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

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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.

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 μm 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 ~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-

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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-endcapped 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 metallacarborane was reported in 1965 [23,24]. The 18-electron low-spin d^6 cobalt bis(1,2- and 1,7-dicarbollides) [*commo*- 3,3′-$Co(1,2\text{-}C_2B_9H_{11})_2]^-$ and [*commo*- 2,2′-$Co(1,7\text{-}C_2B_9H_{11})_2]^-$ were among the very first metallacarboranes synthesized (Fig. 1). Currently, the chemistry of these compounds is considered being one of the most elaborated in metallacarborane field [25].

Although the crystal structure of $Cs[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]$ was determined in 1967 [26], the positions of the carbon atoms, and hence the ligand orientations, were established much later for (Et_3NH) $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]$ [27]. The $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$ anion (Fig. 2) consists of two $(C_2B_9H_{11})^{2-}$ dicarbollide units sandwiched around a formal Co^{3+} ion with the C_2B_9 faces of the two ligands nearly parallel. The vector distances from cobalt to the C_2B_9 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 $(C_2B_9H_{11})^{2-}$ 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_2 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*-$3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$, is reported. To the best of our knowledge this is the first metallacarborane (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). $Cs[(8\text{-dioxane-}1,2\text{-}C_2B_9H_{10})\text{-}3\text{-}Co\text{-}(1',2'\text{-}C_2B_9H_{11})]$ (**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 nucleofile, here amino group of the NH_2-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^{-1}) 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^{-1} resolution. Two repetitive measurements were performed for each sample.

Raman spectra (4000–100 cm^{-1}) 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