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Study of the retention and selectivity of cholesterol bonded phases with different linkage spacers

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ABSTRACT

The chromatographic properties of four cholesterol bonded phases with different structures were studied. The columns used were packed with a stationary phase containing a cholesterol molecule attached to the silica surface using different types of linkage molecules. As a basic characteristic of the bonded phases the hydrophobicity and silanol activity (polarity) were investigated. The presence of the polar amino and carboxyl groups in the structure of the bonded ligand strongly influences the polarity of the bonded phase. Columns were compared according to methylene selectivity using a series of benzene homologues and according to their shape and size selectivity using polycyclic aromatic hydrocarbons (PAHs). The measurements were done using MeOH–water and ACN–water mobile phases. The presented results show that the coverage density of the bonded ligands and length of the linkage strongly influence the retention and selectivity of cholesterol bonded phases.

1. Introduction

The cholesterol bonded phase is a unique separation material [1–3]. This type of bonded phase can be successfully used to separate mixtures by both chromatographic modes: normal phase (e.g. steroids) as well as reversed phase (hydrophobic sample, e.g. PAHs) [1,4–6]. Cholesterol bonded phases have high resolving power in some applications which may be connected to their liquid crystal properties [7–9]. Another advantage of cholesterol stationary phases is the possibility to use it with highly aqueous mobile phases without any evidence of bonded phase collapse that drastically reduces solute retention [1,2]. The bonded phase hydrophobicity, measured by standard tests, (e.g. Engelhardt et al. [10], Walters [11], Galushko [12] and others [13,14]), exhibit similar properties to typical octadecyl bonded phases. However, the control of the coverage density and presence of a polar group in the linkage make it possible to obtain cholesterol packings with different hydrophobic–polar properties.

A few methods of cholesterol bonded phase synthesis have been developed. Those methods include silanization and hydrosilation procedures. During the silanization procedure an organo-silane is attached to the silica surface. The cholesterol ligand may be directly bonded during the silanization procedure [15] or the silica surface may be modified with an aminosilane, which is reacted with cholesteryl chloroformate in the second step [4,9]. Synthesis of a bonded phase on a hydride silica surface, leads to the formation of a direct Si–C bond [2,16]. Regardless of the synthesis methodology, some electronegative atoms (oxygen, nitrogen) are present in the ligand structure. The presence of polar groups in the bonded ligands, e.g. ether, carbamate, ester, has an influence on the retention properties of these phases [17]. Thus the retention mechanism is linked to the bonding type of the unit carrying the cholesterol (i.e., monomeric *vs.* polymeric, carbamate *vs.* ether bonding, etc.) [15].

After chemical modification any non-reacted or non-shielded residual silanol groups present on the silica gel surface may affect the retention of hydrophilic compounds by strong polar interactions. Modification or shielding of the residual silanol groups usually decreases the retention of polar solutes and improves their peak shape. Accurate determination of polar interactions (silanol activity) is important for the characterization of stationary phase properties, but its measurement is less straightforward than the characterization of hydrophobicity [10,12,18].

Hydrophobic selectivity tests are most often based on the relative retention of compounds differing in the size of the non-polar hydrocarbon part of the molecules, e.g. benzene and their homologues) [14,19–22]. The dependence of the logarithm of the retention factor of a solute, $\log k$, on the volume fraction of the organic solvent in a binary aqueous–organic mobile phase, φ, can be described, to a first approximation, by a simple linear solvent

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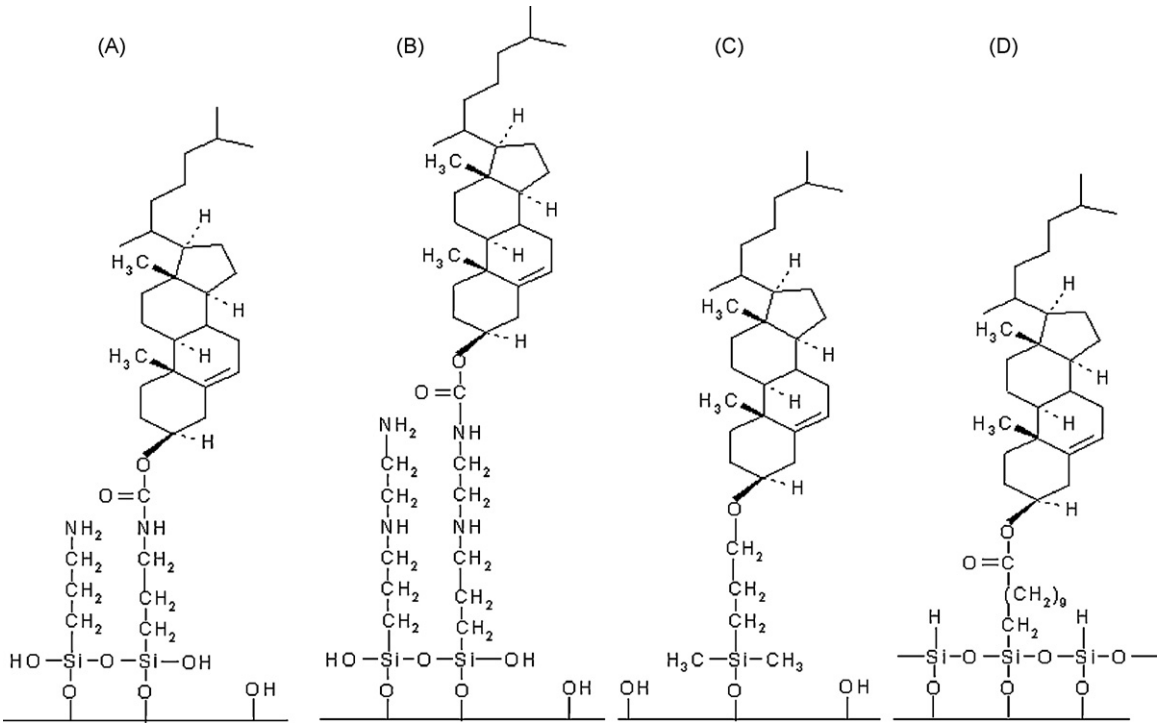

Fig. 1. Structures of the cholesterol bonded phases (A) Amino-cholesterol, (B) Diamino-cholesterol, (C) Cosmosil Cholester, and (D) UDC Cholesterol.

strength model equation [19,23–25]

$$\log\ k = a - m\varphi \quad (1)$$

where a is the logarithm of the retention factor extrapolated to pure water ($\log k_w$) and m is the parameter characterizing the effect of the organic solvent on the retention.

Polycyclic aromatic hydrocarbons are useful solutes for determining the selectivity of stationary phases and their molecular shape recognition [26–30]. The properties of cholesterol provide for sufficient separation of PAHs and other planar molecules [1,31]. Thus, the comparison of different cholesterol bonded phases according to size and shape selectivity is interesting from a practical as well as theoretical point of view.

The goal of our work was to compare the selectivity of stationary phases, which contain a cholesterol molecule attached to the silica surface using different linkage molecules. The influence of the coverage density and length of the linkage molecules on selectivity were determined. Methylene selectivity using a series of alkylbenzenes and shape–size selectivity obtained from PAH separations in MeOH–water and ACN–water were compared.

2. Experimental

2.1. Columns

A series of bonded phases containing cholesterol derivatives was tested. The structures of the bonded ligands are presented in Fig. 1. The first two phases (Fig. 1A and B) are home-made (Chair of Environmental Chemistry & Bioanalytics, Torun, Poland) and contain a cholesterol molecule attached to an amino bonded phase via an amide bond. Synthesis of the Amino-cholesterol and Diamino-cholesterol phases was performed according to methodology described earlier [4]. In the structure of Cosmosil Cholester phase (Nacali Tesque INC., Kyoto, Japan) (Fig. 1C), the cholesterol derivative is attached to a propyl spacer via ether bond. Phase D is UDC Cholesterol (MicroSolv Technology Corporation, Eatontown, NJ, USA) obtained by synthesis on a hydride silica.

The geometric parameters of the tested columns and particle diameter of the silica gels are listed in Table 1. The column void volume was determined by injection of thiourea using 60% MeOH in water as the mobile phase.

The physico-chemical properties of the tested cholesterol phases are listed in Table 2. Stationary phases are synthesized on different types of silica gel. However, the specific surface area and pore diameter of the support are similar in all the tested phases (310–342 m^2/g and 100–120 Å).

2.2. Chemicals

Two different mobile phase systems were used in the measurements: methanol–water and acetonitrile–water. Organic solvents (methanol and acetonitrile) were high-purity "for HPLC" *isocratic grade* from J. T. Baker (Deventer, The Netherlands). Water was purified using a Milli-Q system (Millipore, El Paso, TX, USA) in our laboratory.

Table 1
Column parameters.

	Amino-cholesterol	Diamino-cholesterol	Cosmosil Cholester	UDC Cholesterol
Column length [mm]	125	125	150	150
Column diameter [mm]	4.6	4.6	4.6	4.6
Particle diameter [μm]	5	5	4.5	4.2
Void volume [ml]	1.35	1.29	1.58	1.68

Table 2
Physico-chemical parameter of the stationary phases.

	Amino-cholesterol	Diamino-cholesterol	Cosmosil Cholester	UDC Cholesterol
Carbon load [%]	17.82	22.39	19–21	12.1
Coverage density [μmol/m^2]	2.66 (amino) 1.77 (chol.)	2.30 (diamino) 2.28 (chol.)	2.1–2.4	1.5
Silica gel specific surface area [m^2/g]	310	310	342	340
Pore diameter [Å]	100	100	119	100
Bonding of cholesterol group	Amide bond	Amide bond	Ether bond	Ester bond
Support	Silica	Silica	Silica	Hydride silica

The standard test compounds: aniline, phenol, homologues of benzene and PAHs were obtained from Sigma–Aldrich Chemie (Steinheim, Germany). The concentration of tests compounds was in the range of 10–40 μg/ml. The injection volumes were in the range 2–10 μl.

2.3. Equipment

The liquid chromatograph was an HP Model 1050 (Hewlett Packard, Waldbron, Germany) equipped with a four channel gradient pump, an autosampler with a 100-μl loop, a DAD detector and a data acquisition station (ChemStation software).

The degree of coverage of the surface by alkylsilyl ligands (α_{RP}) was calculated on the basis of the carbon percentage determined on a Model 240 CHN analyzer (Perkin Elmer, Norwalk, USA).

2.4. Methods

Silanol activity (SA_G) and the hydrophobicity (H_G) of the stationary phases were done according to the method described by Galushko [12]. To determine these factors the analysis of aniline, phenol, benzene and toluene retention was done using a mobile phase containing 60% methanol in water. The silanol activity SA_G and hydrophobicity H_G can be calculated as follows using solute retention factors k:

$$SA_G = 1 + 3\left[\left(\frac{k_{\text{aniline}}}{k_{\text{phenol}}}\right) - 1\right] \quad (2)$$

$$H_G = \left(\frac{k_{\text{toluene}} + k_{\text{benzene}}}{2}\right) \quad (3)$$

Methylene selectivity tests were performed in MeOH–water and ACN–water conditions using benzene, toluene, ethylbenzene, propylbenzene and butylbenzene [32,33].

$$\log\ k = \log\ \beta + n \log\ \alpha_{\text{m}} \quad (4)$$

where α_{m} is a measure of methylene (liphophilic) selectivity, n is a number of repeat methylene units and β is a measure of aromatic (phenyl) contribution to the retention [34].

In the shape and size selectivity tests the 8 PAHs were used: 1-naphthalene, 2-phenanthrene, 3-anthracene, 4-pyrene, 5-chrysene, 6-benzo(a)anthracene, 7-perylene, 8-benzo(a)pyrene. The retention of PAHs was performed in pure MeOH and pure ACN as a mobile phase.

3. Results and discussion

3.1. Retention of alkylbenzenes

In Fig. 2, the retention ($\log k$) of a series of homologues is presented. Linear trends are observed using both mobile phases: 70% ACN in water and 70% MeOH in water. The Diamino-cholesterol and Cosmosil Cholester phases have the most similar properties which can be connected with the highest carbon load of these phases. The changes in alkylbenzene retention are parallel on these phases. The retention on the Amino-cholesterol phase is a little bit lower, especially using methanol as organic modifier. On the UDC Cholesterol phase the retention is the lowest which can be connected with its lower hydrophobicity of this phase but the slope of the trend (which corresponds to selectivity) is comparable.

In Fig. 3, the changes of propylbenzene retention ($\log k$) over the mobile phase composition are presented. For all bonded phases a linear dependence was obtained. From the intercept of the trend line with the Y-axis the $\log k_{\text{w}}$ parameter can be obtained. These values correspond with the hydrophobicity of the stationary phases.

The parameters $\log k_{\text{w}}$ of all alkylbenzenes obtained from the linear regression model are presented in Table 3. As can be expected higher $\log k_{\text{w}}$ values were obtained in MeOH–water mobile phases than in ACN–water. The changes of the $\log k_{\text{w}}$ with the organic solvent in the mobile phase depend on the stationary phase. When ACN is replaced by MeOH the highest increase is observed on the Cosmosil Cholester and Diamino-cholesterol

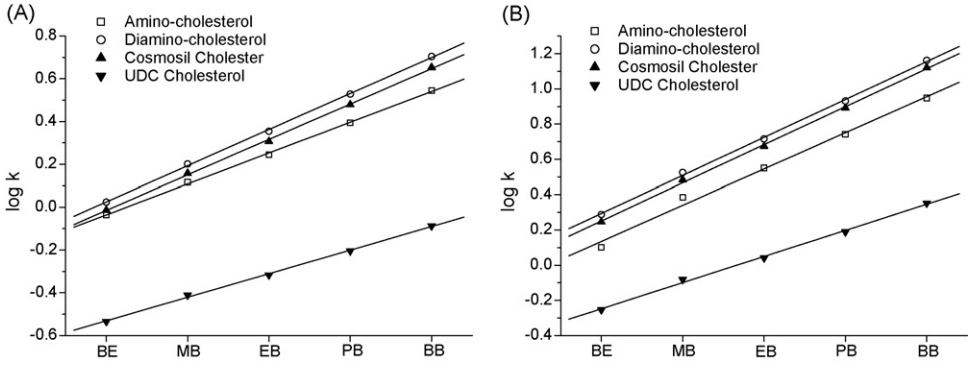

Fig. 2. Changes of the retention ($\log k$) for the homologous series: BE, benzene; MB, methylbenzene; EB, ethylbenzene; PB, propylbenzene; BB, butylbenzene; mobile phases: (A) 70% ACN in water and (B) 70% MeOH in water.

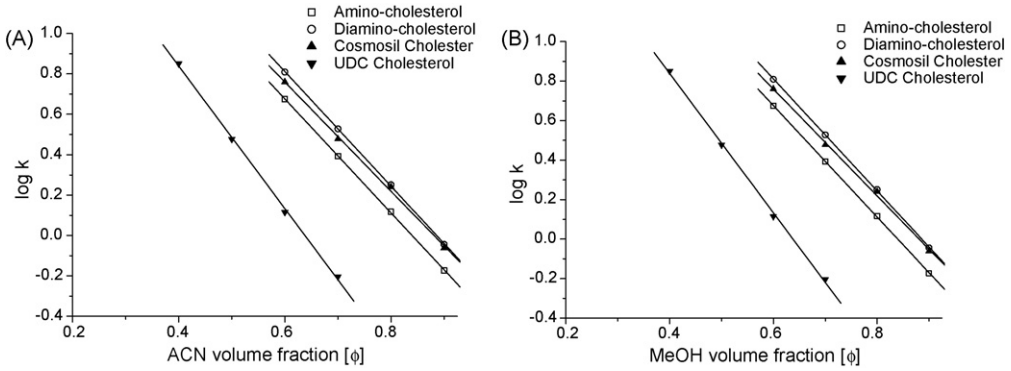

Fig. 3. Changes of propylbenzene retention (log k) with mobile phase composition: (A) acetonitrile and (B) methanol.

Table 3
log k_w values obtained in MeOH–water and ACN: BE, benzene; MB, methylbenzene; EB, ethylbenzene; PB, propylbenzene; BB, butylbenzene.

Column	BE	MB	EB	PB	BB
ACN–water					
Amino-cholesterol	1.64	1.86	2.11	2.37	2.65
Diamino-cholesterol	1.70	1.95	2.22	2.51	2.82
Cosmosil Cholester	1.52	1.79	2.07	2.38	2.70
UDC Cholesterol	1.44	1.69	1.96	2.25	2.55
MeOH–water					
Amino-cholesterol	1.86	2.60	3.03	3.51	4.02
Diamino-cholesterol	2.43	2.90	3.38	3.91	4.46
Cosmosil Cholester	2.23	2.75	3.25	3.81	4.37
UDC Cholesterol	1.65	2.06	2.51	3.01	3.53

bonded phase. The lowest increase was obtained on the UDC Cholesterol bonded phase.

3.2. Stationary phases hydrophobicity/silanol activity test

The retention of four standard compounds (aniline, phenol, benzene and toluene) on the tested stationary phases was compared using mobile phase containing 60% methanol in water. The results are presented in Fig. 4.

Two parameters obtained from Galushko test differentiate cholesterol stationary phases. The lowest polarity (including silanol activity) is observed on the Diamino-cholesterol and Cosmosil Cholester phase (stationary phases with the highest carbon load). Simultaneously, these phases exhibit the highest hydrophobicity. These properties are caused by the relatively high surface coverage of the cholesterol ligands (see Table 2). The decrease of the silanol activity between Amino-cholesterol and Diamino-cholesterol phases may be connected with increase of the coverage density as well as the shielding properties of the longer diamino ligands which can cover the surface of the silica gel because of the conformational changes. The lowest hydrophobicity and relatively high polarity are displayed by the UDC Cholesterol stationary phase. These properties may be connected with the relatively low coverage density of this phase and the hydride surface of the silica support.

Stationary phase hydrophobicity obtained from the Galushko test was correlated with the log k_w parameter of alkylbenzenes. As it can be expected, the strong correlation between log k_w and hydrophobicity is observed (R^2 higher than 0.95). Data for ethylbenzene and butylbenzene log k_w with are presented in Fig. 5. This result confirms that the log k_w parameter represents the hydrophobicity of the stationary phase. However, it is very useful to compare chemically bonded phases according to their relative hydrophobicity or hydrophobic retention.

3.3. Methylene selectivity

In binary mobile phases containing various volume fractions, φ, of organic modifier in water, linear dependencies of log k on φ were found according to Eq. (1), where:

$$a = a_0 + a_1 n \quad (5)$$

$$m = m_0 + m_1 n \quad (6)$$

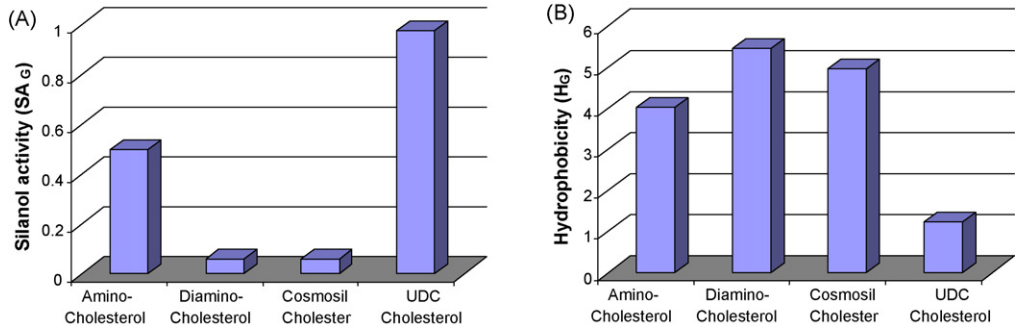

Fig. 4. Stationary phases silanol activity (A) and hydrophobicity (B) according to Galushko test [12].

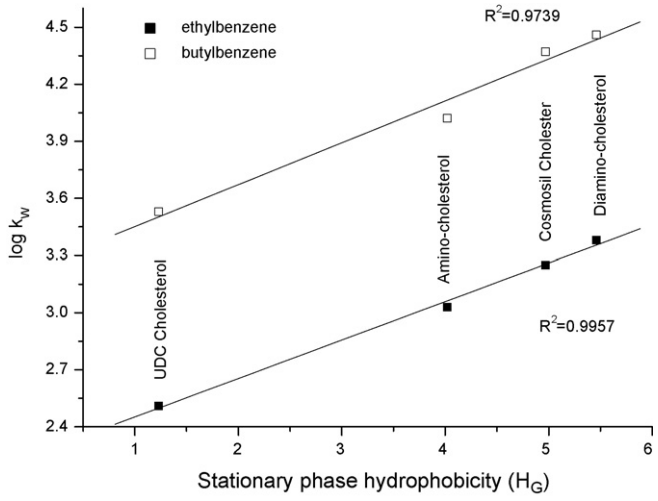

Fig. 5. Correlation between $\log k_w$ of ethylbenzene and butylbenzene from MeOH solution with stationary phase hydrophobicity (H_G).

The increments in the parameters a and m per methylene group, a_0, a_1, m_0 and m_1 were determined using multilinear regression, n is a number of repeat methylene units in alkylbenzenes series. The data are summarized in Table 4.

The parameter a of Eq. (4) is the logarithm of the retention factor extrapolated to pure water ($\log k_w$). The parameter m characterizes the effect of the organic solvent on the retention. Both parameters depend on the hydrophobicity of the sample and of the stationary phase.

The hydrophobicity contribution to retention is characterized using a homologous series with a non-polar repeat methylene group. Parameters a and m of Eq. (4) increase in a linear manner with increasing number of carbon atoms, n, in the alkyl group (Eqs. (5) and (6)) [32,33]. Parameters a_1 and a_0 represent methylene selectivity and phenyl selectivity, respectively. Parameters m_0 and m_1 describe the influence of the mobile phase on the phenyl selectivity and methylene selectivity, respectively.

Despite the difference in the retention of alkylbenzenes, the methylene selectivity of all tested cholesterol phases is very similar. However, the highest methylene selectivity (a_1) is observed for Cosmosil Cholester phase and the lowest for the Amino-cholesterol using both organic modifiers. The UDC Cholesterol column, on which the retention is the lowest, exhibits comparable methylene selectivity. The highest phenyl selectivity (a_0) is obtained on the Diamino-cholesterol phase and the lowest on UDC Cholesterol. Higher coverage density of bonded ligands often results in better selectivity of the stationary phase ($a_1 = 0.30$ and $a_1 = 0.57$ for Cosmosil Cholester in acetonitrile and methanol, respectively). A decrease of the coverage density of bonded ligands often leads to lower selectivity of the packing ($a_1 = 0.26$ and $a_1 = 0.47$ for Amino-cholesterol column in acetonitrile and methanol, respectively). Specific polar groups in the linkage (ether, amide, amino) may also influence the selectivity of the bonded phase. The solvation of the bonded ligands governed by hydrophobic effects [35] or polar interactions [17] also can influence the separation process.

All of the parameters are lower in acetonitrile–water with respect to methanol–water mobile phases except parameter m_0 on the UDC Cholesterol phase. This effect is caused by a higher polarity change in aqueous–organic mobile phases when increasing the concentration of acetonitrile. Parameter m_0, which characterizes the effect of the organic solvent on the contribution to the retention by phenyl, is similar in acetonitrile/water and in the methanol–water mobile phases. It is almost independent of the type of the organic solvent. However, the parameter m_1 is much higher in methanol–water mobile phases.

3.4. Retention of PAHs

In Fig. 6, the chromatograms of the 8 PAH mixture separation on the cholesterol bonded phases using MeOH as a mobile phase are presented. The highest retention of PAHs was obtained on the most hydrophobic bonded phase–Diamino-cholesterol and the lowest retention on the least hydrophobic–UDC Cholesterol bonded phase.

The tested stationary phases differ from each other not only in the retention but also in the selectivity of the separation. One can observe the selectivity based on the size with an increase in the number of the aromatic rings in the molecule and shape selectivity connected with different geometry of the molecules with same number of aromatic rings.

3.5. Shape–size selectivity

For the size selectivity investigation the retention of benzene, naphthalene and phenanthrene were compared. Linear dependencies of the retention ($\log k$) with the number of aromatic rings in the molecule were obtained (Fig. 7). Despite the lower retention on the UDC Cholesterol phase in the test conditions used the shape selectivity (α_s) of all cholesterol packings is similar. Using ACN as a mobile phase the highest size selectivity was obtained on the UDC Cholesterol phase ($\alpha = 2.89$ and $\alpha = 4.82$) and the lowest on the Cosmosil Cholester column ($\alpha = 1.63$ and $\alpha = 1.96$). In pure MeOH as a mobile phase the highest selectivity was obtained on the Diamino-cholesterol ($\alpha = 2.24$ and $\alpha = 2.84$) and the lowest on the Cosmosil Cholester bonded phase ($\alpha = 1.83$ and $\alpha = 2.40$) for naphthalene/benzene and phenanthrene/napthalene, respectively. Stationary phases obtained by modification of corresponding amino-type support exhibit very similar selectivity. The solvation of the cholesterol molecules as well as residual amino ligands and thus creation of the cavities for the solute determine the size selectivity of the stationary bonded phases.

Table 4
The parameters a_0, a_1, m_0, m_1 and correlation coefficients R^2 of Eqs. (5) and (6) in the homologous series of n-alkylbenzenes (C1–C5).

Column	Parameters					
	a_0	a_1	R^2	m_0	m_1	R^2
Acetonitrile–water						
Amino-cholesterol	1.60 ± 0.9%	0.26 ± 2.0%	0.9992	2.33 ± 0.3%	0.16 ± 1.3%	0.9997
Diamino-cholesterol	1.65 ± 1.0%	0.29 ± 2.0%	0.9992	2.33 ± 0.3%	0.17 ± 1.6%	0.9995
Cosmosil Cholester	1.48 ± 1.2%	0.30 ± 2.1%	0.9991	2.12 ± 0.4%	0.19 ± 1.4%	0.9996
UDC Cholesterol	1.40 ± 1.2%	0.29 ± 2.2%	0.9991	2.73 ± 0.4%	0.27 ± 1.5%	0.9994
Methanol–water						
Amino-cholesterol	2.11 ± 1.5%	0.47 ± 2.5%	0.9988	2.74 ± 0.7%	0.41 ± 1.8%	0.9993
Diamino-cholesterol	2.36 ± 1.3%	0.52 ± 2.2%	0.9991	2.94 ± 0.7%	0.44 ± 1.7%	0.9994
Cosmosil Cholester	2.01 ± 1.4%	0.57 ± 2.0%	0.9991	2.76 ± 0.7%	0.47 ± 1.5%	0.9996
UDC Cholesterol	1.55 ± 2.0%	0.49 ± 2.3%	0.9989	2.55 ± 0.7%	0.49 ± 1.4%	0.9996

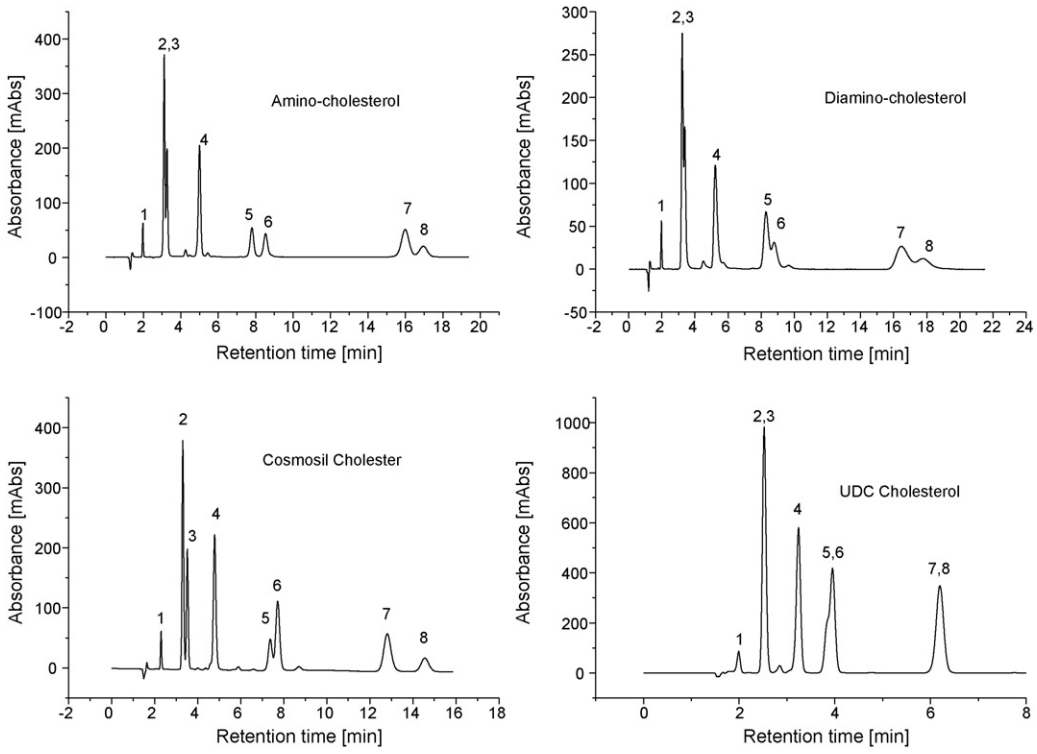

Fig. 6. Chromatograms of the separation of 8 PAHs using pure MeOH as a mobile phase.

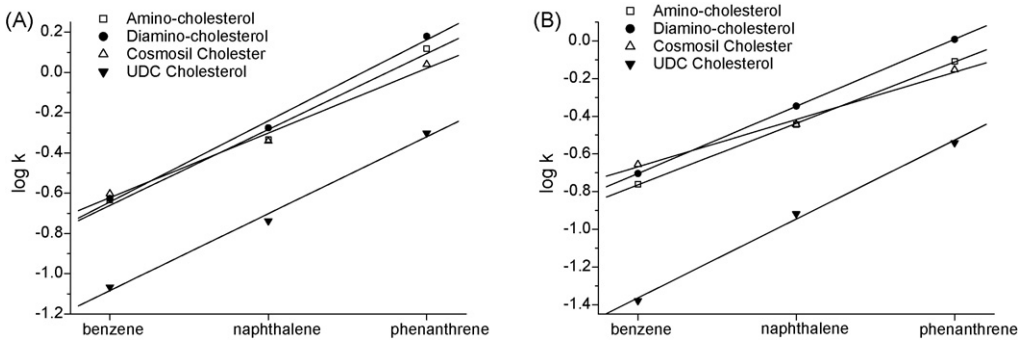

Fig. 7. Changes of the retention (log k) with increase number of aromatic rings using: (A) MeOH and (B) ACN as a mobile phase.

The separations of PAH isomers may be a good parameter for comparison of stationary phase selectivity. In the test mixture three pairs of isomers were evaluated: anthracene/phenanthrene, benzo(a)anthracene/chrysene, benzo(a)pyren/perylene. The selectivity of the separations is listed in Table 5. The highest selectivity of anthracene and phenanthrene was obtained on the Cosmosil Cholester bonded phase using MeOH and on the Amino-cholesterol phase in ACN conditions. The opposite situation was observed for benzo(a)anthracene/chrysene where the highest values of selectivity were observed on the Amino-cholesterol and Cosmosil Cholester in MeOH and ACN environment, respectively. Separation of benzo(a)pyrene and perylene was the best on the Cosmosil Cholester column in both solvents. Under the tested conditions the lowest selectivity was exhibited by the UDC Cholesterol col-

Table 5
Shape selectivity of pairs PAHs isomers.

Column	Anthracene/phenanthrene	Benzo(a)anthracene/chrysene	Benzo(a)pyren/perylene
MeOH			
Amino-cholesterol	1.08	1.11	1.07
Diamino-cholesterol	1.08	1.07	1.09
Cosmosil Cholester	1.12	1.06	1.15
UDC Cholesterol	1.00	1.05	1.00
ACN			
Amino-cholesterol	1.48	1.00	1.10
Diamino-cholesterol	1.08	1.15	1.11
Cosmosil Cholester	1.11	1.26	1.16
UDC Cholesterol	1.00	1.11	1.01

umn which can be correlated with the relatively low retention. A decrease of the mobile phase elution strength should provide much better results.

The main parameter which influences shape selectivity seems to be the coverage density of the bonded phase. An increase of the surface coverage provides better selectivity of isomers. However, the highest shape selectivity obtained on the Cosmosil Cholester and Amino-cholesterol phases can be connected not only on the coverage density but also on the length of the linkage. Stationary phases with a shorter linkage exhibit better selectivity than those with longer spacers. The more rigid structure of Cosmosil Cholester and Amino-cholesterol packing provide better selectivity in separation of planar molecules under the test conditions used in this study.

4. Conclusions

Four bonded stationary phases which contain cholesterol ligands were compared with respect to their hydrophobic–polar properties and separation selectivity. From the tests used for these stationary phases, the highest hydrophobicity and the lowest polarity were exhibited by the Diamino-cholesterol and Cosmosil Cholester bonded phases. The lowest hydrophobicity and highest polarity properties were found on the UDC Cholesterol bonded phase synthesized on the hydride silica gel indicating this phase have the best separation capabilities for hydrophilic species.

The methylene selectivity of all the tested cholesterol phases is very similar. However, the highest methylene selectivity is observed for Cosmosil Cholester phase and the lowest for the Amino-cholesterol using both organic modifiers. The highest phenyl selectivity was obtained on the Diamino-cholesterol phase and the lowest on UDC Cholesterol.

Because of the properties of the cholesterol ligands, the tested stationary phases exhibit good selectivity for PAH separations. Using pure MeOH and ACN as a mobile phase the size selectivity (phenanthrene/naphthalene) is higher than 1.96 on the all tested phases.

A more complicated situation is observed for the separation of PAHs isomers. In this comparison the highest selectivity was obtained on the Cosmosil Cholester bonded phase. However, particular pairs of isomers are separated with the highest selectivity being on different bonded phases. Generally, a higher coverage of cholesterol ligands often results in higher selectivity of the cholesterol bonded phases under the test conditions used in this investigation.

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References

[1] B. Buszewski, M. Jezierska, M. Wełniak, R. Kaliszan, J. Chromatogr. A 845 (1999) 433.
[2] J.J. Pesek, M.T. Matyska, G.B. Dawson, A. Wilsdorf, P. Marc, M. Padki, J. Chromatogr. A 986 (2003) 253.
[3] C. Courtois, C. Allais, T. Constantieux, J. Rodriguez, S. Caldarelli, C. Delaurent, Anal. Bioanal. Chem. 392 (2008) 1345.
[4] B. Buszewski, M. Jezierska, B. Ostrowska-Gumkowska, Mater. Chem. Phys. 72 (2001) 30.
[5] J.J. Pesek, M.T. Matyska, LC–GC North Am. 25 (2007) 480.
[6] B. Buszewski, M. Jezierska-Switala, R. Kaliszan, A. Wojtczak, K. Albert, S. Bachrnann, M.T. Matyska, J.J. Pesek, Chromatographia 53 (2001) S204.
[7] C. Delaurent, V. Tomao, A.M. Siouffi, Chromatographia 45 (1997) 355.
[8] J.J. Pesek, M.T. Matyska, E.J. Williamsen, R. Tam, Z. Wang, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 2747.
[9] M.A. Al-Haj, P. Haber, R. Kaliszan, B. Buszewski, M. Jezierska, Z. Chilmonczyk, J. Pharm. Biomed. Anal. 18 (1998) 721.
[10] H. Engelhardt, H. Low, W. Gotzinger, J. Chromatogr. A 544 (1991) 371.
[11] M.J. Walters, J. Assoc. Off. Anal. Chem. 70 (1987) 465.
[12] S.V. Galushko, Chromatographia 36 (1993) 39.
[13] H.A. Claessens, M.A.v. Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[14] U.D. Neue, HPLC Columns. Theory, Technology and Practice, Wiley-VCH, New York, 1997.
[15] C. Courtois, G. Pagès, S. Caldarelli, C. Delaurent, Anal. Bioanal. Chem. 392 (2008) 451.
[16] J.J. Pesek, M.T. Matyska, J. Sep. Sci. 32 (2009) 3999.
[17] S. Bocian, A. Felinger, B. Buszewski, Chromatographia 68 (2008) S19.
[18] H. Engelhardt, M. Arangio, T. Lobert, LC/GC 15 (1997) 856.
[19] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, John Wiley & Sons, New York, 1997.
[20] A.M. Krstulovic, H. Colin, A. Tchapla, G. Guiochon, Chromatographia 17 (1983) 228.
[21] L.C. Sander, S.A. Wise, J. Sep. Sci. 24 (2001) 910.
[22] M. Jaroniec, J. Chromatogr. A 656 (1993) 37.
[23] P. Jandera, J. Churacek, J. Chromatogr. A 91 (1974) 207.
[24] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. A 165 (1979) 3.
[25] E. Soczewinski, C.A. Wachtmeister, J. Chromatogr. 7 (1962) 311.
[26] A. Radecki, H. Lamparczyk, R. Kaliszan, Chromatographia 12 (1979) 595.
[27] L.C. Sander, S.A. Wise, J. High Resolut. Chromatogr. 11 (2005) 383.
[28] J.G. Dorsey, K.A. Dill, Chem. Rev. 59 (1989) 331.
[29] L. Limsavarna, J.G. Dorsey, J. Chromatogr. A 1102 (2006) 143.
[30] L.C. Sander, S.A. Wise, Anal. Chem. 67 (1995) 3284.
[31] B. Buszewski, R.M. Gadzała-Kopciuch, R. Kaliszan, M. Markuszewski, M.T. Matyska, J.J. Pesek, Chromatographia 48 (1998) 615.
[32] P. Jandera, J. Chromatogr. 352 (1984) 13.
[33] M. Molikova, P. Jandera, J. Sep. Sci. 33 (2010) 453.
[34] K.B. Sentell, J.G. Dorsey, J. Chromatogr. A 461 (1989) 193.
[35] S. Bocian, P. Vajda, A. Felinger, B. Buszewski, Anal. Chem. 81 (2009) 6334.