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Study of solvation processes on cholesterol bonded phases

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ABSTRACT

Four cholesterol bonded phases with different structures were investigated. The columns studied were packed with stationary phase containing cholesterol attached to the silica surface using different types of linkage molecules. The presence of the polar amino and carboxyl groups in the structure of the bonded ligand strongly influence on the solvation process. The possibility of hydrogen bonding, dipole–dipole and $\pi-\pi$ electron interactions lead to preferential solvation of bonded ligands. The coverage density of bonded ligands and length of the linkage strongly influence the adsorption of solvent from the mobile phase. The removal of residual silanols during the hydrosilation procedure significantly influences solvation of the bonded phase. Excess isotherms of the commonly used solvents in RP HPLC (methanol and acetonitrile) were obtained using the minor disturbance method. For comparison of the stationary phases prepared on different silica gels the excess adsorbed amounts were calculated per volume of the stationary phase in the column. The hydrosilated UDC Cholesterol bonded phase is preferentially solvated by methanol whereas the highest coverage Cosmosil Cholester phase exhibit high adsorption of acetonitrile. Polar groups in the Amino-cholesterol type bonded phase are solvated with both solvent but the mechanisms of these processes are different.

1. Introduction

The cholesterol bonded phase is a unique separation material whose analytical capabilities are just beginning to be explored [1–3]. It can be successfully used to separate mixtures by both chromatographic systems: reversed phase (hydrophobic sample e.g. polycyclic aromatic hydrocarbons PAHs) and normal-phase (e.g. steroids) [3–6]. The cholesterol bonded phases have high resolving power for some samples which may be ascribed to its liquid crystal properties [7,8]. In the most interesting and promising cases, the cholesterol packings represent immobilized artificial membranes which are very important in modeling of permeation of drugs and drug candidates through biological membranes [9].

The bonded phase hydrophobicity, measured by standard tests, behave similarly to typical octadecyl bonded phases. The retention mechanism is linked to the bonding type of the unit carrying the cholesterol (i.e., monomeric vs. polymeric one, carbamate vs. ether bonding) [10].

Another advantage of these packing materials is the possibility of using the cholesterol material with highly aqueous mobile phases without any evidence of bonded phase collapse (dewetting) that drastically reduces solute retention [1].

Actually, a few methods of cholesterol bonded phase synthesis are used [1,4,10,11]. Those methods utilize silanization and hydrosilation procedures. Hydrosilation leads to the formation of a direct Si–C bond at the surface [1,11]. In the silanization procedure an organosilane is attached to the surface. The cholesterol ligand may be directly bonded during the silanization procedure [10] or the silica surface can be modified with an aminosilane, which is reacted with cholesteryl chloroformate in the second step [4]. The presence of polar atoms in the structure of the bonded ligands, e.g. ether, carbamate, ester, may have an influence on the solvation properties of those phases [12].

When the binary hydroorganic mobile phase is in contact with the surface of the stationary phase, molecules of the solvents can preferentially solvate the surface [13–15]. The composition of the mobile phase at the interface between mobile and stationary phase is different from its bulk composition. The stationary phase environment is a combination of three components: bonded ligands, adsorbed solvent molecules and residual silanols [16–20].

The organic component of the mobile phase interacts with the hydrophobic ligands on the surface of the stationary phase. In order to understand the distribution of the solvent between the adsorbed phase and a bulk binary solution the excess adsorption of the organic modifier has to be described [21–23]. Solvent adsorption has been measured by many authors using the minor disturbance method [14,24–26]. The excess amount of the adsorbed solvent changes with the surface properties of the stationary phase: the coverage density [16,21,27], the number of carbon atoms

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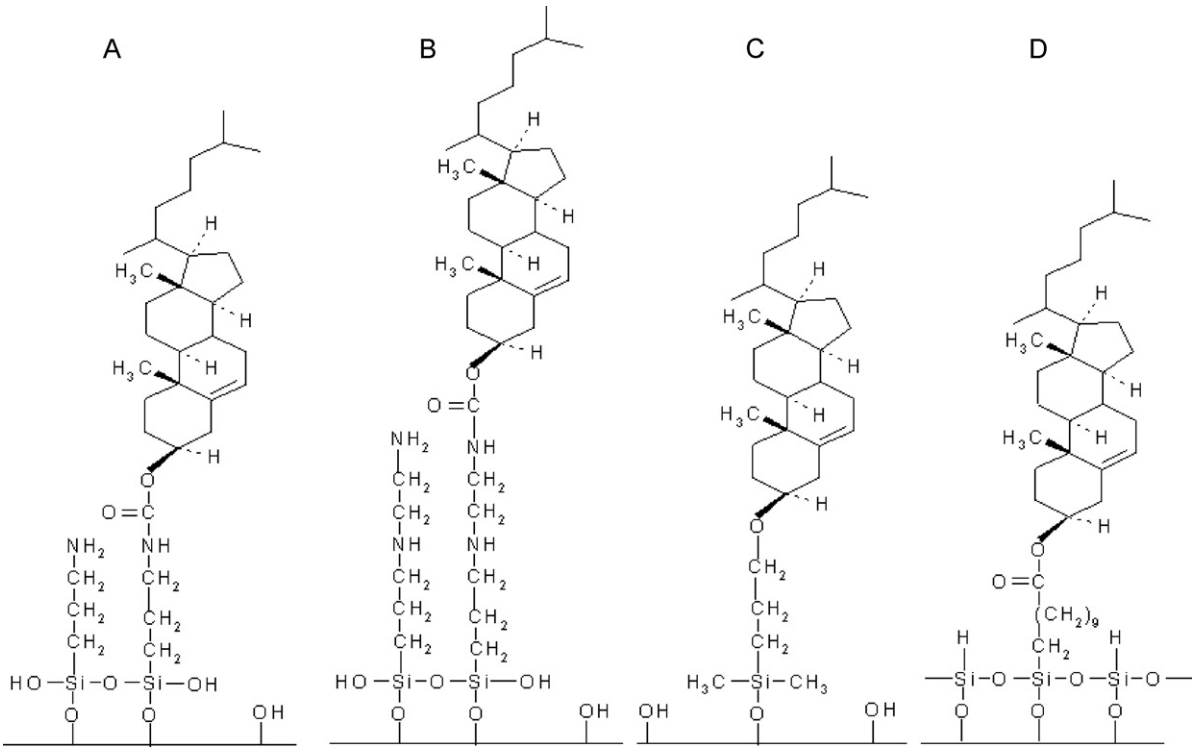

Fig. 1. Scheme of the cholesterol bonded phases used in the study: A – Amino-cholesterol, B – Diamino-cholesterol C – Cosmosil Cholester, D – UDC Cholesterol.

in the organic ligand [12,28,29] and the type of bonded ligand [30,31].

The residual accessible silanols can also interact with the mobile phase components and with the analyte molecules [32]. In the case of hydro-organic mobile phases, the silica is strongly hydrated by water molecules. As a result retention is influenced by both hydrophobic and solvophobic contributions [33]. In the case of the hydrosilation process the residual silanols of the silica have been replaced by Si–H and their influence on adsorption and separation is reduced [1,34]. The bonded phase does not participate in the retention as a homogenous entity but contains multiple sorption centers and changes the composition of the interfacial region [35].

In liquid chromatography, the surface of the stationary phase is in contact with individual components of a mobile phase. The composition of the liquid phase changes in an unknown manner from the adsorbent surface to the bulk liquid [36]. The organic modifier is preferentially adsorbed on the surface of hydrophobic stationary phase, thus it forms a layer in the immediate vicinity of the surface [37,38].

In isocratic chromatography, the stationary phase is in equilibrium with the mobile phase. A small volume of the mixture of the eluent components with different concentration injected onto the column introduces some perturbation and so-called minor disturbance peaks are observed. This perturbation moves through the column and elutes at a given retention volume. The retention volume of disturbance peak can be defined by Eq. (1) [29,37].

$$V_R(C) = V_M + S\frac{d\Gamma(C)}{dC} \quad (1)$$

where V_R is the retention volume of the perturbation [ml], V_M is the thermodynamic dead volume of the column [ml], S is the total surface area of the adsorbent in the column [m^2] and Γ is the excess adsorption isotherm of the analyte [mol/m^2] at concentration C [mol/l].

The integration of the Eq. (1) allows the calculation of excess adsorption values from the dependence of disturbance peak retention on the concentration [29] (Eq. (2)).

In a previous study the solvation process on a bonded phase with specific functionalities was investigated [12]. In this work the four different cholesterol type stationary phases are investigated. The retention and separation selectivity of this series of cholesterol packings were reporter earlier [39]. For determining solvation processes we used the minor disturbance method to measure the excess adsorption isotherms of methanol and acetonitrile from water onto a series of cholesterol bonded phases. The goal of our work was to determine the mechanism of solvation processes on four bonded phases containing cholesterol molecules attached to a silica support. Even thought all phases contains cholesterol molecule, they are connected using spacer with different length and with different functional groups which has to influence the solvation process. Also the impact of the hydrosilation procedure of silica gel to the solvent adsorption was also measured. The solvent adsorption on the carbamate cholesterol phases were compared with a corresponding amino bonded support.

2. Experimental

2.1. Materials

A series of bonded phases containing cholesterol derivatives was tested. The structure of the bonded ligands is presented in Fig. 1. Phases A 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) the cholesterol derivative is attached to the propyl spacer via an ether bond (Fig. 1C). Phase D is an UDC Cholesterol (Micro-

Table 1
Geometric parameters of the columns.

	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	5	5	4.5	4.2
Void volume [mL]	1.35	1.29	1.58	1.68
Phase ratio	0.54	0.61	0.58	0.48

Solv Technology Corporation, Eatontown, NJ, USA) obtained during synthesis on the silica hydride support.

The geometric parameters of the tested columns and particle diameter of the silica gels are listed in Table 1. Phase ration F was calculated from the following equation [40]:

$$F = \frac{1 - \varepsilon_T}{\varepsilon_T} \tag{2}$$

Elementary analysis is the basic method used to describe stationary phase. The amount of carbon and other elements can be measured by sample combustion. The knowledge of percent of the carbon enables to calculate the coverage density of stationary phase. In this case the Berendsen equation is used [41]:

$$\alpha_{RP}{}^{I} = \frac{10^6 P_C}{1200 n_C - P_C (M_1 - n_X)} \frac{1}{S_{BET}} \tag{3}$$

where:

$\alpha_{RP}{}^{I}$ – coverage density [μmol/m^2], P_C – percent of carbon [%], n_C – number of carbon atoms in the ligand, M_1 – molar mass of the ligand, n_x – number of functional group in reactive group of the silan, S_{BET} – specific surface area [m^2/g].

Physico-chemical properties of the tested cholesterol phases are listed in Table 2. Stationary phases are synthesized on different types of the silica gel. However, specific surface area and pore diameter are similar on the all tested phases. Cholesterol molecules are connected with the linkage chain using different functional groups which are the additional polar adsorption center in the structure of the bonded phase. This functional groups will interact with specific interaction with solvents molecules whereas linkage chain can interact only with non-specific van der Waals forces. Types of the bonding groups are listed in Table 2.

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).

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) 1.7 (chol.)	2.30 (diamino) 2.2 (chol.)	1.9-2.2	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	Hydrosilated silica
Number of carbon atom in the linkage	4	6	3	11
Linkage length [Å]	8.8	13.3	5.8	18.1

2.2. Equipment

The liquid chromatograph was an HP Model 1050 (Hewlett Packard, Waldbron, Germany) equipped with a quaternary pump, an autosampler with a 100 μl loop, a DAD detector and a data acquisition station using Chemstation software. The frequency of the detector was 2.5 Hz.

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

Solid-state NMR measurements were performed on a Bruker MSL 300 spectrometer (Bruker AG, Karlsruhe, Germany) with samples of 200–300 mg in double bearing rotors of ZrO_2. Cross-polarization/magic-angle spinning (CP/MAS) NMR were recorded with a pulse length of 5 μs and a pulse repetition time of 2 s. All spectra were externally referenced with liquid tetramethylsilane (TMS) and the chemical shifts (δ) were given in parts per million (ppm).

2.3. Methods

For solvent adsorption measurements, each column was equilibrated with a mobile phase of decreasing concentration of organic solvent in water from 100% to 0% by pumping at least 20 ml of solvent mixture. Perturbation of the baseline was done by 2 μl injections of the mixture with higher concentration of organic solvent than the plateau concentration. The signal was detected with an RI detector and a UV detector at λ = 195 nm. The temperature in all measurements was kept at 298 K.

The thermodynamic void volume of the column (V_M in Eq. (1)) is obtained by integrating the retention times of the perturbation peaks (from 0% to 100% of the organic modifier) [42]:

$$V_M = \frac{1}{C_{max}} \int_{C=0}^{C=C_{max}} V_R(C)\, dC \tag{4}$$

The excess isotherm of the adsorbed organic modifier from water solution per unit amount of stationary phase surface can be calculated with the following equation [29]:

$$\Gamma(C) = \frac{1}{S} \int_0^C (V_R(C) - V_M)\, dC \tag{5}$$

where V_R is a retention volume of the perturbation peak, V_M is the thermodynamic void volume of the column, S is a total surface area of the adsorbent in the column and Γ is the excess adsorption isotherm of the analyte [mol/m^2] at concentration C [mol/L].

The maximum adsorbed amount of solvent (C_{ads}) in/on stationary phase can be found by extrapolating the slope of the excess isotherm in a linear region to the y-axis or it can be calculated as the intercept parameter of the straight line fitted to the linear region of the excess isotherm [29].

The excess adsorbed amount of solvent was calculated per volume of the stationary phase in the column. This assumption is commonly used by Guiochon in the comparison of adsorption phenomena on the different bonded phases [43–46]. However, used silica gels posses different specific surface area but the highest relative difference is up to 10%.

It is known in the literature the methodology for calculation the surface area in the column [47] but it need data from low temperature nitrogen adsorption which in not available without destroying the column. Also the methodology presented by

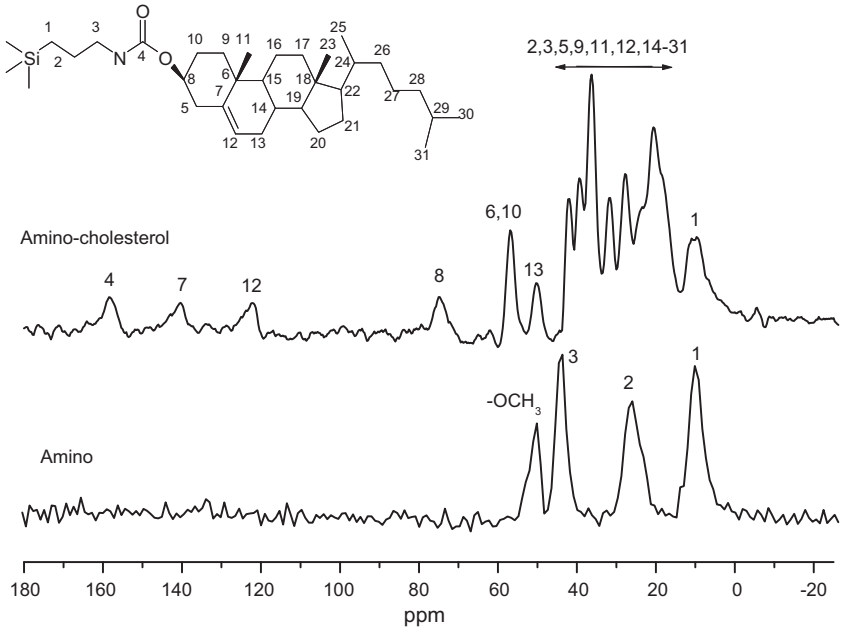

Fig. 2. ^{13}C CP-MAS-NMR spectra of the Amino and Amino-cholesterol bonded phases.

Guiochon [48] need data about the density of bonded ligand s and this calculation in the case of different cholesterol packing will be obtained with significant error. Thus we assume the calculation of excess isotherms per volume of the packing material as a most credible.

3. Result and discussion

3.1. Stationary phase characterization

The carbon loads of the tested phases are from 12.10% for UDC Cholesterol to 22.39% for Diamino-cholesterol. The coverage densities calculated on basis of carbon load are in the range 1.5 μmol/m^2 for UDC Cholesterol up to 2.28 μmol/m^2 for the Diamino-cholesterol bonded phase. Bonded ligands contain spacers with different numbers of carbon atoms which causes irregularity in the comparison of carbon loads and the coverage density of the cholesterol ligands (Table 2).

The structures of the Amino-cholesterol and Diamino-cholesterol bonded phases were confirmed using ^{13}C cross polarization (CP) MAS-NMR spectroscopy. Fig. 2 displays the spectra obtained for the Amino phase and Amino-cholesterol bonded phase.

The carbon atoms of the bonded ligands for each observed signal have been marked. In the spectrum of the Amino phase a signal for the methoxy group (δ = 50.0 ppm) from the methoxysilane is observed. These groups are hydrolyzed during second step of the reaction. The carbon atom connected to silicon gives a signal indicated by peak at δ = 11 ppm.

The absence of bands at δ = 2.5 ppm proved the presence of a polymeric-type of the stationary phase. The signal at δ = 156 ppm corresponds to the carbon atom in the carbonyl groups and signals at δ = 140 ppm and δ = 121 ppm represents carbons connected by a double bond in ligand deposition. The carbon atom connected with the hydroxyl group in the cholesterol molecule has a chemical shift of δ = 74 ppm. Signals in the range δ = 19–57 ppm correspond to the cholesterol moiety bonded to the aminoalkyl ligands. The NMR analysis confirms the presence of the expected functional groups in the structure of bonded ligands, similar to that presented earlier [3,49].

3.2. Excess adsorption of solvent

Acetonitrile forms a thick adsorbed layer on the hydrophobic surface of the stationary phase. As it has been discussed in the literature, acetonitrile is present at an equivalent of four or five molecular layers. Methanol, however, forms only a monomolecular adsorbed layer on the same surface [29]. The location of the maximum on the excess isotherm for acetonitrile shifted towards higher concentration of the organic modifier than in the case of methanol. This behavior is observed for all the tested stationary phases. If the cholesterol molecule in all tested stationary phases is the same, the differences in the solvation processes have to be connected with the structure of the linkage molecule and with the surface of the silica support.

In Fig. 3, the excess isotherms for acetonitrile are shown. The shape of the curves is similar for all the tested stationary phases, with a maximum around 40% of acetonitrile in the mobile phase. Every isotherm has a negative part at high concentration of acetonitrile in the mobile phase. This phenomenon is caused by the excess adsorption of water. Acetonitrile can interact with residual silanols via dipole–dipole interactions which are much weaker than

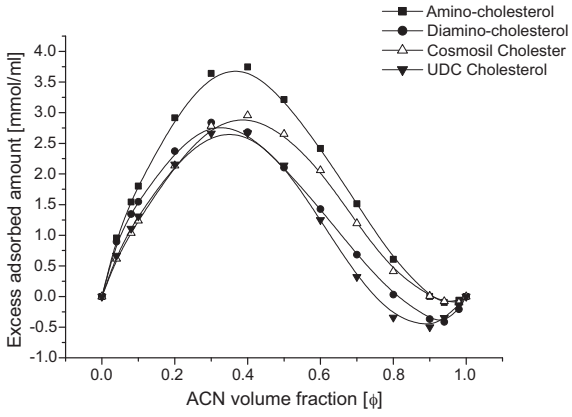

Fig. 3. Excess isotherm of acetonitrile on the series of cholesterol bonded phases calculated per volume of the packing material in the column.

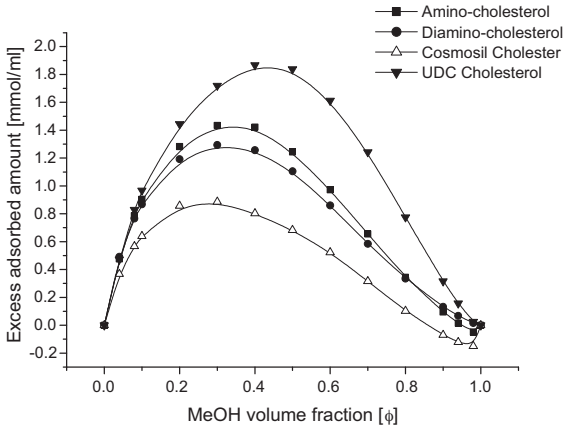

Fig. 4. Excess isotherm of methanol on the series of cholesterol bonded phases calculated per volume of the packing material in the column.

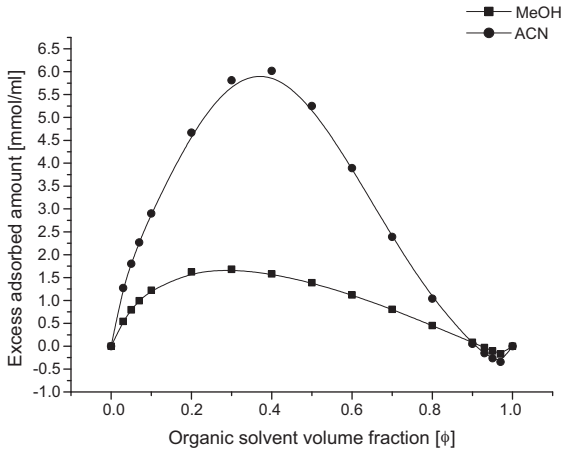

Fig. 5. Excess isotherm of methanol and acetonitrile on the nonend-capped C18 bonded phases with coverage density 3.27 μmol/m^2 calculated per volume of the packing material in the column (data according to Ref. [16]).

the hydrogen bonds created between silanols and water molecules. The water excess adsorption on residual silanols is higher when the amount of water molecules in the mobile phase decreases. The highest excess adsorption of ACN is observed on the Amino-cholesterol phase. On the other stationary phases adsorption is similar and lower by about 30%.

In Fig. 4, the excess isotherms of methanol on the series of cholesterol bonded phases are presented. All the curves behave in a similar manner almost without a negative excess. This indicates that the preferential type of interaction is hydrophobic [42]. The highest negative part is observed for the Cosmosil Cholester column. However, a significant amount of methanol may be adsorbed near the silica surface through the hydrogen bonding with residual accessible silanols [30]. The negative part of the excess isotherms which corresponds to water adsorption is really small or absent. It suggests that residual silanols are covered with methanol molecules, not water. The stationary phases also contain different polar groups (electronegative atoms e.g. nitrogen, oxygen) in the linkage of the cholesterol molecules which can establish preferential interactions with solvent molecules. Recent measurements confirms, that methanol may interact with residual silanols even the coverage density of bonded phase is high [50]. High surface coverage hamper penetration of methanol molecules to the silica surface which is observed on the Cosmosil Cholester column (the highest coverage and the highest water adsorption on the silanols). Additionally, the presence of polar groups in the linkages and amino ligands make the penetration between ligands more easy.

Hydrogen bonding can also be established between water and methanol molecules. The preferential adsorption of water molecules from a methanol–water mobile phase is observed as a negative part of the excess isotherm but it is significant only on the Cosmosil Cholester stationary phase. Relatively lower methanol adsorption on this stationary phase may be connected with relatively high coverage density of bonded ligands which shield the residual silanols. Cholesterol molecules in high density are likely to retain liquid crystal properties which may hamper penetration of the solvent molecules to the silica gel surface and they adsorb mainly on the top of the bonded phase. A lower coverage density of bonded ligands implies relatively better solvation of the ligands by solvent molecules. This phenomenon was also confirmed by a modeling study [51] and microcalorimetric measurements [50].

The highest excess adsorption of MeOH is observed on the UDC Cholesterol phase. The adsorption on Amino- and Diamino-cholesterol phase is lower than 30%. The lowest adsorption (of about 60% than on UDC Cholesterol) is observed on the Cosmosil Cholester bonded phase.

The tested stationary phases contain different types of linkages connecting the cholesterol molecule to the surface. In these spacers oxygen and nitrogen atoms are presented. All phases contain one or two oxygen atoms (carboxyl or ether group). These atoms may be a hydrogen bond acceptor in contact with methanol and water molecules. Cholesterol phases prepared using an amino linkage contains nitrogen atoms. The nitrogen atoms in the amino groups have attached hydrogen and therefore they may function as both a hydrogen bond donor and a hydrogen bond acceptor.

Another property which can differentiate the tested cholesterol phases is structure of the silica surface. The UDC column (Fig. 1D) is prepared on silica hydride. This eliminates silanols as an adsorption center. In attached ligands there are no oxygen atoms on the modified surface, only direct bonds between carbon and silicon atoms. However, the adsorption of MeOH on this stationary phase is relatively higher than on the other cholesterol phases under study.

In Fig. 5 the excess isotherms of methanol and acetonitrile on the octadecyl bonded phase are presented. Adsorption of acetonitrile on the C18 phase with coverage density 3.27 μmol/m^2 is much higher (about 50%) whereas adsorption of methanol is similar than on the cholesterol stationary phases. The adsorption of acetonitrile indicates that on the C18 phases the hydrophobic interactions are preferential and that the C18 phase is more hydrophobic than cholesterol packings. The small negative part of the acetonitrile excess isotherm shows that on the C18 phase there are no so much polar adsorption centers available for interactions with water molecules.

Excess adsorption of methanol on the C18 phase is lower than on UDC Cholesterol column and higher than on other cholesterol packings. There is no so much difference than in the case of acetonitrile. There is no negative part which suggests stronger adsorption of methanol than of water on the silanols. In the case of cholesterol packings the adsorption of water is observed using methanol as an organic solvent.

3.3. *Influence of the coverage density and length of the spacer*

In Fig. 6A the changes of the adsorbed solvents with the coverage density of cholesterol ligands are presented. It shows that the amount of excessive adsorbed solvent is not a function of coverage density of bonded ligands. It confirms that the silica support and the polar group in the linkage strongly influence solvation of the stationary phase. However, excess adsorption is not solely a property of preferential solvation caused by polar and hydrophobic interactions. Another possibility is that solvent molecules can penetrate between the bonded ligands.

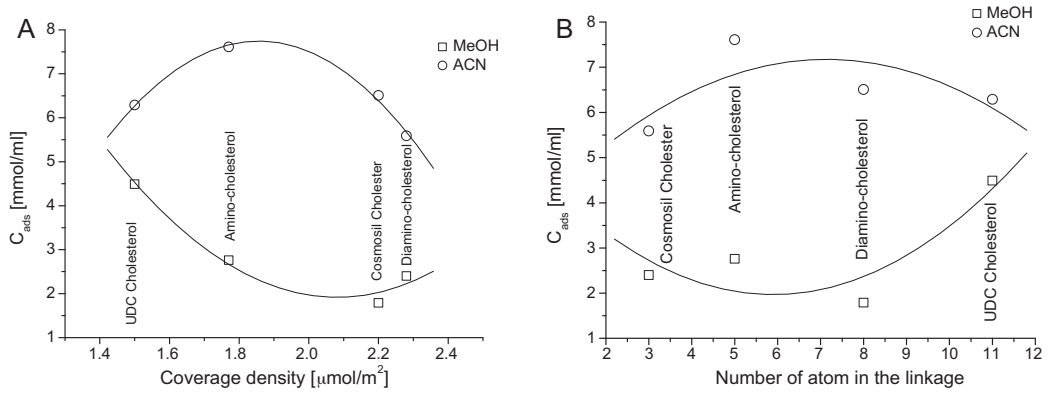

Fig. 6. Changes of the maximum amount of adsorbed solvent (C_{ads}) with coverage density of cholesterol ligands (A) and number of atoms in the spacer (B).

Similar results are obtained when the maximum concentration of adsorbed solvent is plotted against number of atoms in the linkage (Fig. 6B). Despite the lower coverage density in the case of the UDC Cholesterol stationary phase the adsorption of both solvents is higher than on the high coverage density Cosmosil Cholester phase. This may be explained by the increased length of linkage from 3 to 11 carbon atoms. The relatively higher increase of methanol adsorption is probably caused by presence of the carboxyl group in the UDC Cholesterol phase.

3.4. Comparison between amino and cholesterol phases

As seen in Figs. 7 and 8, the excess amount of both solvents increases after the second step of the derivatization of the Amino and Diamino phase with cholesterol ligands. It is accompanied by a large increase of carbon load and increase of the stationary phase hydrophobicity.

Cholesterol molecules attached to the silica gel using a longer linkage have a greater possibility of conformational changes. If cholesterol ligands are collapsed on the silica gel surface, the residual silanols will be totally covered by the ligands and the migration of water and organic solvent molecules between ligands will be more difficult.

The high polarity of the Amino and Diamino bonded phases is caused by the presence of nitrogen atoms in the ligand structure. Due to the low hydrophobicity of these phases (3 or 5 carbon atoms) strong adsorption of water is observed. The highest excess of water adsorption is observed on the Diamino phase which contains two amino groups on the five-carbon chain.

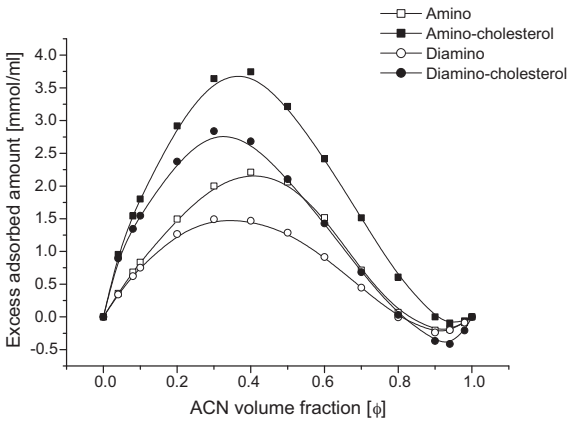

Fig. 7. Comparison of the acetonitrile excess isotherms on the amino-type and cholesterol-type packings calculated per volume of the packing material.

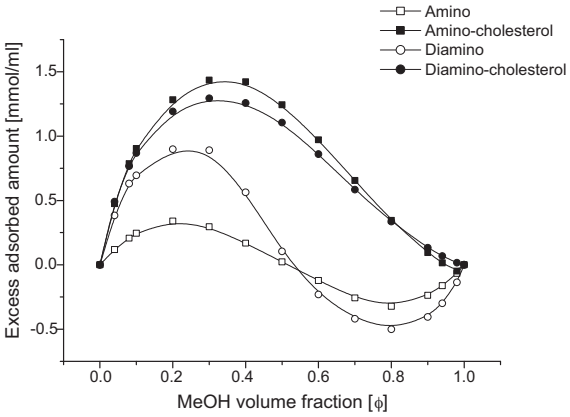

Fig. 8. Comparison of the methanol excess isotherms on the amino-type and cholesterol-type packings calculated per volume of the packing material.

3.5. Relative adsorption of solvents

A comparison of the maximum amount of solvent excess extracted from the mobile phase is presented in Table 3. Values are calculated as an amount of solvent per volume of the packing material in the column. The ratio ACN/MeOH was calculated in order to compare the relative adsorption of both solvents. A higher value reflects preferential adsorption of acetonitrile and a lower value demonstrates preferential adsorption of methanol.

As expected the Amino-cholesterol and Diamino-cholesterol phases exhibit a similar ratio of ACN/MeOH which can be connected with analogous adsorption mechanism. However, the adsorption of methanol on the Diamino-cholesterol phase is slightly higher. This fact is connected with the structure of the Diamino linkage. The presence of the extra amino group in the ligands causes preferential adsorption of methanol via hydrogen bonds with amino groups. These interactions are much stronger than possible dipole–dipole interactions of the acetonitrile molecule with polar group.

As was discussed above, on the UDC Cholesterol phase preferential adsorption of methanol is observed. Relative adsorption

Table 3
Comparison of the maximum amount of adsorbed solvents on the stationary phases calculated per volume of the adsorbent in the column.

Stationary phase	C_{ads}^{MeOH} [mmol/ml]	C_{ads}^{ACN} [mmol/ml]	Ratio ACN/MeOH
Amino-cholesterol	2.76	7.61	2.75
Diamino-cholesterol	2.40	5.59	2.33
UDC Cholesterol	4.49	6.29	1.40
Cosmosil Cholester	1.79	6.51	3.64

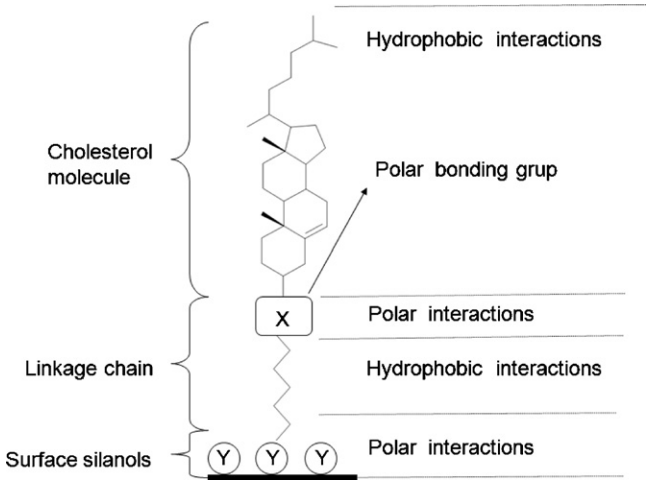

Fig. 9. Model of solvation on the cholesterol bonded phases.

of methanol is the strongest on this stationary phase which can be connected with solvation of the hydride surface of the silica support. On the other hand relatively low methanol adsorption is observed on the Cosmosil Cholester bonded phase. It can be explain by fact that in the structure of this phase only one oxygen atom is present. This limits the possibility for hydrogen bonding between methanol and the stationary phase.

3.6. Model of cholesterol phase solvation

Based on the obtained results, Fig. 9 presents a theoretical model of cholesterol bonded phase solvation. In the structure of bonded ligands four solvation zones may be indentified. First starting from the bottom is the silica surface with polar residual silanols (Y in Fig. 9). On this surface the polar interactions (hydrogen bonds) are preferential thus it is an adsorption zone of water and methanol. The linkage chain may be divided into two zones. On the carbon chain hydrophobic interactions are dominant and the polar bonding group (X = amide, ether or ester) interacts with mobile phase components (MeOH and water) via polar interactions.

The cholesterol molecule is a hydrophobic adsorption center. It is preferentially solvated by organic modifiers via hydrophobic interaction and specific π–π interaction in the case of acetonitrile in the mobile phase. There are two types of adsorption centers in the bonded phase: hydrophobic and polar excess adsorption of solvents from the mobile phase. Excess adsorption of acetonitrile is connected with solvation of the cholesterol moiety and the hydrophobic part of the linkage whereas excess adsorption of methanol may take place on the all units of the bonded phase. Excess adsorption of water is observed mostly in acetonitrile-water mobile phases and is connected with solvation of the silica surface and the polar bonding group. However, differences of coverage density, length of the linkage and type of polar bonding group causes differences in the relative values of excessively adsorbed solvents. The presented model shows that the structure of the cholesterol bonded phase has similar properties to immobilized artificial membranes and thus it can be used for modeling of the process which takes place on the natural cell membrane.

The proposed structure of the solvated stationary phase contains layers of preferentially adsorbed solvents. The solvent layers result in the stability of bonded ligands over a wide range of mobile phase composition, including extremely high water concentrations.

4. Conclusions

Solvation processes on the four different cholesterol bonded phases in methanol and acetonitrile environments were investigated. Adsorption of acetonitrile is higher than adsorption of methanol. However, methanol adsorbs relatively strongly on the UDC Cholesterol bonded phase prepared by a hydrosilation procedure. Relatively low methanol adsorption is observed on the Cosmosil Cholester bonded phase which has the highest surface coverage. The presence of a polar group (electronegative atoms) has a significant influence on solvent adsorption. These groups interact via hydrogen bonds with methanol and water molecules and their interactions with acetonitrile are rather weak. The presence of a carboxyl group causes an increase of methanol adsorption whereas an increase of stationary phase hydrophobicity increases adsorption of acetonitrile.

Preparation of the cholesterol phase via modification of the corresponding amino phase causes an increase in adsorption of the both organic solvents. Simultaneously, a decrease of water adsorption is observed. This phenomenon is connected with an increase of the stationary phase hydrophobicity and reduction of the amino group polarity in the stationary phase.

The properties (silica support, linkage type) of the series of cholesterol packings have a significant influence on the retention and separation selectivity of solutes in methanol–water and acetonitrile–water conditions.

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