FT RAMAN SPECTROSCOPY AS A TOOL FOR CHARACTERIZATION OF DERIVATIZED SILICA GEL SORBENTS

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The potential of Fourier-transform (FT) Raman spectroscopy for verification of individual steps of synthesis of new chromatographic stationary phases was studied. 3-Aminopropylated silica gel was modified with two different optically active compounds ((+)-cloprostenol, (+)-(*R*)-2,2′-dihydroxy-1,1′-binaphthalene-3-carboxylic acid) via amide bonds. In the next step, free silanol groups were protected with trimethylsilyl groups. The FT Raman spectroscopic results compared with the data obtained by elemental analysis enable not only qualitative verification of synthetic steps, but also a semiquantitative determination of covering of the silica gel surface by derivatization agent.

Keywords: FT Raman spectroscopy; Chiral stationary phases; Sorbents; Surface coverage; Elemental analysis; Binaphthyls; Derivatization of silica gel.

Vibrational spectroscopic techniques were often used as diagnostic tools to verify derivatization of chromatographic substrates. The possibilities of infrared spectroscopy are rather limited because silica gel is opaque in quite a broad range; Raman spectra of derivatized materials are more accessible[1]. Raman spectroscopy was further used to characterize some reverse-phase chromatography sorbents[2–4]. Shimizu et al. studied by Raman spectroscopy interaction of (3-aminopropyl)triethoxysilane with silica gel[5]. Doyle et al. characterized conventional chemically bonded chromatographic stationary phases under flow of mobile phase[1,6].

The derivatization of aminopropylated silica gel with compounds containing carboxyl groups[7] is often used for preparation of stationary phases[8–10], because various reagents are quite easily connected to the propyl chains via amide bonds. In our previous studies[9,10] we demonstrate that both the selector linkage and the silylation effect can be identified on the basis of characteristic bands in the Fourier-transform (FT) Raman spectra measured. Variation of intensities of selected characteristic Raman bands

can be related to the changes of the amount of corresponding species. To evaluate such variation at least semiquantitatively, we monitored in this study the individual synthetic steps in the process of sorbent preparation both by elemental analysis and by FT Raman spectroscopy. The amounts of modification reagents were varied in a set of experiments to follow the curve of surface coverage of the silica gel. The aim of this study is to demonstrate two important aspects of the verification of individual synthetic steps. First, some presumptions made for calculation of surface coverage based on elemental analysis are not generally fulfilled and the data obtained are not reliable in the case of multiple step synthesis. Second, FT Raman spectroscopic analysis allows to obtain not only a detailed structural view of individual synthetic steps but also their quantitative parameters.

EXPERIMENTAL

Preparation of Stationary Phases

Two types of conventional 3-aminopropyl silica gels with different particle size (particle diameters 10 and 7 μm) were used for immobilization of both (+)-cloprostenol (i.e. 7-{2-[4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentyl}hept-5-enoic acid) and (+)-(*R*)-2,2′-dihydroxy-1,1′-binaphthalene-3-carboxylic acid. Starting 3-aminopropyl silica has pore volume 1.0 ml g^{-1}, specific surface area 500 m^2 g^{-1} (S_{BET}) and 4.30% carbon. The preparation procedure based on a published method[11] was analogous for both compounds.

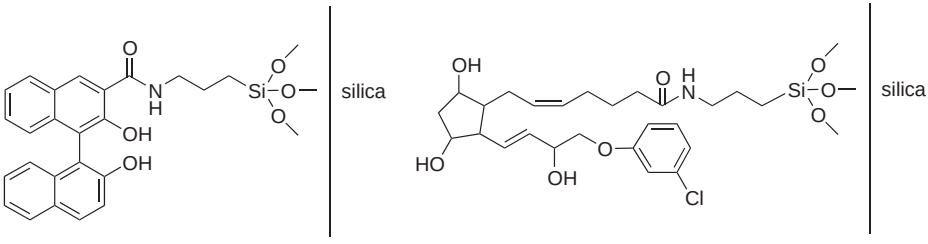
Five specified amounts of cloprostenol were used to prepare a set of sorbents with different surface coverage. A corresponding amounts of cloprostenol (Table I) were dissolved in a

TABLE I
Experimental data of cloprostenol sorbents preparation

Experiment No.	Amounts of cloprostenol, mg	Elemental analysis of carbon, %	Selector coverage μmol m^{-2}
1	6	6.270	0.065
2	20	7.550	0.167
3	20	7.930	0.182
4	40	7.965	0.198
5	40	8.410	0.238
6	82.6	10.010	0.375
7	160	10.860	0.451
8	160	10.860	0.451

10 ml dichloromethane and activated with *N*,*N*′-diisopropylcarbodiimide (4 µl per 1 mg of chloprostenol) and 1-hydroxybenzotriazole (0.05 mg per 1 mg of cloprostenol) at room temperature for 60 min. 4-Dimethylaminopyridine (0.1 mg per 1 mg of cloprostenol) and pyridine (10 µl per 1 mg of chloprostenol) were dissolved in the solution and 3-aminopropyl silica gel (1 g for each sorbent) was then added into each reaction mixture. The resulting reaction mixture was stirred at room temperature for one week. The silica gel was then filtered off, washed with dichloromethane, methanol, water, methanol and again dichloromethane, and dried under high vacuum. The results of the elemental analysis of prepared sorbents and corresponding selector coverages are presented in Table I.

The preparation of binaphthol sorbents was analogous to that of cloprostenol sorbents. Different amounts of (+)-(*R*)-2,2′-dihydroxy-1,1′-binaphthalene-3-carboxylic acid (Table II) were used for modification of sorbent (1 g). The specified amount of binaphtholcarboxylic acid was dissolved in a 10 ml dichloromethane and activated with *N*,*N*′-diisopropylcarbodiimide (7 µl per 1 mg of binaphtholcarboxylic acid) and 1-hydroxybenzotriazole (0.1 mg per 1 mg of binaphtholcarboxylic acid) at room temperature for 60 min. 4-Dimethylamino-



Binaphthol sorbent Prostaglandin sorbent

TABLE II
Experimental data of binaphthol sorbents preparation

Experiment No.	Amounts of binaphtholcarboxylic acid, mg	Elemental analysis of carbon, %	Selector coverage µmol m^{-2}
1	15	6.250	0.108
2	26.3	6.865	0.190
3	100	7.740	0.311
4	100	8.160	0.371
5	150	9.520	0.572

pyridine (0.2 mg per 1 mg of binaphtholcarboxylic acid) and pyridine (10 μl per 1 mg of binaphtholcarboxylic acid) were dissolved in the solution and 3-aminopropyl silica gel (1 g for each sorbent) was then added into each reaction mixture.

The elemental analysis of carbon contents corresponding to the selector coverage are displayed in Table II. Subsequently the modified sorbent was endcapped with silylation agent in the same way as described above.

Free silanol groups were reacted in chloroform solution of chlorotrimethylsilane as silylating agent with pyridine added to the mixture. Derivatized silica (1.0 g) was suspended in a 10 ml of chloroform. Then 0.1 ml of chlorotrimethylsilane and 0.05 ml of pyridine was added to the mixture. The suspension was heated under reflux at 62 °C overnight and then the silica gel was filtered off at room temperature, washed and dried in the same way as described above.

Instrumentation

FT Raman spectra were collected using a FT near-infrared (FT NIR) spectrometer Equinox 55/S equipped with an FRA 106/S Raman module (Bruker). First, the type of the laser beam focus, the magnitude of laser power and the number of scans were tested. Then the samples were irradiated by a focused laser beam with a power of 250 mW from Nd-YAG laser (1064 nm). The scattered light was collected in backscattering geometry. 1024 interferograms were co-added and processed to obtain Raman spectra with 2 cm^{-1} resolution. The OPUS 2.3 software (Bruker) was used to control the spectrometer and to process and evaluate the spectra obtained.

Elemental analysis was performed on a PE Series II CHNS/O Analyser (Perkin–Elmer), which used combustion, column separation of produced gases and thermal conductivity detection.

Optical microscopy was performed using Nikon Optiphot II microscope (objectives 10×, 20×, 40×, 100×) equipped with color CCD camera (Sony) connected to a computer that made it possible to save the visual micro-images.

RESULTS AND DISCUSSION

Spectra of Aminopropylated Silica Gels

FT Raman spectra were measured for both types of 3-aminopropylated silica gels (particle diameters 10 and 7 μm) and for underivatized silica gel. The almost identical spectra of aminopropylated silica gels exhibit typical bands of CH_2 groups (ca. 2930, 2897, 1457 cm^{-1}) (Fig. 1A), weak bands of NH_2 group (ca. 3350, 3310, 1610 cm^{-1}), and broad bands of silica gel itself (ca. 790, 450 cm^{-1}). The high similarity of spectra of both aminopropylated silica gels is in agreement with the calculated coverage of the gel surface by 3-aminopropyl groups based on results of elemental analysis.

The surface coverage was calculated according to Unger et al.[12] from the carbon content and surface area:

$$\alpha_{exp} = \frac{w}{MS_{BET}} \quad [\text{mol m}^{-2}],$$

where w is mass fraction of functional group (g g^{-1} of adsorbent) obtained from carbon elemental analysis, M is molar mass of the bonded functional group (g mol^{-1}) and S_{BET} is specific surface area of the starting sorbent 500 m^2 g^{-1} (see Experimental).

$$w = \frac{(x_f - x_{aps})}{100 A_r n \left(1 - \frac{(x_f - x_{aps})M}{100 A_r n}\right)},$$

where x_f is carbon content in derivatized silica gel, x_{aps} is carbon content in starting 3-aminopropyl silica gel, both are obtained from carbon elemental analysis. A_r is atomic mass of carbon, n is number of carbon atoms in bonded functional group.

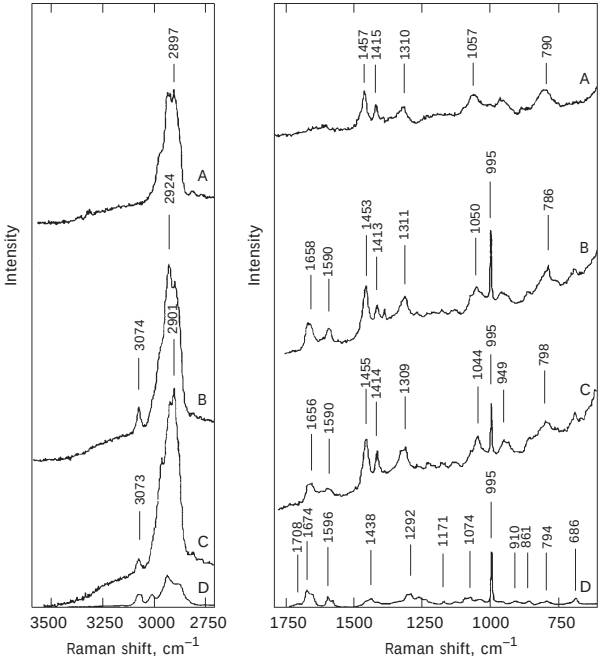


FIG. 1

FT Raman spectra of original aminopropylated silica gel (A), after derivatization with (+)-cloprostenol (B) and after protection of free silanol groups (C) compared with FT Raman spectrum of pure (+)-cloprostenol (D)

Derivatization of Silica Gels with (+)-Cloprostenol

The FT Raman spectra of derivatized silica gels with cloprostenol were compared with spectra of initial aminopropylated silica gel and with spectra of pure cloprostenol (Fig. 1). The most intense very narrow band at 995 cm^{-1} in the spectrum of (+)-cloprostenol (Fig. 1D) can be detected in all spectra of derivatized silica gels without any shift and without any change in its shape. The area of this characteristic band of cloprostenol was finally used for quantitative comparison of sorbent samples (Fig. 2). Nevertheless, also other bands of cloprostenol can be identified in the spectra of modified sorbents (Fig. 1B). For example, the 3079 cm^{-1} band of ν(CH) of aromatic skeleton of pure (+)-cloprostenol is slightly shifted to 3074 cm^{-1} in the spectra of modified sorbent. Another characteristic band of the aromatic skeleton of (+)-cloprostenol (1596 cm^{-1}) with a shoulder at 1581 cm^{-1} is observed as a broadened band at 1590 cm^{-1} in the spectra of derivatized gels. Furthermore, apparent changes are observed in the range 1730–1620 cm^{-1} when comparing the spectrum of modified silica gel (Fig. 1B) with that of pure (+)-cloprostenol (Fig. 1D). The effects observed (e.g., the appearance of 1656 cm^{-1} band in the spectra of modified sorbents) could be attributed to the derivatization reaction of cloprostenol (carboxylic acid) via amide bond.

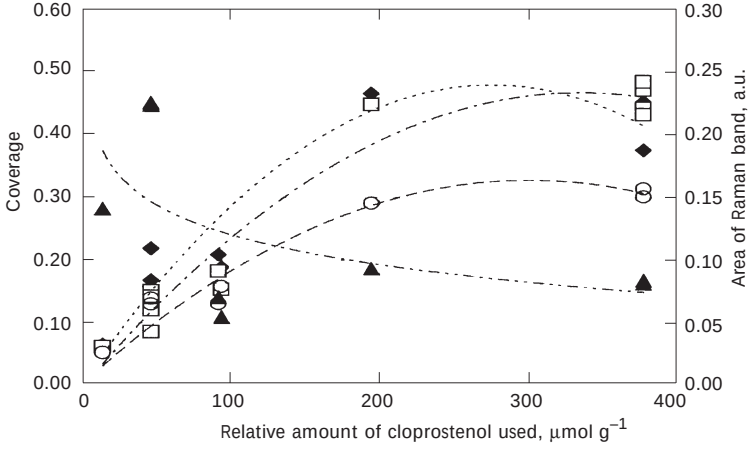


FIG. 2

Comparison of surface coverage calculated from elemental analysis and of integrated areas of Raman bands for silica gels modified by (+)-cloprostenol. The lines show general trends of cloprostenol coverage (μmol m^{-2}) and band areas. ◆ Cloprostenol coverage, □ cloprostenol band area before silylation, ▲ area of bands of silylation agent, ○ cloprostenol band area after silylation

Quantification of cloprostenol was based on integration of area of the 995 cm^{-1} band, because it is the most intense band of cloprostenol as well as the narrowest spectral feature of cloprostenol without apparent overlaps with other bands and with easy correction from background. Figure 2 shows a comparison of Raman and elemental analysis data. The trends of both data in dependence on relative amounts of cloprostenol used for derivatization are analogous showing the saturation effect of the silica gel surface. Nevertheless, the decrease in cloprostenol amount after silylation can be detected only in Raman spectra. In addition, the effectiveness of silylation can be specified only from evaluation of FT Raman spectra.

Derivatization of Silica Gels with (+)-(R)-2,2′-Dihydroxy-1,1′-binaphthalene-3-carboxylic Acid

FT Raman spectra of derivatized gels were compared with the spectrum of pure binaphtholcarboxylic acid to confirm the derivatization step (Fig. 3).

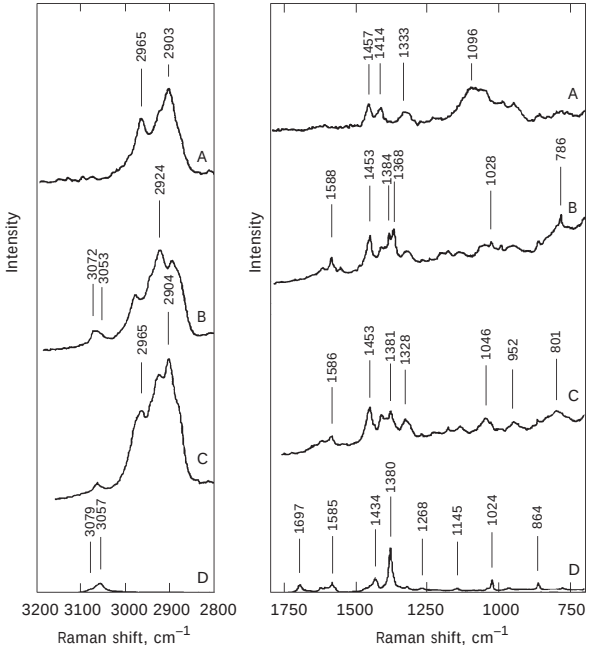


FIG. 3

FT Raman spectra of original aminopropylated silica gel (A), after derivatization with binaphtholcarboxylic acid (B) and after protection of free silanol groups (C) compared with FT Raman spectrum of pure binaphtholcarboxylic acid (D)

The spectral patterns of all derivatized gels in the region 1720–1600 cm^{-1} are very different from those of starting binaphtholcarboxylic acid, e.g. the 1697 cm^{-1} band attributed to carboxylic groups disappears in the spectra of derivatized silica gels. The observed effects are in agreement with the proposed formation of amide bonds. Furthermore, many bands (e.g. 1585, 1434, 1322, 1268, 1024, 864 and 782 cm^{-1}) in the spectrum of pure compound (Fig. 3D) show practically no shifts (1586, 1431, 1328, 1266, 1028, 866 and 786 cm^{-1}) in the spectra of derivatized gels both before (Fig. 3B) and after silylation step (Fig. 3C). Although many bands attributed to the binaphthol skeleton are not shifted after linking the reagent to silica gel, there are several evident differences between the spectra of pure binaphtholcarboxylic acid and those of derivatized silica gels. While the 3057 cm^{-1} band with a weak 3079 cm^{-1} shoulder of aromatic ν(CH) is typical of the pure compound (Fig. 3D), two overlapped bands (3053, 3072 cm^{-1}) of similar intensity are observed for derivatized silica gels before silylation (Fig. 3B), and finally one maximum at 3064 cm^{-1} is typical of silylated samples (Fig 3C). The most intense band of the spectrum of solid binaphtholcarboxylic acid at 1380 cm^{-1} is replaced by two similar intense bands at 1384 and 1368 cm^{-1} in the spectra of gels before silylation. Finally, a maximum at 1381 cm^{-1} is observed after silylation. These effects of reduction in the number of observed bands at 3080–3000 cm^{-1} and 1390–1365 cm^{-1} after silylation are pronounced in the case of large amounts of binaphtholcarboxylic acid used for derivatization. The effects are simultaneously accompanied by significant decreases in intensities of all other bands of the binaphtholcarboxylic skeleton after silylation (exceeding even 50% of the intensity observed before silylation for samples with the highest amount of binaphtholcarboxylic acid used for derivatization). A tentative explanation of the observations is based on the existence of two forms of the binaphthol skeleton attached to silica gel in the first derivatization step: one is strongly bonded to the gel, while the other is removed during silylation step.

Common Effects of Protection of Free Silanol Groups

FT Raman spectra of samples after reaction of free silanol groups with trimethylsilyl chloride groups were compared with the spectra obtained before this step (e.g. Fig. 1 or 3). The increased intensity in the range 3000–2850 cm^{-1} and a new maximum at ca. 2901 cm^{-1} are clearly observed (Fig. 1C). To separate the ν(CH) bands of trimethylsilyl groups, subtraction spectra were calculated. The area of baseline-corrected bands in the range

2995–2889 cm^{-1} was integrated for individual subtracted spectra. The calculated values were related to the amount of derivatization agent (e.g. Fig. 2) to elucidate the existence of a relation between the two synthetic steps. The results obtained show that the two steps of preparation of modified sorbents are not fully independent. In both cases it was observed that for low amounts of the used derivatization reagent the yield of silylation step is significantly higher than for higher coverage of the surface by the modification reagents. Furthermore, the efficiency of silylation is stable when the saturation effect is observed for modification agents.

Each of the sorbents was checked with optical microscopy at every step of preparation. No significant changes of particle size, their structure or shape were observed after any derivatization step (Fig. 4).

CONCLUSION

FT Raman spectroscopy allows verifying of synthetic steps of preparation of new stationary phases based on silica gels. The amount of derivatization agent used can be optimized based on the Raman spectral data so that the gel is covered with the reagent without wasting. Furthermore, the effectiveness of silylation step can be monitored. Although the modification re-

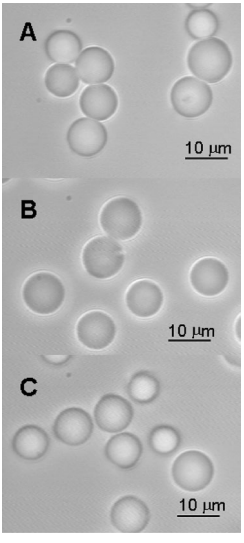


FIG. 4
Example of optical microscopy snapshot: starting aminopropylated silica gel (A), binaphthol sorbent (B), binaphthol sorbent protection of free silanol groups (C)

agents react with amino groups of aminopropylated silica gels and the silylation should protect free silanol groups, the analysis of FT Raman spectra shows that the two synthetic steps are not fully independent, especially when low amounts of modification reagents are used, i.e. at low coverage of the surface by modifying species.

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