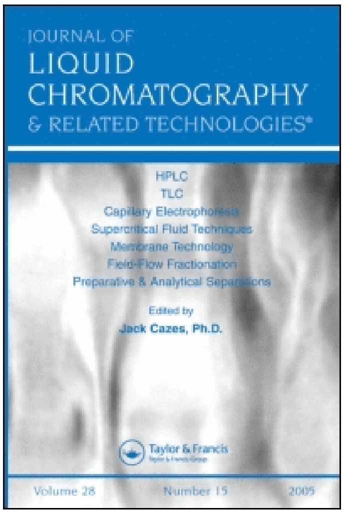

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Preparation of a C_{18} Stationary Phase Immobilized by Gamma Radiation for use in High Performance Liquid Chromatography

M. C. H. da Silva[a]; I. C. S. F. Jardim[a]
[a] Institute de Química Universidade Estadual, Campinas, SP, Brazil

To cite this Article Silva, M. C. H. da and Jardim, I. C. S. F.(1998) 'Preparation of a C_{18} Stationary Phase Immobilized by Gamma Radiation for use in High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 21: 16, 2447 — 2458

To link to this Article: DOI: 10.1080/10826079808003590

URL: http://dx.doi.org/10.1080/10826079808003590

J. LIQ. CHROM. & REL. TECHNOL., 21(16), 2447–2458 (1998)

PREPARATION OF A C_{18} STATIONARY PHASE IMMOBILIZED BY GAMMA RADIATION FOR USE IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. C. H. da Silva, I. C. S. F. Jardim*

Instituto de Química
Universidade Estadual de Campinas
C. P. 6154
13083-970, Campinas, SP, Brazil

ABSTRACT

A liquid stationary phase, poly(methyloctadecylsiloxane), was immobilized on a silica support, in the proportion 50:50 m/m, by gamma radiation doses of 20 to 90 kGy, with the purpose of obtaining a highly stable chromatographic stationary phase. This stationary phase exhibited higher efficiency and shorter analysis times than similar columns prepared with commercial C_{18} packings.

INTRODUCTION

A liquid siloxane polymer, sorbed into the pores of a porous silica support, can be immobilized by producing inter-chain (reticulation) bonds directly to the support surface. Such immobilization normally makes the stationary phase more resistant to solvent attack and to higher temperatures.[1] Also, after immobilization there is better blocking of the silanol groups of the support, due to the reticulation of the bonded stationary phase. The immobilization process is

www.dekker.com

initiated by free radicals.[1] Thus, some means of producing these radicals, such as low energy radiation (heat),[2] chemical agents (peroxides,[3] azocompounds,[4] and ozone[5]), and high energy radiation such as accelerated electrons[6] or gamma radiation from cobalt-60[7] may be used.

Our interest in using gamma radiation to immobilize the polymeric stationary phase in this study comes from the fact that it offers the advantage 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 formation of undesired secondary products. Furthermore, it can be applied directly to the solid substances, due to the high penetration power of the gamma radiation.

Gamma radiation has been used successfully for immobilization of poly(siloxane) liquids for capillary and packed columns for GC.[8-10] A phase synthesized by chemically bonding poly(styrene-divinylbenzene) onto silica with gamma radiation cross-linking, was used for separation of a mixture of nine alkyl aryl ketones in GC.[11] In another work, gamma-immobilized SE-30 on Chromosorb was developed for packed-column GC, where study of the gamma irradiation dose was investigated. The variation of irradiation dose influenced the performance of the columns.[12] A study of cyanoethyl silicone cross-linked by radiation with cobalt-60 gamma-ray radiation at 200 kGy in a glass capillary column for use in GC presented a degree of cross-linking of $\approx 80\%$ and a plate number 4000 m^{-1}.[13]

The use of the gamma radiation, peroxides, or thermal treatment for promotion of the immobilization of poly(siloxane) resulted in HPLC columns with good chromatographic performance .[14] Several new reverse stationary phases for HPLC were developed by covering silica or alumina with polymers (poly(butadienes) and poly(methyloctadecylsiloxane)). Some new reagents for silanization of the silicas were investigated and the results compared with other methods. Immobilization by gamma radiation was effective in producing good quality columns.[15] This paper reports the preparation of immobilized phases possessing the C_{18} group, and the characterization of the resulting packing materials by physical, chemical, and chromatographic methods.

EXPERIMENTAL

Chemicals and Materials

Methanol, chloroform (Merck, HPLC grade), and carbon tetrachloride (Merck, AR grade) were used without further purification. The chromatographic test substances (acetone, aniline, benzene, benzonitrile, naphthalene, nitronaphthalene, toluene) were AR grade and not further purified. The irregular silica Davisil-630 (Alltech Associates, USA) had an average particle size of 10 μm, nominal pore diameter of 6 nm, and a specific surface area (BET) of 387 m^2g^{-1}.

Poly(methyloctadecylsiloxane), PMODS, was obtained from Petrarch Systems Silanes and Silicones (Product PS 130) with a molecular mass of 11000 and a viscosity of 250-300 ctsk (50°). The commercial stationary phase was Partisil ODS-1 (Whatman) with an average particle size of 10 μm, pore size of 8.5 nm and surface area of 350 m^2g^{-1}.

Preparation of Stationary Phase

The silica was dried at 150°C for 24 hours before preparation of the packing material. A defined quantity of silica was added to a solution of known concentration of poly(methyloctadecylsiloxane) in chloroform.

The mixture was submitted to gentle agitation for three hours. After this, the solvent was permitted to evaporate at room temperature.

Irradiation

The prepared phases were sealed into glass ampoules under air at room temperature and irradiated by a Cobalt-60 source (IBRAS-CBO, Campinas, SP, Brazil). The irradiation doses were: 20, 60, 70, 80, and 90 kGy.

Preparation of Columns

The tube material was seamless 316 stainless-steel (125 mm long with an internal diameter of 3.8 mm). The internal surface was polished by means of a technique developed in our laboratory.[16] The packing of the columns was carried out using the high pressure suspension technique at a concentration of 20 % (m/v) in carbon tetrachloride, with a packing pressure of 38 MPa (Haskel Packing Pump). Methanol was the propulsion solvent.

After packing, the columns were conditioned with mobile phase (methanol:water, 50:50, v/v) for approximately 4h before commencing the chromatographic tests.

Instrumentation and Methods

The modular liquid chromatography had a Rheodyne 8125 injector with a 5 μL loop, a Waters 510 pump, an Isco Model V-4 spectrophotometric UV/Vis detector with detection at 254 nm, using a 5.3 μL cell, and a Sargent-Welch Model DSGR recorder. All chromatographic runs were carried out at room temperature. Two mixtures were used for analysis, both dissolved in the mobile phase. Mixture 1 had acetone, benzonitrile, benzene, toluene, and naphthalene while mixture 2 had aniline, nitronaphthalene, and naphthalene. The mobile phase was prepared volumetrically from individual aliquot measures of methanol and water. The optimum flow rate of the mobile phase, determined from van Deemter curves, was 0.1 mL min^{-1}. The chromatographic factors measured were plate number (N), retention time (t_R), asymmetry factor at 10% of peak height (As), resolution (Rs), retention factor (k), and separation factor (α). The values of N, As, and k were calculated for naphthalene, resolution for the acetone-benzonitrile pair, and α for the toluene-naphthalene pair. To verify the chemical stability of the prepared phases, the procedure described by Anazawa et al.[17] was used with MeOH:H_2O 50:50 v/v as mobile phase at a flow of 2.5 mL min^{-1}.

Besides the chromatographic runs, chemical and physical tests were also carried out on the prepared stationary phases, such as solvent extraction, measurements of surface area, and elemental analysis (carbon). The elemental determinations were carried out with a Model 2400 Perkin Elmer CHN analyzer. Soxhlet extraction was carried out using a Soxlhet extractor and submitting the prepared stationary phase to a 6 hour reflux with each of three solvents. After each extraction the solvent was evaporated and the stationary phase dried to constant weight, before initiating the next extraction. Methanol was used for the first extraction as a polar solvent in which PMODS is only slightly soluble followed by benzene in which PMODS is moderately soluble and finally by n-pentane, in which PMODS is much more soluble. The surface areas of the silica and of the prepared stationary phases were determined by the Brunauer-Emmett-Teller (BET) method[18] by means of a Micrometrics Flow Sorb II, model 2300, instrument.

RESULTS AND DISCUSSION

A study to establish the best quantity for the liquid stationary phase to be deposited on the silica support indicated a proportion of 50:50 (m/m) of poly(methyloctadecylsiloxane) and silica, as determined by the procedure described by Anazawa and Jardim.[19] Having established the best proportion of the liquid stationary phase to be deposited on the support, the investigation proceeded to study the influence of gamma radiation on this type of stationary

Table 1

Chromatographic Parameters Obtained with Columns Packed with Non Irradiated Packing Material and With Irradiated Packing Material Prepared with Different Doses of Gamma Radiation

Dose			**Chromatographic Parameters**[a]		
(kGy)	**N/m**[b]	**k**[b]	**As**[b]	**Rs**[c]	α[d]
0	21000	5.1	0.7	3.4	4.2
20	21100	6.2	0.9	4.7	4.5
60	30800	5.5	0.8	5.5	4.0
70	25800	5.4	1.0	4.8	3.8
80	29200	6.5	0.8	6.1	4.2
90	21500	6.3	0.7	4.9	4.4

---

[a] Chromatographic conditions: column 125 x 3.8 mm i.d.; mobile phase: MeOH:H_2O (50:50 v/v); flow rate: 0.1 mL min^{-1}; detection at 254 nm.

[b] Calculated for naphthalene.

[c] Calculated for acetone-benzonitrile.

[d] Calculated for toluene-naphthalene.

phase. Table 1 shows the chromatographic parameters obtained using different gamma radiation doses. It can be seen that after irradiation the columns generally exhibited better asymmetry values. These asymmetry values, besides indicating a higher degree of confidence in the calculated efficiency values, show a better blocking of the residual silanol groups, caused by reticulation promoted by gamma radiation. The values for the retention factor, k, for naphthalene are within the range 2 to 10, indicating a good mass transfer of solute between the mobile phase and the stationary phase.

The resolution values found for acetone-benzonitrile show excellent separation, while the separation factor, α, indicates a good separation of toluene and naphthalene.

Analyzing the number of plates per meter, N/m, it can be seen that, in general, the efficiencies of the columns packed with the irradiated phase were superior to the non-irradiated one. It can be observed that the best efficiencies were obtained with irradiation doses of 60 and 80 kGy, with the maximum value for the 60 kGy dose. This is taken to indicate a good immobilization of this phase without degradation.

Table 2

Chromatographic Parameters Obtained With Columns Packed With Non Irradiated Packing Material and With Packing Material Prepared Irradiated to a Dose 60 kGy

Dose (kGy)	Compound	Chromatographic Parameters[a] t_R (min)[b]	As[b]	Rs[c]	α[c]
0	Aniline	13.9	1.1	---	---
	Nitronaphthalne	48.4	0.8	1.9	1.2
	Naphthalene	56.5	0.8	1.9	1.2
60	Aniline	13.5	1.0	---	---
	Nitronaphthalne	44.9	0.9	2.5	1.3
	Naphthalene	54.2	0.8	2.5	1.3

[a] Chromatographic conditions as inTable 1.
[b] Calculated for naphthalene.
[c] Calculated for nitronaphthalene-naphthalene.

The lowest values were obtained for doses of 20 and 90 kGy, which might indicate, respectively, that not enough cross-linking has occurred to improve the efficiency and that either degradation of the stationary phase is occurring, or excessive cross-linking, in such a way that the sample molecules cannot diffuse into the stationary phase as easily.

To evaluate whether irradiation promotes a better shielding of the silanol groups of the silica support, mixture 2, containing aniline, nitronaphthalene, and naphthalene, was injected onto columns packed with non irradiated and with irradiated (60 kGy) packing materials. The columns were evaluated by means of asymmetry, resolution and separation factor, whose values are found in Table 2.

The data of Table 2 shows that with an irradiation of 60 kGy, a slight increase in the separation factor occurs for the nitronaphthalene-naphthalene pair. This parameter, according to Verzele and Dewaele,[20] is very useful because it allows one to evaluate the column as to its shielding of silanol groups. A separation factor between 1.1 and 1.2 characterizes columns having unprotected surfaces, i.e., with more exposed silanol groups, whereas values above 1.2 characterize columns with more deactivated silanol groups while α higher than 1.4 implies columns with fully covered silanol groups. Thus, it is seen that, for a column packed with irradiated stationary phase, the silanol

Table 3

Chromatographic Parameters Obtained for Mixture 1 with Columns Packed with Irradiated Stationary Phase, SP, and with Partisil ODS

	Chromatographic Parameters				
Column	**t_R (min)[b]**	**N/m[b]**	**k[b]**	**As[b]**	**Rs[c]**
Partisil	39.0	25506	2.9	1.2	3.6
SP irradiated to 80 kGy	32.7	29047	2.5	0.9	2.8

---

[a] Chromatographic conditions: column: 125 x 3.8 mm i.d.; mobile phase: $MeOH:H_2O$ (60:40 v/v); flow rate: 0.1 mL min^{-1}; detection at 254 nm.

[b] Calculated for naphthalene.

[c] Calculated for acetone-benzonitrile.

groups are somewhat more shielded than in the non irradiated stationary phase. Despite the fact that the values for the separation factor show that the non irradiated stationary phase can present exposed silanol groups, the asymmetry values for aniline either in columns packed with non irradiated stationary phase or that irradiated at 60 kGy can be considered good. It can also be observed that the retention times for aniline in the columns with irradiated and non irradiated stationary phase are very similar. This shows that the silanol groups are shielded, even before radiation, that is, with only a polymeric covering of the support surface.

With the purpose of evaluating the potential usefulness of the C_{18} stationary phases immobilized by gamma radiation, the chromatographic performance of a column packed with stationary phase irradiated at 80 kGy was compared with another packed with a non irradiated commercial packing, Partisil ODS. The values of the chromatographic parameters are found in Table 3 and the related chromatograms are shown in Figure 1. A difference in the efficiency of the columns can be observed, with the column filled with the prepared stationary phase irradiated at 80 kGy presenting a higher number of plates per meter. The remaining parameters were slightly lower for the column with the irradiated stationary phase. However this indicates that both columns exhibit good chromatographic performance, that is, good resolution, good values for k and acceptable asymmetries.[21] Another favorable factor for the irradiated column is that it requires a shorter analysis time when compared to the column with the commercial stationary phase. Thus the quality of the stationary phase prepared in this study compares favorably those found commercially.

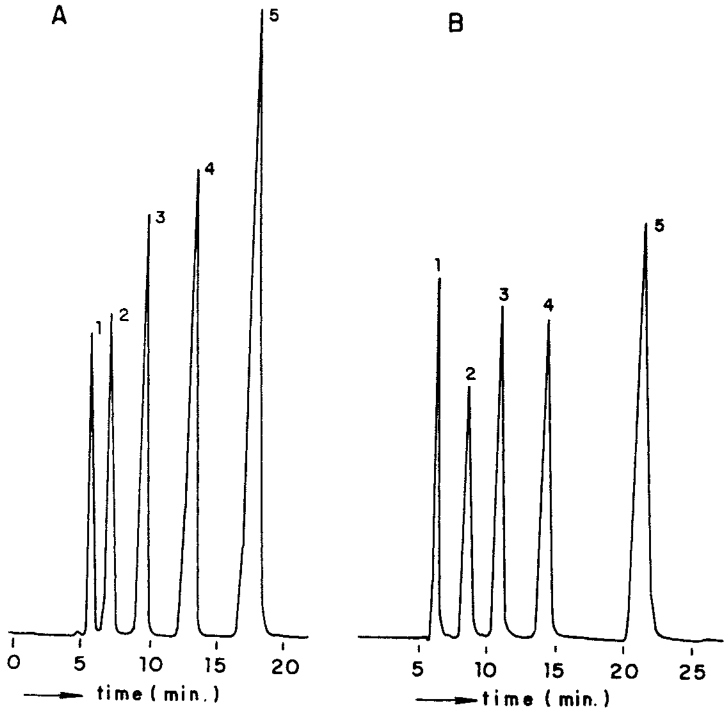

Figure 1. Chromatograms obtained for columns packed with (A) stationary phase (80 kGy) and (B) Partisil ODS; Peaks 1=acetone, 2=benzonitrile, 3=benzene, 4=toluene, 5=naphthalene; Column: 125 mm x 3.8 mm i.d.; Mobile phase methanol:water (60:40 v/v); Flow rate 0.1 mL min^{-1}, Detection UV at 254 nm

Table 4

Results of Extraction of the Prepared Stationary Phases, SP, and with a Commercial Phase

Sample	Total Extracted (%)	% C[a] Non Extracted	% C[a] Extracted
SP 0 kGy	35	39	16
SP 60 kGy	25	41	24
SP 80 kGy	20	39	22
Partisil ODS	10	--	--

---

[a] From elemental analysis.

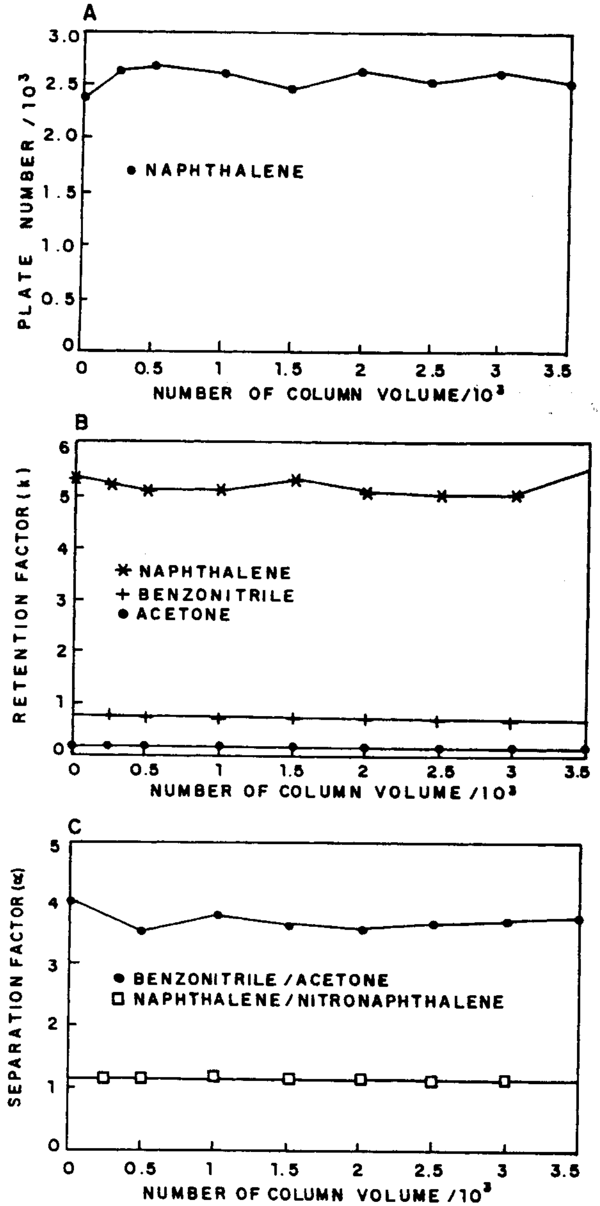

Figure 2. Graphs of column efficiency (N), retention factor (k) and separation factor (α) for columns packed with polymer containing particles irradiated to a dose of 70 kGy.

Table 4 shows the results obtained by physical and chemical measurements. From the data in Table 4 it is seen that, after the irradiation of the material, a decrease in the total quantity of extracted stationary liquid phase occurred. This indicates that significant cross-linking of the liquid stationary phase occurred, because when this happens a decrease in solubility with respect to most solvents occurs. In the elemental analysis, it was found that the non irradiated material after extraction presented a larger decrease in carbon content than the irradiated materials. This confirms the data obtained by solvent extraction and is one more indicator that cross-linking of the stationary liquid phase has occurred. The measured specific surface area shows a sharp decrease after coating with PMODS and this remains after irradiation.

Figure 2 shows graphs that delineate the results obtained by rinsing the stationary phase with mobile phase. It can be seen that the values of N, calculated for naphthalene, and k, calculated for acetone, benzonitrile, and naphthalene, do not change significantly. This indicates that the prepared stationary phase is stable with respect to the mobile phase (methanol/water). The separation factors obtained for the acetone-benzonitrile and nitronaphthalene-naphthalene pairs proved to be essentially stable during the rinsing process. This is an excellent indicator that the silanol groups were not uncovered during the rinsing process, showing that the stationary phase prepared in this study is chemically very stable, and can be used with large volumes of mobile phase without loss in quality of the prepared phase. The results obtained are similar to those of Anazawa et al.[17]

CONCLUSION

This investigation, stressing the use of gamma radiation for immobilization, resulted in a stationary phase of good quality, chemically stable, and with a chromatographic efficiency comparable to that of a commercial ODS phase of the same form and particle size. Thus gamma radiation is an excellent means of immobilization of liquid polymeric stationary phases for High Performance Liquid Chromatography, since cross-linking of the polymer molecules offers higher shielding of the residual silanol groups.

ACKNOWLEDGMENTS

The authors acknowledge financial support from PADCT-SINST and IBRAS-CBO (Campinas, SP, Brazil) for performing the irradiations of the stationary phases and Prof^a. Dr^a. C. H. Collins for suggestions. M. C. H. da Silva thanks CNPq for a fellowship.

REFERENCES

1. E. B., Mano, **Introdução a Polímeros**, 2nd ed., Edgard Blücher Ltda, São Paulo, 1986.

2. N. D. Petsev, G. I. Perov, M. D. Alexandrova, Chi. Dimitov, Chromatographia, **20**, 228-230 (1985).

3. J. Gawdizik, Chromatographia, **31**, 258-262 (1991).

4. A. Bemgard, L. G. Blomberg, J. Chromatogr., **395**, 125-144 (1987).

5. C. H. Chuang, H. Shanfield, A. Zlatkis, Chromatographia, **23**, 169-170 (1987).

6. K. Markides, L. Blomberg, J. Buijten, T. Wännman, J. Chromatogr., **267**, 29-38 (1983).

7. G. Schomburg, J. Köhler, H. Figge, A. Deege, U. Bien-vogelsang, Chromatrographia, **18**, 265-274 (1984).

8. W. Bertsch, V. Pretonius, M. Pearce, J. Thompson, N. G. Schnautz, J. High Resol. Chromatogr. Chromatogr. Commun., **5**, 432-434 (1982).

9. J. A. Hubball, P. R. Dimamo, E. F. Barry, E. A. Lyons, W. A. George, J. Chromatogr. Sci., **22**, 185-191 (1984).

10. G. Schomburg, H. Husmann, S. Ruthe, M. Herraiz, Chromatographia, **15**, 599-610 (1982).

11. R. Abuelafiya, J. J. Pesek, J. Liq. Chromatogr., **12**, 1571-1578 (1989).

12. M. A. Basso, M. J. T. F. dos Santos, K. E. Collins, C. H. Collins, J. High Resol. Chromatogr., **12**, 500-502 (1989).

13. X. W. Kang, Y. C. Zhou, Sepu., **10**, 334-336 (1992).

14. G. Schomburg, A. Deege, J. Köhler, U. Bien-vogelsang, J. Chromatrogr., **282**, 27-39 (1983).

15. U. Bien-vogelsang, H. Figge, J. Köhler, G. Schomburg, Chromatrographia., **19**, 170-179 (1984).

16. T. A. Anazawa, F. Carraro, J. B. de Souza, K. E. Collins, I. C. S. F. Jardim, 14º National Meeting of Brazilian Society of Chemistry, Caxambu, MG, Brazil, 1991, QA-010.

17. T. A. Anazawa, F. Carraro, K. E. Collins, I. C. S. F. Jardim, J. Chromatogr., **697**, 159-164 (1995).

18. S. Brunauer, P. H. Emmett, E. Teller, J. Am. Chem. Soc., **60**, 309-319 (1938).

19. T. A. Anazawa, I.C.S.F. Jardim, J. Liq. Chromatogr., **17**, 1625 -1279 (1994).

20. M. Verzele, C. Dewaele, Chromatographia, **18**, 84-86 (1984).

21. L. R. Snyder, J. J. Kirkland, **Introduction to Modern Liquid Chromatography**, 2nd ed., John Wiley & Sons, New York, 1979.

Received November 1, 1997
Accepted January 29, 1998
Manuscript 4663