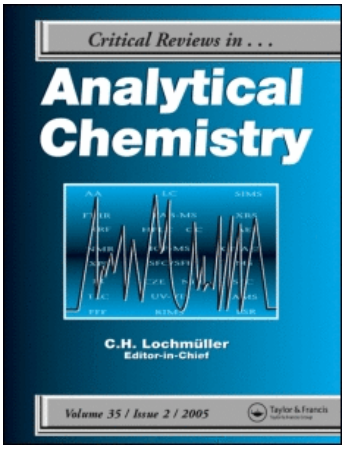

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

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Renata Gadzala-Kopciuch[a]; Krzysztof Pypowski[b]; Ireneusz Chrzaścik[b]; Mariusz Kluska[b]
[a] Chair of the Environmental Chemistry & Bioanalytics, Nicholas Copernicus University, Torun, Poland [b] Institute of Chemistry, University of Podlasie, Siedlce, Poland

Online publication date: 02 August 2010

To cite this Article Gadzala-Kopciuch, Renata , Pypowski, Krzysztof , Chrzaścik, Ireneusz and Kluska, Mariusz(2010) 'Benzylgermanium Compounds as Modifiers of Silica Gel Surface During a Chromatographic Process', Critical Reviews in Analytical Chemistry, 40: 3, 187 — 193
To link to this Article: DOI: 10.1080/10408347.2010.490487
URL: http://dx.doi.org/10.1080/10408347.2010.490487

Critical Reviews in Analytical Chemistry, 40:187–193, 2010

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2010.490487

Benzylgermanium Compounds as Modifiers of Silica Gel Surface During a Chromatographic Process

Renata Gadzała-Kopciuch,[2] Krzysztof Pypowski,[1] Ireneusz Chrząścik,[1] and Mariusz Kluska[1]

[1]*Institute of Chemistry, University of Podlasie, Siedlce, Poland*
[2]*Chair of the Environmental Chemistry & Bioanalytics, Nicholas Copernicus University, Torun, Poland*

In the paper are presented results of investigations concerning modification of silica gel by mono-, di-, and tribenzylgermanium during a chromatographic process. These compounds exhibit biological activity and are characterized by low toxicity. Germanium compounds are widely applied in oncological therapy. The investigations unambiguously show that the above mentioned derivatives can modify silica gel surface. These results have been confirmed by FTIR, ^{13}C CP/MAS NMR, ^{29}Si CP/MAS NMR and scanning microscopy. The strongest bonds are formed by monobenzyl derivative, slightly weaker bonds are formed by dibenzyl and the weakest bonds (because of sterically large group) formed by tribenzyl derivative. The mass ratio of germanium to silica after modification was monobenzyl derivative, for 15.02%, 11.05%, for dibenzyl and 7.10% for tribenzyl.

Keywords organogermanium derivatives, surface modification, silica gel, HPLC

INTRODUCTION

Organic derivatives of germanium belong to relatively new group of compounds; therefore, they are not satisfactory examined. Although their properties are still being investigated, great expectations for applications are entirely justified. Wide access to the source did not decrease the cost of research, but caused quicker development of organogermanium chemistry (1–7).

At present organogermanium compounds arouse interest of the electronic, pharmaceutical, and medical industries. For these reasons, increase of investigations in this area is understandable. Special expectations are connected with the future role of these compounds in anti-cancer therapy because of their low the toxicity, lower than the toxicity of other metalloorganic preparations used in oncology (8–12).

Analyzed organogermanium compounds exhibit biological activity. The highest activity of this kind of compounds shows benzylgermatranes (13, 14). These derivatives were obtained from germanium compounds occurring in nature and from aqueous solutions of trisubstituted amines (15, 16).

Benzylgermatranes are hydrolytically stable. They can be crystallized from water; they are not toxic. They are characterized by high biological activity and have positive influence on living organisms. Their analgetic and anti-convulsive action has been confirmed. They also have a positive influence on memory process (ability to remember); they alleviate the influence of electroshock (17–20).

The development of a wide spectrum of Ge-132 biological activity has stimulated further research, especially in pharmacology concerning anti-cancer therapy (1, 21–24). Ge-132 was treated as a strong analgetic and immunological system-supporting remedy. Further studies of this compound led to the conclusion that its derivatives can penetrate DNA structures. It is very important for the activity of anti-cancer drugs. The investigation results suggest that Ge-132 not only overcomes cancer development but also causes an increase of interferon (the substance inhibiting protein synthesis in cells, including viral proteins) production. At the same time, any negative influence on the cells is observed. These properties formed the idea to insert germanium into a pharmaceutical preparation in order to profit from its anti-cancer activity and its low toxicity. This can lead to less noxious preparations used in cancer treatments.

Ge-132 derivatives show inhibiting activity towards various types of cancer cells (22, 23). It is necessary to remark that the anti-cancer mechanism of organogermanium compounds is not completely known. Presumably they can be an important element of chemotherapy (one of the most often used methods of cancer treatments), which supports surgical cancer treatments.

Address Correspondence to Mariusz Kluska, Institute of Chemistry, University of Podlasie, 3 Maja 54, Siedlce 08-110, Poland. E-mail: kluskam@ap.siedlce.pl

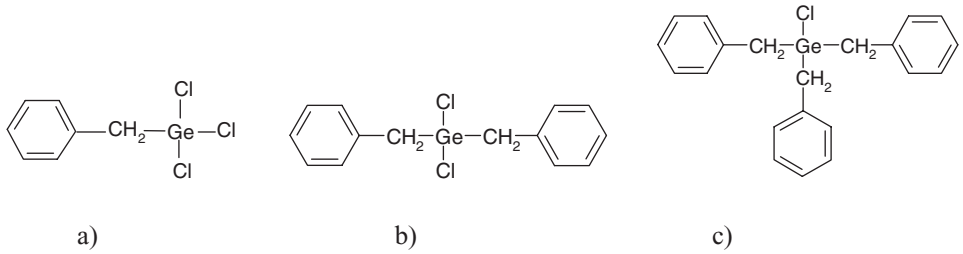

FIG. 1. Scheme of structures of benzyl germanium derivatives: a) benzyltrichlorogermanium, b) dibenzyldichlorogermanium, c) tribenzylchlorogermanium.

Therefore, the main aim of contemporary chemotherapy is to elaborate medicines operating more versatility and more selectivity on degenerated groups of cells.

The most effective preparation inhibiting cancer turned out to be 5-triethylgermyl-2-trifluoroacetylfuran (LD_{50} for Neuro 2A 3.3 mL) (24). This compound is a stronger inhibitor of cancer development than analogous silicon derivative. It was shown that the presence of silyl and germyl groups significantly increases the absorption of grease of the substance. It improves its ability to penetrate by the cell membranes; consequently, biological activity of the preparation is higher than the activity of the carbon analogues. The biological activity, besides the presence of silicon or germanium atoms, is influenced by the presence of heterocyclic rings.

The type and effectiveness of biological activity depends on the nature and structure of heterocyclic compounds and on ring substituents. It was confirmed by a Latvian scientist, who observed that 2-acetyl-5-triethylsilylthiophene is four times more toxic than analogous furan compound. The protective activity of organogermanium compounds against harmful radiation was repeatedly reported. This activity exhibited among others: germanothiazolidyl, germanodithioacetal, germatrane sulfide. These compounds effectively protected mice against gamma radiation emitted by a so-called cobalt bomb (1).

Tests also exhibited that Ge-132 restrains osteoporosis. Germanium derivatives are useful in the therapy of diabetes and malaria (20).

Organogermanes were applied also in agriculture, in cultivating of cereals. A germanium o-chlorobenzamide derivative shows fungi static activity, e.g., fights against Gaecemannomyces graminis (a fungus causing gangrene of corn stalk base). Methyl(2-furyl)- and methyl(2-thienyl)(3-aminopropyl)silane can be used in fighting against rust of wheat, decay of tomatoes caused by pathogenetic fungus Phytophora and cucumber mold (9).

Investigations concerned with this group of compounds are justified by the wide possibility of application. Looking for new, more effective medicines curing 21st century diseases is a priority for numerous scientific centers all over the world. Therefore, new compounds, which are appearing and show biological activity, should be determined and separated in optimum conditions. Organogermanium derivatives belong to this category. However, there is a problem caused by the possibility of stationary phase modification, and then obtained results are burdened with significant error. The main aim of the paper is substantiated behavior of non-modified silica gel during chromatographic processes of determination of mono-, di- and tribenzylgermanium derivatives.

EXPERIMENTAL

Analysis of Silica Gel Modification by Benzyl Derivatives of Germanium

Samples of benzytrichlorogermanium, dibenzyldichlorogermanium and tribenzylchlorogermanium (Fig. 1) were dissolved in acetonitrile (Lab-Scan, Dublin, Ireland) obtaining concentration at about 20 μg/mL. Obtained solutions went through a chromatographic column. Silica gel LiChrosorb Si 60 was used as a stationary phase (E. Merck, Darmstadt, Germany, Fig. 2a). Analyses were carried out at temperature 21°C. Acetonitrile. Served as a mobile phase. Benzyl derivatives of germanium were synthesized according to the literature (6).

Benzyltrichlorogermanium: ^{1}H NMR, δ (ppm) = 3.44 (2H, CH_2Ge) 7.26 – 7.38 (5H, $C_{aromat.}$). ^{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).

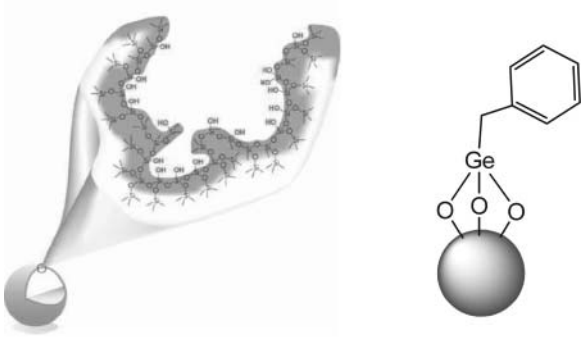

FIG. 2. Scheme of structures: a) unmodified silica gel, b) silica gel surface modified by benzyltrichlorogermanium.

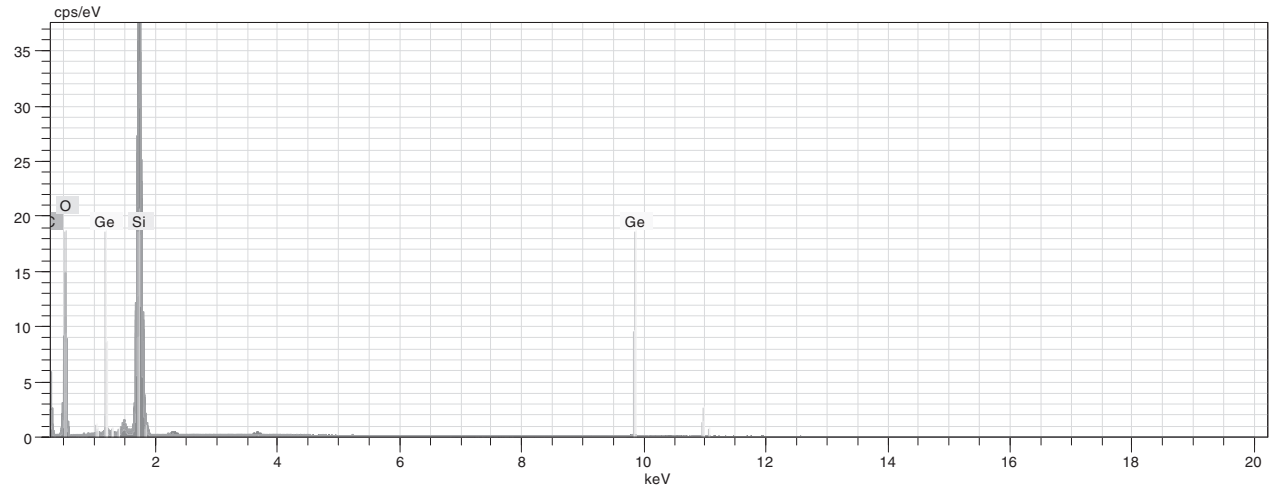

FIG. 3. Diagram of silica gel modified by benzyltrichlorogermanium (average mass ratio of germanium to silicon is after the modification 15.02%, germanium to oxygen 8.30%).

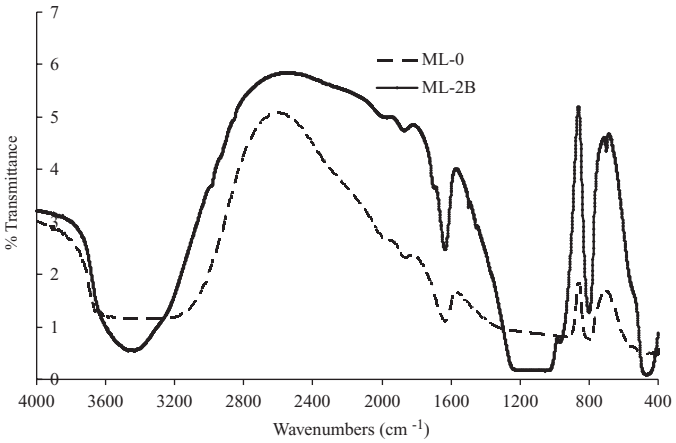

FIG. 4. A comparison of FTIR spectra of unmodified silica gel (ML-0) and silica gel modified by benzyltrichlorogermanium (ML-2B).

Dibenzyldichlorogermanium: ^{1}H NMR, δ (ppm) 3.02 (4H, CH_2Ge), 7.09–7.36 (10H, $C_{aromat.}$). ^{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 (6H, CH_2Ge) 6.84 – 7.31 (15H, $C_{aromat.}$). ^{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.

^{13}C CP/MAS NMR data of modified silica gel surfaces are collected in Table 1.

APPARATUS

^{1}H, ^{13}C, ^{29}Si NMR spectra were recorded on a Varian Mercury 400MHz in DMSO-d_6 (Varian, Inc., Palo Alto, CA, USA), with TMS as internal standard. MS-spectra were performed with a Shimadzu (Kyoto, Japan) mass-spectrometer GC/MS-QP5050, 70 eV, and column Phenomenex BPX-5 (Connecticut, USA) 30 m $\times$ 0.25 mm ID $\times$ 0.25 μm FT, total flow 52.7 mL/min. FTIR spectra were recorded on a Nicollet Magna-IR spectrophotometer (Beckman, USA) in KBr pellets. Scanning electron microscope (SEM) (LEO 1430VP, Zeiss, Germany);

TABLE 1
^{13}C CP/MAS NMR data of modified silica gel surfaces

Modified silica gel surface by	^{13}C NMR, δ (ppm)
Benzyltrichlorogermanium	58.43, (-CH_2-), 122.99 (4 $C_{aromat.}$), 128.11 (2, 3, 5, 6 $C_{aromat.}$), 132.01 (1 $C_{aromat.}$)
Dibenzyldichlorogermanium	58.00, (-CH_2-), 124.62 (4 $C_{aromat.}$), 128.31 (2, 3, 5, 6 $C_{aromat.}$), 134.27 (1 $C_{aromat.}$)
Tribenzylchlorogermanium	58.09 (-CH_2-), 124.32 (4 $C_{aromat.}$), 128.33 (2, 3, 5, 6 $C_{aromat.}$), 137.32 (1 $C_{aromat.}$),

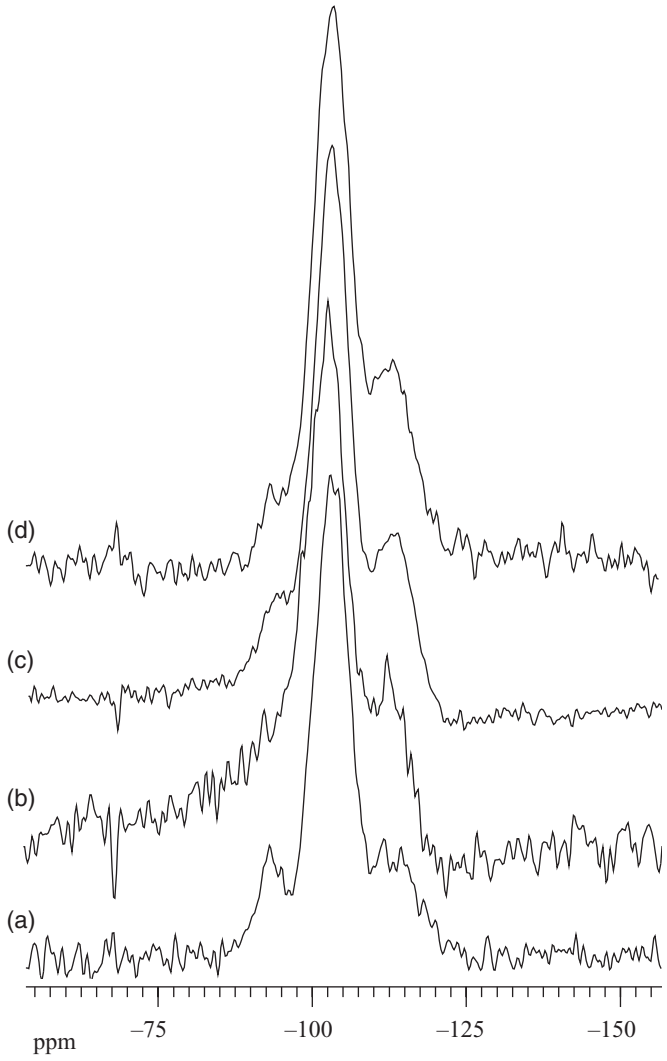

FIG. 5. A comparison of ^{29}Si CP/MAS NMR spectra of modified silica gel surfaces.

was used to evaluate images of the stationary phase modified by mono-, di-, and tribenzylchlorogermanium the EDX analysis was done by XFlash 4010 detector (Bruker AXS Microanalysis, Germany).

RESULTS AND DISCUSSION

Results obtained during investigations are presented in Figs. 3–13. Investigations included behavior of benzyl derivatives of germanium. During chromatography, chloroderivatives of mono-, di- and tribenzylgermanium (Fig. 1) have been observed to have the ability to modify silica gel surface. Taking into consideration chromatographic elution and chemical modification, type and concentration of hydroxyl groups on the silica gel surface is essential. For perfect adsorbents, this surface should be characterized by total silanol concentration at about 8 μmol/m^2. Applied in the investigations silica gel showed total silanol concentration only at about 4 μmol/m^2(25–30).

In order for a precise determination of an architecture of the silica gel modified by benzyl derivatives of germanium, nuclear magnetic resonance (NMR) techniques (^{13}C CP/MAS NMR, ^{29}Si CP/MAS NMR) and infrared spectroscopy with Fourier transform (FTIR) were used. For a more precise characterization of modified surfaces, they were tested by means of SEM.The above mentioned techniques enabled evaluation of a silica adsorbent surface on the molecular level (that is to determine type and concentration of silanol groups).

Spectroscopic investigations, which were carried out, confirmed the assumption of the ability of benzylgermanium derivatives to modify the silica gel surface. It was affirmed that monobenzyl derivatives form the strongest bonds; with the stationary phase surface these bonds were not broken in usual elution processes (Fig. 2b). However, this modification is carried out in 15 percent only (Figs. 3 and 4). This fact can be observed on a diagram obtained from scanning microscope (Fig. 3) as well as after comparison of FTIR spectra of silica gel (before the reaction and after it) modified by monobenzyl derivative (Fig. 4). Modification has also been confirmed by ^{13}C CP/MAS NMR data (Table 1) and ^{29}Si CP/MAS NMR spectra (Fig. 5).

Analysis of the spectrum of starting silica gel (Fig. 4) exhibits a band typical for stretching vibrations of free and twin silanols at ν = 3200–3600 cm^{-1}. On the other hand, at ν = 1600 cm^{-1} are bands characteristic for vibrations of rigid siloxane groups (Si-O-Si). After partial modification of silica matrix by monobenzylgermanium ligands (Fig. 4), new distinct bands appeared that confirm chemisorptions and formation of a new surface (Fig. 2b).

Dibenzyldichlorogermanium modifies the surface of silica gel in similar way. Dibenzylgermanyl substituent is attached to the surface of the stationary phase (Fig. 6) so strongy that the bond can be broken only by the use of extremely polar solvents. Modification of silica gel surface by dibenzyldichlorogermanium is presented in Figs. 7 and 8. The presence of germanium bonded to a silica gel surface was confirmed using scanning

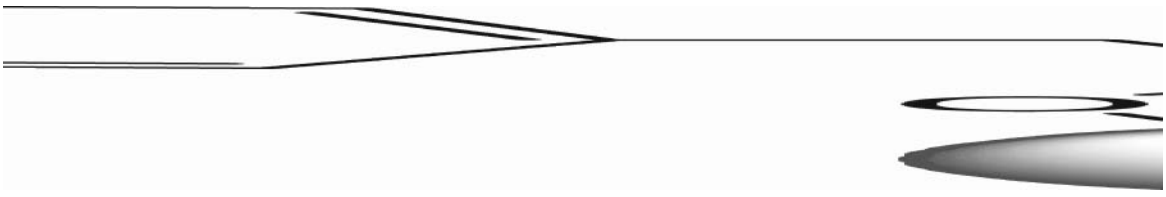

FIG. 6. Scheme of silica gel modification by dibenzyldichlorogermanium.

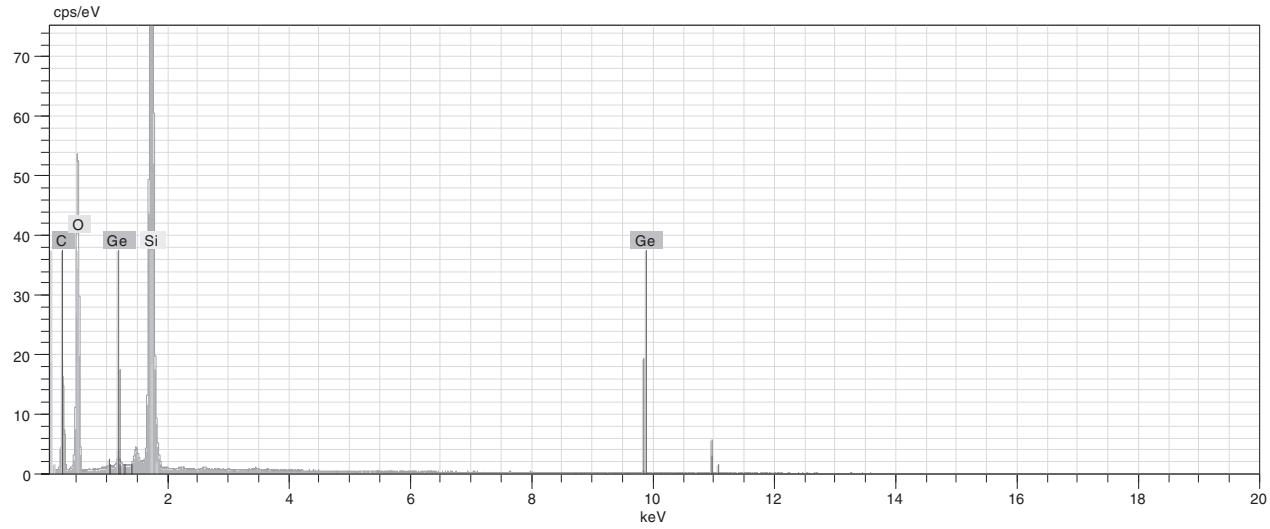

FIG. 7. Diagram ML-3 (average mass ratio of germanium to silicon after modification by dibenzyldichlorogermanium is 11.05%, germanium to oxygen 5.70%).

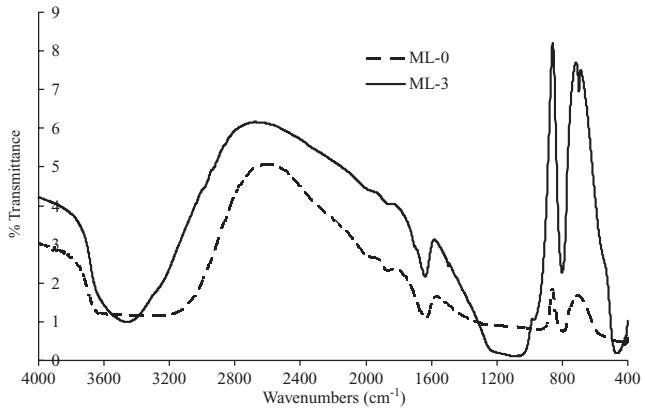

FIG. 8. A comparison of FTIR spectra of unmodified silica gel (ML-0) and silica gel modified by dibenzyldichlorogermanium (ML-3).

microscope (Fig. 7). A comparison of FTIR spectra of silica gel before and after the modification by dibenzyldichlorogermanium (Fig. 8) unambiguously exhibits a participation of this compound in the formation of a new surface. Recorded ^{13}C CP/MAS NMR and ^{29}Si CP/MAS NMR spectra also confirmed chemisorptions of dibenzyl derivative on the silica gel surface. It is interesting that breaking of the bonds by strong polar solvents is accompanied by formation of all three possible structures of dibenzylgermoxanes (A, B, C, and Fig. 9). Each of these three structures can be transformed from one into another. Their identification is very difficult, because they are not stable. In most cases attempts of stabilization lead to structure C.

A dissimilar situation appears after the use of tribenzyl derivatives of germanium (Fig. 10). Tribenzylgermanium, as a sterically large group, is a weaker modifier of the stationary phase surface (Fig. 11 and Fig. 12) than mono- and dibenzyl derivatives of germanium. An effect of modification is confirmed by FTIR spectra as well as ^{13}C CP/MAS NMR and ^{29}Si CP/MAS NMR and scanning microscope display. However, tribenzylchlorogermanium is easily eluted from columns by aqueous organic solvent in the form of germoxane (Fig. 10),

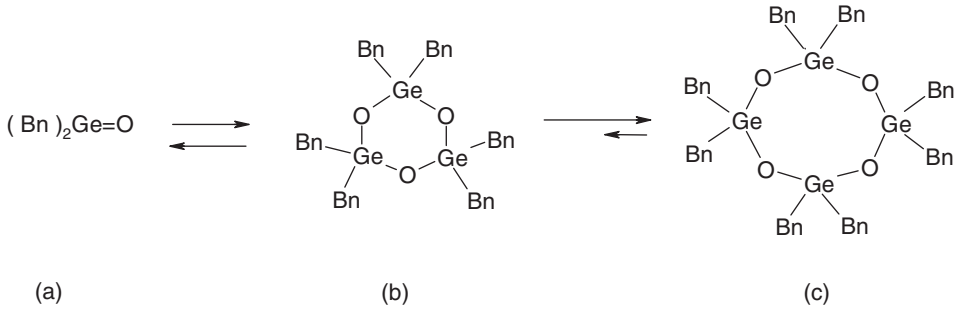

FIG. 9. Possible structures obtained in elution of dibenzyldichlorogermanium (Bn = $PhCH_2$).

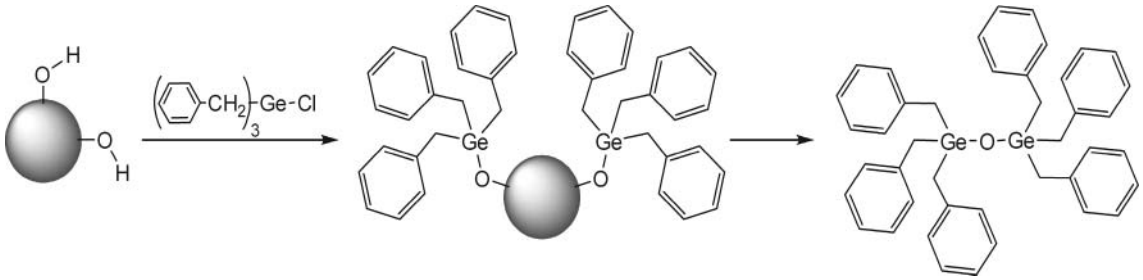

FIG. 10. Scheme of modification by tribenzylchlorogermanium.

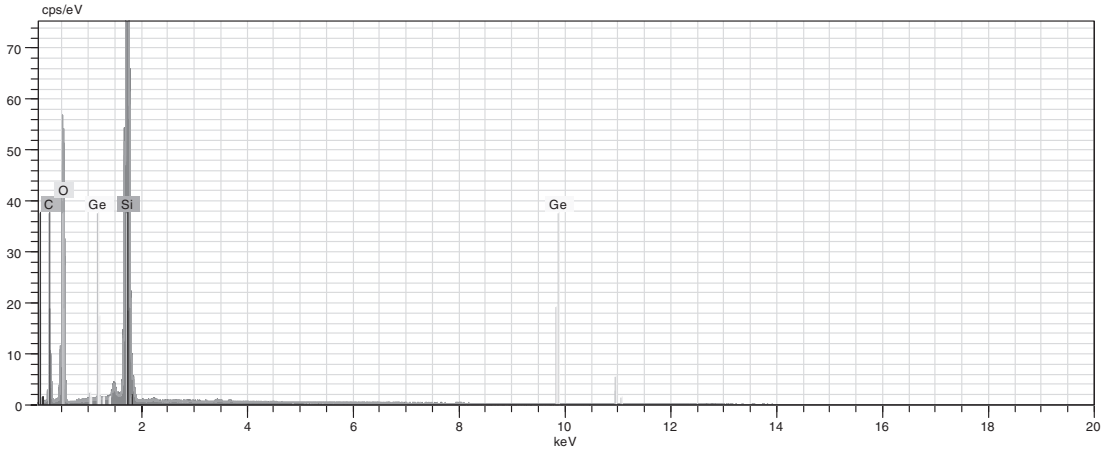

FIG. 11. Diagram ML-4 (average mass ratio of germanium to silicon after modification by tribenzylchlorogermanium is 7.10%, germanium to oxygen 3.6%).

while at about 40% of starting compound is converted (tribenzylchlorogermanium in hexabenzyldigermoxane). On the other hand, elution by polar and anhydrous solvent (e.g. acetonitrile) yields unchanged tribenzylchlorogermanium.

To recapitulate, analyzed mono-, di- and tribenzylgermanium derivatives can modify the silica gel surface (Fig. 5 and 13). This causes numerous difficulties during chromatographic determination. However, because of their applications in oncological therapy, all investigations aimed at deeper knowledge and precise dosage in cancer treatments are justified.

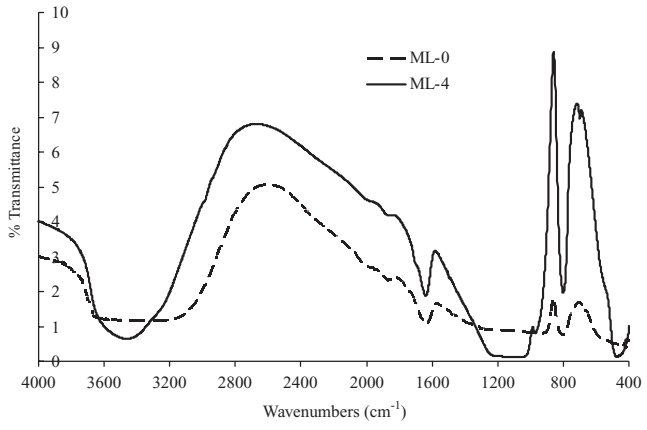

FIG. 12. A comparison of FTIR spectra of unmodified silica gel (ML-0) and silica gel modified by tribenzylchlorogermanium (ML-4).

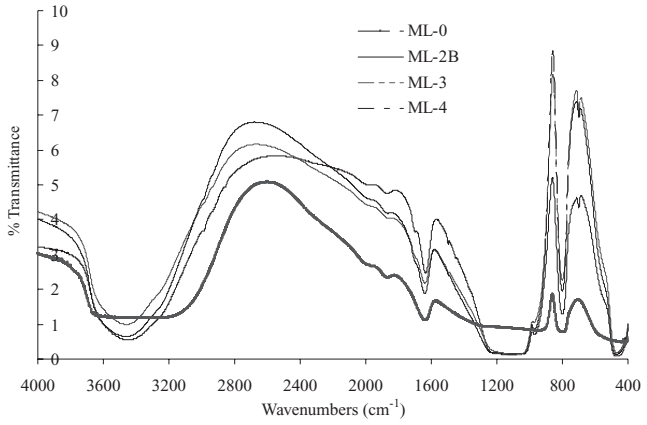

FIG. 13. A comparison of FTIR spectra of unmodified silica gel (ML-0) and silica gel modified by benzyltrichlorogermanium (ML-B), dibenzyldichlorogermanium (ML-3), tribenzylchlorogermanium (ML-4).

SUMMARY

Organogermanium compounds benzyltrichlorogermanium, dibenzyldichlorogermanium and tribenzylchlorogermanium modified silica gel surface when they were subjected to chromatographic investigations. The ability of modification and its strength decreases with an increase of benzyl group number. This information is important from the point of view of analysts, because it warns of possible changes of stationary phases, followed by errors in determinations of various compounds when the column is used.

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