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Modification and Evaluation of Non-Polar Sorbents for Solid-Phase Extraction

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Abstract: Modification of packings for solid-phase extraction through high-frequency low-temperature plasma treatment is a new method to obtain a wide variety of sorbents. This method is still in the process of investigation and application. In this study, several commercial sorbents have been modified in high-frequency low-temperature plasma with the mixed hydrogen/benzene system. The solvation parameter model is used to characterize the sorption properties of commercially available and plasma treated sorbents for solid-phase extraction. Chromatographic test mixtures from the literature were used to probe hydrophobicity, free silanol interactions and shape selectivity of the sorbents. The physical and retention properties of the plasma treated sorbents indicate their suitability to be used in solid-phase extraction. Some suggestions concerning the possible practical application of the newly obtained sorbents have been made.

Keywords: Bonded phases, plasma modification, solid-phase extraction, sorbents

INTRODUCTION

Solid-phase extraction (SPE) is likely the most used method for the sample enrichment prior to separation and detection (1).

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Among the types of high performance liquid chromatography (HPLC) and SPE, it is those employing reversed-phase stationary phases that are the most useful (2, 3). Since the majority of phases for HPLC and SPE consist of silica as the support material, the bonding of various organic moieties to the substrate is based on the chemistry of the silanols on the surface. More importantly, silica contains several types of surface silanols (i.e. free silanol, associated silanols and geminal silanols) (4) that provide the necessary functionality for chemical derivatization to be attained. Different synthetic methods may be employed for the modification of silica surface (5–12). Alkylation of the silica surface for chromatographic purposes is usually carried out using well-established silane coupling agent chemistry (5, 6) and a chlorination/Grignard reaction (7–9). The first approach is readily used, while the second is more tedious and time consuming, but it could lead to synthesis of new phases, which might be difficult to prepare using organosilane reagents. There are some other methods to obtain or modify sorbents for HPLC and SPE described in the contemporary scientific literature (10–12). Hayrapetyan and coworkers (11) synthesized packings for HPLC based on the polymer modification of the surface of wide-pore microspherical silica gel obtained by means of hydrothermal treatment of the mesoporous silica gel under autoclave conditions. Pesek and Matyska (12) reviewed the general approach for fabricating the silica hydride and subsequent bonded phases.

The most important factor that determines the applicability of bonded silica phases in SPE is the presence of silanols. These groups are responsible for the residual silanol activity of bonded silicas, especially for basic compounds, which complicates the retention (13). Clearly, large improvements in the selectivity and stability of chromatographic stationary phases can be realized by the development of a more efficient preparation process.

There has been an increased interest in the use of plasma treatment for polymerization of a number of organic compounds. Plasma polymerization could be used as an alternative method of chromatographic packing synthesis (14, 15). It is a powerful technique for depositing thin films on substrates. The films formed are uniform, crosslinked, and structured in branched networks. Their character depends on the plasma polymerization parameters. These parameters are plasma power, gas flow rate, and the monomer pressure during plasma polymerization. Furthermore, because almost all organic compounds can be polymerized by this technique, many different types of packing can be prepared. These packings are not commercially available.

Recently, the plasma modification method has been also applied to obtain sorbents for SPE (16). The results were encouraging in respect to the change in the sorption properties of the sorbents compared to the raw materials. In this way, the plasma modification method may widen the number and choice of specific and selective sorbents for SPE.

The linear solvation free energy relationship (LSER) have been used to investigate the nature of the sorption properties of sorbents. All basic features of this method have been thoroughly presented together with a large number of examples (17–20). This method was widely used to factorize some given properties of a solute (e.g. log k_s) in terms of structural parameters and the so-called solvatochromic parameters. A solvation property of interest (*log* k_s) for an organic solute is modeled by a linear free energy relationship of the form:

$$\log k_s = c + mV_x/100 + rR_2 + s\pi_2^H + a\alpha_2^H + b\beta_2^H \quad (1)$$

where k_s is the capacity factor for a series of solutes in a given stationary phase-mobile phase system, V_x is the solute's characteristic volume, R_2 is the solute's excess molar refraction, π_2^H is a measure of the solute's ability to stabilize a neighbouring dipole by virtue of its capacity for orientation and induction interactions, and α_2^H and β_2^H are parameters characterizing the solute's hydrogen-bond donor acidity and hydrogen-bond acceptor basicity, respectively. The coefficients in Eq. (1) are found by the method of multiple linear regression analysis and serve to characterize the phase as follows. In conventional reversed-phase liquid chromatography (RPLC), the leading and master terms affecting k_s are the hydrophobicity (*m*) and the ability of solute to accept a hydrogen bond (*b*). The propensity of the phase to interact with solute π-and n- electron pairs (*r*), the contributions of the dipolarity–polarizability term, (*s*) and the ability of solute to donate a hydrogen bond (*a*) are small and negligible. These coefficients are characteristic of the chromatographic system and are independent of the solute. In this work, test solutes based on those described by Mayer et al. (20) were used to characterize two commercially available non-polar sorbents as well as six plasma modified sorbents.

Hierarchical cluster analysis (HCA) is used when large, complex data sets have to be investigated. It is a statistical technique used for exploratory data analysis. Cluster analysis was already involved in HPLC column classification studies (21, 22).

The aim of this study is to evaluate the sorption properties of the modified non-polar sorbents and find out the contribution of plasma modification in reliance to these properties. Besides, the additional aim of this study is to classify the newly obtained sorbents according to their sorption properties. In addition to this, the actual applications of the newly obtained sorbents for SPE will be also studied.

EXPERIMENTAL

Reagents and Materials

The solutes were obtained from commercial sources (Merck, Darmstadt, Germany; Fluka, Buchs, Switzerland; Riedel-de Haen, Seelze, Germany) and in the highest available purity. Other chemical reagents and solvents were obtained from commercial sources and were of reagent grade and higher. Analytical grade methanol was purchased from the Institute for Pure Chemicals (Sofia, Bulgaria). Distilled water was used throughout the study.

A series of packing materials with various chains chemically bonded to a silica matrix were obtained by unpacking the new cartridges of Bond Elut SPE Application Development Kit supplied by Varian Sample Preparation Products (Harbor City, CA, USA). They include ethyl, cyanopropyl, cyclohexyl, phenyl, and octadecyl bonded silicas. According to the manufacturer, they have the following characteristics: 30–60 μm particle size distribution with 40 μm mean particle size; porosity: 60 Å; shape: irregular. The sorbents are endcapped and have typical carbon loading of: ethyl −5.6%, cyanopropyl −8.1%, cyclohexyl −9.6%, etc phenyl −10.7%, and octadecyl −17%. Bulk octadecyl silica (Bakerbond SPE C18) supplied by J.T.Baker (a division of Mallinckrodt Baker, Inc., Deventer, The Netherlands) has similar characteristics, namely 40 μm irregularly shaped silica, 60 Å mean porosity, endcapped, and it has carbon loading of 17.2%.

These sorbents were the object of the plasma modification study.

Plasma Polymerization

Prior to plasma treatment, the silica samples were immersed in an organic compound used as monomer in the plasma reaction. Thus, efficient penetration of the monomer into the silica pores was ensured. Benzene was of analytical grade and was used as monomer without further purification. The silica samples were dried overnight. This dried sample was loaded into the upper reactor container. The plasma polymerization apparatus used in this study has been described previously (15). The apparatus consisted of a tubular reactor made of glass (750 mm × 30 mm) equipped with the pairs of ring electrodes (Fig. 1). A radio-frequency generator (RFG) (13.5 MHz) was used for the plasma polymerization reactions. The radio-frequency power was capacitively coupled to the plasma reactor. The reduced pressure (0.3 Torr) in the apparatus was maintained by means of a rotary vacuum pump. The flow rate of the monomer vapors was adjusted by a needle valve. The flow rate of the discharge gas was controlled by a flow meter. Hydrogen was used as discharge gas. The monomer vapors were continuously introduced into the reactor during the plasma process and pressure was maintained at 0.3 Torr. When the system reached steady state at the desired flow rate and pressure, the power was turned on, and glow-discharge polymerisation was initiated and continued at a level of 40 W power input. Exposure time was 40 s. and all other operational parameters were kept constant. Sorbent particles were fed to the reactor through the container which contained a sieve. The sieve served as a distribution plate. Thus, an effective particle exposure was achieved. Effluent sorbent particles from the reactor were

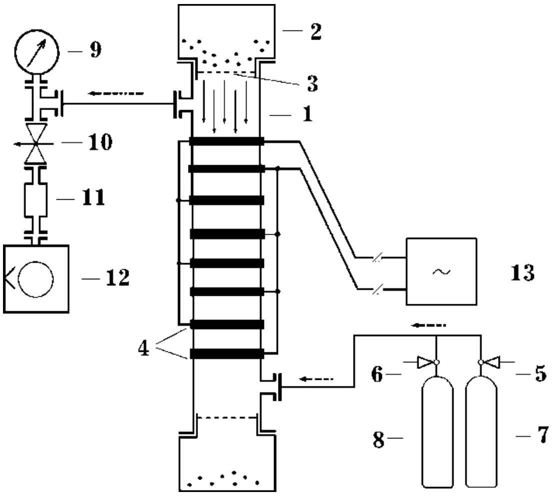

Figure 1. Schematic of the plasma apparatus. 1 – Plasma reactor; 2 – Container; 3 – Sieve; 4 – Ring electrodes; 5, 6, 10 – Vacuum valves; 7, 8 – Gas supplies; 9 – Pressure gauge; 11 – Trap; 12 – Rotary vacuum pump; 13 – Radio-Frequency Generator.

collected in a lower container by gravitation. After plasma treatment, the packings were extracted in a Soxhlet consecutively with hexane and methanol for 24 h each to remove the impurities. Table 1 lists the numerical notation and the preparation method of each sorbent.

Instrumentation and Chromatographic Conditions

HPLC analyses were performed on a Waters model Delta Prep 3000 HPLC equipped with a Waters model System Controler 3000, a Rheodyne model 7125 injector with 20 μL loop and Knauer variable wavelength UV

Table 1. Preparation method of sorbents

Sorbent	Preparation method
C2	Plasma-treated Ethyl bonded silica from Bond Elut cartridge.
CN	Plasma-treated Cyanopropyl bonded silica from Bond Elut cartridge.
CH	Plasma-treated Cyclohexyl bonded silica from Bond Elut cartridge.
PH	Plasma-treated Phenyl bonded silica from Bond Elut cartridge.
VC18	Plasma-treated Octadecyl bonded silica from Bond Elut cartridge.
BC18	Plasma-treated Bekerbond Octadecyl silica.
CN*	Cyanopropyl bonded silica from Bond Elut cartridge.
VC18*	Octadecyl bonded silica from Bond Elut cartridge.

detector. The UV detector was operated at 254 nm. Elution was performed at room temperature using a flow rate of $1.0\ \mathrm{ml} \cdot \mathrm{min}^{-1}$. Chromatograms were recorded using a Star ADC Board Kit running under Star Chromatography Software Rev. 4.1. The bonded and plasma treated phases were dry packed by the tap-and fill method into 250 × 2.6 mm i.d. column blank. The column was preconditioned by flushing overnight with methanol at a flow rate of $1\ \mathrm{ml}\ \mathrm{min}^{-1}$. The void volume was determined using sodium nitrate as an unretained solute. Each run was repeated at least three times.

Calculations

The solute capacity factor (k_s) was derived from the equation:

$$k_s = \frac{t_\mathrm{R} - t_0}{t_0} \tag{2}$$

where t_R is the solute retention time, t_0 is the retention time for an unretained solute.

The total porosity of a column can be determined by the equation:

$$\varepsilon_t = \frac{F_v t_0}{\pi r^2 L} \tag{3}$$

where F_v is the volumetric flow rate through the column, t_0 is the retention time of an unretained substance, r is the internal column radius, and L is the column length. Injection of sodium nitrate at a concentration of $26\ \mathrm{mg}\ \mathrm{ml}^{-1}$ was used to determine the total porosity. For the interparticle porosity, an injection of $1\ \mathrm{mg}\ \mathrm{ml}^{-1}$ sodium nitrate was used. Intraparticle porosity is presented by the difference between the total and interparticle porosity values (17).

The least mean squares regressions fits and hierarchical cluster analyses were executed with Statistica 5.0 software (StatSoft, Tulsa, USA). Excel program was used in the other calculations.

RESULTS AND DISCUSSION

Packing Density

The typical SPE columns have low packing density, due to a relatively large particle size distribution (40 μm on average) compared to analytical HPLC columns. As it could be seen from Table 2, with the increase of the carbon content of the commercial sorbent, the mass in grams packed in the column also increases. Since the geometric volume of the column is the same in all cases, the packing density normally decreases in the following order: BC18 > VC18* > VC18 > CN* > CN > PH > CH > C2. The lowest degree of

Table 2. Packing density

Sorbent	Weight of packing (g)	Packing density (g/cm^3)
C2	0.8051	0.9307
CN	0.9475	1.0953
CH	0.9024	1.0431
PH	0.9112	1.0533
VC18	0.9846	1.1382
BC18	1.0017	1.1579
CN*	0.8702	1.0059
VC18*	0.9871	1.1411

packing density is observed at the ethyl plasma treated sorbent. The plasma treated cyanopropyl and octadecyl packings manifest packing density values very close to the respective values of plasma non-treated sorbents.

Porosity

The total and interparticle porosities have been established for each of the studied columns at mobile phase methanol/water (50:50 v/v). The data about the total porosity of the column (ε_t), the interparticle porosity (the empty volume among the particles) (ε_u) and intraparticle porosity (ε_i) at mobile phase methanol/water (50:50 v/v) are presented in Table 3. Injection of sodium nitrate at concentrations of 26 mg ml-1 and 1 mg ml-1 was used to determine the total and interparticle porosity, respectively. The hold-up times of sodium nitrate solutions were determined one by one at the apex of the respective chromatographic peaks. The intraparticle porosity is presented by the difference between the total and interparticle porosity values (17). Considering the data presented in the table, the values of the porosity of

Table 3. Sorbent porosity at mobile phase methanol/water (50:50 v/v)

Sorbent	Total porosity ε_t	Interparticle porosity ε_u	Intraparticle porosity ε_i
C_2	0.902	0.805	0.097
CN	0.997	0.963	0.034
CH	0.991	0.871	0.121
PH	0.853	0.770	0.083
VC18	0.908	0.831	0.077
BC18	0.831	0.722	0.109
CN*	0.810	0.667	0.143
VC18*	1.012	0.840	0.172

intraparticles ε_i are of interest. The highest values have been obtained for octadecyl and cyanopropyl bonded silica gels (VC18* and CN*). According to the manufacturer's data, the diameter of the pores for both sorbents is 60 Å. The porosity of the particles of plasma treated sorbents is approximately twice lower. This refers mostly to cyanopropyl plasma treated sample, where this decrease is even more significant. Thus, we may assume that the plasma reaction leads to a decrease in ε_i. However, it is obvious that the volume of VC18* pores is larger compared to the one of VC18 packing. The latter has been produced based on the same silica gel with defined pore volume and radius, which allows us to contribute the obtained differences to the plasma treatment. Still, the plasma treated packings have similar porosity values. Probably, the similar values could be explained only by the fact that the mixed hydrogen/benzene system is used in all plasma experiments at constant conditions. In comparison to this result, the porosity value of ε_i particles at the cyanopropyl plasma treated sample (the lowest one in the order of Bondesil derivatives) looks unusual. Evidently, the obtained sorbent is quite different form the other sorbents and this is because the bed contains cyano groups, whose presence changes the plasma reaction.

Capacity Factors

The data about the values of the studied solutes capacities, obtained upon columns, packed with solid-phase sorbent, at composition of mobile phase methanol/water (50:50 v/v) are represented in Table 4. The capacity factors (k_s) were calculated according to Eq. (2). A hold-up time (t_0) for sodium nitrate at concentration of 1 mg ml^{-1} was used in calculations. The samples of bonded cyanopropyl (CN*) and octadecyl (VC18*) groups are comparative in respect of the plasma-modified sorbents.

The highest retention of the test compounds is observed on the columns packed with octadecyl bonded silica gel and octadecyl plasma-modified sorbents. It must be noted that in this case the plasma treated Bondesil sample (VC18) retains more strongly the analyzed compounds compared to the plasma non-treated one (VC18*). The highest capacity values are observed at Bakerbond octadecyl modified silica gel (BC18). With the decrease of the carbon content on the surface of the studied sorbents, the capacity factors normally decrease in the order: BC18 > VC18 > VC18* > CH > PH > C2 > CN* > CN. This is in accordance with the decrease of the non-polar component of the solute-sorbent interaction. The reversion at C2 and CN may be because the cyano group in the cyanopropyl sorbents introduces a specific component into the solute/sorbent interaction, which makes the retention weaker at CN modified sorbents.

2-Chlorophenol retains upon all studied columns stronger than phenol and weaker than chlorobenzene. The difference, in the first case, is due to the chlorine atom. Having in mind that chlorine is an electron acceptor, its

Table 4. Capacity factors (k_s) for sorbents studied

	Sorbent							
Test Compound	C2	CN	CH	PH	VC18	BC18	CN*	VC18*
2-Pentanone	0.509	0.181	1.041	1.005	1.528	2.410	0.239	0.999
3-Pentanone	0.495	0.227	1.032	1.005	1.540	2.265	0.285	1.019
2-Hexanone	0.688	0.261	1.880	1.474	3.336	4.920	0.378	1.538
Phenol	0.414	0.313	1.101	0.881	1.460	1.907	0.490	0.924
Caffeine	0.398	0.277	0.640	3.144	0.698	0.926	0.357	0.428
1-Phenylethanol	0.577	0.311	1.722	1.206	2.437	3.143	0.447	1.551
Benzaldehyde	0.684	0.469	1.740	1.651	2.688	3.327	0.567	1.702
Benzamide	0.338	0.240	0.620	0.662	0.613	0.764	0.388	0.376
Acetanilide	0.491	0.356	1.227	1.086	1.352	1.693	0.487	0.833
Acetylaceton	0.444	0.261	0.779	0.816	1.761	0.995	0.300	1.268
Benzonitrile	0.766	0.466	1.897	1.907	2.683	3.314	0.672	1.692
Anisole	0.891	0.581	3.059	2.359	8.332	9.258	0.873	4.845
Acetophenone	0.760	0.472	2.160	2.011	3.503	3.926	0.695	2.063
Benzyl alcohol	0.436	0.273	1.073	0.856	1.422	1.791	0.386	0.897
o-Cresol	0.615	0.416	1.824	1.304	3.302	3.828	0.637	1.943
p-Cresol	0.595	0.375	1.716	1.297	3.022	3.459	0.596	1.721
2-Chlorophenol	0.647	0.494	2.012	1.493	3.375	3.506	0.756	2.018
Chlorobenzene	1.396	0.811	5.582	3.351	18.402	21.065	1.271	10.653
Bromobenzene	1.561	0.921	6.832	4.283	22.749	28.278	1.418	13.007
Nitrobenzene	0.868	0.703	2.616	2.559	5.224	5.730	0.961	3.343
Benzene	0.852	0.540	2.874	1.942	8.143	9.328	0.794	5.046
Toluene	0.851	0.691	5.001	3.035	18.228	21.227	1.022	10.677
Ethylbenzene	1.832	0.797	9.212	4.794	36.467	43.797	1.313	20.608
Propylbenzene	3.075	1.056	17.894	7.854	83.128	97.343	1.724	42.766
Butylbenzene	4.776	1.326	35.168	13.150	—	—	2.445	—
Pentylbenzene	7.883	1.671	70.373	22.499	—	—	3.346	—
Naphthalene	1.990	1.362	9.113	5.907	39.980	47.305	2.132	20.802
Nitronaphthalene	2.635	1.654	9.195	8.771	25.985	29.623	2.420	13.808
Anthracene	5.557	3.314	30.952	19.789	—	—	6.015	—
1,3-Dibromo benzene	2.223	1.134	11.050	5.673	46.490	56.713	1.826	26.292
1,4-Dibromo benzene	2.011	1.058	9.751	5.082	38.468	45.519	1.705	21.054

presence polarizes the 2-chlorophenol molecules to a higher extent and therefore the polar interactions are preferred. At all studied columns, chlorobenzene retains weaker than bromobenzene. There is no significant difference in the behavior of this test pair in the different columns. Nevertheless, the differences at the sorbents with a higher carbon content are well established, whereas the cyanopropyl modified sorbents retain chlorobenzene and bromobenzene at the same degree. It is known that, the bromine atom is heavier and more polarizable than the one of chlorine, whereas chlorine is more electronegative, which determines the behavior of test compounds.

Nearly all benzene derivatives retain considerably weaker than benzene itself, except the halogen derivatives and alkyl benzenes. The retention times of nitrobenzene are smaller compared to these of benzene upon the octadecyl modified sorbents (VC18, VC18* and BC18). The case is quite the opposite for phenyl, cyclohexyl, ethyl, and cyanopropyl modified samples, where nitrobenzene retains stronger. We may deduct the conclusion that the cyano-group, with its nitrogen atoms determines higher retention because of the possibility for specific interaction with the aromatic system. Within the group of substituted alkyl benzenes, the retention increases with respect to the number of the carbons in the chain of the substitute and reaches its maximum at pentylbenzene. Since the alkyl substitutes possess a weak induction effect, if it is accepted that the polar component of interaction is due only to the aromatic system, the increase of retention in the homologue order may be entirely attributed to the increase in the non-polar component, which is dominating the retention. This dependency is valid mostly for the octadecyl modified sorbents, and it is weaker for the ethyl and cyanopropyl samples.

A significant difference in the retention at 1,3-dibromobenzene and 1,4-dibromobenzene is not observed at the cyanopropyl and ethyl bonded silics. To a certain degree, differences are observed at the packing with phenyl bonded phase. However, with increasing the number of the carbons in the grafted chain, as is the case with cyclohexyl, the difference in retention becomes significant and its maximal value is observed at octadecyl modified sorbents (VC18 and BC18). It is obvious (Table 4) that the cyclohexyl and octadecyl bonded (VC18*) phases have the same ability to separate position isomers compared to the plasma modified octadecyl bonded silicas. Therefore, the reason for the retention difference is probably hidden in the surface chemistry of plasma treated sorbents.

3-Pentanone and 2-pentanone have similar capacity factors in all studied sorbents. Apparently, the difference in the location of the $>C{=}O$ group in both samples does not affect their retention. The picture gets clearer if the value of the capacity factor for 2-hexanone, which is higher, is considered. The molecule of 2-hexanone is similar to the one of 2-pentanone. The difference is the methylene group. Therefore, the presence of this group determines the higher retention of 2-hexanone. Phenol and benzyl alcohol also have similar k_s. While in the above case the presence of the methylene group is of great significance, in this specific case this difference is insignificant. The aromatic system, which both samples possess, in combination with the hydroxyl group, determines the interaction forces for both compounds and their retention is approximately the same.

Acetylacetone (2,4-pentanedione) has the lower retention times upon all studied sorbents. And here the octadecyl bonded silics retain it at the highest degree.

A fact to be mentioned is the increase of the retention of caffeine upon the plasma modified sorbents. This increase is higher at the plasma treated phenyl sorbent. Having in mind the chemical properties of the surface, polar interactions favour retention.

Separation Factors

The values of the separation factor of the test pairs are presented in Table 5. The value of the separation factor α for the test pair caffeine/phenol (α_{SI}) is determined at the composition of mobile phase methanol/water (30:70 v/v). For octadecyl bonded silicas with low silanol activity, the sorbent silanophilic index shall be between 0.4 and 1.29 (23). The results show that almost all commercially available sorbents have values indicating low silanol activity. The exception to this is phenyl plasma treated silica. The high value of the separation factor shows that the phenyl aromatic rings located on the surface contribute to the specific interactions. If it is accepted that the manufacturers use similar techniques of suppressing the silanol activity, the high values of the separation factor for caffeine/phenol at phenyl modified sorbent may be attributed only to the aromatic system. The other higher value of the separation factor may be observed at the ethyl modified sorbent. This fact is not surprising. As it is in the previous case, since the ethyl groups bonded on the surface have the smallest length compared to the other sorbents, which favors stronger specific interactions between the solute and the silanols.

In the order of the studied octadecyl modified sorbents, the differences between the plasma treated packings and the plasma non-treated ones are insignificant. Apart from this, the studied values are the lowest ones among all the other sorbents. The circumstance that the difference between plasma treated and plasma non-treated sorbents is practically none is probably due to the fact that plasma treatment does not affect the silanols.

Table 5. Separation factors α

Sorbent	Caffeine/Phenol α_{SI}	Naphthalene/Nitro-naphthalenea α_{NO2}	n-Pentylbenzene/ n-Butylbenzene α_{CH2}
C2	0.964	0.987	1.215
CN	0.762	0.867	0.953
CH	0.711	1.072	1.451
PH	4.369	0.811	1.280
VC18	0.604	1.475	1.729
BC18	0.607	1.546	1.825
CN*	0.726	0.886	0.920
VC18*	0.618	1.497	1.712

A common goal of treatment of the silica surface is the reduction of the number of residual silanols after the bonded phase has been attached. Residual silanols can be reduced after the main ligand been bound to the surface by endcapping. The separation factor of the pair naphthalene/nitronaphthalene (α_{NO2}) at the mobile phase methanol/water (60:40 v/v) is used in the HLPC (24) for estimating the success of deactivation of bonded C18 packings by endcapping. A separation factor of at least 1.4 or more is characteristic of a well deactivated packing. Lower values between 1.1 and 1.2 are characteristic of a phase without endcapping. This approach fails to give conclusions of similar utility for other types of bonded silicas. The results show that almost all commercially available and plasma modified sorbents have values lower than 1.1. The relatively high α value for octadecyl sorbents (VC18, BC18 and VC18*) may be explained with a deactivation of bonded C18 packings by endcapping. Indeed, according to the producers, they are endcapped. The plasma treated cyanopropyl silica and the non-treated one have similar separation factors. On the one hand, the cyano groups, which are on the silica gel surface, will relate in a similar way to the test compounds of this pair, and on the other hand, the second sample has a plasma covering. The fact that commercial packings have roughly the same values as the plasma treated ones indicate that plasma modification does not affect the degree of deactivation.

Literature data show that sorbents with higher content of grafted organic ligands on the surface have a hydrophobicity index of the test pair pentylbenzene/butylbenzene (α_{CH2}) between 1.3 and 1.5 (determined at mobile phase content of methanol/water 80:20 v/v) (23). As may be seen from the obtained data presented in Table 5, only two samples match the limits mentioned above. These are ethyl and phenyl plasma treated sorbents. The cyanpropyl sorbents, both non-modified and plasma treated, have the lowest α value. Obviously, the presence of cyano groups on the surface determines the mentioned lowest values.

At the octadecyl modified sorbents, the increase at Bondesil packing value is due to the increased carbon content, as it was mentioned when discussing the capacity factors (k_s).

The study of retention and the sorbent/solute interaction sometimes does not lead to a logical result. That is why the use of another approach to the estimation of these interactions is appropriate. One of the variants most often used in the contemporary scientific literature, allowing estimation of the various types of interactions, is the determination of system coefficients in the equation of the solvation parameters.

System Constants from the Equation of Solvation Parameters

The solvatochromic parameters of the test solutes were collected from the literature (20). Application of the LSER model to k_s was performed by multifactor least-squares regressions to Eq. (1). The results are summarized in Table 6, and the following conclusions could be drawn.

In general, the statistical measures of the goodness-of-fit for all the equations are equally good. The average residuals are about 0.07 (*se*) and the correlation coefficients are better than 0.98. Hence, the fits shown in Table 6 are adequate to indicate that the LSERs method can be used to identify the sorbent/solute retention interactions in the light of the sorbent evaluation. In all instances, intercepts are negative for all commercial and plasma treated packings.

As seen from Table 6, larger intercepts are observed in CN, CN*, and C2 sorbents, which have a lower amount of carbon load, while smaller intercepts are observed in VC18, VC18*, and BC18 sorbents, which have a higher amount of organic carbon load.

The sign of the coefficient *m* shows that increasing the solute size will extend its residing over the sorbent. The *m*-coefficient represents hydrophobicity of the sorbent. The lowest values of *m*-coefficient are observed at cyanopropyl modified sorbents, and the highest–at octadecyl modified phases. In this specific case, it is obvious that the octadecyl modified ones are most hydrophobic, and cyanopropyl–the least hydrophobic ones. Cyclohexyl and phenyl bonded phases occupy an intermediate position and both sorbents have almost equal hydrophobicity. Hydrophobicity of the combined modified sorbent VC18 is higher compared to the non-modified VC18*. This is a result of the additional plasma modification, which increases the carbon content on the surface of the sorbents. The opposite phenomenon is observed at CN, where the plasma treated sorbent is less hydrophobic than the non-treated one. The fact that the ethyl modified sorbent is more hydrophobic than the CN-packing was discussed above, and the reason for this was attributed to the cyano group.

An important conclusion derived from studying the *m*-coefficient is that plasma treatment leads to an increase in hydrophobicity of the raw sample because of the plasma reactions and polymer covering.

The *r*-coefficient is a measure for the stationary phase trend to interact with π-and n- electronic pairs. The positive sign of *r* results in increasing of the studied compounds' retention. The lowest (negative) values are observed at the ethyl plasma modified phase, and the highest ones at the octadecyl sorbents. It is interesting to notice that cyanopropyl packings have higher *r*-coefficient values than the ethyl modified silica. Obviously, the cyano group has its contribution to the increase of the interactions of the type mentioned above. The comparison between C18 non-treated and plasma-treated sorbents is also interesting. The latter always have a lower ability to interact with n-and p-electronic pairs. This is due to the plasma reaction, which eliminates the possibility of such interactions.

The *s*-coefficient has a negative sign. According to the *s*-coefficient, sorbents may be divided into two groups: the first one has a positive sign, and CN, CN* sorbents belong to it. The other group consists of C2, CH,

Table 6. System constants and statistics for the fit of the solvation parameter model to the capacity factor data with methanol/water (50:50 v/v) as the mobile phase

	c	m	r	s	a	b	R	F	Se	N
C2	−0.951	1.396	−0.039	−0.062	−0.301	−0.920	0.9891	180.52	0.053	26
	(0.070)	(0.080)	(0.073)	(0.079)	(0.061)	(0.104)				
CN	−1.002	0.850	0.240	0.054	−0.275	−0.859	0.9767	82.68	0.069	26
	(0.091)	(0.102)	(0.094)	(0.102)	(0.079)	(0.134)				
CH	−0.678	1.959	0.041	−0.322	−0.315	−1.307	0.9937	315.16	0.059	26
	(0.078)	(0.088)	(0.080)	(0.087)	(0.067)	(0.115)				
PH	−0.668	1.507	−0.012	−0.010	−0.439	−1.078	0.9897	190.50	0.061	26
	(0.080)	(0.090)	(0.083)	(0.090)	(0.069)	(0.118)				
VC18	−0.398	2.394	0.216	−0.675	−0.513	−1.819	0.9914	206.47	0.088	23
	(0.167)	(0.201)	(0.128)	(0.138)	(0.102)	(0.182)				
BC18	−0.316	2.433	0.140	−0.666	−0.524	−1.763	0.9901	179.44	0.092	23
	(0.176)	(0.211)	(0.134)	(0.145)	(0.108)	(0.191)				
CN*	−0.943	1.031	0.218	0.048	−0.189	−1.003	0.9784	89.64	0.072	26
	(0.095)	(0.108)	(0.099)	(0.107)	(0.083)	(0.141)				
VC18*	−0.486	2.157	0.278	−0.672	−0.527	−1.718	0.9900	177.49	0.092	23
	(0.174)	(0.210)	(0.133)	(0.144)	(0.107)	(0.190)				

R-multiple linear regression coefficient, Se-standard error in the estimate, F-Fisher statistic, and N-number of, solutes. Numbers in parentheses indicate the standard deviation in the system constants.

VC18, VC18*, and BC18 and has negative values. The phenyl modified sample has *s*-coefficient values of about zero, which reveals that these interactions are not dominating at this sorbent. The octadecyl bonded phase has the most negative values of *s*. A high *s*-coefficient generally favors the retention of non-polar compounds. On the other hand a low *s*-coefficient favors the retention of compounds capable of participating in orientation and induction interactions.

The values of *a*, characterizing hydrogen bond basicity of the sorbent/mobile phase system, change insignificantly. This determines its equal contribution to the retention at all studied sorbents. Only the non-modified cyanopropyl sorbent shows the opposite tendency, i.e. it decreases with the increase of the percentage content of the organic modifier. Generally, the negative *a*-coefficients indicate that mobile phase is more basic at the formation of hydrogen bonds than stationary phase. The octadecyl modified and non-modified sorbents have the lowest and approximately the same values of the *a*-coefficient.

The sign of the *b*-coefficient indicates that increasing the solute hydrogen-bond accepting ability will reduce its retention on the sorbents and is a second most important factor in determining the retention. The values of the *b*-coefficient for the different sorbents vary in the following order: VC18 > BC18 > VC18* > CH > PH > CN* > C2 > CN. It is interesting to notice that the non-modified cyanopropyl sorbent has higher values compared to the plasma treated one. This again confirms the fact that plasma treatment increases the retention of the test compounds.

These results give a clear overview of the retention mechanism in SPE that solute hydrophobicity is the dominant factor in determining the retention, and the solute hydrogen-bond accepting ability is the next-most important factor. The characteristic coefficients of about thousand organic compounds are tabulated (17–20), and it makes possible that the system coefficients in the equation of the solvation parameters, which were determined in the present work, are used to calculate k_s for compounds, which have not been the subject of this work.

Hierarchical Cluster Analysis

The aim of clustering is to classify objects so that similar objects are grouped together and dissimilar objects are found in different groups called clusters. This method is used to classify the multitude of stationary phases in chromatography (22).

Many stationary phases are available to SPE, which differ in their properties. Selecting those that would be good starting points for the development of a specific method is far from evident and it is usually based on the trial and error method. That is why the classification of the sorbents according to groups with similar properties increases the efficiency of the selection of a sorbent for the specific analytical task. In order to specify the similarity between test sorbents, the cluster analysis method was applied to the autoscaled coefficients of the equation of the solvation parameters (Table 6) so that they could be compared to each other in the same scale. This method is very important because each variable is equally weighted, i.e. all variables in a dataset are equally important in describing the underlying structure of the data. Euclidean distance was accepted as a measurement for the distance between the sorbents, on the condition that Euclidean distance may be used in the cases, when the classes form a compact cluster. The differentiation of the groups is made at a significance level of 60%, i.e. the links between the groups are considered weak above this level.

As it may be observed from the dendogram (Fig. 2), the samples form three groups, which are clearly differentiated from one another. The first group includes the two cyanopropyl modified sorbents. The second one contains the octadecyl modified packings. Besides, there is a third group containing ethyl, cyclohexyl, and phenyl samples.

Some general conclusions may be drawn from the conducted cluster analysis: the obtained picture of similarities clearly revealed that the plasma modified sorbents form a different group together with the sorbent they initiate from. For example, the cyanopropyl non-modified packing and cyanopropyl plasma treated one are grouped together. This is due to the cyano group, which contributes a specific component to the sorbent/solute interaction. The octadecyl modified samples are significantly

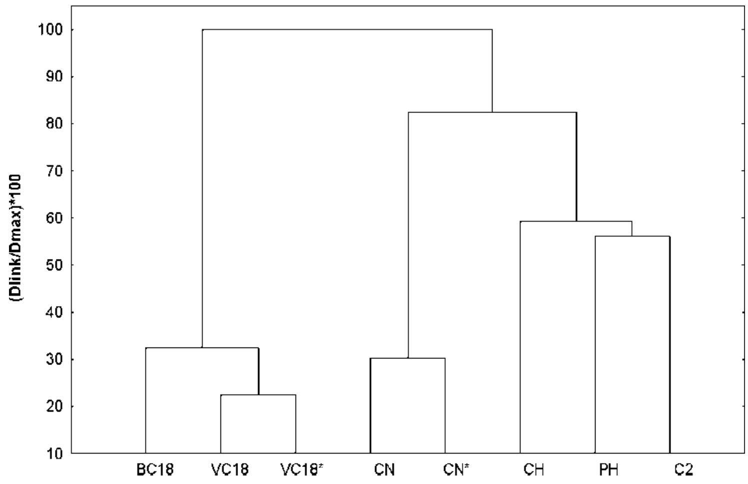

Figure 2. Dendrogram for cluster analysis of sorbents studied. The vertical axis indicates the degree of similarity between sorbents. Sorbents are listed across the horizontal axis.

different from the rest of the sorbents, according to the types and capacity of the sorbent/solute interactions, which determine the retention at reverse phase chromatography, and according to the ability to retain strongly the test compounds with a high break-through volume. According to the retention mechanism, Bondesil octadecyl non-modified packing (VC18*) and octadecyl plasma treated one (VC18) are grouped together with Bekerbond octadecyl modified sorbent (BC18). On the other hand, the last sample has the highest sorption capacity values at the introduction of the sample.

The behavior of the third group of sorbents is interesting. In Fig. 2, where the basis is the coefficients of the equation of solvation parameters, cyclohexyl, phenyl, and ethyl plasma treated packings are grouped together due to the similar coefficients values. In this case, the group occupies an intermediate position, as the types of the sorbent/solute interactions. On one hand, we have the group of the octadecyl derivatives with their hydrophobicity, and on the other, the group of the cyanopropyl samples with their characteristic in the interactions, i.e. the studied group of sorbents (phenyl, cyclohexyl, and ethyl) is the joining unit.

The obtained dendogram clearly shows the plasma treated sorbents cover, as types of sorbent/solute interactions—from polar to non-polar, and as sorption capacity—from low to high. This extends and develops further the capacity of SPE as a modern method of sample isolation and preconcentration.

CONCLUSIONS

Stationary phases based on plasma deposited polymers have been compared with the conventional commercial stationary phases for SPE. The plasma modification method has shown to be an appropriate method for the modification of sorbents for SPE. Based on LSER studies, five plasma modified sorbents (C2, CH, PH, VC18, and BC18) are reversed stationary phases, whereas cyanopropyl plasma modified sorbent is a normal phase packing. Plasma modification of bonded octadecyl silica increases retention. The effect may be explained by the increased carbon content on the surface of the sorbent, resulting from the plasma treatment. It has been demonstrated that octadecyl plasma modified sorbents are effective sorbents for SPE. These packings may be used in preference to or have advantages not available from typical octadecyl bonded stationary phases for the retention (isolation) of alkyl benzenes. The obtained coefficients in the equation of the solvation parameters once derived on a given sorbent can be used to predict the retention of any analyte of the known characteristic constants. The scope of applications of such stationary phases can be extended to the isolation of 1,3-dibromobenzene, 1,4-dibromobenzene, and other benzene derivatives.

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