



# Immobilized metallacarborane as a new type of stationary phase for high performance liquid chromatography

David Sykora\*, Magda Vosmanska, Pavel Matejka, Vladimir Kral

*Institute of Chemical Technology, Department of Analytical Chemistry, Technická 5, 16628 Prague, Czech Republic*

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## ABSTRACT

A new type of high performance liquid chromatography (HPLC) stationary phase was prepared, and its chromatographic properties were evaluated. The sorbent was composed of metallacarborane covalently bound to silica. Because of the chemical structure of the immobilized metallacarborane, the synthesized stationary phase was able to interact with nonpolar analytes *via* hydrophobic interactions. The chromatographic behavior of several low-molecular-weight hydrocarbons on the sorbent under typical reversed-phase conditions was compared with octadecyl-, sulfo phenyl- and aminopropyl-modified silica stationary phases. Moreover, as a consequence of the synthetic protocol employed, the immobilization of the metallacarborane led to the development of a zwitterionic chemically bonded phase, which demonstrated excellent resistance to “phase collapse” in a 100% aqueous environment. Finally, preliminary experiments indicated that the new stationary phase has the potential for utilization in hydrophilic interaction chromatography (HILIC) mode for the separation of polar compounds.

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## 1. Introduction

For many years high performance liquid chromatography (HPLC) has represented an indispensable tool for research and routine analysis in various fields. Despite the fact that HPLC is considered a relatively developed technique, the advancement of separation methods is still necessary and includes both instrumental and stationary phase development.

Innovative stationary phase formats, monolithic media [1,2] and highly efficient superficially porous packing materials [3–5], have been introduced and successfully implemented. Furthermore, the commercialization of ultra HPLC (UHPLC) technology has led to a broad application of highly efficient totally porous sub-2  $\mu\text{m}$  particles [6–8].

In the quest to increase selectivity and to extend the analytical capacity of particulate LC phases, many investigators have worked hard on the preparation of novel chemically bonded phases (CBPs) with improved properties. This has been also the case of the most broadly used packing materials in HPLC, i.e., silica-based alkyl bonded phases C8 and C18. They have been a subject of a very intense research and innovations in the past years. The “classical” octadecyl and octyl bonded phases exhibited a decreased and poorly reproducible retention times at aqueous conditions with water content above  $\sim 95\%$ . This feature was explained for many

years as being a consequence of the hydrophobic ligand (C18, C8) collapsing or chain folding. It was speculated that, if the organic solvent content of the mobile phase was too low, the stationary phase would tend to collapse onto itself in a low-energy conformation. As a result, the collapse could lead to abnormal chromatographic behavior and often the complete loss of retention. A more realistic explanation was proposed in 2002 [9,10]. Several scientists clearly demonstrated that classic reversed-phase HPLC columns show a decrease in retention under 100% aqueous conditions because, under such conditions, the mobile phase is expelled from the pores of the packing material, which results in decreased contact between the stationary and mobile phase. Consequently, partial or complete loss of retention of the analytes occurs. This hypothesis has been supported by numerous experiments, e.g., [10–12].

Two alternatives of column chemistry have been developed to overcome the wetting–dewetting phenomenon, the polar-embedded and the polar-endcapped sorbents [13–15]. These phases are modifications of classic C18 chemistry with the incorporation of a polar (in some cases, ionizable or zwitterionic) functional group within the alkyl chain itself, or they use a polar group as an endcapping agent, respectively. As a result, the wettability of such stationary phases in 100% aqueous environments is significantly increased and they do not undergo “phase collapse”. This allows them to be used in conjunction with highly aqueous mobile phases without the mobile phase being driven out of the pores. Such phases have been successfully applied to RP separation of polar analytes requiring 100% aqueous mobile phases. Moreover, they exhibit improved peak shapes for basic analytes, a different sepa-

\* Corresponding author. Tel.: +420 220444062; fax: +420444352.

E-mail address: [David.Sykora@vscht.cz](mailto:David.Sykora@vscht.cz) (D. Sykora).

ration selectivity and decreased hydrophobicity in comparison to the standard C18 and C8 phases.

However, the separation of highly polar compounds can still be a significant challenge even on polar-embedded/polar-encapped phases. Such compounds are among the most problematic solutes in chromatography. They have insufficient retention on reversed-phase sorbents, and they lack the ability to interact on ion-exchange stationary phases by Coulombic interaction. The polar functional groups usually render the solutes non-volatile and unsuitable for GC separation. Hydrophilic interaction chromatography (HILIC) has proven to be a suitable alternative, and its application has increased tremendously especially in the last few years [16–20]. Here, the analyte retention is caused by partitioning of the analyte between a water-enriched layer of a stagnant eluent on a hydrophilic sorbent and a relatively hydrophobic mobile phase; the main component is usually a mixture of water in acetonitrile [21]. In this case, water is the strong solvent, and it gives HILIC significant advantages over conventional normal phase chromatographic modes because total control over the solvent water content is omitted. The elution order in HILIC is roughly the opposite of that seen in RP separations. This orthogonality is one of the inherent advantages of HILIC, and it allows for possibilities of coupled separations, where the strong eluting solvent used to desorb the analyte from the RP sorbent is a weak eluting solvent for HILIC or *vice versa*. The number of commercially available columns intended for HILIC is continuously growing. The chemistry of such stationary phases varies significantly. The most common stationary phases are underivatized-, aminopropyl amide-, diol-, poly(succinimide)-, cyanopropyl-, cyclodextrin-, sulfoalkylbetaine-based silicas, nonsilica-based amino packings and sulfonated styrene-divinylbenzene copolymers. In addition, the ZIC-HILIC, which is based on a covalent modification of silica with a zwitterionic moiety [22], has proven to be applicable in the HILIC mode.

Boron cluster chemistry includes a variety of synthetic compounds with unique properties. The synthesis of the first metallocarborane was reported in 1965 [23,24]. The 18-electron low-spin  $d^6$  cobalt bis(1,2- and 1,7-dicarbollides) [*commo*-3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>]<sup>-</sup> and [*commo*-2,2'-Co(1,7-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>]<sup>-</sup> were among the very first metallocarboranes synthesized (Fig. 1). Currently, the chemistry of these compounds is considered being one of the most elaborated in metallocarborane field [25].

Although the crystal structure of Cs[3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>] was determined in 1967 [26], the positions of the carbon atoms, and hence the ligand orientations, were established much later for (Et<sub>3</sub>NH) [3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>] [27]. The [3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>]<sup>-</sup> anion (Fig. 2) consists of two (C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub><sup>2-</sup> dicarbollide units sandwiched around a formal Co<sup>3+</sup> ion with the C<sub>2</sub>B<sub>9</sub> faces of the two ligands nearly parallel. The vector distances from cobalt to the C<sub>2</sub>B<sub>9</sub> planes are virtually identical, and the metal is approximately equidistant from the facial boron and carbon atoms, although the Co–C distances are slightly shorter than the Co–B vectors. The two (C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub><sup>2-</sup> ligands are both rotated by 37°. This produces a staggered orientation of the carborane cages with the two C–C edges in close proximity; the molecule is approximated to have an overall C<sub>2</sub> symmetry [25].

The exceptionally high stability of polyhedral boron hydrides and their derivatives has numerous applications in practical use [28,29]. Boron neutron capture therapy and carborane-based superacids are probably the best-known examples [30].

Polyhedral boron hydrides are characterized by electron-deficient bonding because there are too few valence electrons for bonding to be described exclusively in terms of a two-centered, two-electron bond. Such electron-deficient structures easily aggregate to form unusual three-centered, two-electron bonds, which typically result in the formation of trigonal faces and hyperconjugation [30]. Investigation of the properties of these compounds has

resulted in the conclusion that these compounds have aromatic properties. The structures represent the first examples of non-planar, three-dimensional aromatic compounds. This has resulted in the development of the generally accepted concept of three-dimensional aromaticity [30,31].

Researchers have found numerous interesting applications for boron cluster chemistry. For instance, it has been found recently that cobalt bis(dicarbollide) and some of its derivatives act as potent and specific inhibitors of the HIV-1 protease [32].

Here, a new type of CBP based on the covalent immobilization of cobalt bis(dicarbollide), [*commo*-3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>]<sup>-</sup>, is reported. To the best of our knowledge this is the first metallocarborane (MCB) CBP designed for utilization in HPLC. This paper describes the synthesis and characterization of the CBP by spectroscopic and chromatographic techniques with the focus on the evaluation of its hydrophobicity. Moreover, the capacity of the new CBP to work in a 100% aqueous environment and its potential utilization in HILIC mode were also studied.

## 2. Experimental

### 2.1. Chemicals and reagents

Aminopropyl silica, Separon SGX NH2; sulfo phenyl silica, Separon SGX CX; and octadecyl silica, Separon SGX C18; were all of 7 μm particle size, and they were purchased from Tessek (Prague, Czech Republic). Cs[(8-dioxane-1,2-C<sub>2</sub>B<sub>9</sub>H<sub>10</sub>)-3-Co-(1',2'-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)] (1) was obtained from Katchem (Prague, Czech Republic). Chloroform, dioxane, toluene, dichloromethane, methanol and acetone (all p.a. grade) were obtained from LaChema (Brno, Czech Republic).

### 2.2. Preparation of the MCB CBP, Sil-MCB

The synthetic protocol was based on a widely applicable and well-described method of the ring opening procedure utilized to attach the cobalt bis(dicarbollide) moiety to the aminopropyl silica support [32–35]. The ring opening reaction of (1) is carried out by a suitable nucleophile, here amino group of the NH<sub>2</sub>-silica, yielding a zwitterionic structure (Fig. 2).

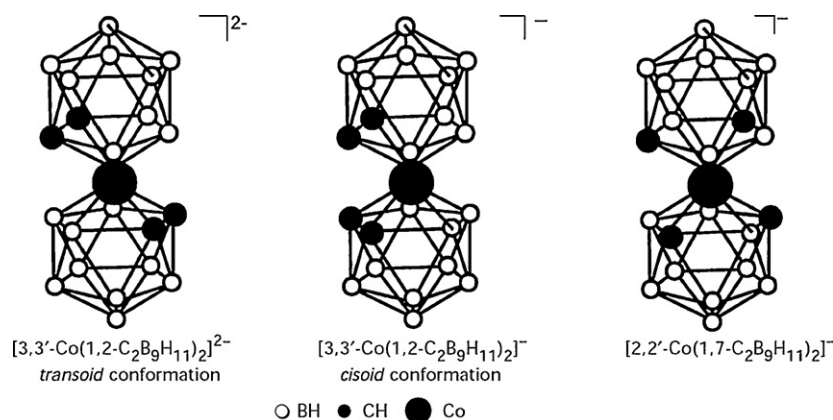
Briefly, aminopropyl silica (1.1 g) was washed with chloroform on the frit and then left to dry. Next, 20 mL of dioxane/toluene, 1/1 (v/v) was added to the aminopropyl silica and placed in a boiling flask, and (1) (0.3 g) was added. The resulting suspension was refluxed for 30 h, and then the product was washed with dichloromethane, methanol and acetone. Subsequently, the product was left to dry on the frit. The reaction scheme is shown in Fig. 2.

Three columns were packed with the prepared sorbent. The sorbent was suspended in methanol and packed by a slurry method into 4 mm × 50 mm stainless steel blanks. Methanol at 50 MPa was employed as a packing liquid.

### 2.3. Vibrational spectroscopy

Diffuse reflectance infrared Fourier-transform (DRIFT) spectra (4000–400 cm<sup>-1</sup>) were measured with Nicolet 6700 FTIR spectrometer (Thermo Scientific, U.S.A.) equipped with a Smart Diffuse Reflectance Accessory. Powdered samples were placed in a steel cup. Afterwards 256 scans were accumulated per spectrum at 4-cm<sup>-1</sup> resolution. Two repetitive measurements were performed for each sample.

Raman spectra (4000–100 cm<sup>-1</sup>) were recorded with Bruker FT Raman system (FT-NIR spectrometer Equinox 55/s plus FT Raman module FRA 106/S, Germany). The excitation source was Nd: YAG laser (Coherent, 1064 nm, laser power 250 mW). The samples were examined in Al cup as powders. The scattered light was collected in



**Fig. 1.** Structure of cobalt bis(dicarbollide) anions. Reprinted with permission from Ref. [25]. Copyright (1999) Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic.

backscattering geometry. 1024 separate interferograms per spectrum at  $2\text{-cm}^{-1}$  resolution were accumulated four times and thirty times for pure compound and silica based samples, respectively. The averaged spectra and standard deviation records were calculated.

#### 2.4. Chromatographic equipment

Chromatography experiments were carried out using an HPLC Agilent 1100 Series system consisting of a G1379A degasser, G1329A and G13308 thermostatted autosamplers, a G1312A binary pump, a G1318A thermostatted column compartment, and a G1315B diode array detector (all Agilent, Germany).

### 3. Results and discussion

#### 3.1. Characterization of Sil-MCB

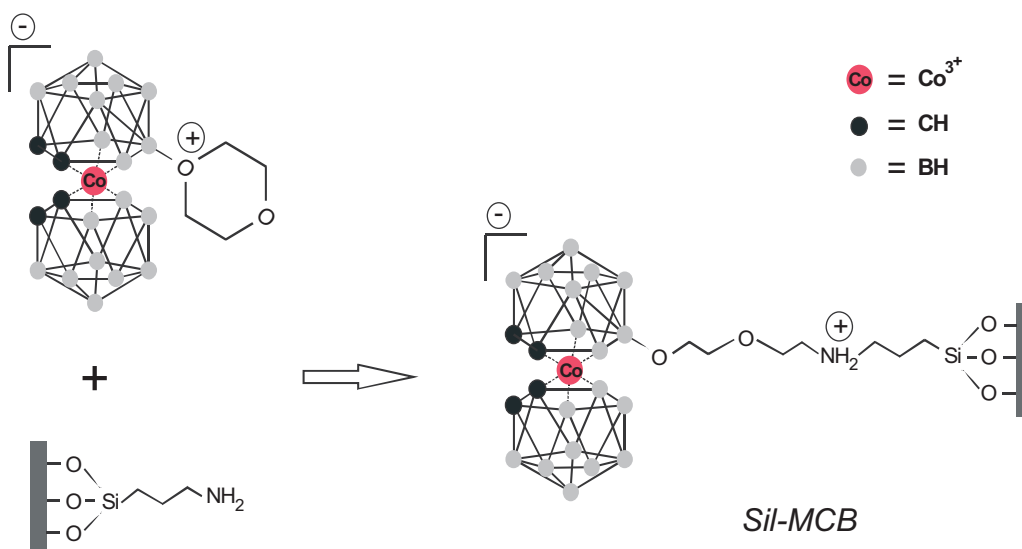
##### 3.1.1. Vibrational spectroscopy of Sil-MCB

The successful immobilization of the compound (**1**) on the aminopropyl silica was confirmed by vibrational spectroscopic techniques measuring DRIFT and FT Raman spectra.

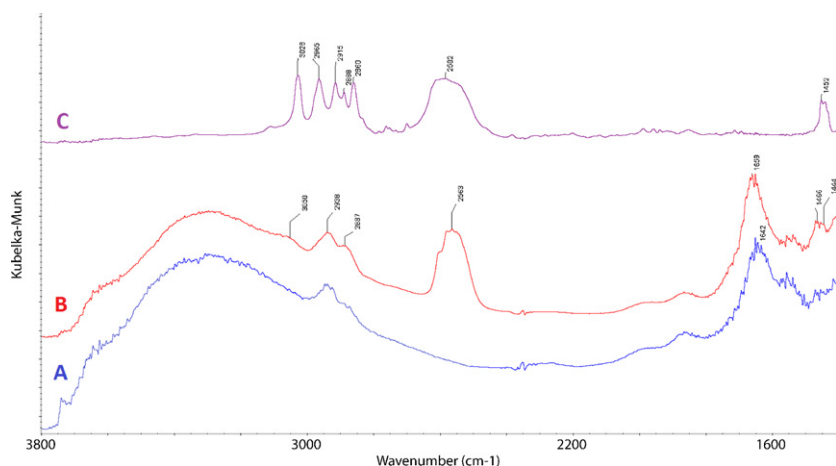
The region  $1400\text{--}400\text{ cm}^{-1}$  of DRIFT spectra is overlapped by strong and broad spectral features of the silica matrix. Hence,

the spectra of the aminopropyl silica, the Sil-MCB and the metallacarborane (**1**) were compared in the range  $4000\text{--}1400\text{ cm}^{-1}$ . The spectrum of the aminopropyl silica (Fig. 3A) exhibits several features of aminopropyl groups; apparent bands of C–H stretching modes in the range  $2950\text{--}2850\text{ cm}^{-1}$ , weak and noisy bands of C–H bending modes around  $1460\text{ cm}^{-1}$ , both stretching modes ( $3600\text{--}3200\text{ cm}^{-1}$ ) and the deformation one (at ca.  $1642\text{ cm}^{-1}$ ) of  $\text{NH}_2$  groups are overlapped partially with analogous modes of O–H groups and humidity of the silica matrix.

The modification of the aminopropyl silica with the derivative (**1**) is evidenced by comparing the resulting corresponding spectrum (Fig. 3B) with the spectra of both the aminopropyl silica (Fig. 3A) and the plain metallacarborane derivative (**1**) (Fig. 1C). The characteristic broad band of B–H stretching modes appears in the region  $2630\text{--}2470\text{ cm}^{-1}$ . The maximum (at  $2563\text{ cm}^{-1}$ ) is somewhat shifted to lower frequencies compared to the original derivative (**1**) ( $2582\text{ cm}^{-1}$ ); this can be explained tentatively by the fixation of the metallacarborane moiety on the silica matrix considering the described effect of reduction of B–H vibrational frequency by an appearance of negative charge on carborane rings [36]. The well-resolved bands of CH stretching vibrations of the crystalline compound (**1**) in the range  $3100\text{--}2850\text{ cm}^{-1}$  are broadened, slightly shifted and weakened upon fixation onto the aminopropyl silica due to (i) formation of less ordered system compared to crystal



**Fig. 2.** The reaction scheme used for the preparation of the Sil-MCB stationary phase.



**Fig. 3.** DRIFT spectra of the aminopropyl silica, SGX NH<sub>2</sub> (A); the metallacarborane modified aminopropyl silica, *Sil-MCB* (B); and metallacarborane derivative (**1**) (C). The spectra A and B are displayed in the same scale with offset of zero-line.

structure and (ii) opening of the dioxane ring causing higher conformation flexibility of the aliphatic chain formed. The similar effect is observed on the bands of bending C–H vibration around 1450 cm<sup>-1</sup>. The fixation of metallacarborane skeleton to the aminopropyl silica via NH<sub>2</sub><sup>+</sup> group is suggested by the shift and intensity increase of the band in the range around 1659 cm<sup>-1</sup> (Fig. 3B) attributed to an overlap of bands of deformation vibrations of both NH<sub>2</sub> and NH<sub>2</sub><sup>+</sup> groups.

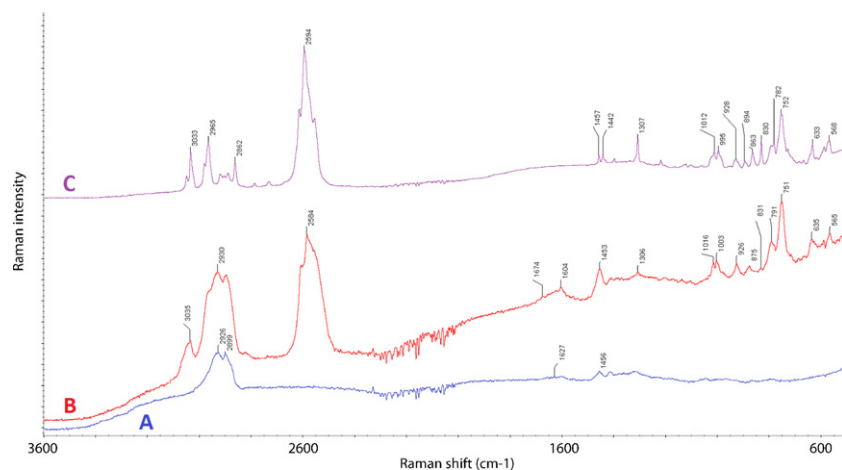
The corresponding Raman spectra obtained (Fig. 4) match very well the results obtained by DRIFT spectroscopy. First, the broad band of B–H stretching vibrations is clearly evident in the Raman spectrum of the modified aminopropyl silica and the peak position is shifted slightly to lower frequency compared to the original crystalline compound (**1**) (from 2594 to 2584 cm<sup>-1</sup>). Second, broadened bands in the range of CH stretching vibrations (3050–2850 cm<sup>-1</sup>) are observed related to a less ordered assembly of molecules on the aminopropyl silica matrix compared to the crystalline compound (**1**). Third, new bands appear in the range 1680–1600 cm<sup>-1</sup> which can be attributed to the NH<sub>2</sub><sup>+</sup> linking group. Furthermore, FT Raman spectroscopy enables to compare spectra below 1400 cm<sup>-1</sup>. In this spectral region a series of bands are observed for the modified aminopropyl silica, which have its counterparts in the spectrum of the crystalline initial compound (**1**) (e.g., 1306/1307, 1016/1012, 1003/995, 926/928, 751/752, 565/568 cm<sup>-1</sup>). Thus, the fixation of metallacarborane moiety on the aminopropyl silica was confirmed unambiguously.

### 3.1.2. Hydrophobicity of *Sil-MCB*

Modified polyhedral boron hydrides are known to have hydrophobic properties; therefore, it was expected that the newly synthesized stationary phase would also exhibit hydrophobic properties. Thus, the first chromatographic tests concentrated on the comparison of hydrophobicity between the new CBP with respect to three structurally related stationary phases. The important characteristics of the sorbents are summarized in Table 1. Sorbent SGX C18 represents the typical reversed-phase stationary phase based on a silica matrix, which was prepared by the covalent modification of naked silica with a hydrophobic octadecyl chain. SGX CX is a strong cation exchanger based on the same silica matrix. Because the sulfo group was connected to an aromatic phenyl moiety, a certain affinity for hydrophobic compounds was also expected for this sorbent. Aminopropyl silica phase, SGX NH<sub>2</sub>, was included in the experiments because it served as the starting material for the preparation of the metallacarborane-based CBP, *Sil-MCB*. Aminopropyl silica is a sorbent that exhibits a medium level of

hydrophobicity, which can be used successfully in both normal and reversed-phase chromatographic modes and can be employed as a weak anion exchanger. All of the sorbents were slurry-packed into stainless steel blanks in a 4 mm × 50 mm format.

Neutral low-molecular-weight aromatic compounds, specifically alkyl derivatives of benzene, were used as the testing probes for hydrophobic interactions on the sorbents. The mobile phase consisted of mixtures of acetonitrile with water in various ratios, which is a typical solvent combination in reversed-phase chromatography. The dependence of log *k* versus % of acetonitrile in the mobile phase is shown in Fig. 5a–d for SGX C18, SGX CX, *Sil-MCB* and SGX NH<sub>2</sub>, respectively. Several conclusions can be made based on the results. Fig. 5a illustrates the “classic” reversed-phase SGX C18, where the retention of neutral analytes is dependent on the hydrophobicities. The highest retention value was obtained for butylbenzene, whereas the lowest value was for benzene, as expected. The values of log *k* decreased linearly with increasing amounts of acetonitrile in the mobile phase for all of the tested compounds. Additionally, the slopes of the lines for all of the analytes were very similar. Despite the fact that SGX CX is primarily used in ion exchange chromatography, it can also be used in a reversed-phase mode because the strongly acidic sulfo-group is bound to the phenyl ring that is available for hydrophobic interaction. In Fig. 5b, the linear log *k*–% acetonitrile dependence is shown for SGX CX. However, the lines show diverse slopes, and the dependence converges at approximately 60–65% acetonitrile in the mobile phase. Retention of the analytes was generally lower for SGX CX than with SGX C18. This behavior corresponds well with the significantly lower carbon load for SGX CX (Table 1). Different behaviors with respect to SGX C18 and CX was observed for *Sil-MCB* (Fig. 5c). As expected, the retention factors increased with the hydrophobicity of the separated analytes. The log *k* versus acetonitrile amount (%) dependence was linear for butylbenzene, propylbenzene and ethylbenzene; however, for the compounds of lower hydrophobicity, toluene and benzene, a slight but evident curvature was apparent. The carbon loads for SGX CX and *Sil-MCB* were similar (9.9 versus 11.3% C, respectively) (Table 1), and the retentions of the analytes on both sorbents were comparable at 20% acetonitrile in the mobile phase. However, at more than 40% acetonitrile in the mobile phase, the retention behaviors of these two stationary phases began to differ, and higher log *k* values were obtained for the *Sil-MCB* sorbent. Nevertheless, the overall retention was generally rather low on *Sil-MCB* in comparison to SGX C18 considering both the concentration of acetonitrile in the mobile phase and the carbon load of the sorbents. This might be a consequence of a different type of aromaticity



**Fig. 4.** Raman spectra of the aminopropyl silica, SGX NH<sub>2</sub> (A); the metallacarborane modified aminopropyl silica, *Sil-MCB* (B); and metallacarborane derivative (**1**) (C). The spectra A and B are displayed in the same scale with offset of zero-line.

**Table 1**

Properties of the evaluated stationary phases. (All sorbents were packed in 4 × 50 mm stainless steel columns.).

Sorbent	Source	Carbon [%]	Nitrogen [%]	Boron [%]	Cobalt [%]	Particle size [μm]	Pore size [nm]	Functional group
Separon SGX C18	Tessek, Czech Republic	16.4	–	–	–	5	8	Octadecyl
Separon SGX CX	Tessek, Czech Republic	9.9	–	–	–	5	8	Sulfo phenyl
Separon SGX NH <sub>2</sub>	Tessek, Czech Republic	5.9	1.9	–	–	7	8	Aminopropyl
<i>Sil-MCB</i>	ICT, Prague, Czech Republic	11.3	1.7	2.61 ± 0.07 <sup>a</sup>	0.77 ± 0.07 <sup>a</sup>	7	8	Metallacarborane

<sup>a</sup> Data obtained by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS) (*n* = 3); C and N contents were determined by elemental analysis.

of *Sil-MCB* (the three-dimensional aromaticity as mentioned in Section 1). Finally, the behavior of the tested analytes on SGX NH<sub>2</sub> is illustrated in Fig. 5d. The hydrophobicity of the sorbent SGX NH<sub>2</sub> (5.9% C) (Table 1) was much lower than for SGX C18, and thus, even at 30% acetonitrile in water, very low retention of the compounds was obtained. The elution order of the alkylbenzenes at low acetonitrile concentration in the mobile phase corresponds to a usual reversed-phase pattern; however, at approximately 35% acetonitrile in the solution, the curves of the individual compounds started to cross, and all dependence values were significantly curved in the whole measured range.

Fig. 6 depicts the methylene selectivity for all silica-based sorbents at 40% acetonitrile in water. SGX CX and *Sil-MCB* provided data that were similar and consistent with the hydrophobicity results discussed above.

### 3.1.3. Separation efficiency and peak shape for *Sil-MCB*

The newly synthesized sorbent has the potential to be used for practical purposes; therefore, it was important to study not only the retention factors of the analytes, related to the thermodynamics of the separation process, but also the kinetic properties were of high interest. Representative chromatograms of alkylbenzenes on SGX C18 are shown in Fig. 7a. For comparison, the same standard

mixture was analyzed on *Sil-MCB* (Fig. 7b). Because of the overall lower hydrophobicity of the *Sil-MCB*, no separation of compounds was apparent at 70% acetonitrile, unlike SGX C18, where signs of peak separation were evident in 100% acetonitrile. However, considering the separation efficiency and peak symmetry, the results obtained on both columns are comparable, as shown in Table 2. Thus, the mass transfer characteristics of SGX C18 and *Sil-MCB* were similar.

### 3.1.4. Stability of *Sil-MCB* and column-to-column repeatability

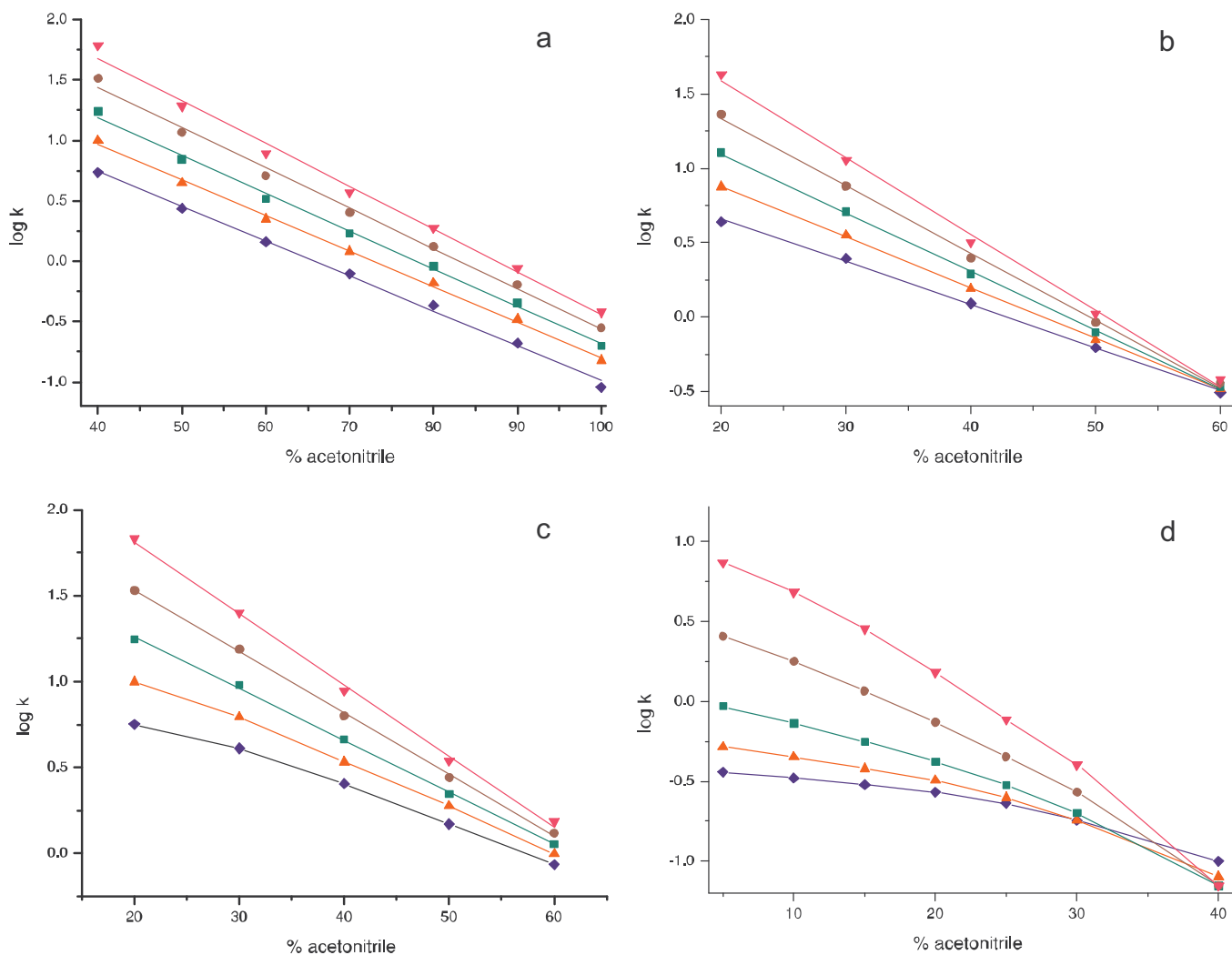
The chemical stability of any newly prepared CBP is always a significant point of interest because real-time applications require the use of durable stationary phases. The sorbent *Sil-MCB* was used for more than one month, and approximately 5 L of various mobile phases ran through the column. Besides acetonitrile/water mixtures, sodium acetate and formate buffers ranging from pH 3 to 6 were also used. Almost no changes in retention factor or separation efficiency of the alkylbenzenes were recorded (data not shown). Thus, it can be concluded that metallacarborane was firmly anchored on the silica matrix and that no “bleeding” occurred. Similarly, the back-pressure on *Sil-MCB* remained constant for the duration of the experiments.

**Table 2**

Separation efficiency and peak asymmetry calculated for butylbenzene on SGX C18 and *Sil-MCB* (column size 4 × 50 mm).

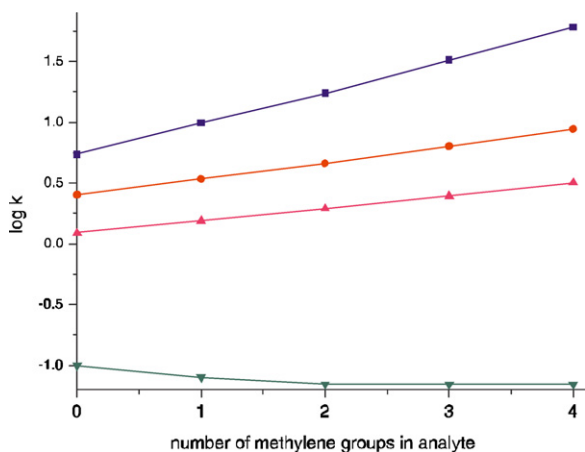
	Acetonitrile/H <sub>2</sub> O ratio in mobile phase (v/v)				
	80/20	60/40	40/60	40/60	20/80
	SGX C18			<i>Sil-MCB</i>	
Chromatographic efficiency, <i>N</i>	3220	3080	2040	2350	2390
Peak asymmetry <sup>a</sup>	1.57	1.41	1.13	1.20	0.94

<sup>a</sup> Calculated at 10% of peak height.



**Fig. 5.** Dependence of the logarithms of the retention factor,  $k$ , on the amount of acetonitrile in water for benzene  $\blacklozenge$ , toluene  $\blacktriangle$ , ethylbenzene  $\blacksquare$ , propylbenzene  $\bullet$  and butylbenzene  $\blacktriangledown$  on SGX C18 (a), SGX CX (b), *Sil-MCB* (c) and SGX NH2 (d). Mobile phase: acetonitrile/ $\text{H}_2\text{O}$ , flow rate:  $0.5 \text{ mL min}^{-1}$ .

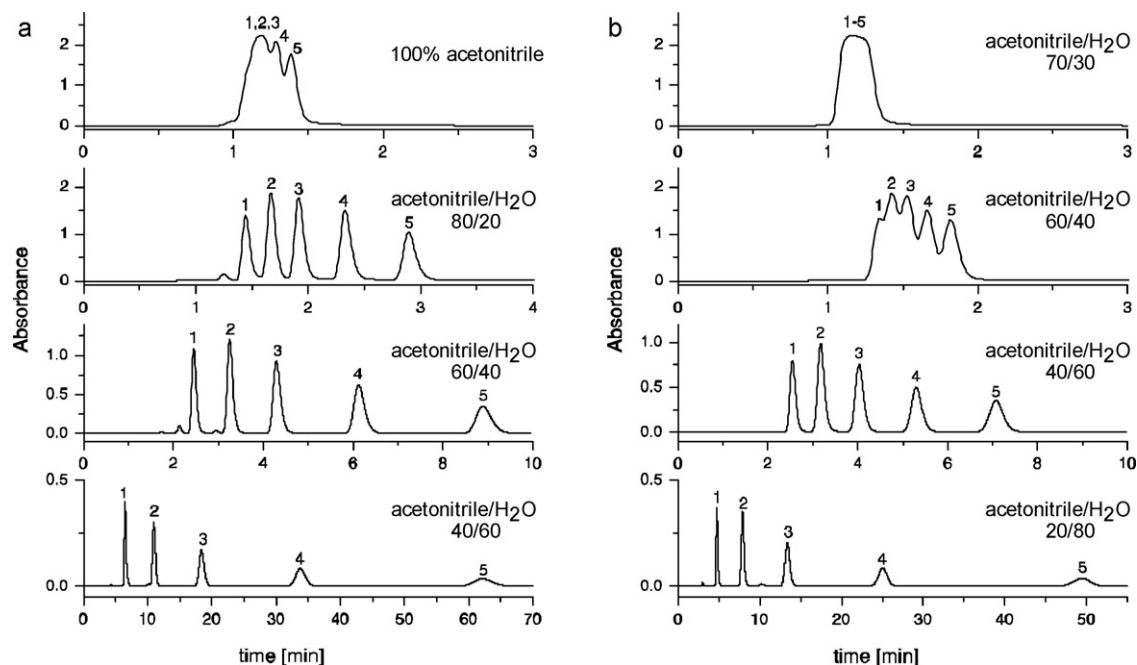
Three columns were packed with the *Sil-MCB* sorbent and their chromatographic efficiency, peak symmetry and retention factors compared. It was found that column-to-column variability in both the separation efficiency and peak symmetry was within about 10% and the retention factors better than 5%.



**Fig. 6.** Methylene selectivity for SGX NH2  $\blacktriangledown$ , SGX CX  $\blacktriangle$ , *Sil-MCB*  $\bullet$ , SGX C18  $\blacksquare$ . Mobile phase: acetonitrile/ $\text{H}_2\text{O}$ , 4/6 (v/v), flow rate:  $0.5 \text{ mL min}^{-1}$ .

### 3.1.5. Compatibility of *Sil-MCB* with a 100% aqueous mobile phase

The chemical structure of our new *Sil-MCB* stationary phase is similar to some well-known polar-embedded phases. Specifically, it resembles the commercially successful zwitterionic sorbent designed for HILIC applications, ZIC-HILIC (Merck, Germany) [22]. This inspired us to evaluate the resistance of *Sil-MCB* to the dewetting phenomenon in a 100% aqueous environment. For that purpose we followed a test described by Przybyciel and Majors [10]. They used amoxicillin, a polar antibiotic, as a test probe and a classic C8 endcapped column. The column was first washed with an acetonitrile/water mobile phase. Then, the mobile phase was switched to 0.1% acetic acid in water (with no organic solvent) without stopping flow. The initial chromatogram of amoxicillin in 0.1% acetic acid showed a peak with a retention time of 8.6 min. In the next step, the flow was stopped for 10 min, and the pressure was left to decrease to atmospheric level. Subsequently, the flow was resumed with the same mobile phase, and the probe was again injected; in this instance, the retention time for amoxicillin was 3.5 min. This drastic decrease in the retention time was directly related to the exclusion of the 100% aqueous mobile phase from the pores, i.e., dewetting of the sorbent. Restoring the retention time of amoxicillin to its original state was only possible by re-pressurization of the system or resolution of the sorbent with an organic solvent containing the mobile phase.

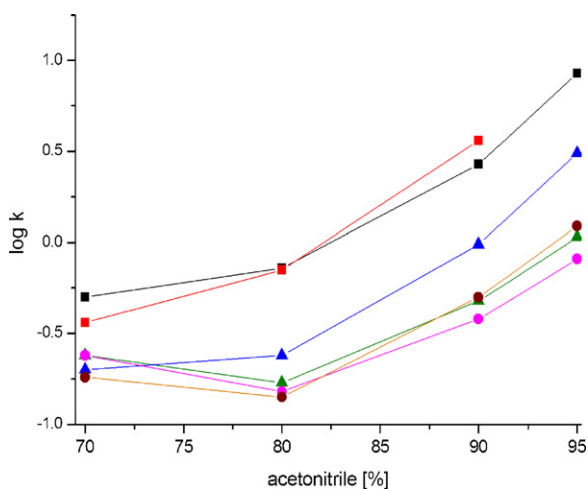


**Fig. 7.** Chromatograms of a hydrophobic interaction test mixture consisting of benzene (1), toluene (2), ethylbenzene (3), propylbenzene (4) and butylbenzene (5) on SGX C18 (a) and *Sil-MCB* (b) sorbents, respectively. Mobile phase: acetonitrile/ $\text{H}_2\text{O}$ , flow rate:  $0.5 \text{ mL min}^{-1}$ , detection UV–vis at 210 nm.

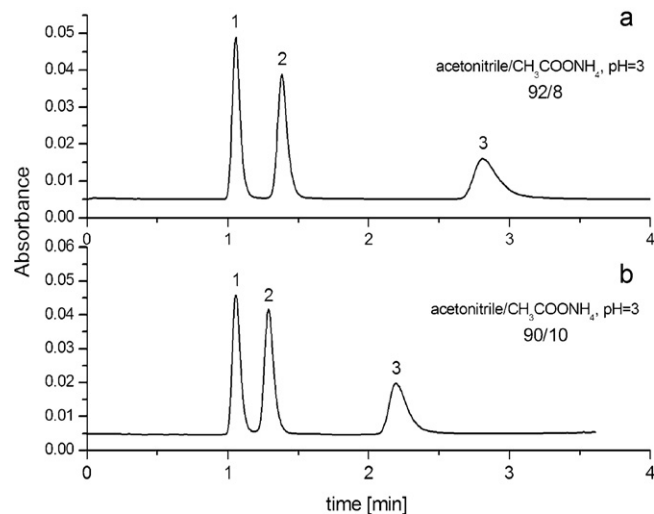
Here we adapted the above mentioned procedure as follows. First, the *Sil-MCB* column was thoroughly washed with an acetonitrile/ $\text{H}_2\text{O}$ , 2/8 (v/v) mobile phase for 20 min at  $0.5 \text{ mL min}^{-1}$ . Then, the mobile phase was switched, without flow interruption, to 0.1% acetic acid in water, and equilibration took place for 20 min at  $0.5 \text{ mL min}^{-1}$  flow rate and 3.1 MPa back-pressure. After the specified time a diluted benzene solution was injected onto the column and the retention time and peak shape for benzene were determined. It was found that benzene eluted at 5.06 min in the form of a slightly tailing peak (the void time of the column was equal to 1.05 min). Subsequently, the flow was shut off for 10 min and then resumed followed by the immediate injection of the benzene probe onto the column. The resulting elution time and peak shape of benzene remained the same as before stopping the flow. Thus, the experiment confirmed full compatibility of *Sil-MCB* with a 100% aqueous mobile phase and an excellent resistance to dewetting.

### 3.2. Possible utilization of *Sil-MCB* in HILIC mode

Finally, preliminary studies were conducted on the potentially new sorbent *Sil-MCB* in HILIC mode. Typically, retention factors of hydrophilic compounds increase with the addition of an organic solvent to the aqueous mobile phase in HILIC mode. Exactly this was the trend observed with the *Sil-MCB* phase for six nucleobases and nucleosides. Fig. 8 presents data obtained for these compounds in an acetonitrile/water mixture in the range of 70–95% acetonitrile in aqueous buffer. The ability of *Sil-MCB* to provide strong HILIC-based interactions with the hydrophilic solutes is further demonstrated in Fig. 9. The probe mixture contained toluene (as a representative of a low-molecular-weight hydrophobic compound), uracil and adenine. Toluene eluted with the shortest time of the three compounds and the two hydrophilic analytes showed a significantly greater



**Fig. 8.** Dependence of the logarithms of the retention factor,  $k$ , on the amount of acetonitrile in buffer for thymidine (●), thymine (●), uridine (▲), uracil (▲), cytidine (■), and cytosine (■) for the column packed with *Sil-MCB*. Mobile phase: acetonitrile/ $5 \text{ mmol L}^{-1}$  ammonium acetate, flow rate:  $0.5 \text{ mL min}^{-1}$ .



**Fig. 9.** Chromatogram of the separation of toluene (1), uracil (2), and adenine (3) on the column packed with the sorbent *Sil-MCB*. Mobile phase: acetonitrile/ $5 \text{ mmol L}^{-1}$  ammonium acetate, flow rate:  $0.5 \text{ mL min}^{-1}$ , detection UV–vis at 210 nm.

retention on the stationary phase (Fig. 9a). This is an inverse elution pattern in comparison to the retention on classical RP stationary phase where the most hydrophobic compound elutes as the last component of the analyzed mixture. Finally, Fig. 9b shows that even a slight decrease in the content of acetonitrile in the mobile phase was immediately followed by a pronounced reduction in retention times of the hydrophilic probes, which demonstrates again the capability of *Sil-MCB* to be utilized in HILIC mode.

#### 4. Conclusion

A new stationary phase with metallacarborane bonded onto a silica matrix was prepared, packed into columns and chromatographically tested. Under RP conditions, the sorbent manifested hydrophobic interactions with nonpolar solutes and reasonable separation efficiency and peak symmetry. The immobilized chemical structure exhibited hydrophobic and zwitterionic properties. The expected resistance against a phase collapse was also studied, and no loss of retention for benzene in a 100% aqueous mobile phase was observed. The synthesized *Sil-MCB* sorbent demonstrated good chromatographic stability for several months as retention factors, and the other peak characteristics, remained unchanged. Finally, *Sil-MCB* showed promising potential in HILIC mode, which is useful for strongly polar and charged solutes.

Several other metallacarborane-based CBPs varying in chemical structure will be prepared in the future, and various means of immobilization on silica will be examined. Additionally, attention will be given to a detailed study of the three-dimensional aromaticity of MCB-based CBPs because this might offer different chromatographic selectivities for some compounds relative to octadecyl silica RPs. Finally, more practically oriented applications on the new stationary phases in HILIC mode will be developed.

Despite the relative maturity of the stationary phase science and the many great achievements reported in recent times within the field, we strongly believe that there is still plenty of room for novel materials to be discovered with different and unusual selectivities, as well as for tailor-made stationary phases for specific applications and complex analyses.

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