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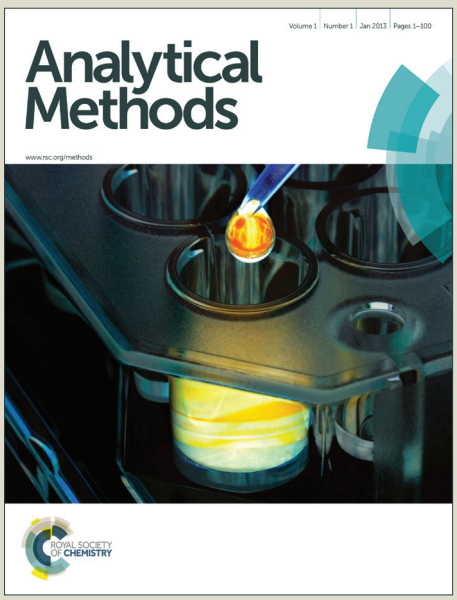

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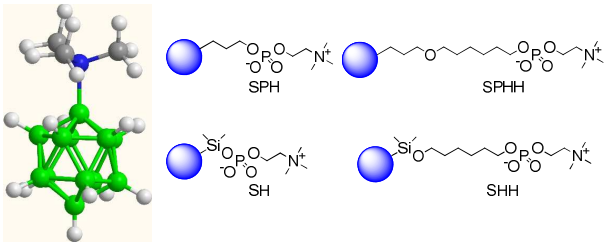

New matrices were prepared in order to understand the interaction of dodecaborate clusters with liposome surfaces. Interaction is not only through ionic forces, although the clusters are anions.

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ARTICLE TYPE

Interaction of organic compounds and boron clusters with new silica matrices containing the phosphatidylcholine headgroup

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Silica chromatography matrices containing the phosphatidylcholine headgroup have been prepared in order to allow the study of solutes interacting with the surface of lipid membranes. The headgroups were attached to the silica gel with two different methods, and with and without a hexyl spacer. Different organic compounds and dodecaborate clusters were investigated on these matrices, and compared to phosphatidylcholine liposomes covalently immobilized on silica gel. The correlation of elution when comparing the matrices pairwise was in general good for organic compounds, and excellent for dodecaborate clusters. Brominated and iodinated dodecaborate could not be eluted from phosphatidylcholine liposomes, while their retention on the other matrices was not exceptionally strong. For applying the linear free energy relation formalism to boron clusters, further descriptors appear to be required.

A Introduction

The low chemical reactivity, the high stability and solubility in biological systems as well as the ability of the ^{10}B isotope to absorb neutrons and emit α particles (neutron capture therapy of inoperable tumors) make ionic boron cluster attractive compounds for pharmaceutical and medical applications. In the context it appears crucial to understand their interactions with biological molecules and surfaces. Chromatographic methods have been applied to investigate the retention characteristics of ionic boron clusters using different matrices, including DEAE-cellulose [1],hydroxymethyl metacrylate [2], and RP18 reverse phase material [3, 4].

Recently, we have found that ionic boron clusters present an unusually strong retention on carbohydrate-containing matrices such as Superdex, Sepharose, and Sephadex [5]. This phenomenon was unexpected, and it precluded to investigate the interaction of these clusters with liposomes immobilized in Superdex, which had been suggested before as a suitable method to study interactions between drugs and lipids [6, 7]. Previously, we have used this method to study the retention of organic molecules of pharmaceutical interest [8]. In separate studies, we had found that interaction of boron clusters with liposomal membranes is quite strong, and the interaction leads to leakage of liposome contents and structural changes [9-12].

The interaction of the ionic boron clusters with liposomes could be due to an interaction with the headgroups of the lipids, or with a distribution of the clusters into the hydrophobic fatty acid tails of the lipids. Our initial thought was that the interaction of the headgroups would be crucial, and perhaps dominant, for the interaction.

We therefore considered to study the interaction between boron clusters and phospholipid headgroups by immobilizing the headgroups of phosphatidylcholine, and introducing different aliphatic carbon chains as hydrophobic linkers. With such matrices, we hoped to resolve the question how strongly the clusters interacted with the surface of lipid bilayers.

In our study on the chromatographic behavior of boron clusters on different matrices, we had found that the interaction of the clusters with silica was weak [5]. Therefore, as matrix for immobilization of the headgroups, silica was chosen. Suitable matrices were prepared, where the headgroup was attached to the silica gel with different spacers.

On these new matrices, we compared the chromatographic behavior of the clusters with those of organic compounds, in order to see whether the data from one class of compounds could be applied to the other class of compounds, and whether the retention behavior could be described in a rational manner. The organic compounds were chosen as they had been analyzed by us for chromatographic behavior on immobilized lipids before [8]. We also used egg phosphatidylcholine (EPC) liposomes immobilized on silica as comparison [13].

B Materials and Methods

Preparation of column materials

Silica gel (0.060-0.200 mm, 60 Å, surface area 500-600 m^2/g) was from Fisher Chemicals, all other chemical from Sigma-Aldrich or Acros Organics. Egg phosphatidylcholine (EPC) was a gift of Lipoid GmbH.

Phosphate was determined as described by Mao et al. [14].

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All structures of newly synthesized matrices and their abbreviations are given in Figure 1.

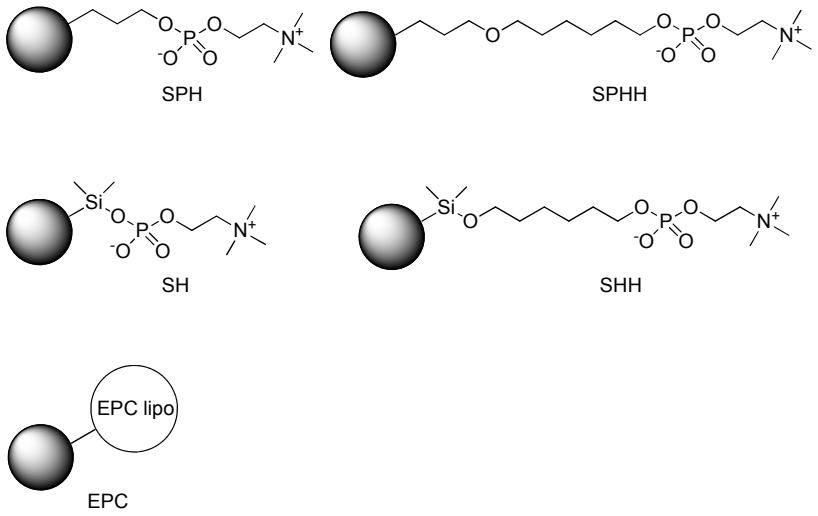

Figure 1. Structure of newly synthesized matrices and their abbreviations.

Covalent coupling of liposomes to silica gel to form EPC

The procedure described by Mao et al. [14] was followed. Silica gel was boiled in 20% (v/v) aqueous HCl for 4 hours and then washed with bidistilled water until the eluent was neutral. The gel was dried under vacuum at 120°C for 8 h.

The acid-treated silica gel (4 g) was added to a solution of 0.5 g (2.49 mmol) 4-nitrophenylchloroformate in 30 mL acetone. Then 0.34 g (2.48 mmol) p-dimethylaminopyridine was added, and the suspension was stirred for 1.5 h at 35°C. The gel was recovered by filtration and washed with acetone and water and dried at 120°C.

The activated silica gel (0.2 g) was added to 0.75 mL of a suspension of unilamellar liposomes (concentration of lipid 100 mM) prepared from EPC by extrusion through a 100-nm membrane. The amount of phosphate was found to be 0.171 mmol/g for the batch used for organic compounds, and 0.271 mmol/g for the batch used for boron clusters.

Preparation of SPH gel

Silica gel was activated according to Sales [15] by first heating it to 150°C under a stream of N_2 for 10 h. The activated gel (10 g) was immediately suspended in 50 mL dry toluene, and 10.0 mL (54.0 mmol) 3-chloropropyltrimethoxysilane was added. The suspension was stirred at RT under N_2 for 3 d, the gel was filtered off, washed with toluene, and dried in vacuo for 3 h.

The Bu_4N salt of choline phosphate was prepared from the Ca salt by dissolving 1.0 g Ca salt in 5 mL water and adding 0.39 g oxalic acid hydrate dissolved in 5 mL water. The milky suspension was filtered through a membrane filter (0.25 µm), and the clear filtrate was titrated to pH=7.4 with an aqueous solution of Bu_4NOH. The solution was evaporated, and remaining water was removed by azeotropic distillation with toluene. The yellowish oil was dried in a desiccator over P_4O_{10} [16].

Of this preparation, 1.27 g (3.0 mmol) was dissolved in 25 mL dry toluene, 1.3 g of the activated silica gel was added, and the mixture was refluxed under stirring for 2 d. The gel was filtered off, washed with toluene, MeOH and Et_2O and air-dried.

The phosphate content was 0.268 mmol/g.

Preparation of SPHH gel

The activated silica gel described above (1.2 g) was reacted with 0.5 mL (0.521 g or 3.75 mmol) 6-chlorohexanol in 20 mL dry toluene, the mixture was refluxed under stirring for 12 h, then another 6 h after 0.5 mL (3.59 mmol) triethylamine was added, the new gel was filtered off, washed with toluene, MeOH and Et_2O and air-dried. To this new gel, 1.27 g (3.0 mmol) Bu_4N salt of choline phosphate in 25 mL dry toluene was added, and the mixture was refluxed for 2 d, followed by filtration and washing with toluene, MeOH and Et_2O. The phosphate content was 0.280 mmol/g.

Preparation of SH gel

Activation of silica gel was carried out as described by Durrani et al. [17] Silica gel (1.2 g) was added slowly over 1 h to 4 mL dichlorodimethylsilane while N_2 was passed through the suspension, which was stirred until no liquid was left. To this gel, 1.27 g (3.0 mmol) Bu_4N salt of choline phosphate in 25 mL dry toluene was added, and the mixture was refluxed for 2 d, followed by filtration and washing with toluene, MeOH and Et_2O.

The phosphate content was 0.120 mmol/g.

Preparation of SHH gel

Activation of silica gel was carried out as described by Durrani et al.[17] Silica gel (1.2 g) was added slowly over 1 h to 4 mL dichlorodimethylsilane while N_2 was passed through the suspension, which was stirred until no liquid was left. The obtained gel was then reacted with 0.5 mL (0.521g or 3.75 mmol) 6-chlorohexanol in 20 mL dry toluene, the mixture was refluxed under stirring for 1 d, the gel was filtered off, washed with toluene, MeOH and Et_2O, and air-dried. To this gel, 1.27 g (3.0 mmol) Bu_4N salt of choline phosphate in 25 mL dry toluene was added, and the mixture was refluxed under stirring for 2 d, followed by filtration and washing with toluene, MeOH and Et_2O.

The phosphate content was 0.091 mmol/g.

Compounds investigated

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Clusters

The clusters investigated (the general structure is shown in Figure 2) are listed in Table 1. Their preparation was according to literature procedures given in [5]. All cations of the boron cluster compounds were either Na or K.

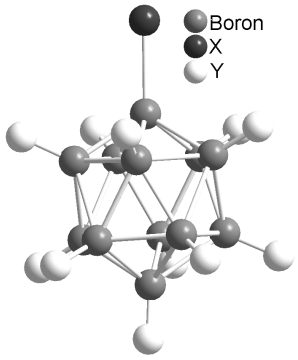

Figure 2. Structure of the dodecaborate cluster with substituents. The kind and type of X and Y are given in Table 1. The cluster carries two negative charges; ammonio- and sulfonio-substituents reduce the total charge by 1 to a single negative charge.

Table 1. Cluster compounds investigated

Name*	Y	X	Charge	Abbreviation
Dodecahydrododecaborate	H	H	2-	BH
Dodecachlorododecaborate	Cl	Cl	2-	BCl
Dodecabromododecaborate	Br	Br	2-	BBr
Dodecaiododododecaborate	I	I	2-	BI
Hydroxoundecaiododododecaborate	I	OH	2-	BIOH
Ammoniododecaborate	H	NH_3	1-	BNH3
N,N,N-Trimethylammonioundecahydrododecaborate	H	NMe_3	1-	BNMe3
N,N,N-Triethylammonioundecahydrododecaborate	H	NEt_3	1-	BNEt3
N,N,N-Tripropylammonioundecahydrododecaborate	H	NPr_3	1-	BNPr3
N-Benzyl-*N,N*-diethylammonioundecahydrododecadecaborate	H	NEt2Bn	1-	BNEt2Bn
Mercaptoundecahydrododecaborate	H	SH	2-	BSH
S,S-Dimethylsulfonioundecahydrododecaborate	H	SMe_2	1-	BSMe2
p-Tolylundecahydrododecaborate	H	C_6H_4Me	1-	BTol
N-Benzylammonioundecahydrododecaborate	H	$NH_2CH_2C_6H_5$	1-	BNBn

[a] All salts had either K or Na as counterions.

Organic compounds

The following polar or ionizable organic compounds were used (see Fig. 3): 4-aminobenzoic acid, 4-hydroxybenzoic acid, 4-chlorobenzoic acid, 4-amino-2-hydroxybenzoic acid, 4-bromobenzoic acid, methyl 4-(hydroxymethyl)benzoate, N-((4-aminophenyl)sulfonyl)acetamide (sulfacetamide), N-((4-aminophenyl)sulfonyl)benzamide (sulfabenzamide), 4-aminobenzenesulfonamide (sulfanilamide), 4-amino-N-(5-methylisoxazol-3-yl)benzenesulfonamide (sulfamethoxazol), 4-amino-N-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (sulfamethazine), 2-(4-isobutylphenyl)propanoate (ibuprofen), 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid (diclofenac), 2-hydroxybenzoic acid, 1,3-dimethyl-7H-purine-2,6-dione (theophyllin), 2-((2,3-dimethylphenyl)amino)benzoic acid (mefenamic acid), 2-(3-benzoylphenyl)propanoic acid (ketoprofen), 1-(4-aminophenyl)ethanone (aminoacetophenon), 3-phenylpropanoic acid, 5-phenylpentanoic acid, 8-phenyl-octanoic acid.

Chromatography

All newly synthesized material was packed into glass columns with an inner diameter of 5 mm and a bed length of 5 cm.

Chromatography of cluster compounds was performed on an HPLC system Series 1100 (Agilent, Germany), with gradient pump, online degasser, autosampler and a Bruker Esquire ESI-MS ion trap detector (Bruker, Germany). The device was run at a flow rate of 0.2 mL/min with 10 mM ammonium formate solution in water, and the injection volume of each sample was 20 µL. Back pressure was minimal, due to the relatively large size of the gel particles used.

Chromatography of organic compounds were performed by a Merck® L-6200A Pump System with an UV-Vis-Detector L-4250 (Merck-Hitachi Ltd., Tokyo, Japan), the wavelength of detection was 220 nm. The device was run at a flow rate of 0.2 mL/min with 10 mM ammonium formate solution in water as eluent, and the injection volume of each sample was 20 µL.

Slopes, intercepts, and correlation coefficients r were calculated from linear regression analysis, using SigmaPlot software.

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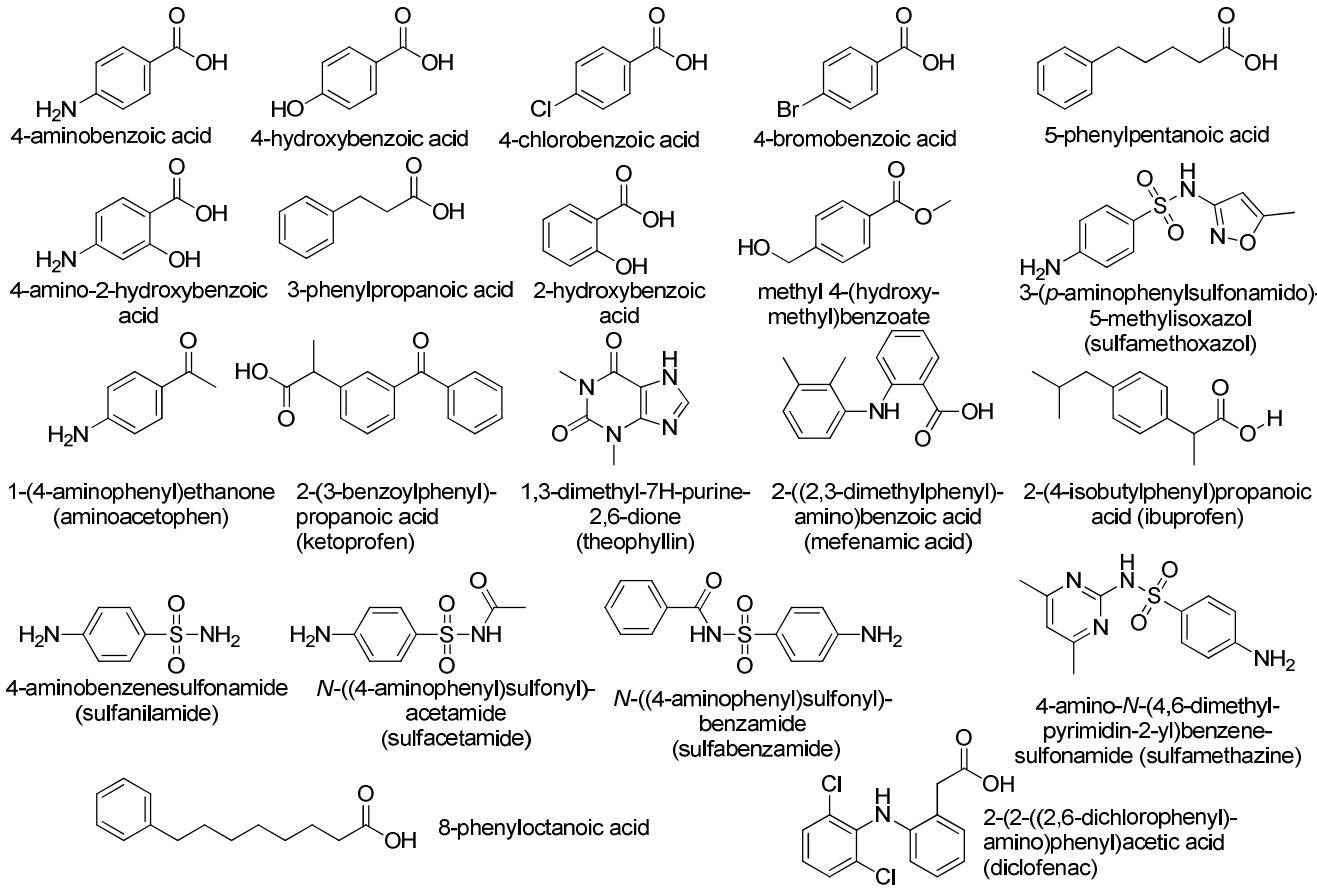

Fig. 3 Organic compounds used

C Thermodynamic description

For comparison of newly synthesized matrices, the energetics of retention were investigated following the approach of Melander et al. [18], with further adaptation.

The retention factor k is given by

(1) $k = \varphi K$

with φ the phase ratio and K the thermodynamic equilibrium constant for the association between substance and matrix.

Then, the logarithm of k is

(2) $\log(k) = \log(\varphi) + \log(K) = \log(\varphi) - \frac{\Delta G^0}{2.3RT}$

with R and T being the gas constant and the temperature.

For two matrices 1 and 2, equation (2) will be

(3) $\log(k1) = \log(\varphi 1) - \frac{\Delta G_1^0}{2.3RT}$

and

(4) $\log(k2) = \log(\varphi 2) - \frac{\Delta G_2^0}{2.3RT}$

Subtraction and rearrangement of equations (3) and (4) gives

(5) $\log(k1) = \log(k2) + \log\frac{\varphi 1}{\varphi 2} + \frac{\Delta G_2^0 - \Delta G_1^0}{2.3RT}$

Melander et al. [18] differentiate between two different behaviors for isothermal chromatography:

a) In solute/matrix combinations where ΔG^0 for both matrices are equal, slope is unity for a plot according to equation (5), and the intercept is the logarithm of the ratios of the two phase ratios. Such behavior is named *homoenergetic*.

Thus, for two matrices, a plot of log(k1) versus log(k2) should give a straight line with a slope of unity and an intercept corresponding to the logarithm of the ratios between the two phase ratios.

b) If the ΔG^0 values for the two matrices are not identical, but proportional, as in eq. (6), then a plot of log(k1) versus log(k2) (eq. (7) gives a straight line with a slope α and an intercept being the same as in the homoenergetic behavior. This behavior was called *homeoenergetic*.

(6) $\Delta G_1^0 = \alpha \Delta G_2^0$

(7)

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$$\log(k1) = \alpha\log(k2) + \log\frac{\varphi 1}{\varphi 2^{\alpha}} = \alpha\log(k2) + \log(\varphi 1) - \alpha\log(\varphi 2)$$

with $\alpha = \frac{\Delta G_1^0}{\Delta G_2^0}$

When replacing in eq. (7) log(k1) with κ1, log(k2) with κ2, log(φ1) with Φ1, log(φ2) with Φ2, the equation is

(8) $\kappa 1 = \alpha\kappa 2 + \Phi 1 - \alpha\Phi 2$

(eq. 8 in [18])

In the paper by Melander et al. [18], different column materials were compared pairwise. Table I of that paper shows that all butyl groups, compared to any of the other materials, gave slopes of 2, whereas the other comparisons had slopes of around 1. For aqueous eluent solvents, Table II of that paper shows that slopes of down to 0.37 were found when comparing RP-2 to other matrices.

Further analysis was not considered in the paper by Melander et al. [18]. The case b) of Melander et al. can, however, be extended: The term introduced by them (see eq. (6)) is the first of a series of terms with virial coefficients

(9) $\Delta G_1^0 = \alpha\Delta G_2^0 + \beta(\Delta G_2^0)^2 + \gamma(\Delta G_2^0)^3 + \ldots$

Inserting equation (9), limited to the second virial coefficient, into equation (3) gives equation (10)

(10) $\kappa 1 = \Phi 1 - a(\alpha\Delta G_2^o + \beta(\Delta G_2^0)^2)$

with $a = (2.3RT)^{-1}$.

Rearrangement of equation (4) for ΔG_2 gives

(11) $\Delta G_2 = -\frac{1}{a}(\kappa 2 + \Phi 2)$

Inserting equation (11) into equation (10) gives (12)

$$\kappa 1 = -\frac{1}{a}\beta(\kappa 2)^2 + (\alpha + 2\frac{1}{a}\beta\Phi 2)\kappa 2 + \Phi 1 - \alpha\Phi 2 - \frac{1}{a}\beta(\Phi 2)^2$$

When κ1 is plotted against κ2, a non-linear, upward bending line should be seen.

As shown below, the data obtained by us can be fitted well with equation (8), and for the instances where the fit is not very good, a systematic upward bending cannot be seen. Thus, the data were evaluated only with equation (8).

Results

Retention of compounds.

Synthesis of matrices

For the gels where attachment of the organic moiety was through dimethylsiloxy groups (gels SH and SHH), the amount of phosphate on the gel (Table 2) was about half of that found for the other gels (EPC, SPH, SPHH). Surface coverage could be calculated for the gels, using the data for surface area of the silica gel of 550 m^2/g as provided by the supplier; the coverage is about 10% of the values reported for commercial RP-18 supports. The amount of ligand bound in SPH and SPHH and the surface coverage is about half of what has been reported by Sales et al. [15] for gels prepared by the same activation procedure.

Table 2. Amount of phosphate on the different matrices

column material	amount of choline phosphate mmol/g material	surface coverage µmol/m^2
EPC batch 1 [a]	0.271	
EPC batch 2 [b]	0.171	
SPH	0.268	0.487
SPHH	0.280	0.509
SH	0.120	0.218
SHH	0.095	0.173

[a]: Used for chromatography of clusters

[b]: Used for chromatography of organic compounds

Stability of matrices

The stability of the matrices was checked through column performance. The materials showed a gradual decrease in retention with time, and after storage for two weeks in the chromatography buffer, some gels showed a reduction in the retention times of between 4% and up to 46%, depending on the gel and the compound. The same was found for the EPC gel. Chromatographic results reported here were obtained with gels within a few days after their synthesis, and reproducibility of performance was checked before and after the experiment. Results of columns with larger deviations at the end of the experiment were not used.

Performance of matrices

The matrices showed a slight tailing of compounds. A sample chromatogram for selected boron clusters (which had been injected as a mixture simultaneously) on the EPC column is shown in Figure 4.

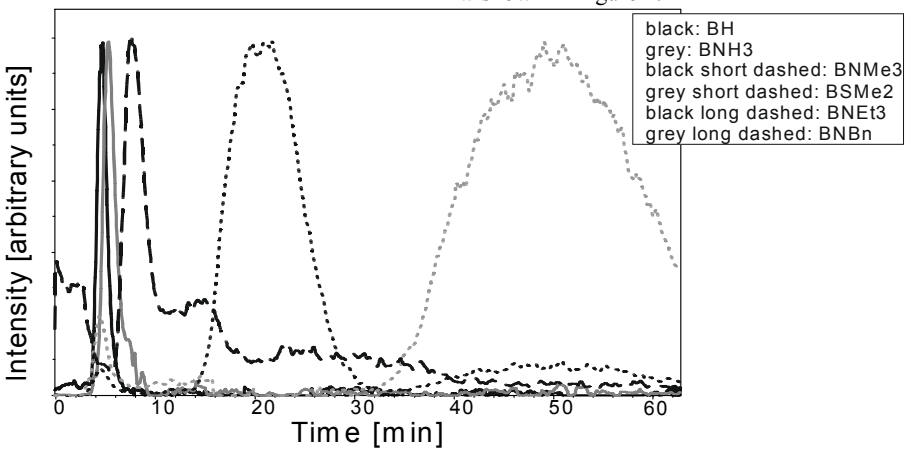

Figure 4. Chromatogram of selected boron clusters on the EPC column, detected by ESI-MS

Table 3 lists all retention factors for all compounds on the five

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different gels.

Table. 3. List of the compounds and their log(k) values on the respective matrices.

Compound	EPC	SPH	SPHH	SH	SHH
BH	-0.1581	0.1903	-0.6892	-0.4667	-0.6335
BTol	1.3401	0.3010	-0.3882	-0.2326	-0.5195
BNH3	0.0648	0.2788	-0.4393	-0.3118	-0.4873
BNMe3	0.9623	0.4393	-0.0994	0.0207	-0.2717
BNEt3	1.8692	0.6580	0.3478	0.5111	-0.1150
BNBn	1.6623	0.3802	-0.1963	-0.0107	-0.2533
BNPr3	2.6441	0.9243	0.8644	1.2793	0.4421
BNEt2Bn	3.0425	0.8949	1.0212	1.4238	0.7898
BSH	0.4493	0.2041	-0.5643	-0.4087	-0.6335
BSMe2	1.3333	0.4472	-0.0524	0.0404	-0.2355
BCl	0.3784	0.3802	-0.0994	-0.0330	-0.3112
BBr		0.3979	0.0969	0.1431	-0.1421
BI		0.5185	0.5819	0.7938	0.2530
BIOH		0.3892	-0.0307	0.1431	-0.1563
Salicylate	-1.1903	-0.7884	-0.5819	-0.5195	-0.7202
4-Aminobenzoate	-1.4914	-1.3324	-1.3222	-1.0314	-1.3222
4-Hydroxybenzoate	-1.7924	-1.1563	-1.6232	-1.1563	-1.3222
4-Chlorobenzoate	-1.0142	-0.9345	-1.0212	-0.6335	-1.0212
4-Amino-2-hydroxybenzoate	0.0589	-0.0653	-0.055	-0.2355	-0.4771
4-Bromobenzoate	-0.8381	-0.7884	-0.8451	-0.5543	-1.0212
Ibuprofen	0.1267	-0.5195	-0.1761	0.3665	-0.5441
Ketoprofen	-0.3774	-0.4574	-0.3222	0.7245	-0.2430
Theophyllin	-0.3010	-0.3547	-0.4472	0	-0.3010
Diclofenac	1.1041	0.0293	0.2093	0.9740	-0.0669
3-Phenylpropanoate	-1.0934	-0.7884	-1.0212	-0.5921	-0.9243
Sulfacetamide	-1.4914	-1.1563	-1.3222	-0.9345	-1.1461
Sulfabenzamide	-1.0934	-0.7884	-0.7782	-0.4873	-0.9243
Sulfanilamide	-0.1903	-0.2185	-0.1919	-0.2355	-0.4771
Sulfamethoxazol	-0.6463	-0.4873	-0.5441	-0.1283	-0.7202
Sulfamethazine	-0.9838	0.4234	0.6223	0.8771	0.3680
Methyl(4-hydroxymethyl)benzoate	-0.6901	0.1300	0.3724	0.5368	-0.0435
4-Amino-acetophenon	0.1666	0.2358	0.4412	0.3918	-0.0435
5-Phenylpentanoate	-0.5883	-0.6335	-0.6690	-0.2355	-0.7202
8-Phenyloctanoate	0.7566	-0.2185	0.0395	0.7030	-0.0792
Mefenamate	1.2630	0.1300	0.6198	0.9990	0.0102
Salicylate	-1.1903	-0.7884	-0.5819	-0.5195	-0.7202
4-Aminobenzoate	-1.4914	-1.3324	-1.3222	-1.0314	-1.3222
4-Hydroxybenzoate	-1.7924	-1.1563	-1.6232	-1.1563	-1.3222
4-Chlorobenzoate	-1.0142	-0.9345	-1.0212	-0.6335	-1.0212
4-Amino-2-hydroxybenzoate	0.0589	-0.0653	-0.0550	-0.2355	-0.4771
4-Bromobenzoate	-0.8381	-0.7884	-0.8451	-0.5543	-1.0212

Correlation between log(k) values

Organic compounds

Table 4. Coefficients of regression lines of Figure 5.

ordinate	abscissa	intercept	slope	r^2
EPC	SPH	-0.26	0.41	0.48
EPC	SPHH	-0.12	0.59	0.57
EPC	SH	0.26	0.64	0.63
EPC	SHH	-0.36	0.40	0.49

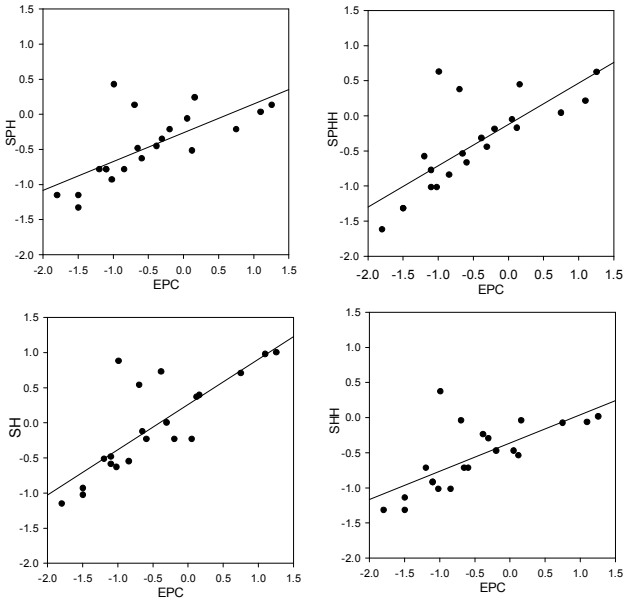

Fig. 5. Correlation of log(k) for organic compounds between EPC and the different matrices. (Data in this and the subsequent diagrams are plotted with equal scaling of x- and y-axis, thus allowing a fast identification of slope and intercept differences. The lines are regression lines through all data of each graph.)

Figure 5 shows the correlation of the log(k) values for the organic substances on the four newly synthesized matrices with EPC as the matrix to be compared with. A few compounds do not fit onto the regression lines. These are: sulfamethazine, 4-hydroxymethylbenzoic acid, ketoprofen (on the SH gel) and 4-aminoacetophenone (on both SPH and SPHH). No particular structural feature of the compounds could be identified as an obvious cause of the deviating behavior in chromatography.

Substances eluting after a certain time from a new matrix will elute about twice as late from EPC. Thus, α in equation (8) is around 0.5±0.1 for these matrix and compound combinations.

The intercepts of the lines vary between +0.26 and -0.36 (Table 4). These values are small in comparison to those of the boron clusters described below. Thus, the compounds would behave homeoenergetically (i.e. according to equation (8)).

The spreads between the compounds in the log(k) values are biggest for EPC and decrease in the order

EPC>SPHH≈SH>SHH≈SPH

The strongest retention was found on SH (despite the relatively small degree of modification). While EPC showed strongest retention for mefenamic acid and diclofenac, these two

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substances were retained almost as strongly on SH. On SH, sulfamethazine had a log(k) value of +0.88, while it had a log(k) value of only -0.98 on EPC. Also 4-hydroxymethylbenzoate was much more strongly retained on SH (log(k) value of +0.54 as compared to -0.69 for EPC). EPC was not the matrix where all compounds were retained most strongly.

When the elution from one of the new matrices is compared to that from any other of the new matrices (Figure 6), correlation of elution between different pairs of matrices is in most cases better than between the matrices and EPC. For the comparison of SH with SPHH, the correlation for the organic compounds is very poor. For the other pairs, the slopes vary between 0.8 and 1.3, with intercepts between +0.5 and -0.5 (Table 5).

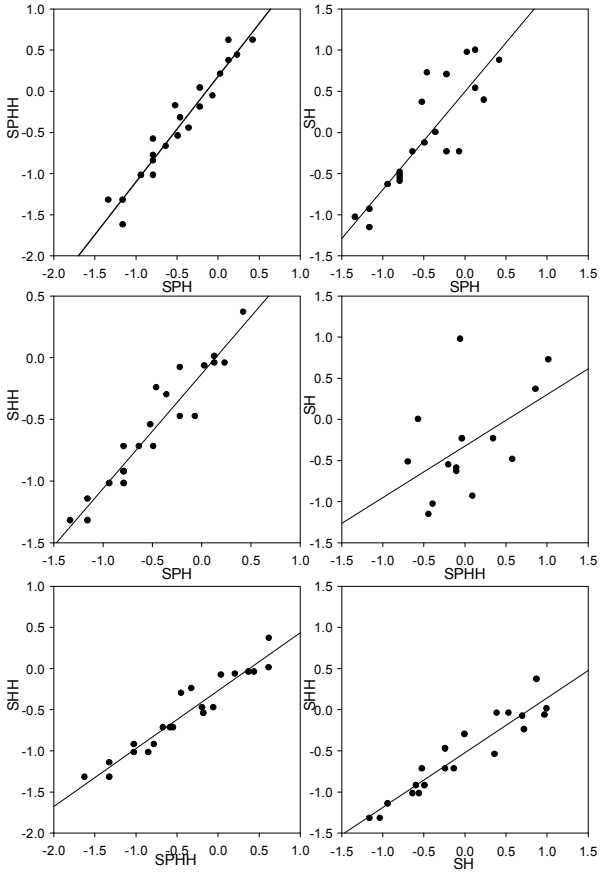

Fig. 6. Correlation between log(k) values between the different matrices for drugs.

Table 5. Coefficients of regression lines of Figure 6.

ordinate	abscissa	intercept	slope	r^2
SPH	SPHH	0.18	1.28	0.94
SPH	SH	0.50	1.19	0.75
SPH	SHH	-0.13	0.93	0.91
SPHH	SH	-0.32	0.63	0.26
SPHH	SHH	-0.27	0.70	0.92

The two gels SPH and SPHH with the same attachment, and an intervening hexyl chain for SPHH, have about the same amount of phosphorus bound (Table 2). As the number of non-hydrogen atoms in SPHH is 50% larger than in SPH, its phase ratio should also be about 50% larger. Then, equation (8) would predict that the slope is α and the intercept is (1.5-α). With the slope of 1.28, an intercept of 0.22 would be expected, which is close to the found value of 0.18 (Table 5). For SHH, the number of heavy atoms per chain is also about 50% larger than for SH, but the amount of phosphorus is smaller by 20%. With this relation between the phase ratios, an intercept of 0.51 would be expected for the observed slope of 0.67, which is not in agreement with the found value of -0.52. For the other combinations, the relation between the phase ratios cannot be estimated with confidence, as their chemical structures are too different.

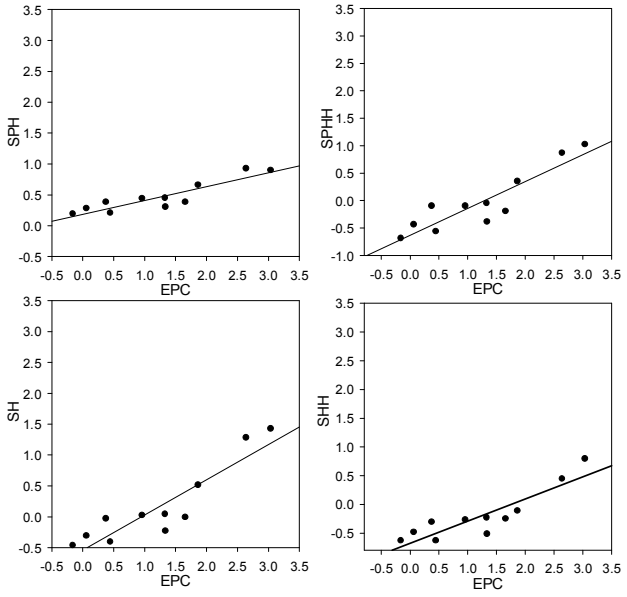

Fig. 7. Correlation between log(k) values on EPC and the matrix on the abscissa for boron clusters

Cluster compounds

Figure 7 shows the correlation between log(k) value of clusters on EPC and those obtained for the different headgroup gels. The values for the slopes and intercepts are found in Table 6.

Table 6. Coefficients of regression lines from Figure 7.

ordinate	abscissa	intercept	slope	r^2
EPC	SPH	0.19	0.22	0.82
EPC	SPHH	-0.63	0.49	0.82
EPC	SH	-0.54	0.57	0.82
EPC	SHH	-0.68	0.39	0.79

For the clusters, slopes between 0.22 (SPH) and 0.57 (SH) are found, which is a bigger spread than for the corresponding comparisons for organic molecules. The intercepts are around -0.6 for SPHH, SH, and SHH, and +0.19 for SPH.

The comparison between the different matrices shows a much better correlation (r^2 values of 0.79 and 0.82) Table 6) than what was found for organic compounds (0.63 and lower, Table 4). For SPH, the increase in retention was much smaller for the clusters

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than for the organic compounds, whereas the correlation of EPC with other matrices was around as that found for the organic compounds.

Clusters with bromine and iodine as substituents (BI, BBr, BIOH) behave quite differently than other clusters. They should elute within reasonable times from EPC, based on their retentions on the other matrices, but they were not detected on EPC, indicating that their log(k) was considerably bigger than 3.0. This could be an indication of a different retention mechanism of these clusters on the EPC column.

Correlations between the elution of clusters on the different headgroup gels are shown in Figure 8, with the values for intercept and slope tabulated in Table 7.

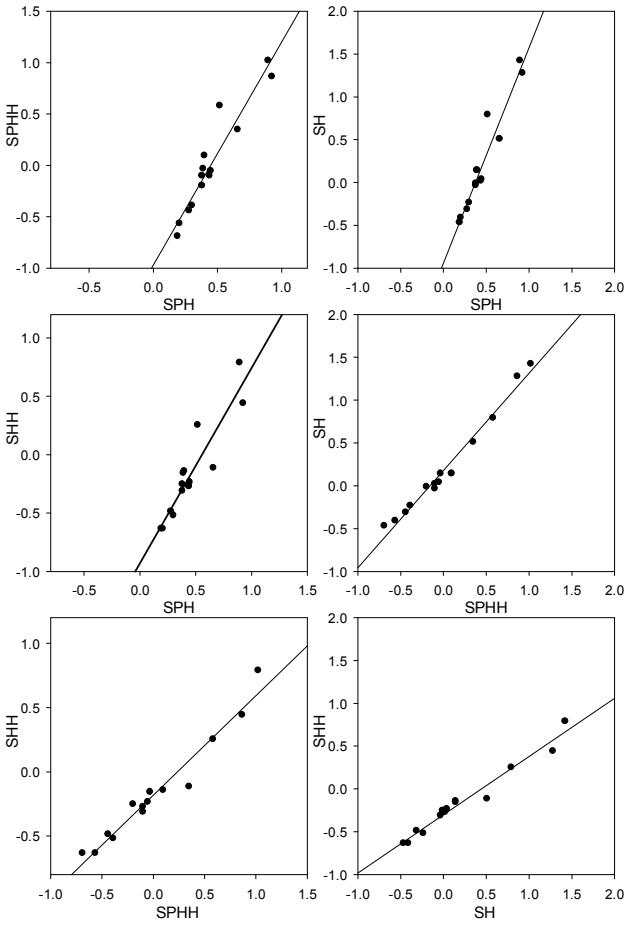

Figure 8. Correlation between log(k) of clusters for pairs of different matrices

Table 7. Coefficients of regression lines of Figure 8.

ordinate	abscissa	intercept	slope	r2
SPH	SPHH	-0.97	2.17	0.91
SPH	SH	-0.94	2.52	0.93
SPH	SHH	-0.93	1.67	0.86
SPHH	SH	0.18	1.14	0.98
SPHH	SHH	-0.18	0.78	0.95
SH	SHH	-0.30	0.68	0.97

The correlations of the slopes for all six combinations are very good, with SH to SPHH showing the best correlation (see Table 7, in contrast to the organic compounds, see Table 5)

The three slopes of SPH compared to the other matrices are all above 1, thus the ΔG° value for interaction with SPH appears to be much less than that for the other gels. The intercepts for all three pairs with SPH are around -1.0, which is much smaller than what would be expected from equation (8), even when the different phase ratios are taken into account.

SPHH and SH show very similar retention, both for the intercept (0.18) and the slope (1.14) for clusters. In contrast, intercepts and slopes for organic compounds on these two matrices correlate very poorly (Table 5).

The spread in log(k) was by far biggest for EPC.

EPC>>SH≈SPHH>SHH>SPH.

The maximum log(k) values of clusters on EPC and all other matrices were much larger than that of the organic compounds. BH was the compound retained most weakly on all the matrices, while BNPr3 and BNEt2CH2Ph were retained most strongly. On the other hand, there were numerous organic compounds which eluted earlier than BH from the matrices.

Interestingly, the brominated cluster BBr and both iodinated clusters BI and BIOH, which could not be eluted from EPC because of their strong affinity to the matrix, showed no particular retention on the other gels, and eluted with a log(k) value in the middle of the observed range for the other clusters.

Despite the fact that SPH showed no particularly strong retention even of BNPr3 and BNEt2CH2Ph (log(k) values of around 0.9), the compound eluting first, BH, had a log(k) value of +0.19 on SPH, whereas BH had negative log(k) values (between -0.16 and -0.69) on all other matrices including EPC.

Comparison between organic compounds and clusters on the same matrix pairs

According to equation (8), slopes and intercepts for a collection of one group of compounds on the same matrix should be identical to that of another group of compounds, and a ratio of the slopes should be unity. We have therefore compared the group of drug and drug-like compounds with the group of clusters by calculating the ratios of the intercepts and of the slopes between the groups (see Table 8).

For the matrices SH, SHH, SPH and SPHH, the phase ratios between the phases were constant for chromatography of organic compounds and clusters, as the same gels were taken for chromatography. For the comparison of EPC with the other matrices, a gel with less phosphate was taken for the organic compounds than for the clusters, and therefore the intercepts (where the phase ratios enter according to eq. (8)) could not be compared directly.

When considering the slopes, the ratio of the slopes is around 1 for all comparisons, except for the five pairs involving SPH as matrix. For these pairs, the slopes with SPH on the ordinate are only around half of the values expected (slopes around 0.5), whereas the other slope ratios are in the range of 0.90 to 1.20

Intercepts between the two groups do not correlate at all. The ratios vary between -1.78 and +1.73.

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Table 8. Comparison of chromatographic parameters for organic molecules and clusters.

		organ-ics		clusters			
ordin-ate	ab-scissa	inter-cept	slop e	inter-cept	slop e	inter-cept organic com-pounds /cluster s	slope organic com-pounds /cluster s
EPC	SPH	-0.26	0.41	0.19	0.22	-1.37[a]	1.86[a]
EPC	SPHH	-0.12	0.59	-0.63	0.49	0.19[a]	1.20[a]
EPC	SH	0.26	0.64	-0.54	0.57	-0.48[a]	1.12[a]
EPC	SHH	-0.36	0.4	-0.68	0.39	0.53[a]	1.03[a]
SPH	SPHH	0.18	1.28	-0.97	2.17	-0.19	0.59
SPH	SH	0.5	1.19	-0.94	2.52	-0.53	0.47
SPH	SHH	-0.13	0.93	-0.93	1.67	0.14	0.56
SPHH	SH	-0.32	0.63	0.18	1.14	-1.78	0.55
SPHH	SHH	-0.27	0.7	-0.18	0.78	1.50	0.90
SH	SHH	-0.52	0.67	-0.3	0.68	1.73	0.99

[a]: As the amount of EPC immobilized in the matrix used for chromatography of clusters was different from that used for organic compounds, these values cannot be compared directly.

If the different slope ratios are interpreted in the formalism of equation (8), the phase ratios should be different for the two groups, for identical matrices. As they are physically the same, different mechanisms of interaction must be assumed. This is probably also the case for the halogenated clusters on EPC as matrix.

Discussion

The synthesis of the matrices could be achieved without large problems. The coverage of the gel is, however, smaller than for commercial reversed-phase material. As, however, the interaction in water of the drugs and the clusters with non-modified silica gel is minimal [5], differences seen during the chromatography of compounds are not caused by interaction with the uncovered parts of the gel. Retention of the compounds as observed here must therefore be attributed to the interaction of the boron clusters with the organic groups present on the different matrices.

For organic compounds, the retention on the headgroup-modified matrices, when compared pairwise, matched quite well, with the exception of the pair SH/SPHH. SH contains the shorted linker between the headgroups, and one cannot exclude that the interaction of the analytes with the headgroup is influenced, e.g. sterically, by the close neighborhood of the silica matrix.

In contrast, boron clusters showed a good correlation also with the pair SH/SPHH, and in general, the correlation coefficients were higher than for the organic compounds. This might be due to the smaller structural and chemical variations of the clusters in comparison to the organic compounds tested.

We had previously studied the retention of boron clusters on reversed phase gels in mixed solvents in the presence of ion pair reagents [19]. On RP18, elution in the absence of an organic solvent (methanol, or acetonitril) was not possible, pointing to a strong interaction of boron cluster compounds with silica gels substituted with hydrophobic chains. The gel pairs SPH and SPHH, as well as SH and SHH, allow to study the reversed phase contribution to retention, as the SPHH and SHH both have a hexyl spacer, which is absent in SPH and SH. For all compounds tested, SPHH shows a slightly stronger retention than SPH, which lacks the hexyl spacer. For SHH, this is reversed, and compounds are retained more strongly on SH. The contribution of the hexyl spacer in SPHH is stronger for clusters than for organic compounds.

When comparing SH *versus* SPH, the changed mode of attachment and the extra propyloxy linker of SPH have only little influence on the ratio of the ΔG values for organic compounds, but a considerable influence for clusters. With an additional hexyl spacer (SPHH *versus* SHH), the influence of the different types of attachment to the silica gel and the extra propyloxy group is small for both types of compounds, with SPHH having a slightly bigger ΔG than SHH.

The interaction of the halogenated clusters with EPC appears to be of a different quality than those of non-halogenated clusters and organic compounds. These compounds did not elute at all from immobilized EPC. This might be a result of the close packing of both headgroups and hydrophobic chains in the liposome, whereas the density of the groups substituting the gel in the newly prepared matrices is much lower. Interaction with the headgroup-containing gels could therefore occur with one or at most only a few chains at a given time, whereas in liposomes, simultaneous interaction with several headgroups and hydrophobic chains is bound to occur with certainty.

The formalism developed by Melander et al. was applied to the matrices produced here [18]. The results show that the formalism appears to be applicable. Thus, an expansion of equation (8) to include higher virial coefficients, as formulated in equation (12), appears not to be necessary. The data points off the regression line in Figure 5 for the pair SH/SPHH appear to be too scattered to assume that a second virial coefficient would be required.

One of the goals which we had pursued, and for which the synthesis of new matrices was initiated, was the observation that boron clusters interact very strongly with the surface of phospholipid liposomes [11, 12, 20]. We were initially inclined to see this as a specific ionic interaction of the clusters with the quaternary ammonium groups of the phospholipids, an observation which is used widely to isolate boron clusters from aqueous solutions [21]. With this being the major goal of the work, we were not concerned very much with the stability of the matrices, which we found to be limited; this could be expected due to the presence of phosphate ester groups which can be hydrolyzed. Our results indicate that the interaction is not solely due to the interaction with the quaternary ammonium group, but also with other parts of the lipid headgroups.

We have recently shown that the hydration of boron clusters differs from that of other ions [22], and that the attractive force between water and cluster is influenced by the substituents of the cluster [22]. For halogen-substituted clusters, no model of interaction with solvent has been proposed so far. It might be speculated that hydration is different from that of hydrogen-substituted clusters. Molecular dynamics simulations of the interaction of boron clusters with the matrix modifiers prepared

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here might shed more light on the specific interactions between the analyte and the stationary phase.

The matrices prepared contain polar, ionic, and hydrophobic organic moieties; also, the surfaces of the silica gel are different due to different activation and attachment methods. Still, if similar solute descriptors would apply for both classes of compounds, a reasonable correlation between them should have been obtained. The poor correlation which we found indicates that the forces leading to retention on each of the matrices are different for the two classes of compounds.

It might be possible to characterize the molecular interaction potentials of the boron clusters with the linear free energy relationship (LFER), i.e. the Abraham model [23]. Our work shows that new solute descriptors for boron clusters appear to be required. Chromatography on a series of matrices with known system parameters (as recently also applied for ionic compounds [24]) would result in the appropriate solute descriptors. This will be part of further research.

Conclusions

We have shown that the chromatographic behavior of boron cluster compounds on the newly synthesized gels mimicking the headgroups of phosphatidylcholine lipids differs from that of organic, drug-like molecules. This might find its explanation in the hydration of the boron anions, which is different from that of other inorganic and organic ions. For complete understanding of the interaction of the clusters with organic matrices, new solute descriptors for the LFER formalism might be required.

Notes and references

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† Electronic Supplementary Information (ESI) available: Figures of individual log(k) values. See DOI: 10.1039/b000000x/

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