columns. In this instance chromatographic tests are very useful. Many tests defining the quality of HPLC columns were proposed in literature. They are based on empirical, statistical, and thermodynamic methods.[1,3] There is not one, ideal procedure which gives the possibility of evaluation of column and packing in regard to physicochemical properties. Various authors propose test mixtures containing compounds with different chemical character depending on packing and mobile phase composition.[1,4,19] The comparison of accessible tests suggests that in spite of test analytes, eluents, experimental conditions, and computational procedure, there is no one ideal test procedure for simultaneous demonstration of positive and negative column properties.[1]

As it was mentioned above, analyte retention depends on the mobile phase and stationary phase composition but also the temperature of the chromatographic system. The effect of temperature on retention is largely determined by the enthalpy changes of analyte interactions with the stationary phase. A transfer of solute from the mobile to the stationary phase is associated with decreasing Gibbs energy of the mobile phase and increasing the Gibbs energy of the stationary phase. The sum of the change in both phases is identical to the change in the total Gibbs energy of the overall system. From the Gibbs theory, it could be substituted that the Gibbs free energy corresponds to the thermodynamic equilibrium constant (K) for the distribution of the solute between the bulk mobile phase and the stationary phase:

$$\Delta G^{\circ} = -RT \ln k = \Delta H^{\circ} - T\Delta S^{\circ} \quad (1)$$

Where ΔG° is the Gibbs free energy change (the standard chemical potential for the stationary phase minus that for the mobile phase),

ΔH° is the standard enthalpy change, ΔS° is the standard entropy change of the compound transfer from mobile to the stationary phase, T is the absolute temperature, R is the gas constant.

The calculation of thermodynamic and kinetic contribution to retention is based on retention data in fact on capacity factor (k).

$$k = t_R - t_0/t_0 \tag{2}$$

Where: t_R and t_0 are respectively elution times of retained and non-retained solute.

The retention factor is related to the equilibrium constant K:

$$k = K\beta \tag{3}$$

Combination of Equation (2) and (3) gives van't Hoff equation, so the dependence of the solute logarithmic retention factor on the temperature:

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \beta$$

Where: R is the universal gas constant, β is the phase ratio ($\beta = V_S/V_M$ is the volume ratio of stationary phase (V_S) and mobile phase (V_M) in the column.

When the chromatographic surface, analyte, and solvent properties do not depend on temperature enthalpy and entropy changes, also phase ratio becomes temperature independent. In this instance the plot of ln k versus $1/T$ is linear. In this case, the enthalpy change is determined from the slope of van't Hoff graph, and entropy change can be evaluated from the intercept.

In this paper, aliphatic ketones and alcohols were used as test compounds to evaluate silica gel surface coverage homogeneity by chemically bonded organic groups. All studies were made for home made octadecyl stationary phases.

EXPERIMENTAL

Reagents and Materials

The solid support of home made phases was Kromasil®100 AT 0191 (Akzo Nobel, Bohus, Sweden). Table 1 shows the physicochemical characteristics of bare silica gel.

Octadecyl stationary phases for HPLC were received in our laboratory as a result of the well controlled substitution reaction of silica gel with mono- and difunctional octadecylochlorosilanols.[19]

The following reagents were used for the chemical modification of the silica support material: octadecyldimethylchlorosilane (Johnson

Table 1. Characteristic of bare silica gel Kromasil®100

Parameter	Abbreviation	Unit	Value
Particle shape	–	–	Spherical
Mean particle size	d_p	μm	5
Specific surface area	S_{BET}	m^2/g	295
Pore volume	V_p	cm^3/g	0.92
Mean pore diameter	D	A	113
Concentration of OH groups	μ_{OH}	$\mu mol/m^2$	7.1
Trace amount of metals	C_M	ppm	<20

Mathey ALFA Products, Karlsruhe, Germany), octadecylmethyldichlorosilane (Petrarch Systems Inc. Levittown, Pennsylvania, USA). Organic solvents were of HPLC-grade (Scharlau Chemie S.A., Barcelona, Spain). The test solutes were of various origins.

INSTRUMENTATION

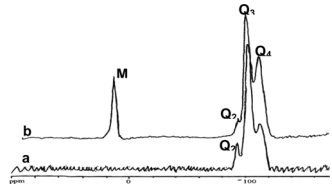
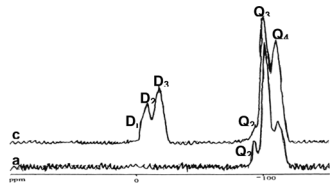
The degree of coverage density of silica support with bonded ligands for home made packings was calculated from the carbon content (P_C), determined by an elemental analysis with a CHN analyzer Model 240 (Perkin-Elmer, Norwalk, CT, USA) (Table 2). The ^{29}Si solid state NMR experiments were performed on a ASX Bruker spectrometer, model 300 (Rhenstteten, Germany) in the magic angle spinning (MAS) module.

Home made stationary phases were packed into 125 mm × 4.6 mm i.d. stainless steel columns. The columns were packed under a pressure of 50 MPa using a home made set based on a DSF-122 packing pump (Haskel INC, Burbanck, CA, USA).

Chromatographic measurements were made using an HP 1050 liquid chromatograph system (Hewlett Packard, Waldbronn, Germany), equipped with a UV-Vis detector and a HP ChemStation-2 for data collection and control of the process.

Ketone and alcohol molecular structures were modeled by the use of HyperChem v. 5.1 (HyperCube, Waterlo, Canada) and are presented in Figure 2. The CNDO method permits plotting the map of electrostatic potential field due to electronic charge distribution and nuclear charges. This is the simplest method for semiempirical quantum mechanics calculations. These calculations solve the Schrödinger equation, with certain approximations, and describe the electron properties of atoms and molecules. HyperChem v.5.1 was also used to calculate molecule volume and surface area determination (Table 3). These calculations were performed after structure modeling in the way described above.

Table 2. Character of chemically bonded stationary phases

Packing	Type of phase	P_C (%)	Coverage density ($\mu m/m^2$)	Percent of surface coverage	29 Si CP/MAS NMR spectra
MC_{18}	monomer	10.66	1.75	23.3	
DC_{18}	polymer	16.02	3.27	43.8	

where: M – monomeric phase, D – polymeric phase, (a) – bare silica gel, (b) – MC_{18}, (c) – DC_{18}.

Table 3. Capacity factors and molecule volume for alcohols and ketones

Analyte	Molecule volume (A^3)	Molecule surface are (A^2)	30/70 v/v ACN/H_2O		40/60 v/v $MeOH/H_2O$	
			K C18 mono-meric	*K* C18 poly-meric	*K* C18 mono-meric	*K* C18 poly-meric
n-butyl alcohol	348.7	251.7	1.43	1.29	2.09	1.82
i-butyl alcohol	341.06	246.7	1.28	1.15	2.01	1.75
t-butyl alcohol	338.04	243.7	0.94	0.89	1.50	1.31
n-amyl alcohol	402	279.8	2.99	2.67	4.91	4.22
i-amyl alcohol	391.3	272.8	2.69	2.43	4.36	3.72
t-amyl alcohol	380.8	267	1.80	1.69	3.19	2.76
n-hexyl alcohol	454.9	310.9	6.55	5.76	11.78	9.99
n-propyl alcohol	294.8	219.96	0.73	0.68	0.98	0.85
methyl n-propyl ketone	380.71	265.8	2.69	2.34	2.58	2.34
methyl isobutyl ketone	392.7	270.4	5.29	4.53	5.33	4.72

RESULTS AND DISCUSSION

A substitution reaction with mono- and difunctional octadecylchlorosilanols leads to different materials, depending on the modifier type (Table 2, Figure 1).

Stationary Phase Surface Characterization

The steric effect plays a predominant role (deciding homogeneity and ligand location on the surface) in the reaction of exchange between the silica surface and the modifier molecule.

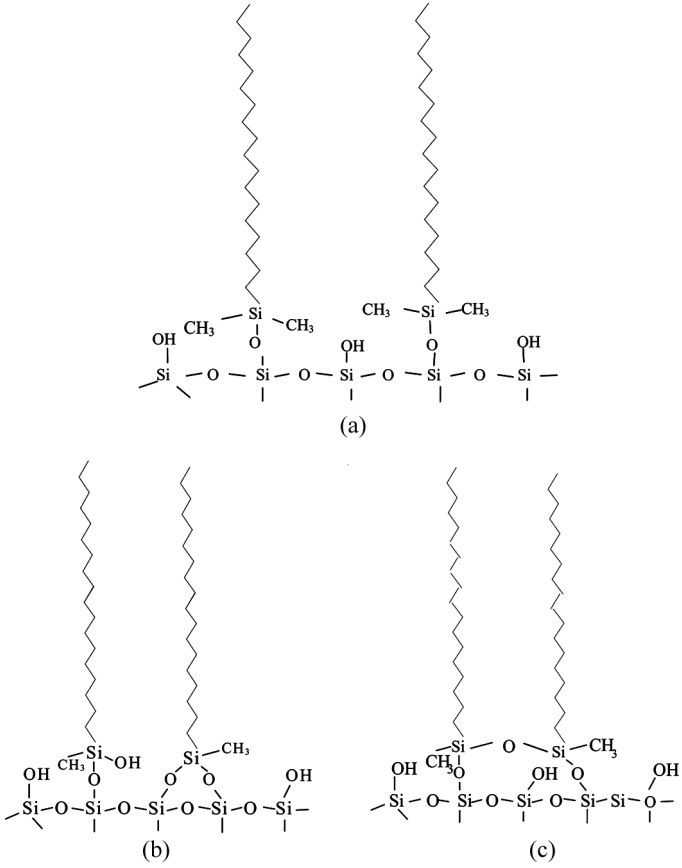


Figure 1. Possible structures of chemically bonded octadecyl stationary phases *monomeric* (a), *polymeric* (b) & (c).

Physicochemical methods such as nuclear magnetic resonance, infrared spectroscopy are useful to estimate synthesis efficiency and chemically bonded film structure prediction; but mainly, chromatographic tests provide retention data, which confirm and complete spectroscopic data and also study retention mechanism.

CP/MAS NMR spectroscopy for ^{29}Si, gives quantitative information about the density of silica gel coverage with organic ligands (Table 2). For bare silica, three characteristic signals correspond with geminal

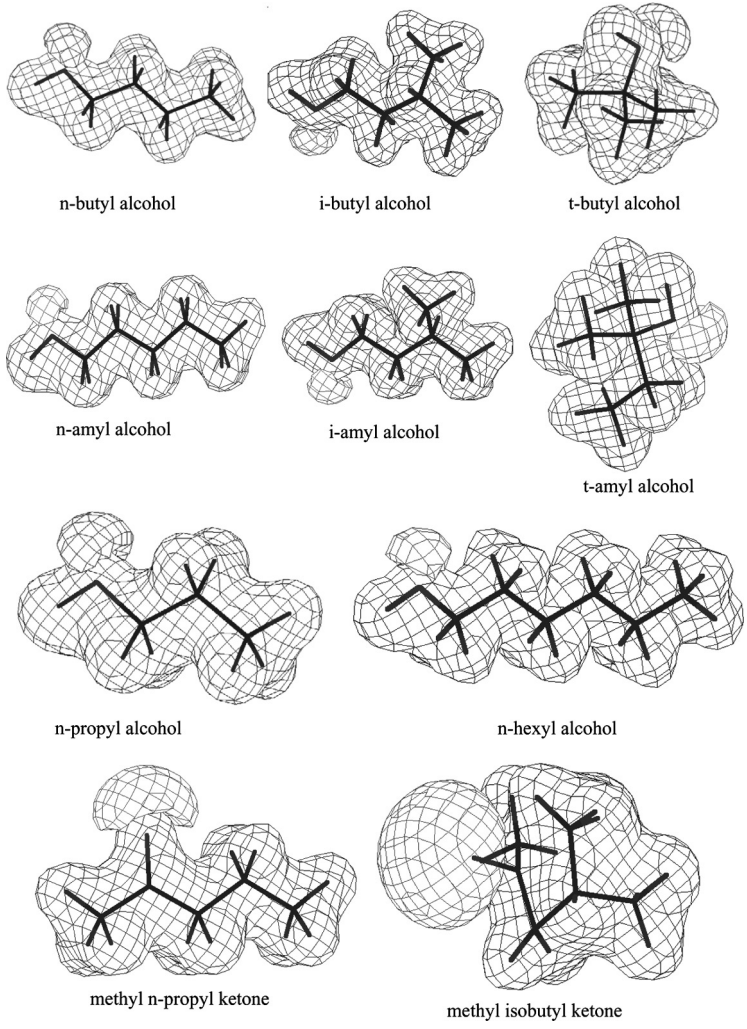


Figure 2. Alcohols and ketones structures with electrostatic potential maps.

(Q^2) $\delta = -91$ ppm and free/bonded (Q^3) $\delta = -100$ ppm silanol groups and oxosilanes (Q^4) $\delta = -108$ ppm.[15,16]

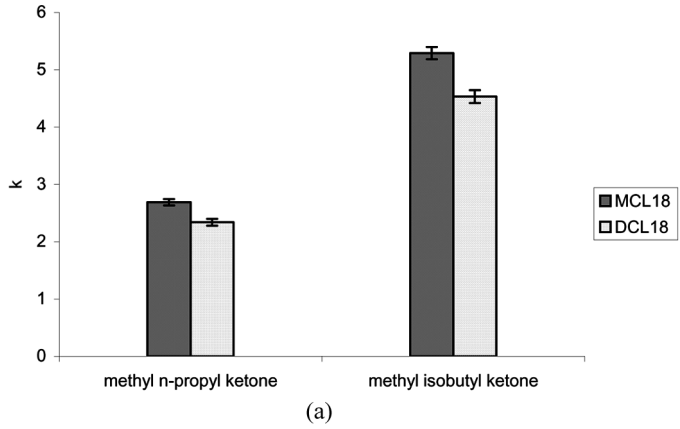
An analysis of ^{29}Si CP/MAS NMR spectra for the materials from Table 2, shows that along with chemical modification of silanols the intensity of signals of particular Q^2 and Q^3 bands decreases, whereas the intensity of signal Q^4 increases. Band M corresponds with the monomeric structure of the chemically bonded phase (one-point bonding ligand-support) and appears on the spectrum within a chemical shift $\delta = 12.5$–13.0 ppm. The spectra for packings obtained using difunctional silane shows that geminal groups are not blocked completely. Bands correspond to one-point ($D^{1;}$ $\delta = -2.5$ ppm and/or $\delta = -6$ ppm) and multiple bonding (D^2; $\delta = -10$ ppm, D^3; $\delta = -16$ ppm) to the silica support are observed on the spectrum and show small differences in the values of chemical shifts (signal D^{1-3}).[16]

Carbon content on the polymeric octadecyl stationary phase surface is *ca.* 50% higher than on the monomericone. However, surface coverage is higher for polymeric material prediction of the chemically formed structure is more complex because difunctional octadecylochlorosilane can create single bonds with one or two neighboring silanol groups on the silica surface (Table 2).

Retention Mechanism

Aliphatic compounds such as ketones (methyl isobutyl and methyl n-propyl ketone) and alcohols (n-hexyl, n-propyl, t-amyl, i-amyl, n-amyl, t-butyl, i-butyl alcohol) were used as simple test analytes for evaluation of chromatographic column shape selectivity (Figure 2). Measurements were performed for two water-organic mobile phases: methanol/water (40/60 v/v) and acetonitrile/water (30/70 v/v).

Mobile phase composition, because of interactions with stationary phase's chemically bonded groups and analytes, influences their conformation. Hydrophobic interactions are stronger for methanol than for acetonitrile. Comparison of retention factors (k) for methyl-n-propyl ketone and methyl isobutyl ketone show that more branched molecules have twice as much retention as straight ones (Figure 3). This is a result of steric effects and, in fact, there is better stationary phase accessibility for analyte molecules; also, they exhibit an ability to penetrate between chemically bonded octadecyl groups. Partition between mobile and stationary phase is more effective and faster for straight methyl n-propyl ketone than for methyl isobutyl ketone. Higher concentration of octadecyl groups on the silica gel surface (*ca.* 45%) (Table 2) changes the ketone's retention time (*ca.* 10%). It is a consequence of different analyte penetration among bonded to the surface C_{18} groups and interactions



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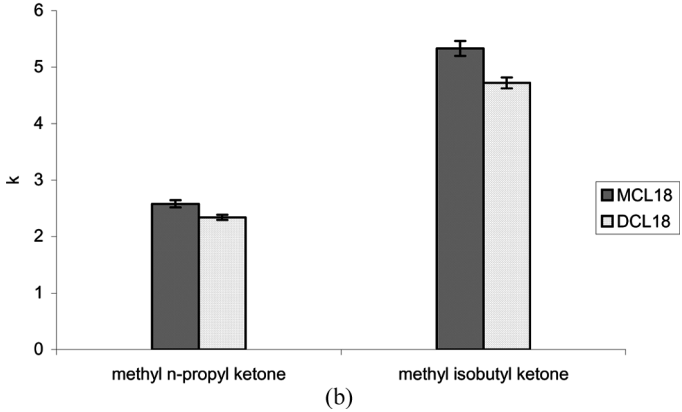


(b)

Figure 3. Ketones retention on octadecyl stationary phases, mobile phase acetonitrile/water (30/70 v/v) (a), methanol/water (40/60 v/v) (b).

between molecules and surfaces groups. Methyl isobutyl ketone because of the branched structure has stronger retention in the column than methyl *n*-propyl ketone. The same tendency is observed for monomeric- and polymeric octadecylstationary phase.

The opposite situation was noticed for alcohols. Analyte molecule shape, volume, and surface area seems to be predominant in retention (Table 3, Figure 4). Retention of *n*-isomers is stronger than for iso- and tert-isomers. This means that normal molecules (*n*-alcohols) interact strongly with stationary phase octadecyl chains through hydrophobic interactions chain $\Longleftrightarrow$ chain type. Planar and rigid n-isomers have a bigger surface area and therefore more contact area for interactions with octadecyl ligands. Bulky iso- and tert-structures with lower molecule

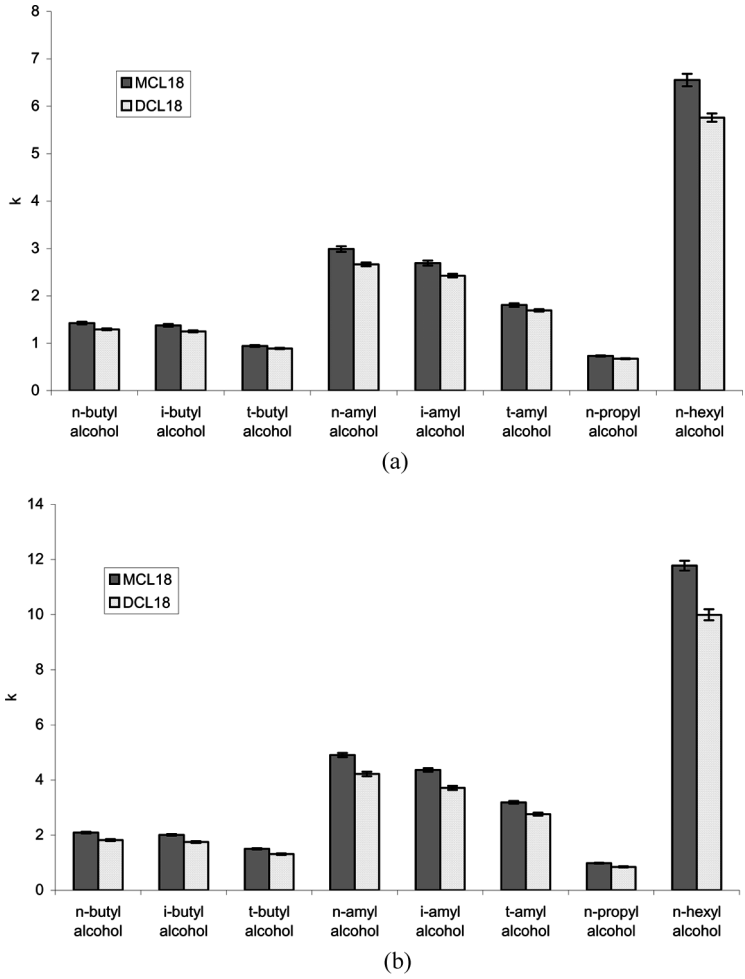


Figure 4. Alcohols retention on octadecyl stationary phases, mobile phase acetonitrile/water (30/70 v/v) (a), methanol/water (40/60 v/v) (b).

surface area need more space for partitioning within the stationary phase. This is why homogenous and dense surface coverage makes the entry of branched molecules into the stationary phase impossible. Analytes are stopped on the top of octadecyl chains and, in fact, slide on the support surface because of the conformation and solute size. When we compare retention just for *n*-isomers it could be noticed that *n*-propanol, the shortest alcohol with the smallest surface area, has retention *ca.* 80% weaker than *n*-hexanol. The same profile is observed for monomericand polymeric-stationary phase. The electrostatic potential map is almost the same for

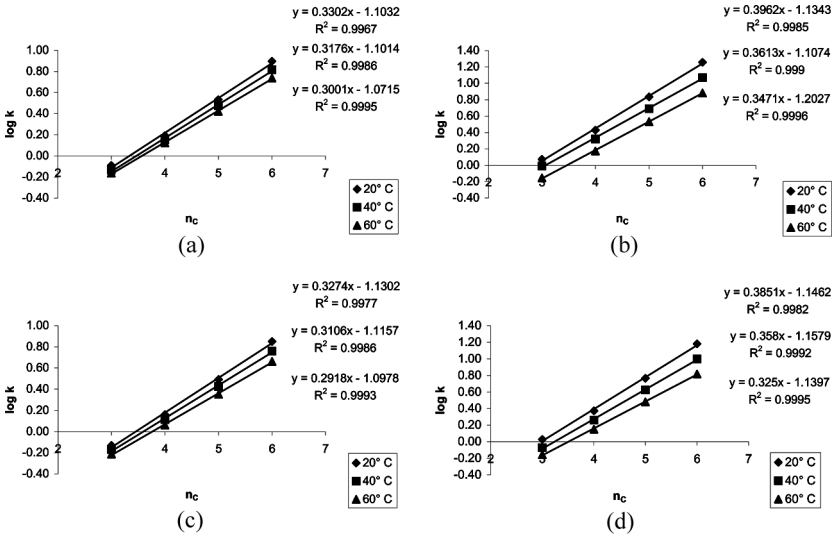


Figure 5. Correlation between log k *vs.* number of carbon atoms in alcohol molecule (n_C): (a) MC_{18}, mobile phase: 30/70 v/v acetonitrile/water; (b) DC_{18}, mobile phase: 30/70 v/v acetonitrile/water; (c) MC_{18}, mobile phase: 40/60 v/v methanol/water; (d) DC_{18}, mobile phase: 40/60 v/v methanol/water.

each *n*-alcohol. hence, hydrophobic interactions chain $\Longleftrightarrow$ chain are responsible for retention.

The above considerations stay in agreement with selectivity study for alcohols. The shape selectivity is different in systems with monomeric and polymeric stationary phases and depends, also, on the type of organic modifier applied in the mobile phase (Figure 5). Conclusions are based on slope value for linear correlations between log k *vs.* number of carbon atoms in alcohol molecule (n_C). All correlations are linear ($R^2 = 0.996 \div 0.9967$). The best correlations were obtained for 60°C, which suggests a more orderly stationary phase structure. Selectivity for acetonitrile is worse than for methanol (ca. 20%). This means that surface solvation with methanol molecules is better and the octadecyl chains order is better than in acetonitrile. The best selectivity is observed for monomeric stationary phase (Figure 5c), which suggests more efficient mass transfer and, in fact, partition analyte molecules between mobile phase $\Longleftrightarrow$ stationary phase. This is opposite to Sander and Wise results for polyaromatic hydrocarbons where the best shape selectivity was obtained for polymeric stationary phase.[17] The worst results for the polymeric stationary phase with acetonitrile as mobile phase point at weak interactions of solvent and analyte with packing. Acetonitrile as the more polar solvent (dipolarity/polarisability equal 0.60) will interact easier with low

coverage C_{18} stationary phase than methanol (dipolarity/polarisability equal 0.45), which testifies to collapsed chemically bonded groups more effectively than in methanol.[21] Separation selectivity was also compared for chromatographic systems with methanol and acetonitrile in mobile phase at 20, 40, 60°C (Figure 5). Differences are more significant in methanol as the mobile phase than in acetonitrile. Probably this phenomenon is observed because collapsed octadecyl ligands in acetonitrile are not as susceptible to conformation changes than are more rigid chains in methanol. In fact, temperature causes mass transfer changes, therefore, the standard entropy (ΔS°) and enthalpy (ΔH°) changes in the chromatographic system. Enthalpy change is determined from the slope and entropy change can be evaluated from the intercept of van't Hoff graph (lnk *vs.* 1000/T) (Figure 6, Table 4). Decreased mass transfer in alcohol isomers series n- > iso- > tert- causes decreased standard enthalpy (ΔH°) changes (ca. 95%) and increased standard entropy (ΔS°) changes (ca. 95% for butyl alcohols and ca. 50% for amyl alcohols) (Table 3). Lower ΔH° values indicate weaker interactions, analyte $\Longleftrightarrow$ hydrophobic chains of stationary phase. Higher ΔH° values obtained for methanol (increase more than 100%) confirm better arrangement of stationary phase chains in methanol than in acetonitrile. This phenomenon finds confirmation and reflection in selectivity measurements (Figure 5).[9]

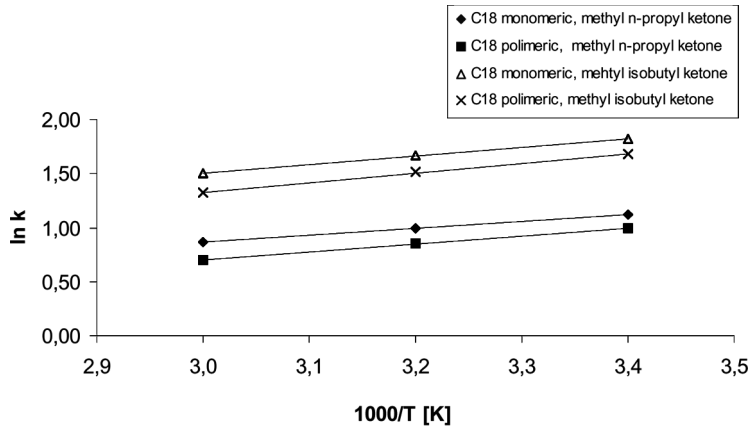
A reverse situation is observed for ketones. Here, standard enthalpy changes increased for more branched methyl isobutyl ketone, which suggest better mass transfer than for methyl *n*-propyl ketone. It suggests that the carbonyl group prevents hydrophobic interactions. Methyl isobutyl ketone has an additional methylene group which increases molecule hydrophobicity (Figure 2).

CONCLUSIONS

Application of aliphatic alcohols and ketones isomers and homologues as test compounds permits fast stationary phases characterization. Molecule shape and size and also physicochemical properties enable prediction of stationary phase structure, conformation changes, and, in consequence, homogeneity and density of surface coverage.

Size and conformation of the analyte molecule decide interactions with stationary phase moieties and good mass transfer, which was confirmed by enthalpy and entropy changes measurements. Temperature study shows that retention changes are lower for chromatographic systems with a different modifier at 60°C in comparison to 20°C.

Branched ketone molecules are retained longer than linear ones, which suggest easier partition between mobile and stationary phase. The



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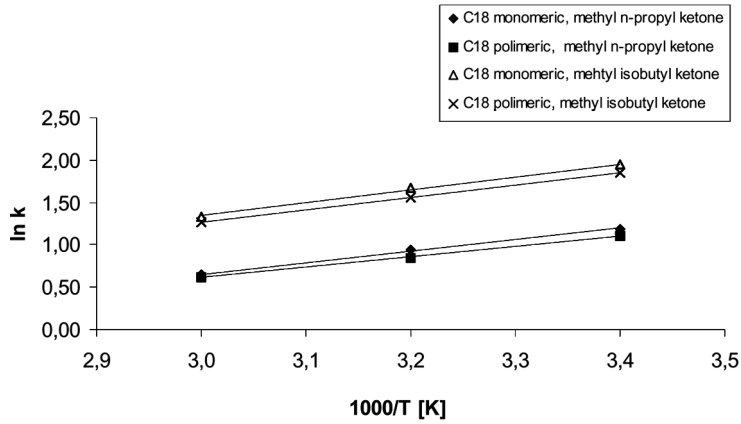

(b)

Figure 6. Van't Hoff correlation for ketones: 30/70 v/v acetonitrile/water (a), 40/60 v/v methanol/water (b).

opposite situation observed for alcohols testifies about less accessibility of space between chemically bonded C_{18} groups for branched isomers. Stationary phase surface accessible for planar *n*-alcohols is bigger and brings higher retention as a consequence of hydrophobic interactions. Selectivity tests for alcohols prove significant effects of the mobile phase, probably because of different solvation of the stationary phase and, in consequence, chemically bonded ligands conformation changes. This is distinctly visible for the compound with the longest and the shortest chain – *n*-propanol and *n*-hexanol for which the difference in retention is *ca.* 85%.

Table 4. Values of $-\Delta H^{\circ}$ [kcal/mol] and - ΔS° [cal/mol K] for test analytes

	293–313 K							
	30/70 v/v ACN/H_2O				40/60 v/v MeOH/H_2O			
	C_{18} monomeric		C_{18} polymeric		C_{18} monomeric		C_{18} polymeric	
Analyte	$-\Delta H^{\circ}$	$-\Delta S^{\circ}$	$-\Delta H^{\circ}$	$-\Delta S^{\circ}$	$-\Delta H^{\circ}$	$-\Delta S^{\circ}$	$-\Delta H^{\circ}$	$-\Delta S^{\circ}$
n-butyl alcohol	0,79	−1,83	1,13	−3,10	2,91	−7,90	2,55	−6,98
i-butyl alcohol	0,71	−1,65	1,01	−2,80	2,77	−7,55	2,47	−6,78
t-butyl alcohol	−0,02	−0,06	0,32	−1,27	2,27	−6,56	1,89	−5,50
n-amyl alcohol	1,27	−1,88	1,59	−3,16	3,46	−7,93	3,24	−7,53
i-amyl alcohol	1,08	−1,49	1,43	−2,83	3,32	−7,75	3,12	−7,38
t-amyl alcohol	0,06	0,96	0,46	−0,45	2,60	−6,06	2,37	−5,56
n-hexyl alcohol	1,83	−2,12	2,16	−3,45	4,28	−8,80	4,16	−8,75
n-propyl alcohol	0,41	−1,97	0,96	−3,85	2,59	−8,43	2,10	−7,03
methyl n-propyl ketone	1,28	−2,14	1,45	−2,97	2,72	−6,85	2,42	−6,06
methyl isobutyl ketone	1,57	−1,71	1,76	−2,65	3,00	−6,33	2,85	−6,03

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