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Preparation of a polybutadiene stationary phase immobilized by γ radiation for reversed-phase high-performance liquid chromatography

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Abstract

Polybutadiene (PBD) has been immobilized on HPLC silica by γ radiation doses in the range from 5 to 180 kGy. Columns prepared from these reversed-phase materials, as well as from similar non-irradiated materials, were tested with standard sample mixtures and characterized by elemental analysis (% C) and infrared spectroscopy. A low dose of 5 kGy is sufficient to produce a layer of immobilized PBD which functions as an efficient and stable stationary phase. Higher doses give thicker immobilized layers having less favorable chromatographic properties.
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1. Introduction

Undoubtedly, silica continues to be the most widely used chromatographic support for the preparation of reversed-phase HPLC materials. It is available in a wide range of particle sizes and porosities (pore diameters of 5–400 nm) [1]. Most RP-HPLC separations in use today are based on silica surfaces covered with chemically bonded organic materials [2].

Polybutadiene (PBD) coatings on silica have also been investigated as stationary phases since the early 1980s [3]. The PBD used for this purpose is obtained from the polymerization of 1,3-butadiene, which gives a polymer containing double bonds, which may exist as a mixture of three isomeric units: *cis*-1,4, *trans*-1,4 and vinyl, in different proportions [4]. By way of these groups, PBD can attach to the silica surface and minimize or eliminate adsorptive effects of the matrix, thus improving the chemical stability and lifetime of the packing material whilst maintaining a high mechanical stability [5]. A model for the distribution of PBD on porous silica [6] does not assume a homogeneous polymer film but rather an inhomogeneous loading where the polymer is mainly sited in a non-uniform manner in the pores of the silica [7]. During the past decade, other metal oxides, such as zirconia (ZrO_2) or alumina (Al_2O_3), have been tested as substitutes for the traditional silica support materials because the chemical stability of these oxides is higher than that of silica. Some of the best stationary phases are those having PBD coated onto zirconia [8–14] or alumina [15].

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The immobilization process of a polymer on the silica surface can be initiated by free radicals [16]. Different cross-linking methods have been used to immobilize PBD onto the support material but, in most cases, PBD was immobilized using chemical agents such as dicumyl peroxide, followed by thermal treatment [3,5,7–14].

Other sources of free radicals can also be used. For example, γ radiation from cobalt-60 has been used successfully to immobilize the polymers: poly(methyloctadecylsiloxane) (PMODS) [17], and poly(methyloctylsiloxane) (PMOS) on silica supports [18–20]. Also γ radiation has been used to immobilize PMOS onto zirconized silica support particles [21] or titanium-grafted silica support particles [22]. In the present work we have used γ radiation to immobilize PBD onto silica surfaces because this process offers the advantages of initiating bond formation at room temperature, with no need for the addition of reagents, which might alter the subsequent performance of the column, and no undesired secondary products are formed. Furthermore, γ radiation can be applied directly to the solid substances, due its high penetration power. Therefore, this work has investigated the effect the different doses of γ radiation for immobilization of PBD on silica particles for use in reversed-phase HPLC and compared the stability of irradiated stationary phase with that of non-irradiated stationary phase.

2. Experimental

2.1. Materials

Hexane (Mallinckrodt, HPLC grade) was used as solvent in the preparation of the stationary phase. Filtered methanol (Mallinckrodt, HPLC grade) and water (Milli-Q) were used to prepare the mobile phases. Filtered chloroform (Merck, analytical-reagent grade) and methanol were used for the extraction of excess polymer. The compounds used for the chromatographic testing (acetone, benzene, benzonitrile, naphthalene, toluene and uracil) were analytical reagent grade and were not further purified. The silica support used was Rainin (100-5) (Varian–PK.101-K5), having 5 μm spherical porous particles. The polybutadiene (PBD) polymer (molar mass of 5000, 80% 1,4-*cis*/*trans* and 20% vinyl) was obtained from Aldrich Chemical Company.

2.2. Preparation of stationary phase

2.2.1. Preparation of PBD coatings

Preliminary tests were carried out using the proportions of PBD and silica particles (g PBD to g silica) of 40, 50 and 60%. To load the PBD into the silica pore system a 12% (w/v) solution of PBD in hexane was used to suspend the silica particles. The suspension was stirred for 3 h then sonicated for 10 min. Then the solvent was allowed to evaporate, without stirring, at room temperature (6 days). The best proportion was found to be 50%, which was then used in the subsequent experiments.

2.2.2. Immobilization of PBD

Portions of the prepared stationary phase were sealed into glass ampoules under air at room temperature and irradiated by a Cobalt-60 source (IBRAS-CBO, Campinas, Brazil). The irradiation doses were: 5, 10, 20, 30, 60, 80 and 180 kGy.

2.2.3. Extraction procedure

After immobilization of the stationary phases, tubes containing the irradiated phase were connected to a pump for solvent extraction of the non-immobilized (excess) PBD. First, chloroform and then methanol were passed through the irradiated material at 1 ml min^{-1} for 3 h at ambient temperature, for each solvent. A portion of non-irradiated stationary phase was also submitted to the extraction procedure. After this procedure, the residual solvent in the stationary phase was allowed to evaporate at room temperature.

2.3. Column packing

Columns (60×3.6 mm) were made from type 316 stainless-steel tubing. The internal surface was polished using a technique developed in our laboratory [23]. The columns were downward packed using 10% (w/v) slurries of each stationary phase in chloroform. A constant packing pressure of 34.5 MPa (Haskel packing pump) was used, with methanol as propulsion solvent. Columns were conditioned

for 2 h with a mobile phase consisting of methanol–water (70:30, v/v) at 0.3 ml min^{-1}. Each phase was evaluated chromatographically using two different columns packed with that phase.

2.4. Chromatographic evaluation

The chromatographic evaluation of column performance was done with a modular HPLC system equipped with a Shimadzu LC-10 AD pump, a Rheodyne Model 7125 injection valve (5 μl loop) and a Shimadzu Model SPD-10 AV UV–Vis detector (at 254 nm). Data acquisition used Chrom Perfect for Windows, version 3.52 and Report Write Plus software (Justice Innovations).

The standard sample mixture used in this study contained the solutes: uracil, acetone, benzonitrile, benzene, toluene and naphthalene. The mobile phase was methanol–water (60:40, v/v) or (70:30, v/v) at 0.2 ml min^{-1} for the non-irradiated or irradiated stationary phases, respectively. The optimal flow-rate was determined by a Van Deemter plot. The column dead time, t_M, was determined using uracil as an unretained compound. Chromatographic performance was evaluated by means of efficiency [plates per meter (N/L)], retention factor (k), resolution (R_s) and asymmetry (A_s) measured at 10% of the peak height.

2.5. Stability testing

Stability testing of the non-irradiated stationary phase and the best irradiated stationary phase was carried out using mobile phases of methanol–water (60:40, v/v) and (70:30, v/v), respectively, which were passed through the columns at a flow-rate of 1.0 ml min^{-1}. Chromatograms of a standard sample mixture were obtained periodically with the same mobile phases as used for washing, at flow-rates of 0.2 ml min^{-1}. Chromatograms were taken at 500 ml intervals until 20 000 ml (32 800 column volumes) had passed.

2.6. Characterization of stationary phases

2.6.1. Percent carbon, surface area and mean pore size

Elemental analyses (% C and % H) were obtained of the prepared stationary phases, the extracted stationary phases and the packing materials from the various test columns. These data permitted the determination of the real immobilized PBD content in each prepared stationary phase. Duplicate determinations were made with a Model CHN-2400 Perkin-Elmer Analyzer. Specific surface area ($S_{BET}-N_2$) determinations were carried out with a Model 2300 Micromeritics FlowSorb II instrument. The mean pore size of the silica particles was obtained with a Model 9320 Micromeritics Poresizer mercury intrusion porosimeter.

2.6.2. Infrared spectroscopy

Infrared spectra were obtained of the PBD, the silica and the stationary phases (immobilized by γ radiation and non-irradiated) using a Perkin-Elmer model FT-IR 1600 instrument to evaluate the presence of residual silanols and possible alterations caused by γ radiation on the stationary phase.

3. Results and discussion

3.1. Characterization of stationary phases

The mass of PBD on the silica can be assessed by elemental analysis (% C). Several parameters, which are obtained from the stationary phase mass data (m_{SP}) and the elemental analysis (% C_{SP}) can be obtained as follows:

In a sample of PBD the fraction of the PBD mass (m_{PBD}) that is carbon (m_C) is 0.889, so we can write $m_{PBD}=m_C/0.889$. This equation, plus that for the % C of the sample, $\%\,C=100m_C/(m_{SiO2}+m_{PBD})$, gives $m_{PBD}=(m_{SiO2}+m_{PBD})\cdot\%\ C/(100\cdot 0.889)$. Solving for m_{PBD} gives $m_{PBD}=m_{SiO2}\cdot\%\ C/(88.9-\%\ C)$. Normalizing to specific mass ($\bar{m}_{PBD}$), i.e. mass per gram of silica, gives:

$$\bar{m}_{PBD}=\frac{m_{PBD}}{m_{SiO2}}=\frac{\%\,C}{(88.9-\%\,C)}$$

Thus, the specific mass of PBD, $\bar{m}_{PBD}$, can be obtained directly from the %C.

For special case of filling 100% of the pore system with PBD, the full volume of PBD will be equal to the specific volume of the pore system (v_p) as determined by another procedure:

Table 1
Percent carbon and PBD layer thickness of the stationary phases after extraction and after column testing of the non-irradiated phase and phases immobilized by γ radiation at different doses

γ Radiation dose (kGy)	Carbon (%)		Layer thickness (τ) (nm), after column testing
	After extraction	After column testing	
0	11.1	7.6	0.6
5	15.3	14.0	1.2
10	17.0	14.9	1.3
20	19.5	16.4	1.5
30	19.8	17.3	1.7
60	24.1	22.5	2.2
80	25.1	23.9	2.4
180	28.4	27.7	4.1

$$v_{\text{fullpores}} = v_{\text{p}} = \frac{0.55\ \text{ml}}{\text{g SiO}_2}$$

If we now assume that the density of PBD sorbed and PBD immobilized in the pore system are very nearly equal, we can use the volume–mass–density relationship ($v = m/\delta$) to obtain the fraction (F_{V}) of the pore volume occupied by immobilized PBD as follows:

$$F_{\text{V}} = \frac{\bar{m}_{\text{PBDimm}} \times \delta_{\text{imm}}}{\bar{m}_{\text{PBDfullpores}} \times \delta_{\text{PBD liq.}}} \approx \frac{\bar{m}_{\text{PBDimm}}}{\bar{m}_{\text{PBDfullpores}}} = \frac{\%\,\text{C}_{\text{imm}}}{(88.9 - \%\,\text{C}_{\text{imm}})/0.55}$$

Using this fraction a simple geometric argument [18,19,25,26] gives the expression:

$$F_{\text{imm}} = \frac{\bar{m}_{\text{PBDimm}}}{\bar{m}_{\text{PBDfullpores}}} = \frac{(11.4)^2 - (11.4 - 2\tau)^2}{(11.4)^2},$$

from which the thickness, τ, of an assumed uniform immobilized polymer layer can be obtained. Values of τ, in nm, are included in Table 1, along with the corresponding % C data.

The dependence of layer thickness, τ, on the radiation dose is shown in Fig. 1. At least two important observations come from this figure. First, a very small absorbed dose of 5 kGy or less causes the

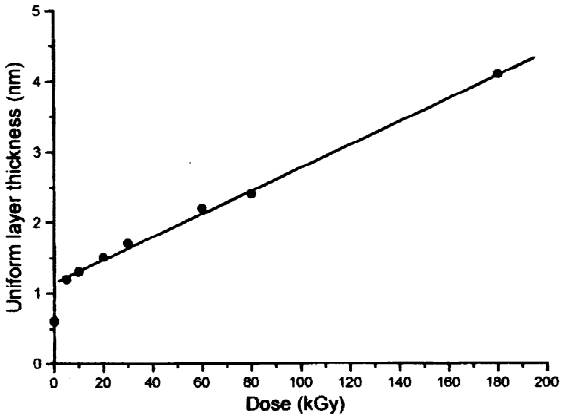

Fig. 1. Dependence of specific mass of radiation-immobilized PBD on the absorbed dose.

Table 2
Mean pore size, specific pore volume and specific surface area (S_{BET}) of silica and phase immobilized by 5 kGy of γ radiation

Type of material	Mean pore size (nm)	Specific pore volume (ml/g)	S_{BET} (m^2/g)
Silica	11.4	0.55	188
Phase immobilized by 5 kGy of γ radiation	13.4	0.30	89

radiation immobilization to arrive at a pseudo-plateau that corresponds to a layer thickness of 1.1–2.2 nm. Second, additional absorbed doses cause a much less pronounced increase in layer thickness. Tentative interpretations which come from these two observations are as follows: First, the pseudo-plateau value is so well defined by the linear dependence, at ~1.15±0.05 nm, that we can tentatively conclude that some sort of well-defined immobilized PBD monolayer of this thickness is involved. Second, the

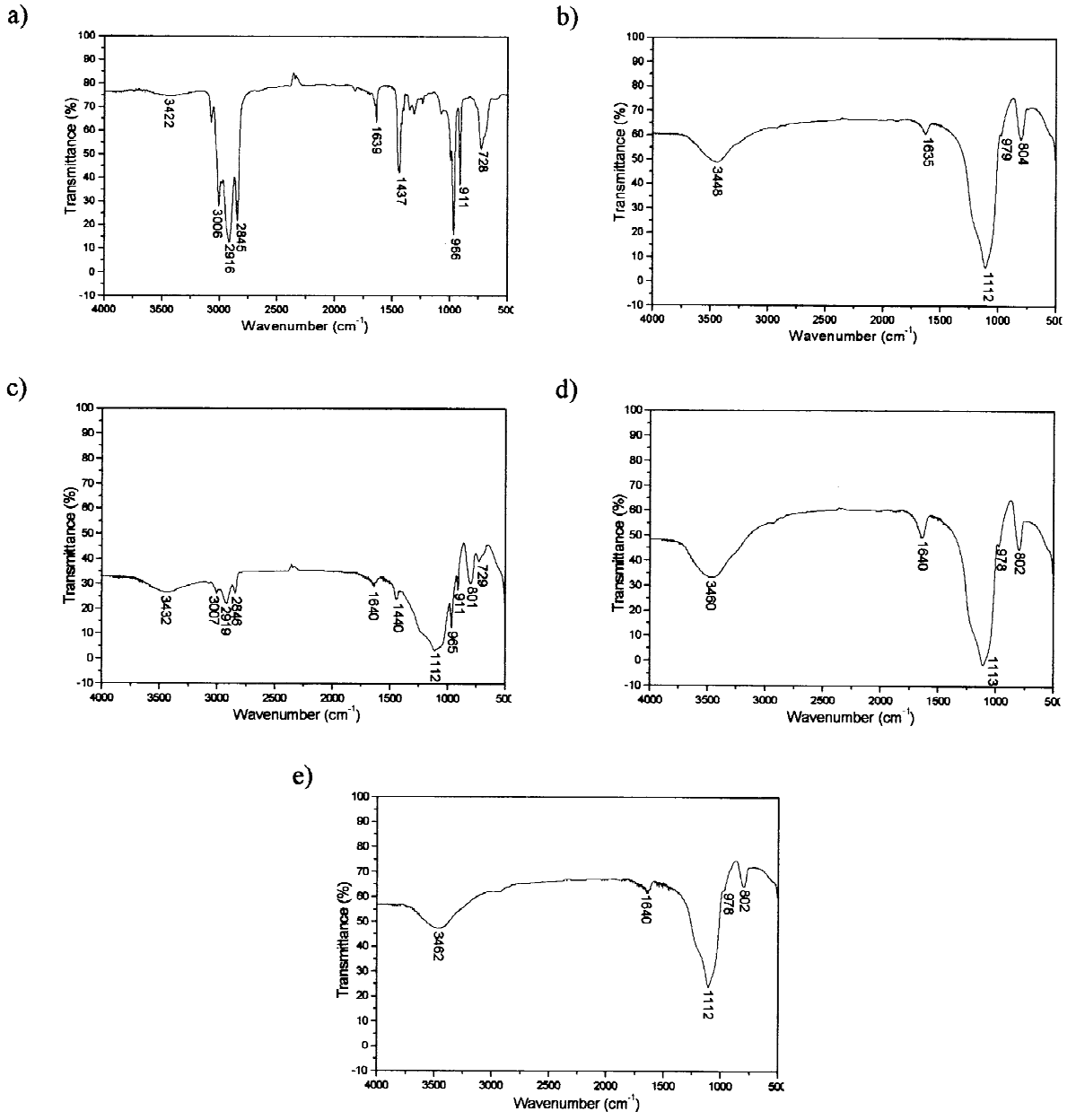

Fig. 2. IR spectra of (a) PBD; (b) silica; (c) non-irradiated stationary phase before extraction; (d) non-irradiated stationary phase after extraction; and (e) stationary phase immobilized by 5 kGy of γ radiation.

''slower'' linear increase in thickness with absorbed dose indicates strongly that it is much more difficult to immobilize more PBD on top of the immobilized PBD surface layer. Third, subsequent ''layers'' may not have independent layer character, i.e., may not be involved in such descriptions as 2nd layer, 3rd layer, etc. The uniform linear character of Fig. 1 argues against a set of independent layers.

Yet another aspect of the Fig. 1 dose dependence deserves comment here. As the addition of more and more immobilized PBD to the pores of the silica support particles occurs, the average open-pore diameter must decrease, giving less transport space for whatever HPLC application of a test column. For example, in the range of Fig. 1 (from 2.2 nm to 4.1 nm) the average open-pore volume decreases from 0.35 ml/g SiO_2 to 0.044 ml/g SiO_2—a factor of 8. Based on this fact alone we expect to observe a decrease in chromatographic efficiency and perhaps other properties from columns packed with high-dose irradiated phases, whether or not some of the pores are blocked.

Direct evidence for blockage of small pores is presented in Table 2. The mean pore size of the pore system apparently increases when PBD is present (immobilized) in the pore system due to selective blockage of small pores. Corresponding decreases in surface area and pore volume due to the presence of immobilized PBD are also shown in Table 2.

Identification of the infrared bands associated with *cis* and *trans*-CH=CH and vinyl $CH=CH_2$ structural groups of the PBD can be seen in Fig. 2. The spectrum of PBD (Fig. 2a) shows absorption in many regions. The regions of 3330–2600 cm^{-1} and of 1700–1600 cm^{-1} are assigned to the =CH stretching and C=C stretching modes, respectively. The regions of 1500–1000 cm^{-1} and of 1000–500 cm^{-1} are assigned to in-plane =CH and out-of-plane =CH modes, respectively [27]. This last region is very characteristic of PBD. The *cis*-1,4, *trans*-1,4 and vinyl structures give rise to sharp bands at 728 cm^{-1}, 966 cm^{-1} and 911 cm^{-1}, respectively, and are thereby amenable to quantitative measurement.

The spectra of the silica and the stationary phases (Fig. 2) show a band near 3400 cm^{-1}, due to vibrations of the hydroxyl groups having a hydrogen bridge to physically adsorbed water. Another band, around 1100 cm^{-1}, is attributed to the siloxane groups and 978 cm^{-1} is assigned to the stretching vibration of free silanols [28]. The spectrum of the stationary phase before extraction of the excess PBD is shown in Fig. 2c. On the other hand, after extraction, the bands characteristic of PBD are greatly reduced due to the lesser quantity of polymer present (Fig. 2d).

Table 3
Chromatographic parameters of the stationary phases prepared with different proportions of PBD on silica supports

% PBD on silica	Chromatographic parameters[a]			
	N/L[b]	A_s[b]	k[b,c]	R_s[d]
40	19 800	1.5	2.4	6.5
50	74 200	1.2	3.8	7.0
60	57 900	1.8	4.0	7.5

[a] Chromatographic conditions: mobile phase: methanol–water (60:40, v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 5 μl and detector: UV at 254 nm.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene-naphthalene pair.

3.2. Chromatographic evaluation

Table 3 shows the chromatographic parameters of the columns obtained using stationary phases with different loadings of PBD on the silica, using methanol–water (60:40, v/v) as mobile phase. The

Table 4
Chromatographic parameters of stationary phases immobilized by γ radiation at different doses

Gamma radiation dose (kGy)	Chromatographic parameters[a]			
	N/L[b]	A_s[b]	k[b,c]	R_s[d]
0	58 700	1.5	1.3	4.2
5	84 800	1.3	3.8	6.8
10	80 000	1.3	3.8	6.8
20	77 800	1.5	4.1	6.9
30	70 800	1.3	3.9	6.2
60	24 800	1.4	7.1	5.3
80	18 300	1.2	7.5	4.8
180	39 200	1.2	9.3	5.8

[a] Chromatographic conditions: mobile phase: methanol–water (70:30, v/v), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 5 μl and detector: UV at 254 nm.
[b] Calculated for the naphthalene peak.
[c] Column dead time was measured with uracil.
[d] Calculated for the toluene–naphthalene pair.

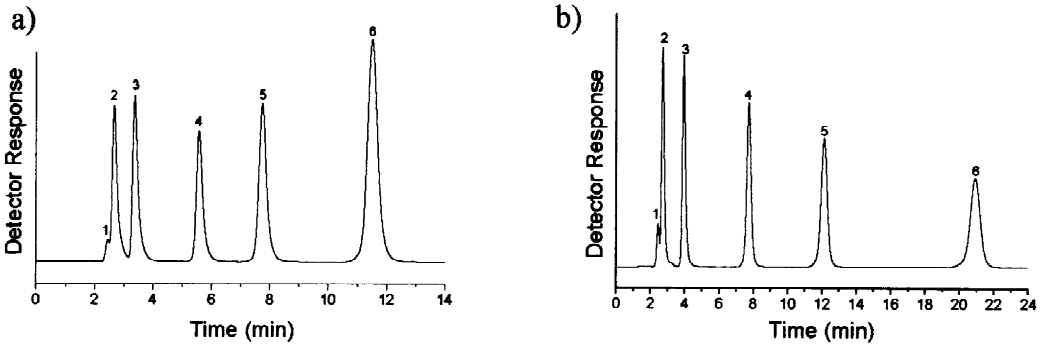

Fig. 3. Chromatographic behavior of stationary phases immobilized by 5 kGy of γ radiation. Test mixture: 1=uracil, 2=acetone, 3=benzonitrile, 4=benzene, 5=toluene and 6=naphthalene. Chromatographic conditions: mobile phase: methanol–water (70:30, v/v) (a) or (60:40, v/v) (b), flow-rate: 0.2 ml min^{-1}, volume of injected sample: 5 μl and detector: UV at 254 nm.

stationary phase prepared with a loading of 50% gives the best chromatographic performance.

The chromatographic parameters of the columns obtained using stationary phases immobilized with different γ radiation doses, using methanol–water (70:30, v/v) as the mobile phase, are shown in Table 4. The immobilization of PBD on the silica with γ radiation at 5 kGy produced columns that exhibited better efficiency values than were obtained at the other absorbed doses of γ irradiation. For the non-irradiated phase, the best separation of all the compounds was obtained when the composition of the mobile phase used was methanol–water in the proportion (60:40, v/v). For the irradiated phases, this mobile phase resulted in higher efficiencies and more symmetrical peaks, but the analysis time was increased from 13 min to 23 min. For the non-irradiated phase the analysis time was 16 min for the same test mixture. This result corresponds to the average data from two columns packed using the same batch of packing material. Typical chromatograms obtained using the different compositions of mobile phase are shown in Figs. 3 and 4.

The low efficiency values obtained for the high γ radiation doses probably result from diminished pore diameters or from pore blockage, which seriously affect the diffusion of sample molecules within the stationary phase.

3.3. Stability testing

Fig. 5 shows the corresponding chromatographic test results obtained for the naphthalene solute of the stationary phase immobilized by γ radiation at 5 kGy

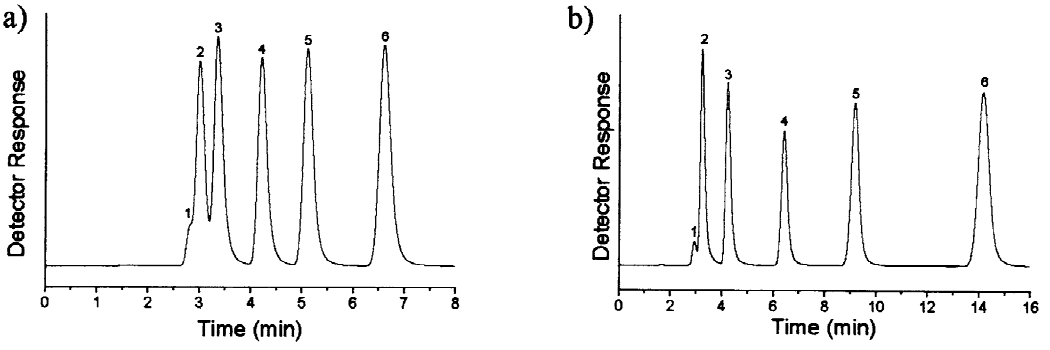

Fig. 4. Chromatographic behavior of non-irradiated stationary phases. Test mixture: 1=uracil, 2=acetone, 3=benzonitrile, 4=benzene, 5=toluene and 6=naphthalene. Chromatographic conditions: same as Fig. 3.

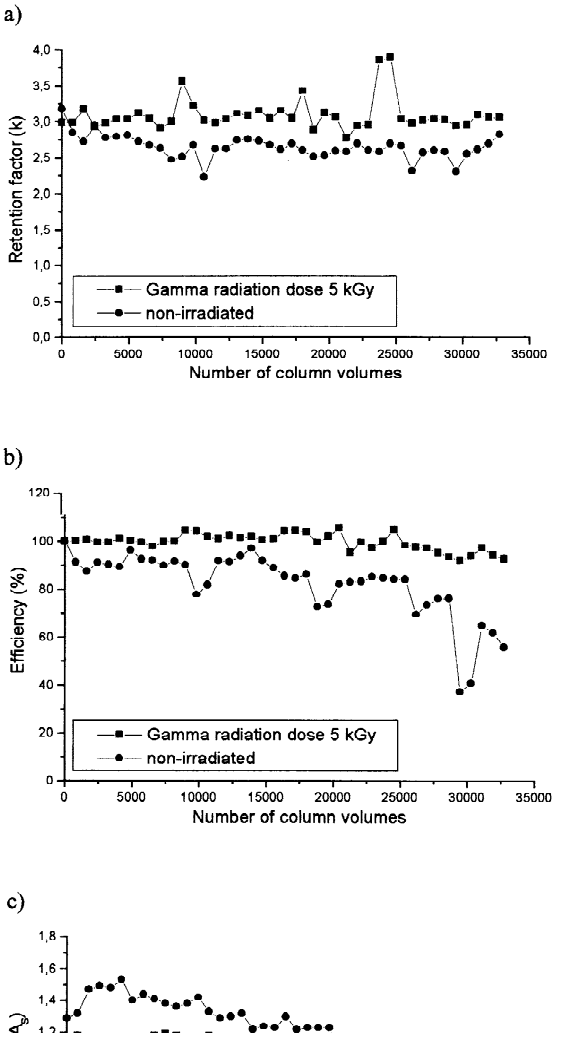

Fig. 5. Stability testing of stationary phase immobilized by 5 kGy of γ radiation and of non-irradiated phase in terms of (a) retention factor (b) relative efficiency and (c) asymmetry peak, calculated for the naphthalene peak, as functions of volume of mobile phase passing through column packing. Chromatographic conditions: same as Fig. 3.

absorbed dose and non-irradiated stationary phase. The retention factor, Fig. 5a, does not change throughout the stability testing. The passage of mobile phase apparently does not affect the polymeric layer, which continues to interact chromatographically with the test solute. As the retention factor is related to solute interaction with the stationary organic phase, stability is better evaluated by observing the variation in efficiency [24,25]. Fig. 5b shows that the irradiated stationary phase gives better efficiency during the entire test, showing greater stability compared with the non-irradiated stationary phase. After passing 32 800 column volumes, the irradiated phase had only a small decrease in column efficiency while the column prepared with non-irradiated phase had a larger (~ 50%) decrease in efficiency. The irradiated phase shows a small decrease in asymmetry of the naphthalene peak (Fig. 5c).

3.4. Comparison with radiation immobilized poly(methylalkylsiloxanes)

Radiation immobilization and thermal immobilization of poly(methyloctadecylsiloxane) (PMODS) and poly(methyloctylsiloxane) (PMOS) in the pores of HPLC silica have been studied by several research groups since the early 1980s [17–22,25,26]. From these and related studies it has been tentatively concluded that immobilized monolayer and bilayer structures covering the pore surfaces were obtained. The thickness of such layers apparently correlates with the length of the alkyl side-chains attached to the principal polymer-chain of the surface-attached molecules.

As an example, an immobilized monolayer of PMOS is thought to be formed [18,25,26] under appropriate conditions with a layer thickness of ~1.1 nm (obtained via calculations similar to those used in the present paper) which corresponds to a structure in which the main polymer chain lies flat on the pore surface and the C_8 chains extend directly into the open pore space.

In the present PBD case the essentially identical layer thickness (1.15±0.05 nm) also suggests a uniform structure of some sort, but no obvious simple substructure (e.g., side-chain) is present. Thus the assumed layer ‘‘structure’’ for PMOS and PBD