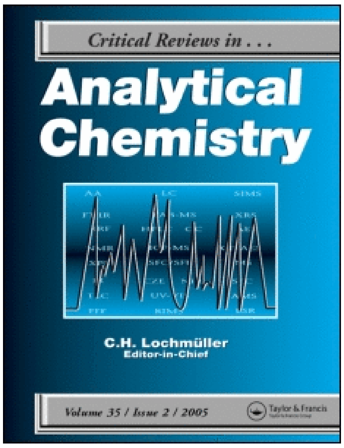

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Problems Associated with the Chromatographic Determination of Chlorobenzylgermanium Derivatives Using an Octadecyl Stationary Phase

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Results of investigations regarding the difficulties of benzylgermanium determination by high performance liquid chromatography using standard octadecyl stationary phases have been substantiated on the molecular level. The analyzed organogermanium compounds are biologically active and some are used in oncology. Investigations showed modifications of the octadecyl stationary phase caused by the above-mentioned derivatives. These modifications have been confirmed by infrared spectroscopy with Fourier transform (FTIR), ^{13}C CP/MAS Nuclear Magnetic Resonance techniques (NMR), ^{29}Si CP/MAS NMR, and scanning microscopy. The strongest bonds are formed by benzyltrichlorogermanium but the tribenzyl derivative with a sterically large group and the long, 18-carbon chains, is bound only weakly. It also interacts to the smallest extent with residual silanols of the octadecyl phase. Mass ratio germanium to silica calculated on the base of scanning display after modification was 12.63% for monobenzyl derivative, 12.49%, for dibenzyl, and 5.6% for tribenzyl.

Keywords Benzylgermanium derivatives, octadecyl stationary phase, surface modification

INTRODUCTION

Despite continuous development of organogermanium chemistry, properties of numerous germanium compounds are insufficiently known. The benzyl and furyl derivatives of germanium serve as good examples. Furyl derivatives can show similar properties as furylsilanes. Yet, germanium and its organic derivatives show different properties when are compared with their silicon analogues, especially concerning biological activity. At the same time, some germanium derivatives, e.g., benzylgermanium are very similar to silicon analogues, because of ability to modify stationary phases in high performance liquid chromatography (HPLC). This fact causes errors and difficulties in chromatographic determinations, independently of the type of column used.

Almost all organogermanium compounds show biological activity. Among benzyl derivatives of germanium, the highest activity shows benzylgermatranes (1–4). These derivatives exhibit anesthetic and anticonvulsive properties. Some of these compounds completely prevent amnesia of animals induced by an electric shock, e.g., benzylgermatrane and o- and p-bromobenzylgarmatrane (3). So, for these reasons, organogermanium derivatives are used first of all in the pharmaceutical and medical industry.

Organogermanium compounds' protection against harmful radiation has been confirmed in numerous cases. Such activity showed, among others, germanothiazolidil, germanodithioacetal, and germatrane sulfide. They efficiently protected mice against gamma radiation emitted during investigations by the so-called cobalt bomb.

Recently, an increase of popularity concerning organogermanium chemistry could be observed. Particular hopes are connected with the use of these compounds in anticancer therapy (e.g., Ge-132). It is of great importance because of their toxicity, low in comparison with other metalorganic preparations now used in oncology (7–10).

The anticancer mechanism of organogermanium compounds is not fully known. Their use is supposed in chemotherapy, which is the most frequent, supporting surgery, method of cancer treatment. Now, the basic aim of contemporary chemotherapy is an elaboration of drugs characterized by wider

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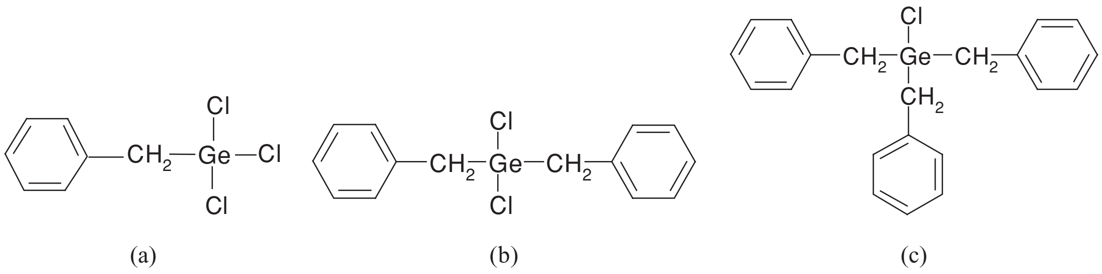

FIG. 1. Structures of: a) benzyltrichlorogermanium, b) dibenzyldichlorogermanium, c) tribenzylchlorogermanium.

versatility and higher selectivity on degenerated groups of cells. Until now, 5-triethylgermyl-2-trifluoroacetylfuran was regarded as the most effective preparation in a fight against cancer (10, 11). This compound is a stronger inhibitor of tumor development than analogous silicon compound. Biological activity of organic derivatives is influenced by the presence of germanium or silicon atoms as well as the presence of heterocyclic rings in the structures.

Organogermanium compounds are a little harmful for a human organism, however halogen derivatives of germanium are toxic (5, 6). Germanium is accumulated in kidneys, liver, spleen, and bones and can cause fatty liver, inflammation, and cancerous changes (12, 13).

The organic form of germanium is in daily nourishment applied as Ge-132. The average content of germanium in the human body is 0.15 mg/kg in the liver and over 0.1 mg/kg in muscles. However, official standards of daily demand are not fixed. Average daily intake depends on diet and is at about 0.35 mg/kg. In vegetarian diet, it is at about 3 mg/kg, and in a highly proteinic diet only 0.8 mg/kg. To achieve a therapeutic level of germanium normal diet, supplementation is necessary (14–16).

The extent of germanium toxicity is not high. Toxicological investigations exhibited that a majority of tested organogermanium preparations is less toxic than their silicon and tin analogues (1, 17), but long application and high doses of these compounds can cause damage of kidneys. Their extent of toxicity mainly depends on germanium substituents. Tetraalkylgermanium derivatives, and germatranes, and geranols belong to not toxic compounds ($LD_{50} = 3000$–5000 mg/kg). On the other hand, thienylgermatrane is very toxic ($LD_{50} = 15$–20 mg/kg) (11).

Tetraorganogermanium preparations R_4Ge (where R = Et, Pr, Bu, $PhCH_2$, Ph) exhibited low toxicity in mice tests. A toxic dose for n-alkyl was between 2000 and 10000 mg/kg. Tetraisopropylgermanium is more toxic (620 mg/kg) than tetra(n-propyl)germanium (5690 mg/kg). Tricyclohexylgermanol and hexaphenyldigermoxane were shown to be not toxic to mice $^{(3)}$ during tests. Toxicity of hexaorganodigermoxane strongly depends on a sort of germanium substituent, e.g., hexaethyldigermoxane is more toxic ($LD_{50} = 240$ mg/kg) than hexabutyldigermoxane ($LD_{50} = 6000$ mg/kg).

To recapitulate, benzylgermanium derivatives exhibit interesting biological activity. Various prospects of applications justify wide-ranging investigations concerning this group of compounds. Research in the field of new, more efficient medicines treating cancer diseases is especially important. So, properties of such biologically active compounds and their determination must be known on the molecular level. In the case of benzylgermanium derivatives there appears to be a problem of their determination, because some of them modify the stationary phase surface during chromatographic processes. This causes great difficulties during determination and results are burdened with significant errors. This problem concerns standard silica gel as well as chemically modified stationary phases. Detailed information concerning modification of octadecyl phase by chloroderivatives of mono-, di-, and tribenzylgermanium are presented in the work. This problem was the main aim of this

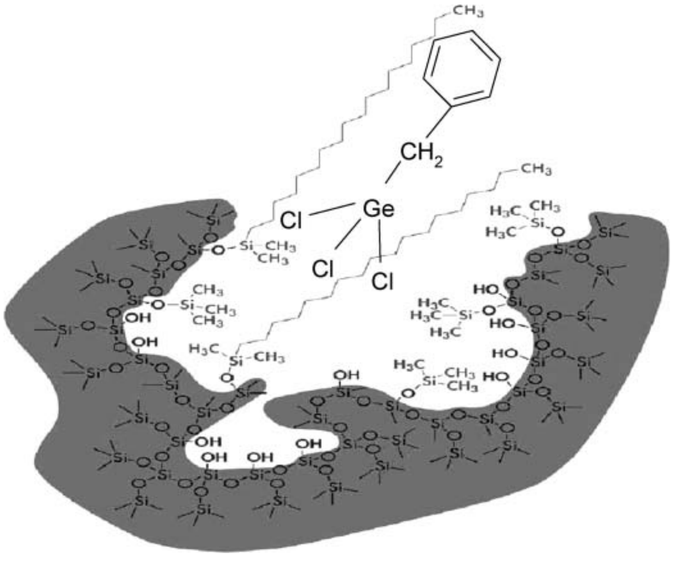

FIG. 2. Scheme of the structure of chemically bonded octadecyl phase and their modification by benzyltrichlorogermanium.

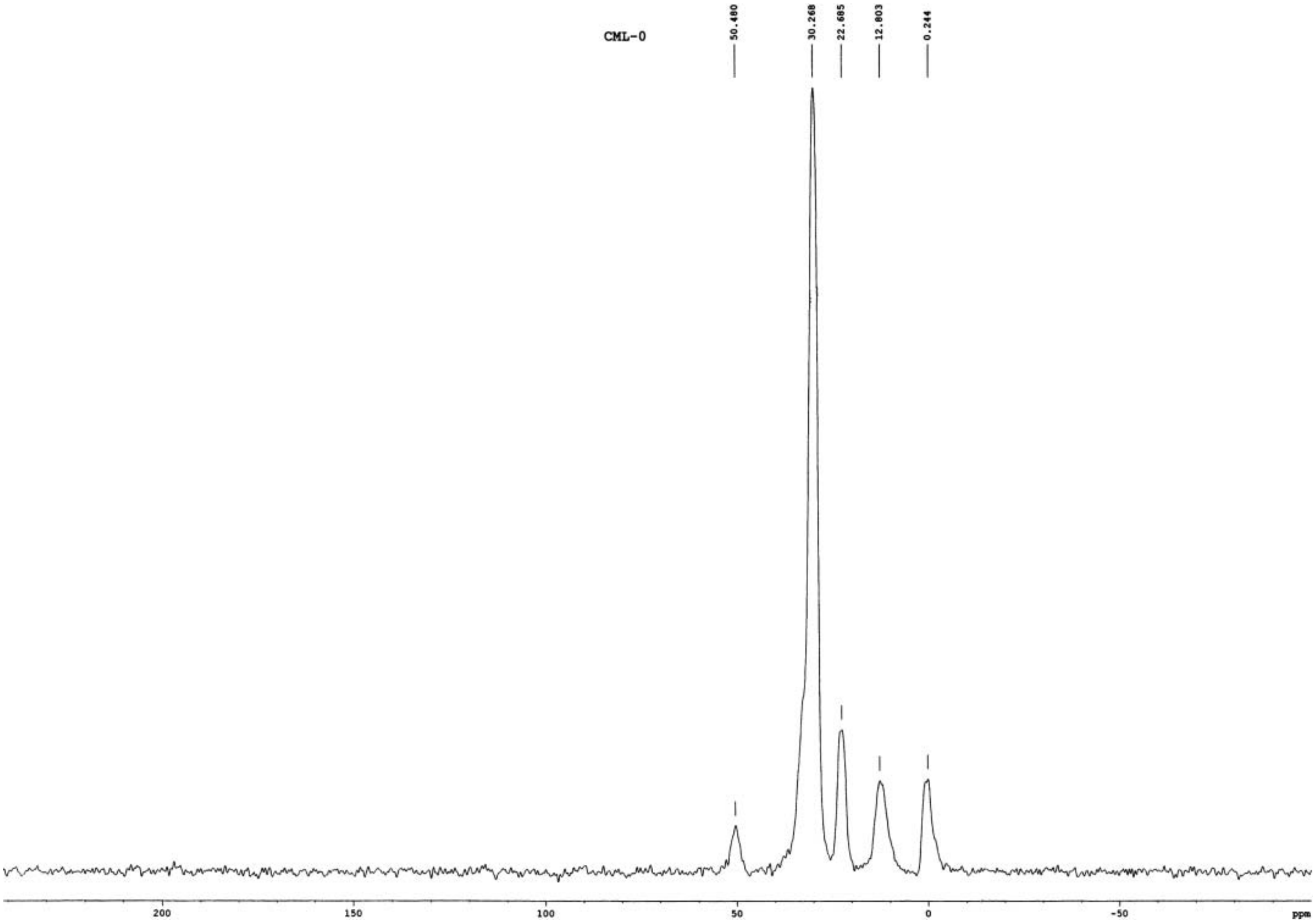

FIG. 3. A comparison of ^{13}C CP/MAS NMR spectra of octadecyl phase.

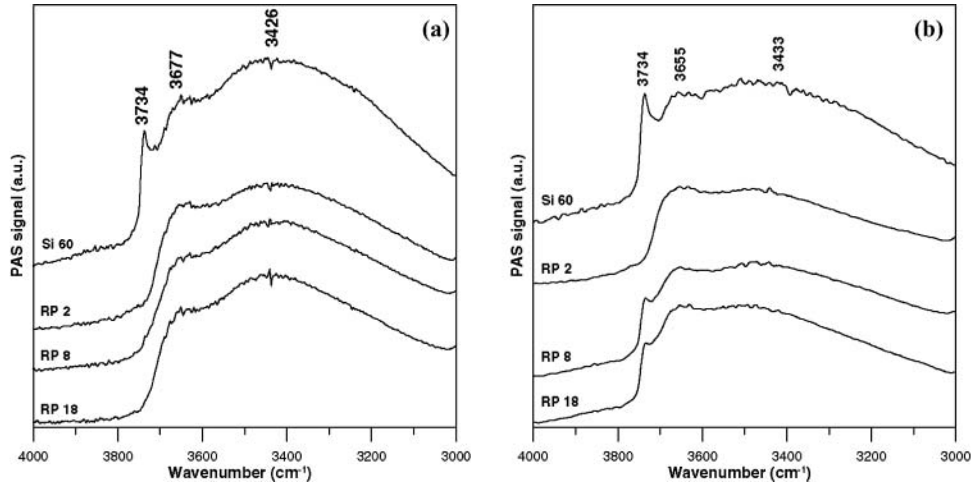

FIG. 4. FT-IR/PAS spectra of the hydroxyl groups for thermally treated liwestigated samples at: a) 423 K, b) 573 K (29).

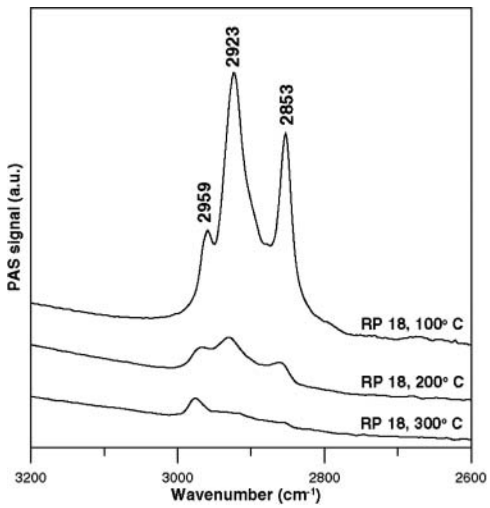

FIG. 5. FT-IR/PAS spectra for octadecyl phase at different temperatures (29).

work. In order to characterize results in detail, spectra of ^{13}C NMR and ^{29}Si NMR for solid and scanning microscopy pictures were used.

EXPERIMENTAL

Modification of chemically bonded octadecyl stationary phase by chlorobenzyl derivatives of germanium in the chromatographic process:

Samples of benzytrichlorogermanium, dibenzyldichlorogermanium, and tribenzylchlorogermanium (Fig. 1) were dissolved in acetonitrile (Lab-Scan, Dublin, Ireland) obtaining a concentration of about 25 μg/mL. Obtained solutions were passed through chromatographic columns. Chemically bonded octadecyl phases were used as a stationary phase (Figs. 2, 3). Analyses were carried out at a temperature of 23°C. Acetonitrile and acetonitrile/water served as mobile phases. Obtained modified octadecyl packings were subjected to investigations by FTIR , ^{13}C CP/MAS NMR , ^{29}Si CP/MAS NMR, and scanning microscopy. Benzyl derivatives of germanium were synthesized according to the literature.$^{(18)}$

Benzyltrichlorogermanium: ^{1}H NMR, δ (ppm) = 3.44 (s, 2H, CH_2Ge) 7.26–7.38 (m, 5H, aromat.). ^{13}C NMR, δ (ppm) = 39.48 (-CH_2-), 127.67, 129.09, 129.31, 130.35 ($C_{aromat.}$). MS (EI), m/z (%) = 273 (0.36), 272 (1.2), 270* (1.5), 268 (0.77), 266 (0.46), 179 (0.34), 144 (0.25), 125 (0.37), 111 (0.75), 109 (2.13), 107 (1.21), 105 (0.68), 92 (6.99), 91 (100), 89 (5.57), 65 (23.85).

Dibenzyldichlorogermanium: ^{1}H NMR, δ (ppm) 3.02 (s, 4H, CH_2Ge), 7.09–7.36 (m, 10H, aromat.). ^{13}C NMR, δ (ppm) 33.92 (-CH_2-), 126.53, 128.82, 129.07, 133.22 ($C_{aromat.}$). MS (EI), m/z (%): 328*(3.46), 182 (1.80), 104 (2.15), 91 (100).

Tribenzylchlorogermanium: ^{1}H NMR, δ (ppm) 2.61 (s, 6H, CH_2Ge) 6.84–7.31 (m, 15H, aromat.). ^{13}C NMR, δ (ppm) 26.73 (-CH_2-), 125.41, 128.59, 128.62, 136.41 ($C_{aromat.}$). MS (EI), m/z (%): 382* (2.29), 291 (10.95), 255 (3.55), 165 (3.11), 109 (1.67), 91 (100).

The infrared spectra of benzylgermanes have been examined in the region 4000–50 cm^{-1} to assign the characteristic group frequencies in the compounds synthesized.

Benzyl derivatives: (Abbreviations: w–weak; m–medium; s–strong; b–broad)

3099 w, 3082 w, 3066 m, 3050 s, 3018 s, 2936 m, 2899 m, 2293 w, 1948 w, 1874 w, 1816 w, 1754 w, 1595 s, 1578 s, 1491 s, 1450 s, 1414 m, 1334 m, 1317 m, 1210 s, 1181 s, 1146 s, 1056 s, 1030 m, 999 w, 908 m, 805 s, 761 s, 698 s, 559 m, 542 m, 460 bs, 444 bs, 342 w, 207 w, 204 w, 150 m, 144 m.

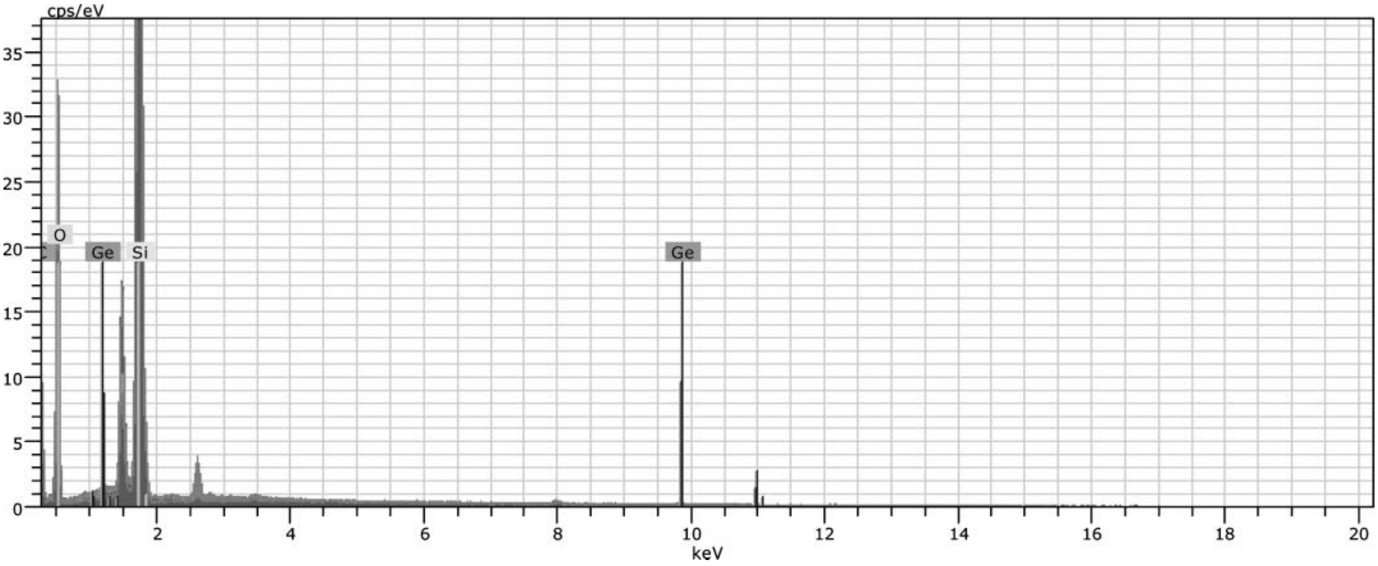

FIG. 6. Diagram of octadecyl phases modified by benzyltrichlorogermanium.

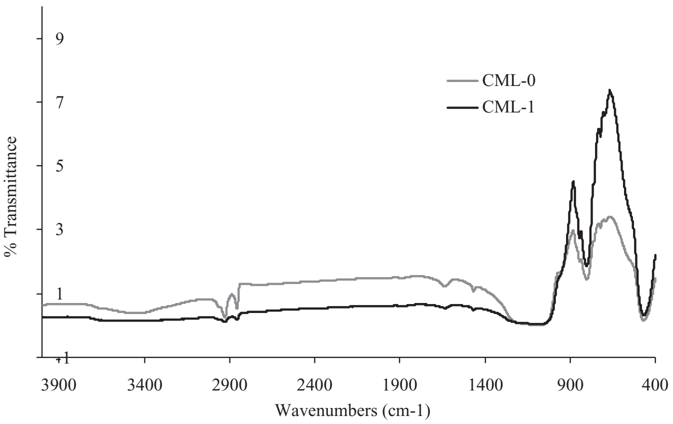

FIG. 7. A comparison of FTIR spectra of octadecyl phases (CML-0) and octadecyl phases modified by benzyltrichlorogermanium (CML-1).

Apparatus

^{1}H, ^{13}C, and ^{29}Si NMR spectra were recorded on a Varian Mercury 400 MHz in DMSO-d6 (Varian, Inc., Palo Alto, CA, USA), with TMS as an internal standard. MS-spectra were performed with a Shimadzu Mass-Spectrometer GC/MS-QP5050, 70 eV, and a Phenomenex BPX-5 column 30 m × 0.25 mm ID × 0.25 μm FT, total flow 52.7 mL/min. FTIR spectra were recorded on a Nicollet Magna-IR spectrophotometer in KBr pellets. Scanning electron microscope (SEM) was used to evaluate images of the octadecyl stationary phase modified by mono-, di-, and tribenzylchlorogermanium (LEO 1430VP, Zeiss, Jena, Germany); the EDX analysis was done by XFlash 4010 detector (Bruker AXS Microanalysis, Berlin, Germany).

RESULTS AND DISCUSSION

The main aim of the investigations was to show on a molecular level the ability of modification of the octadecyl phase

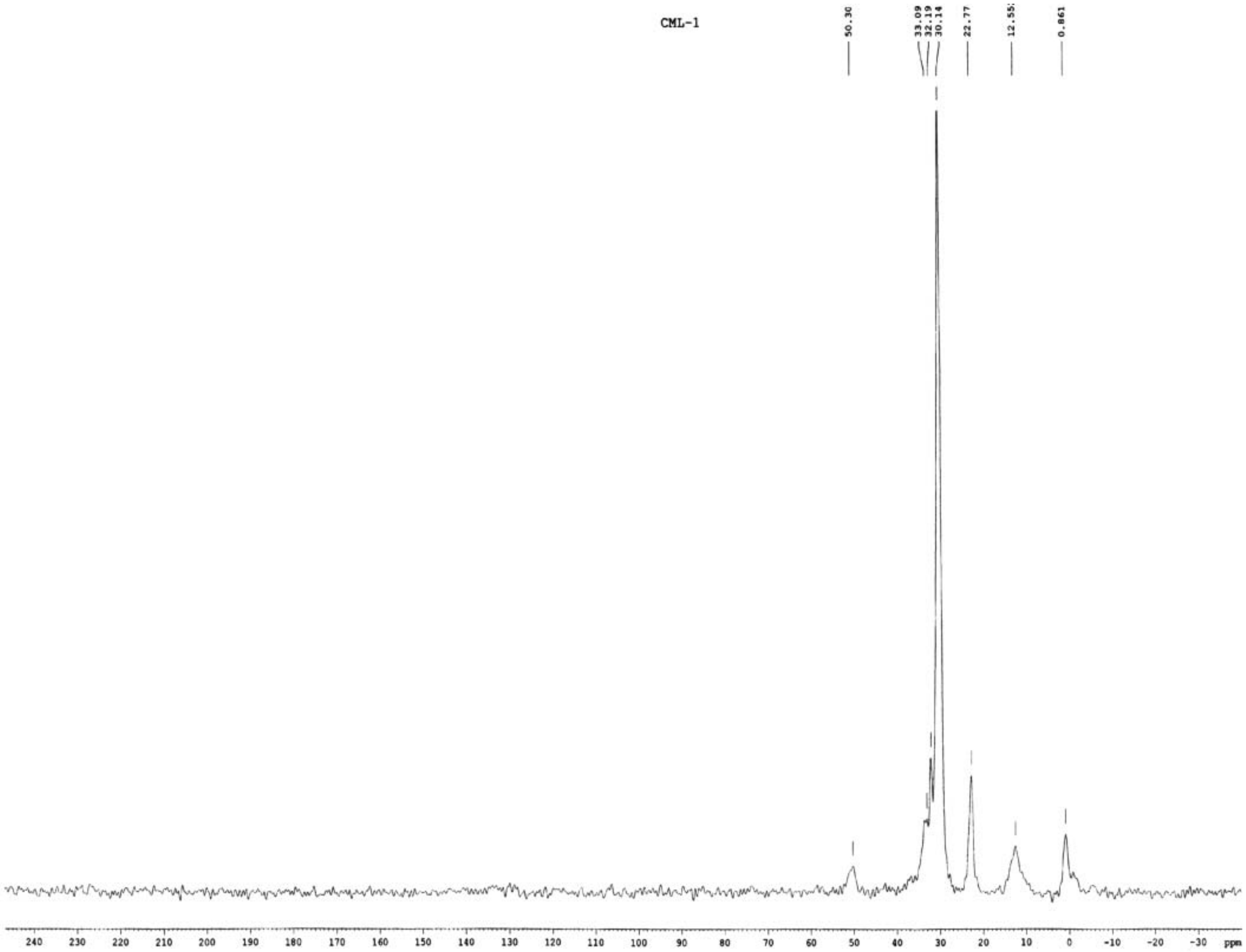

FIG. 8. A comparison of ^{13}C CP/MAS NMR spectra of modified octadecyl phases surfaces by benzyltrichlorogermanium.

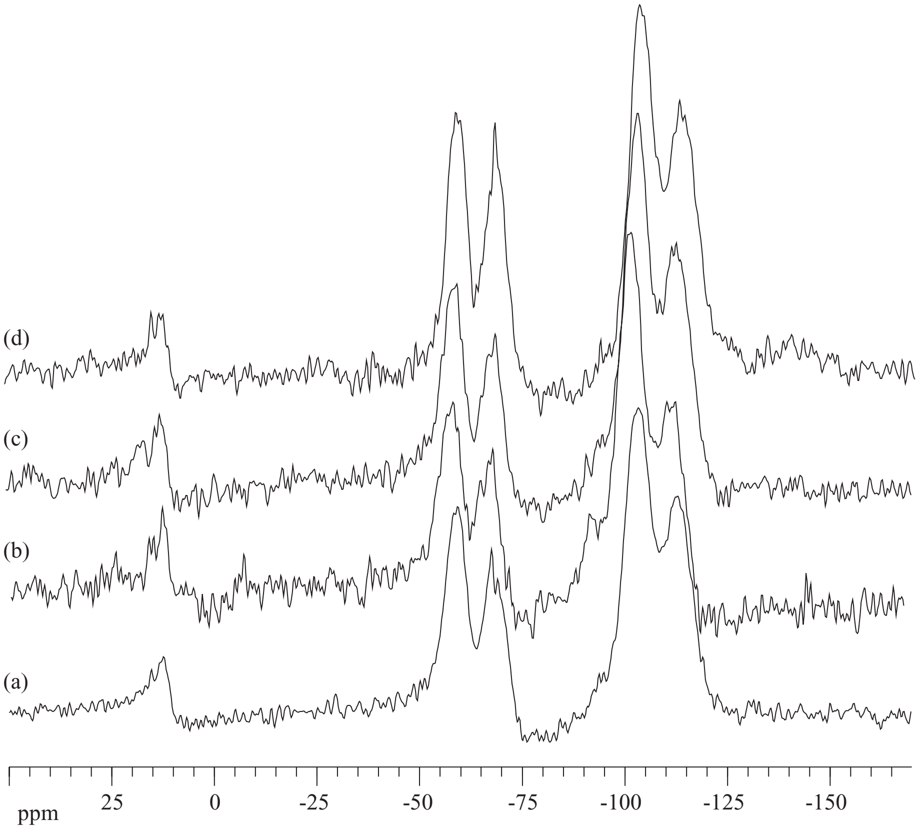

FIG. 9. A comparison of ^{29}Si CP/MAS NMR spectra of a) unmodified octadecyl phases surfaces, b) modified by benzyltrichlorogermanium, c) modified by dibenzyldichlorogermanium, d) modified by tribenzylchlorogermanium.

during benzylgermanium determination and to evaluate their participation in the modification into percentage. In order to do this FTIR, ^{13}C CP/MAS NMR, ^{29}Si CP/MAS NMR, and scanning microscopy were used. Obtained results are presented in Figs. 3–18. The work is a continuation of earlier investigations, which exhibited modification of silica gel surface by mono-, di-, and tribenzyl derivatives of germanium (11, 19, 20). Originally, the chemically bonded (standard) octadecyl stationary phase was used in order to solve problems of phase modification during the determination of chlorogermanium derivatives. This assumption was shown to be false. Despite long chains of 18 carbon atoms, chlorobenzyl derivatives of germanium showed an ability to modify residual (that is not blocked) silanols.

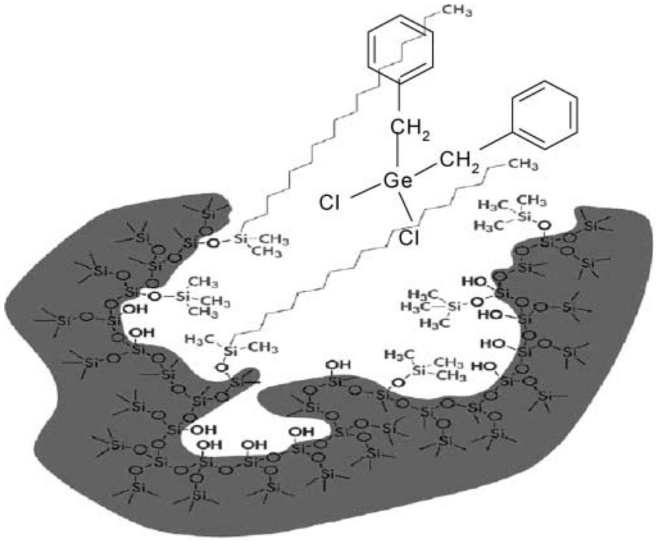

FIG. 10. Scheme of the structure of chemically bonded octadecyl phase and their modification by dibenzyldichlorogermanium.

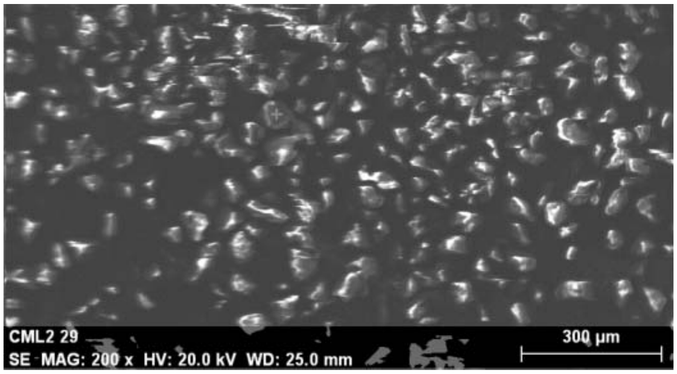

FIG. 11. Image of the octadecyl phase modified by dibenzyldichlorogermanium obtained by SEM microscope.

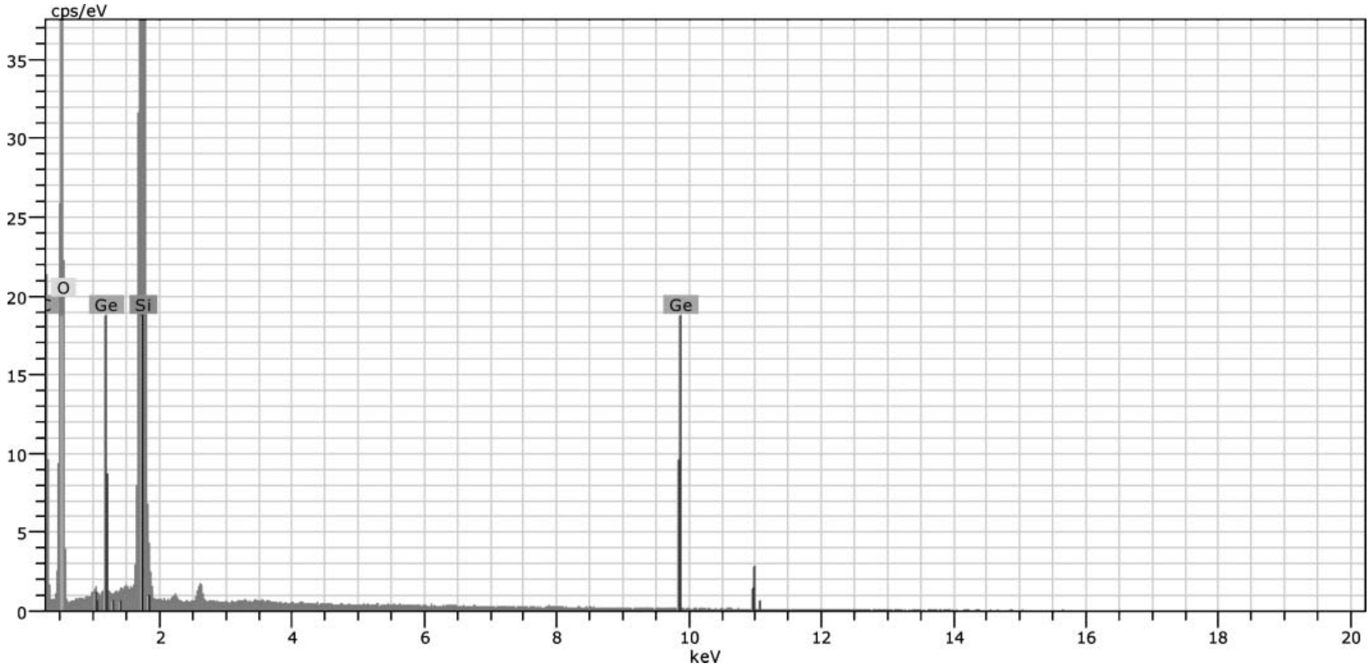

FIG. 12. Diagram of octadecyl phases modified by dibenzyldichlorogermanium.

The type and concentration of surface hydroxyl groups are essential factors connected with chemical modification and chromatographic elution. For ideal adsorbents a good hydroxylated surface should be characterized by total silanol concentration at about 8 μmol/m^2 (21). However, it is an assumption that only 50% can be effectively chemically blocked. In the case of octadecyl phase synthesis, because of long carbon atom chains, the number of blocked silanols can be even less. It means that some unblocked silanols can be bonded by other reactive molecules with small size. This situation appears also in the case of chromatographic determination of chlorobenzyl germanium derivatives.

Investigations concerning the silanols included in the octadecyl phase were repeatedly performed (22–28). Stationary phases were analyzed (Fig. 4) taking into consideration, for example, resistance to high temperature and accessibility of silanols (29). The resistance to high temperature was shown to be lower with an increase of hydrocarbon chain length. The C2 phase showed higher thermal stability than C8 and C18. Moreover, temperature degradation of chemically bonded C8 and C18 phase caused surface silanols (isolated -OH groups) to become accessible on the silica surface (Fig. 5).

In order to precisely define the architecture of the modified surface of octadecyl phase by chlorogermanium, derivatives applied included techniques of nuclear magnetic resonance (^{13}C, ^{29}Si), infra red spectroscopy in modus Fourier transformation, and displaying of the surface by scanning electronic microscope (SEM). These techniques allow the characterization of the adsorbent surface on the molecular level.

Spectroscopic investigations, which were carried out, confirmed modification of the octadecyl phase surface by mono-, di-, and trichlorobenzyl derivatives of germanium. This modification includes from several up to 12% of the surface. It was affirmed that during the chromatographic process

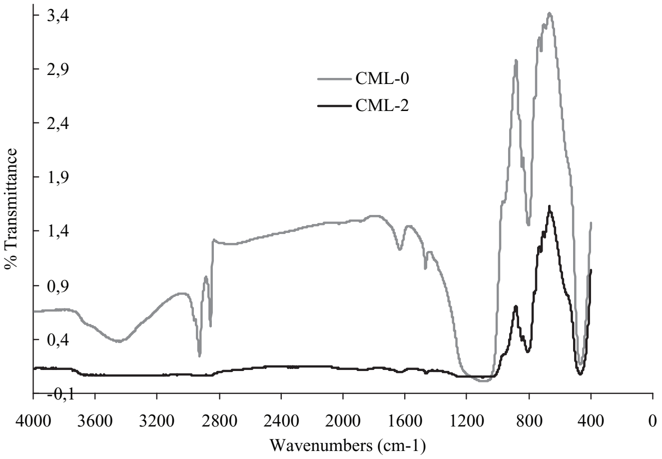

FIG. 13. A comparison of FTIR spectra of octadecyl phases (CML-0) and octadecyl phases modified by dibenzyldichlorogermanium (CML-2).

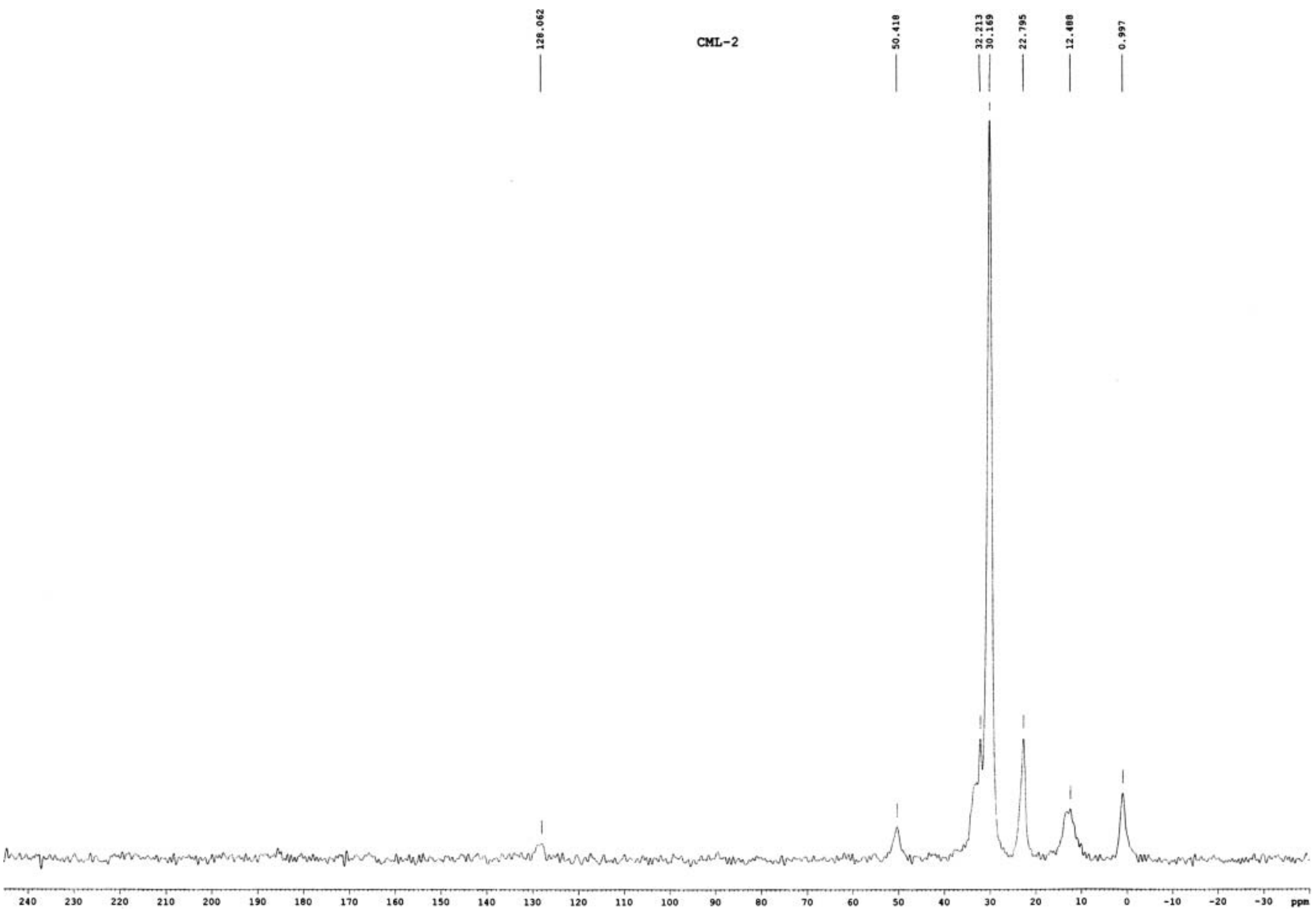

FIG. 14. A comparison of ^{13}C CP/MAS NMR spectra of modified octadecyl phases surfaces by dibenzyldichlorogermanium.

benzyltrichlorogermanium blocked a part of free residual silanol groups and in this way modified octadecyl phase surface (Figs. 6–9). The modification of the octadecyl phase surface was confirmed by a diagram obtained by the use of the scanning microscope (Fig. 6), FTIR spectra of the octadecyl phase before modification and after the modification by monobenzyl derivative (Fig. 7). The surface modification can also be observed on spectra ^{13}C CP/MAS NMR (Fig. 8) and ^{29}Si CP/MAS NMR (Fig. 9).

After partial blocking of silanols on the matrix surface by monobenzylgermanium ligands some signals in spectra appeared or were changed (32.19, 33.09 ppm, Fig. 8). It is an evidence of a chemisorptions and a new surface formation (Figs. 7–9). Monobenzyl derivatives form strong bonds with the stationary phase surface, which are not broken in usual elution processes. It causes serious problems during their chromatographic determination. Only the use of concentrated inorganic acids, e.g., hydrochloric acid or sulfuric acid, breaks the bonds and leads to inorganic germoxanes, similarly as in the case of usual silica gel (30). Mass ratio germanium to silica calculated

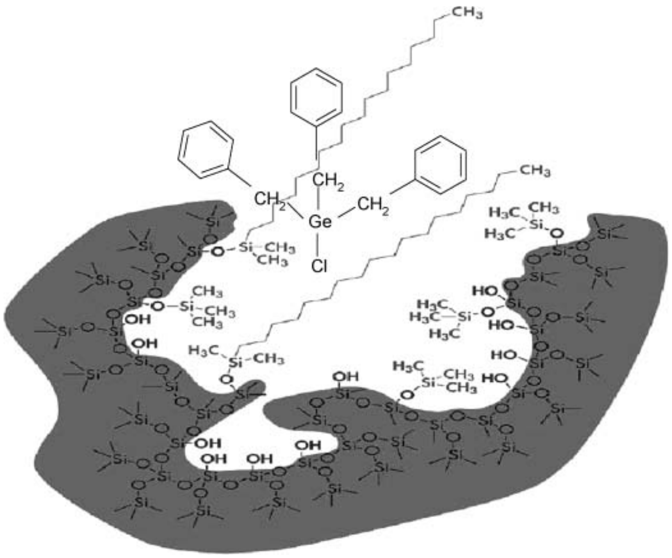

FIG. 15. Scheme of the structure of chemically bonded octadecyl phase and their modification by tribenzylchlorogermanium.

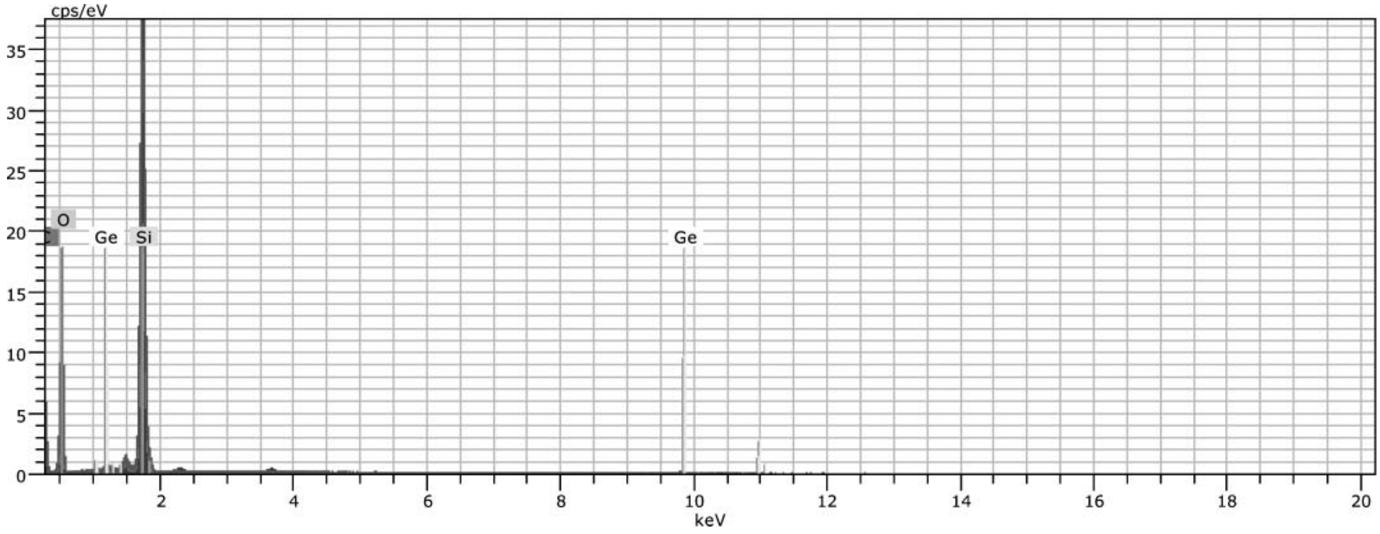

FIG. 16. Diagram of octadecyl phases modified by tribenzylchlorogermanium.

on the base of scanning display after modification was 12.63 % for monobenzyl derivative.

Dibenzyldichlorogermanium is blocking the part of residual silanols of octadecyl phase in a similar way. The dibenzylgermanyl substituent has a slightly weaker attachment to the surface of the stationary phase; the bonds can be broken only by the use of extremely polar solvents. Modification of the surface is presented in Figs. 9–14. The presence of germanium bonded to the octadecyl phase surface was confirmed using scanning microscope (Figs. 11, 12). The mass ratio of germanium to silica calculated on the base of scanning display after modification was 12.49% for dibenzyl derivative. A comparison of FTIR spectra of silica gel before and after the modification by dibenzyldichlorogermanium (Fig. 13) confirms the role of this derivative in the formation of a new surface. Recorded spectra ^{13}C CP/MAS NMR data (Fig. 9) and ^{29}Si CP/MAS NMR (Fig. 14) also showed the chemisorptions of dibenzyl derivative at the phase surface.

A dissimilar situation appears after the use of tribenzyl derivatives of germanium (Fig. 9, 15–18). Because of the sterically large group and long hydrocarbon chains consisting of 18 carbon atoms, tribenzylchlorogermanium is the weakest modifier of the stationary phase surface in comparison with mono- and dibenzyl derivatives of germanium. This fact is confirmed by FTIR spectra (Fig. 17) as well as ^{13}C CP/MAS NMR (Fig. 9) and ^{29}Si CP/MAS NMR (Fig. 18, bands 32.222, 33.124 ppm) and scanning microscope display (Fig. 16). However, tribenzylchlorogermanium is easily eluted from columns by aqueous organic solvent in the form of hexabenzylgermoxane. On the other hand, elution by an anhydrous solvent yields unchanged tribenzylchlorogermanium. The mass ratio of germanium to silica calculated on the base of scanning display after modification by tribenzylchlorogermanium was 5.6%.

To recapitulate, analyzed mono-, di-, and tribenzylgermanium derivatives exhibit the ability to block residual silanols of chemically bonded octadecyl stationary phase. Because during chemical modification of stationary phases a considerable part of silanols remain not blocked, such difficulties can also appear on other modified phases used for determination. We hope that problems connected with the determination of benzyl germanium derivatives and substantiated in the work may be useful to numerous analysts. Despite many difficulties, because of their applications in oncology, all investigations aimed at a deeper knowledge and precise dosage in cancer diseases treatment are justified.

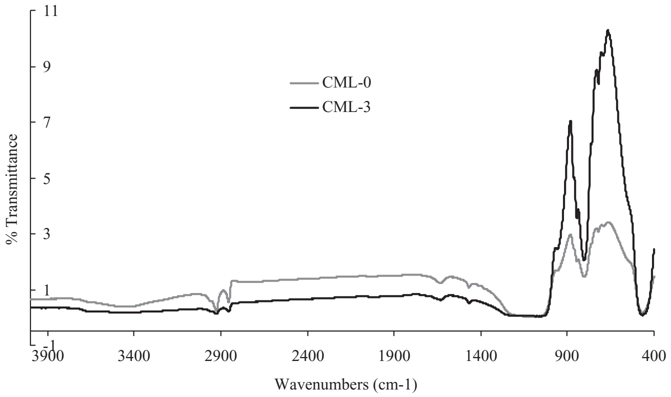

FIG. 17. A comparison of FTIR spectra of octadecyl phases (CML-0) and octadecyl phases modified by tribenzylchlorogermanium (CML-3).

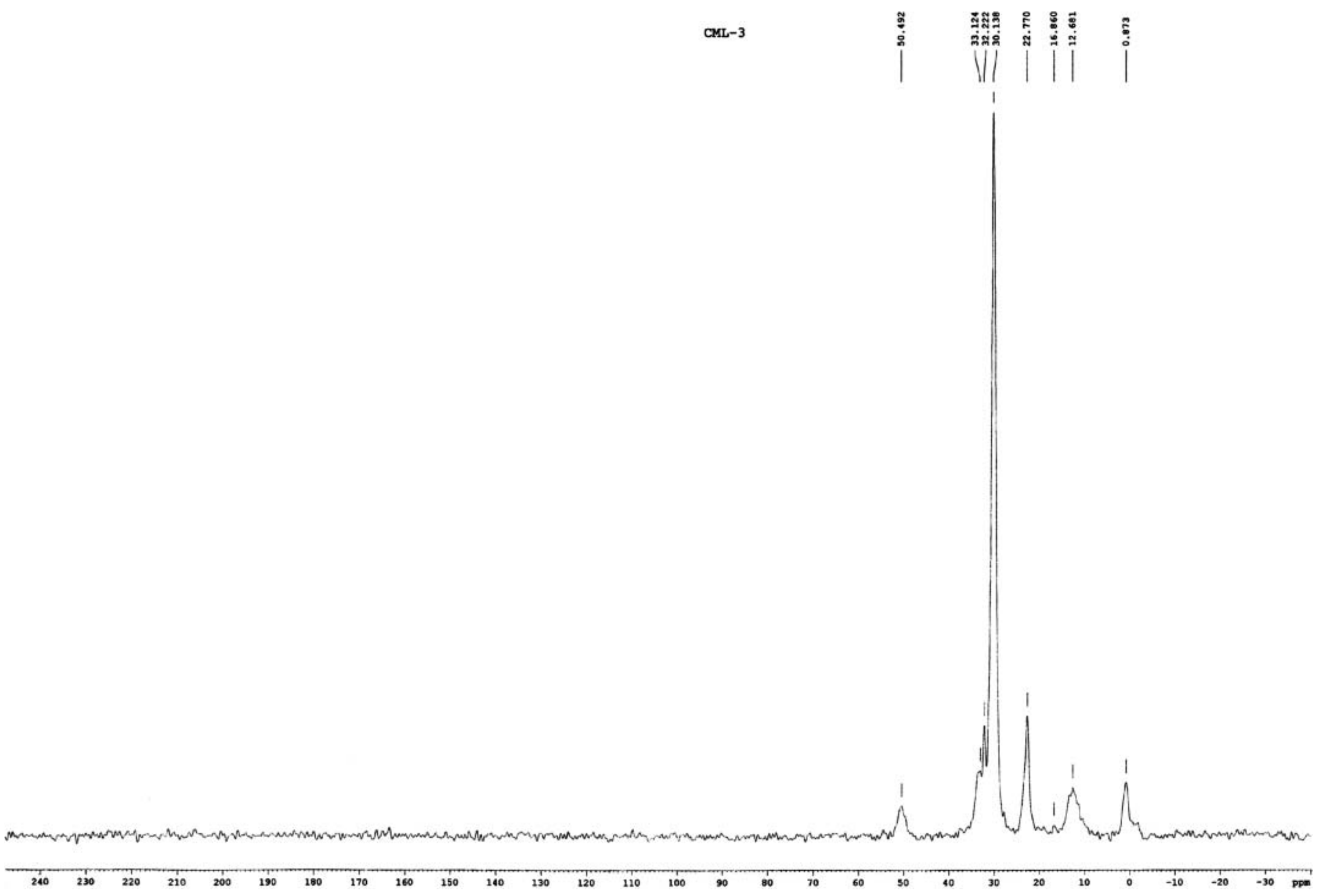

FIG. 18. A comparison of ^{13}C CP/MAS NMR spectra of modified octadecyl phases surfaces by tribenzylchlorogermanium.

CONCLUSIONS

Benzylgermanium derivatives show an ability to modify the octadecyl phase surface (monobenzyl derivative modifies it in the highest degree and the formed bonds are the strongest). The ability of modification and its strength decreases with an increase of benzyl group number. Tribenzyl derivative is the weakest modifier of the octadecyl phase surface because of sterically large group and long hydrocarbon chains (C18). This information is important from the point of view of analysts, because it warns about possible changes of stationary phases, followed by errors in determinations of various compounds, when the column is used.

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