



ELSEVIER

Journal of Chromatography A, 849 (1999) 299–307

JOURNAL OF  
CHROMATOGRAPHY A

# Radiation immobilization of poly(methyloctylsiloxane) on silica for use in HPLC: a uniform layer model

Isabel C.S.F. Jardim, Kenneth E. Collins\*, Tania A. Anazawa

Instituto de Química, Universidade Estadual de Campinas – UNICAMP, Cx Postal 6154, 13083-970 Campinas (SP), Brazil

Received 2 December 1998; received in revised form 1 March 1999; accepted 21 April 1999

## Abstract

Poly(methyloctylsiloxane) (PMOS) was sorbed into the pores of HPLC silica by a solvent evaporation procedure, then irradiated with gamma rays from a cobalt-60 source to absorbed doses in the range from 0 to 200 kGy (1Gy=1J kg<sup>-1</sup>). Non-irradiated and irradiated samples were characterized by solvent extraction, specific surface area determination, infrared spectroscopy and reversed-phase column performance. Solvent extraction data reveal that about 40% of the PMOS is not extractable prior to irradiation and this increases to about 75% with radiation doses of 50 kGy or higher. Column performance was improved by the radiation treatment, reaching a maximum efficiency in the dose range of 80–140 kGy while the peak symmetry changed from As=1.7 to 1.1. The improvement is attributed to the increased mass of polymer immobilized by the radiation treatment and to a more uniform distribution of the immobilized polymer in the silica pore system. A multi-layer stationary-phase model is presented in which the first layer consists of an adsorbed monolayer of PMOS and the second layer is immobilized by gamma radiation. © 1999 Elsevier Science B.V. All rights reserved.

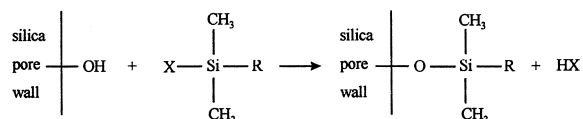
**Keywords:** Immobilization; Radiation immobilization; Stationary phases, LC; Polymethyloctylsiloxanes

## 1. Introduction

### 1.1. Stationary phases: general

Packing materials for reversed-phase High Performance Liquid Chromatography usually consist of porous silica support particles having a layer of organic material attached to the pore walls. Such coatings (stationary phases) can be prepared in many

ways [1–3]. These can be discussed in terms of three categories of layer structure. One of these (Type I) utilizes condensation reactions of reagents such as chlorodimethylalkylsilane with silanol (Si-OH) groups on the silica pore walls:



In this way *individual* R-groups, such as C<sub>4</sub>, C<sub>8</sub> and C<sub>18</sub> are immobilized on the surface by covalent bonds. Type I stationary phases have been reviewed by Kirkland [4] and by Sander and Wise [5].

\*Corresponding author. Tel.: +55-197-883-061; fax: +55-197-883-023.

E-mail addresses: icsfj@iqm.unicamp.br (I.C.S.F. Jardim), kec@iqm.unicamp.br (K.E. Collins)

Type II coatings utilize a quasi-continuous two dimensional (2-D) layer of polymeric organic material on the pore walls. The desired R groups can be incorporated on the monomer units during the construction of the 2-D polymer layer – or can be added later [6]. Type II materials are discussed by Engelhardt et al. [7] and by Petro and Berek [3].

Type III materials are based on the formation of a polymer layer from “strips”, consisting of *linear polymer* molecules, which are sorbed into the silica pore system [3,8,9]. Features of this approach, relative to the other stationary phase preparations, are: (a) it is not necessary to attach the desired functional (R) groups directly to the silica surface by covalent bonds; (b) each polymer molecule can carry a large number of R groups (for example, one on each monomer unit of the polymer) with a separation between them that is near optimum for multipoint adsorption of the principal siloxane chain  $[-O-Si-]_n$  onto the surface [10]; (c) the same, or similar, immobilization-by-crosslinking procedures that work for type II phases also work for producing stable layers of variable thickness on the silica surfaces, as Schomburg and collaborators demonstrated in a series of papers during the 1980's [11–13]. They found that some polymers, such as poly(methylcytolsiloxane) and poly(metyloctadecylsiloxane), could be sorbed into the pores of HPLC silica by the static solvent evaporation procedure but that the polymer tended to wash off into strong solvents such as dichloromethane or hexane unless additional “immobilization” reactions were used [14]. Based on their experience in the development of polymer coated capillary columns for high resolution GC, they developed several successful procedures, including the use of gamma radiation, for the immobilization of various linear polymers sorbed in the pores of silica particles [11]. During this period, another useful procedure was developed; that of *pre-silanizing* the silica surface *prior* to coating and immobilization [14,15]. Columns prepared from these polymer-coated packing materials were efficient and stable under most use conditions. Indeed, they were better than most of the Type I packings of that time.

At present, after many years of development, the best of the Type I [16] (and Type II [17]) packings give excellent performance and have useful lifetimes

even when used with fairly aggressive mobile phases. Today's columns with Type III packings may be as good, or even better, for some applications but few such columns are commercially available [18]. Presumably much development of concepts and procedures will be necessary before the potential of Type III packings can be realized.

Our decision to emphasize the  $\gamma$ -irradiation procedure for immobilization of sorbed polymers comes from an awareness that this procedure has advantages over other immobilization procedures that, as Charlesby expresses it, “...include the ability to promote changes reproducibly and quantitatively, without the introduction of chemical reagents, and with complete control of temperature, environment and additives” [19].

In the present work we have examined the chromatographic properties of a sorbed polysiloxane following irradiation by gamma radiation in an oxygen-containing environment.

## 1.2. Radiation effects: general considerations

Gamma radiation consists of very high energy photons that interact (“collide”) mainly with individual electrons of a material being irradiated. The interaction occurs very rapidly, as it takes only  $\sim 10^{-18}$  s for a photon to traverse an atom such as Si or O. The interaction can transfer an impulse that removes an electron from an atom or molecule (causing ionization) or can transfer a lesser quantity of energy, producing “excited” centers in atoms or molecules [20]. The subsequent dispersion of energy in such events gives rise to many possible ionic and radical reactions and a wide variety of possible products [19,20], including those also produced by other cross-linking agents.

When bulk polymers are irradiated, the predominant reactions/products depend strongly on the transport of excitation energy (and charge) within and between molecules, on the strengths of individual chemical bonds, and on the geometrical arrangements of polymer molecules. Usually one of two general processes dominates: cross-linking or degradation [19,20]. Irradiated bulk poly(dimethylsiloxane) mainly undergoes cross-linking because the yields for degradation products (into small product species such as  $H_2$ ,  $CH_4$ ,  $H_2O$ , etc.) are low

[21]. On the other hand, bulk poly(methyloctylsiloxane), irradiated to a moderately high dose of 100 kGy [22], *did not* show the expected increase in viscosity or decrease in solubility which are indicators of cross-linking. Furthermore, 20–30% of the polymer sorbed into the silica pore system remained solvent extractable, hence was not immobilized, following absorption of 100 kGy of radiation dose.

These results raise the question of whether or not the observed degree of polymer immobilization resulting from irradiation was due to cross-linking between the (alkyl) chains of the polymer – or to some other phenomenon. Relevant literature references to alternative processes in a silica pore system are difficult to come by, but at least one alternative explanation can be imagined: the silica support also absorbs radiation energy – even more than does the sorbed polymer. Energy transport from excited and/or charged centers of the silica to the polymer on the surface might initiate formation of silica-polymer chemical bonds, even without high yields of cross-linking *within* the polymer layer – or perhaps lead to high degradation yields of the polysiloxane (polymer) skeleton.

The above discussion is intended to emphasize the point that we do not know in any detailed way what structural/chemical changes take place when a polymer coating, located in the nanometer-scale pores of HPLC silica, is subjected to ionizing radiation. It is convenient to assume that a polymer in the pores behaves the same as bulk polymer, but this might not be the case.

## 2. Experimental

### 2.1. Reagents and materials

Methanol (HPLC grade), dichloromethane (HPLC grade) and carbon tetrachloride (AR grade), obtained from Merck (Rio de Janeiro, Brazil), were used without further purification. The chromatographic test substances were analytical reagent grade and not further purified. Poly(methyloctylsiloxane) (PMOS) with an average molecular mass of 6200 was obtained from Hüls America (USA).

Davisil 630 silica, having a mean particle size of

10  $\mu\text{m}$ , a mean pore size of 8.7 nm and a 387  $\text{m}^2 \text{g}^{-1}$  specific surface area, was obtained from Alltech Associates (USA). Lichrosorb RP 8, 10  $\mu\text{m}$  particle size (Merck, Germany), was used in some comparison tests.

### 2.2. Preparation of polymer coated stationary phase

A known quantity of silica (dried at 150°C for 24 h) was added to a solution of PMOS in dichloromethane to prepare a PMOS-loaded material,  $\text{SiO}_2(\text{PMOS})$ , having an initial loading of 40% PMOS. This mixture was slowly agitated at room temperature for three hours, after which the dichloromethane was evaporated using a 150 mm diameter Petri dish.

### 2.3. Immobilization by gamma irradiation

Five gram samples of  $\text{SiO}_2(\text{PMOS})$  were placed in glass ampoules which were then sealed under air. The sealed samples were irradiated, using an industrial Cobalt-60 source (IBRAS-CBO, Campinas, SP, Brazil) to absorbed doses of 40, 50, 80, 100, 120, 150, 180 and 200 kGy (1 Gy = 1  $\text{J kg}^{-1}$  of absorbed dose).

### 2.4. Column preparation

Columns (125 × 3.4 mm I.D.) were made from type 316 stainless-steel tubing whose inner surface was highly polished in our laboratory.

The columns were slurry packed, in the downward direction, using a 10% slurry (w/v) of the packing material in carbon tetrachloride. Packing was done at 38 MPa with a Haskel Model 51769 packing pump using methanol as pressurizing solvent.

### 2.5. Chromatographic experiments

Chromatographic experiments were performed with a modular HPLC instrument, equipped with a Waters Model 510 pump, SSI Model 3XL pneumatic injector with a 10  $\mu\text{l}$  loop, a Waters Model 481 spectrophotometric detector (14  $\mu\text{l}$  cell volume) and a Waters Model 740 integrator.

A test mixture of acetone, benzonitrile, benzene,

toluene and naphthalene was analyzed isocratically with a mobile phase consisting of 70:30 (v/v) methanol–water. The test solutes were dissolved in the mobile phase. Injections of 10  $\mu\text{l}$  of this mixture produced satisfactory chromatographic peaks with  $\times 128$  attenuation at 254 nm. Efficiency ( $N/L$ ), resolution ( $R_s$ ), retention factor ( $k$ ) and asymmetry factor at 10% of peak height ( $A_s$ ) were determined for each chromatogram. The number of plates of a peak was calculated using the equation:  $N = 5.545 (t_R/w_h)^2$ .

All columns having PMOS stationary phases were operated at ambient temperature with a mobile phase flow-rate of 0.2  $\text{ml min}^{-1}$  (optimized using a van Deemter plot). Column dead times,  $t_M$ , were determined using methanol as a non-retained marker.

### 2.6. Solvent extraction

A series of three independent extractions (with methanol, benzene and dichloromethane), of six hours each, were carried out on each sample, in a Soxhlet extractor using a modification of the method of Sanchez et al. [23]. After each extraction the solvent was evaporated from the sample and the residual mass was determined before initiating the next extraction.

### 2.7. Physical measurements

The specific surface areas of the various packings were determined by the conventional BET procedure, with nitrogen as adsorbent gas, on a Micromeritics Model 2300 Flow II instrument. Infrared

absorption spectra were obtained with a Perkin-Elmer Model 1600 FT-IR spectrophotometer.

## 3. Results and discussion

PMOS (MW=6200) can be sorbed into the pore system of HPLC silica by the static evaporation procedure, nearly to the limit posed by the specific pore volume of the silica [12]. For the silica used in this work, the specific volume of 0.76  $\text{ml g}^{-1}$  silica limits the mass of PMOS (density 0.91  $\text{g ml}^{-1}$ ) that can be sorbed to about 0.68 g per g of silica. Thus a PMOS loading of 40% ( $= 100 \times 0.68/1.68$ ) was chosen for the experiments presented here.

A series of  $\text{SiO}_2$  (PMOS) samples were irradiated by a Co-60 gamma ray source to absorbed doses from zero to 200 kGy. These samples were then exhaustively extracted, first by methanol, then by benzene and finally by dichloromethane. Table 1 summarizes the dry-mass data from these samples.

The first solvent, methanol, extracts very little PMOS from  $\text{SiO}_2$ (PMOS), irradiated or not. This is not an unexpected result since PMOS is only slightly soluble in methanol. The second solvent, benzene, in which the PMOS is moderately soluble, extracts a much larger quantity. However, the third solvent, dichloromethane, in which PMOS is very soluble, removes only a small fraction of the remaining PMOS. It is also seen that 42% of the PMOS is not extracted from *non-irradiated*  $\text{SiO}_2$ (PMOS), as already reported [24].

Irradiation immobilization, as represented by the percentage of initial polymer retained by the silica

Table 1  
PMOS extraction from irradiated  $\text{SiO}_2$ (PMOS) as a function of radiation dose

Dose (kGy)	Initial mass $\text{SiO}_2$ (PMOS) (g)	Initial mass PMOS (g)	% of initial PMOS extracted with:			% of initial PMOS extracted (retained)
			methanol	benzene	dichloromethane	
0	0.3180	0.1272	0.8	53.6	3.6	58.0 (42.0)
40	0.3035	0.1214	1.1	29.3	3.7	34.1 (65.9)
50	0.2972	0.1189	1.1	25.3	3.3	29.7 (70.3)
80	0.2944	0.1177	0.8	18.1	2.1	21.0 (79.0)
100	0.2965	0.1186	0.9	19.7	2.5	23.1 (76.9)
120	0.3109	0.1244	1.0	21.3	2.1	24.4 (75.6)
150	0.3008	0.1203	1.4	22.0	2.4	25.8 (74.2)
180	0.2993	0.1197	1.3	22.0	1.9	25.2 (74.8)
200	0.2957	0.1183	1.3	22.3	2.4	26.0 (74.0)

Table 2  
Chromatographic parameters obtained using a neutral test mixture<sup>a</sup>

Dose (kGy)	Chromatographic parameters			
	<i>N/L</i> <sup>b</sup>	<i>A<sub>s</sub></i> <sup>b</sup>	<i>k</i> <sup>a</sup>	<i>R<sub>s</sub></i> <sup>c</sup>
0	24800	1.7	3.2	2.2
40	26000	1.2	3.6	2.2
50	29600	1.1	4.5	2.4
80	32000	1.1	4.3	2.5
100	33200	1.1	4.3	2.5
120	33600	1.1	4.3	2.5
150	31600	1.1	4.7	2.5
180	28000	1.1	5.0	2.2
200	27200	1.1	5.1	2.2
Lichrosorb RP8	33200	1.1	3.0	3.4

<sup>a</sup> Chromatographic conditions: mobile phase: methanol: water (70:30, v/v), flow-rate: 0.2 ml min<sup>-1</sup>, volume of injected sample: 10 μl and detector: UV, 254 nm.

<sup>b</sup> Calculated for the naphthalene peak.

<sup>c</sup> Calculated for the toluene-naphthalene pair.

support following the series of extractions, takes the form of a rapid increase from 42% to 75% with ~50–80 kGy of radiation dose. Apparently no additional immobilization occurs at higher doses. The fact that ~25% of the PMOS resists further radiation immobilization, first noted by Schomburg [22], is an important one which will be discussed later.

Table 2 presents values of chromatographic per-

formance parameters for SiO<sub>2</sub>(PMOS) as a function of absorbed dose. Representative chromatograms are shown in Fig. 1. The efficiency (*N/L*) of columns packed with this material goes through a maximum which is more clearly shown in Fig. 2. The efficiency of columns packed with irradiated SiO<sub>2</sub>(PMOS) increases with absorbed dose up to about 50 kGy, a change which seems to parallel the increase in %PMOS shown in Table 1. But the similarity does not extend to higher doses. The %PMOS retained by the silica support remains nearly constant following the initial increase while the efficiency falls at higher doses. One interpretation of the data in Fig. 2 is that all of the immobilization of PMOS that can take place does so with the initial 50–70 kGy of absorbed dose. The decrease in *N/L* at higher doses may be the result of polymer degradation; however, the % immobilization data of Table 1 apparently does not support this possibility. Our tentative conclusion is that the increase in column efficiency with the first 50–70 kGy of absorbed dose is a direct result of the extra PMOS that becomes available as a result of radiation immobilization and that the high dose drop-off is a result of degradation processes. (Note: the presence of air during the irradiation treatments would support degradation reactions involving oxygen [20]). An alternative possibility, the formation of

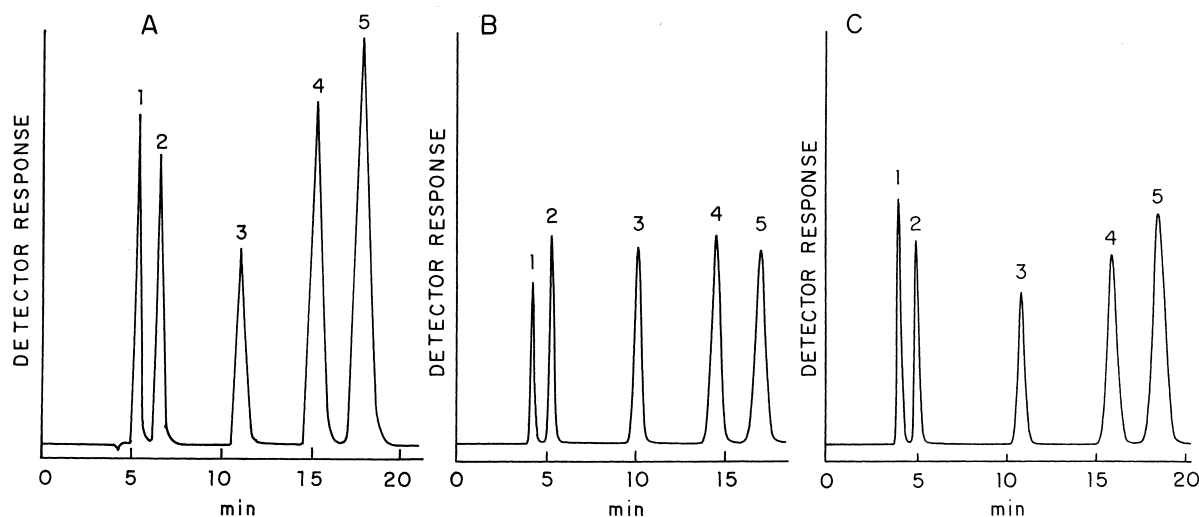


Fig. 1. Chromatograms of test mixture: 1=acetone, 2=benzonitrile, 3=benzene, 4=toluene and 5=naphthalene, obtained with the columns packed with packing material irradiated with: (A) 0, (B) 100 and (C) 150 kGy doses. Chromatographic conditions: mobile phase: methanol: water (70:30, v/v), flow-rate: 0.2 ml min<sup>-1</sup>, volume of injected sample: 10 μl and detector: UV, 254 nm.

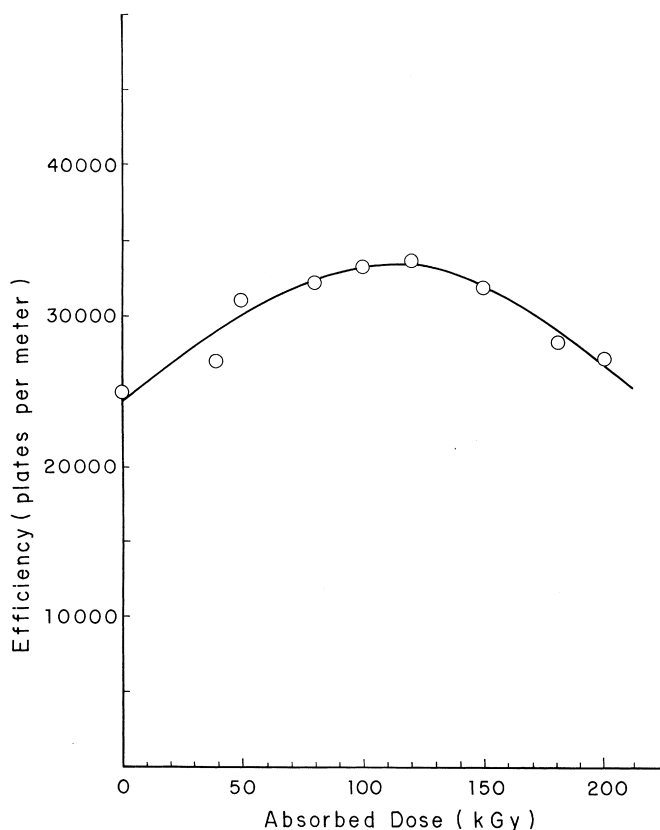


Fig. 2. Column efficiency versus absorbed dose of  $\gamma$ -radiation.

too high a density of cross-links, which could diminish the rate of diffusion of solutes into the polymer layer, is difficult to exclude but would seem to be a minor contributor due to the surprisingly low production of cross-links in bulk PMOS [22].

The effect of radiation on peak asymmetry (Table 2) is dramatic. The drop from  $As=1.7$  at zero dose to 1.1 at higher doses is real, as we have consistently obtained values of 1.5–2.0 for non-irradiated  $SiO_2$ (PMOS) samples having various initial loadings. The Table 2 values show remarkable consistency at  $As=1.1$  for doses up to at least 200 kGy. This result informs us that, not only is our column packing procedure reproducible, there is also no indication that cross-linking or degradation reactions (even those involving oxygen) cause significant changes in the polarity (hydrophobicity) of the irradiated polymer coating once  $\sim 80$  kGy of radiation dose has been absorbed.

Peak asymmetry factors from samples of  $SiO_2$ (PMOS) prepared from a different batch of silica (Davisil-640) are presented in Table 3. Peaks from acidic, neutral and basic test compounds also show decreases in  $As$  upon irradiation to 80 kGy of absorbed dose. If the similar decreases in  $As$  for the different test solutes are indeed attributable to the irradiation treatment (and not to differences in the quality of the column packing operation), then this could be a potentially useful phenomenon, worthy of further investigation. One interpretation of the “phenomenon” would be that cross linking from doses of 0 to 80 kGy occurs in a very efficient way to decrease access to the silanol and metal sites on the surface of the support silica, thus decreasing these important contributions to peak asymmetry.

The retention factor,  $k$ , for naphthalene, (Table 2) undergoes a rapid rise from 3.2 to 4.5 upon receiving 50 kGy of absorbed dose, then rises to 5.1 with 200

Table 3  
Chromatographic parameters obtained using a test mixture of benzylic alcohol, benzene and *N,N*-dimethylaniline<sup>a</sup>

Dose (kGy)	Compounds	Chromatographic parameters			
		Efficiency ( <i>N/L</i> )	<i>As</i>	<i>k</i>	<i>Rs</i>
0	benzylic alcohol	13 600	2.3	0.4	7.4
	benzene	21 400	1.8	1.7	
	<i>N,N</i> -dimethylaniline	20 700	2.0	2.1	
80	benzylic alcohol	14 500	1.8	0.4	9.9
	Benzene	28 100	1.4	2.1	
	<i>N,N</i> -dimethylaniline	26 700	1.4	2.6	

<sup>a</sup> Chromatographic conditions: mobile phase: methanol: water (70:30, v/v), flow-rate: 0.2 ml min<sup>-1</sup>, volume of injected sample: 10 µl and detector: UV, 254 nm.

kGy. This early increase presumably reflects the increase in immobilized PMOS, as shown in Table 1. It is perhaps significant that the zero dose value of 3.2 is close to that (2.9) observed for the case of a similar column prepared from LiChrosorb C-8 (a Type I C<sub>8</sub> monolayer stationary phase).

The resolution parameter for the toluene-naphthalene pair, *Rs*, appears to be nearly independent of absorbed dose all the way from zero to 200 kGy. This result shows that whatever the effect of radiation on the naphthalene elution may be (see the *k* data of Table 2), there is a similar effect on the toluene elution. However, in this case there is a real difference between the nearly constant value (about 2.3) of *Rs* for the irradiated SiO<sub>2</sub>(PMOS) phase and the LiChrosorb C-8 phase. This difference in *Rs* may be taken as evidence that the *structures* of the two kinds of stationary phase are, indeed, different.

It would be desirable to have control of the stationary phase thickness and composition [8]. It should be possible to control the thickness of Type III coatings, as has been done with capillary GC columns, and, to a limited extent, in HPLC ([3,5,6,25] and the present work). One limitation is the very small size (5–12 nm) of the pores: very few, perhaps only 2–3 “mono-layers”, can be arranged into such a tight space. Another limitation is the lack of understanding of how the polymer molecules move and arrange themselves within the pores.

As a point of departure for developing a better understanding of in-pore processes we assume that

the static evaporation procedure results in partially or completely filling the silica pore system with *liquid* PMOS [26]. We then assume that some sort of rearrangement occurs in which some of the PMOS molecules become attached (“immobilized”) by several or many physisorption contacts on the silica surface. Subsequent irradiation then causes a complicated series of bond-breaking and bond-making reactions which result in a larger “immobilized” polymeric structure. This concept is elaborated via a *uniform-layer model* as follows:

Fig. 3a represents the cross-section of a typical pore which is filled with *liquid* PMOS by the solvent evaporation procedure to give liquid PMOS sorbed into the pores (Fig. 3b). Thermal rearrangement then gives an adsorbed monolayer of PMOS (Fig. 3c) and residual liquid PMOS which can be extracted to give pores with a single, presumably uniform, monolayer of “immobilized” PMOS on the wall. Alternatively, the liquid PMOS of Fig. 3c can be partially immobilized by gamma irradiation, as shown by Fig. 3e. Subsequent extraction can remove the small amount of residual liquid PMOS to give pores with a thicker layer of radiation-immobilized PMOS.

Fig. 3d shows the mean pore size (8.7 nm) and the layer thickness, *t*<sub>1</sub> or *t*<sub>2</sub>. With constant pore size, a uniform thickness layer of PMOS, and the same density (0.91 g ml<sup>-1</sup>) for bulk, sorbed and immobilized PMOS, the geometry of the system tells us that the fraction of the cross-section of the pores that is identified with PMOS is equal to the fraction of the

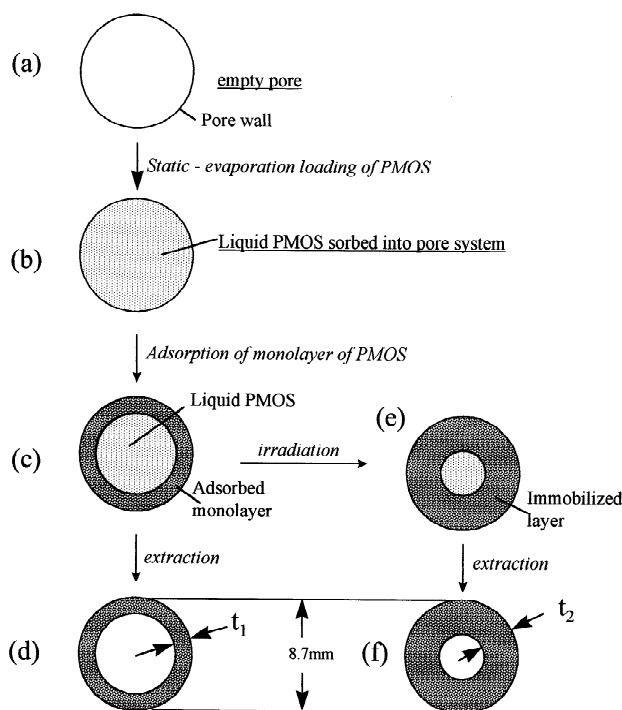


Fig. 3. The *Uniform-Layer-Model* summarizing the relationships of the immobilized layers of PMOS obtained in irradiated and non-irradiated  $\text{SiO}_2$  (PMOS).

mass of PMOS which is immobilized; i.e.,  $F_1 = 0.42$ . Thus we calculate the thickness  $t_1$  to be 1.04 nm, a value which is quite reasonable for a fully covered pore system having the polysiloxane skeleton of PMOS lying on the surface, with the  $\text{C}_8$  groups more or less projecting (“brush”-like) away from the surface *or* an incomplete coverage of the pore walls with PMOS, but with “loops” and “tails” [27], also extending away from the surface. We assume here that the coverage is complete.

A similar analysis for the irradiated  $\text{SiO}_2$ (PMOS) case, Fig. 3f, utilizes the 75% immobilization value ( $F_2 = 0.75$ ) to yield a layer thickness  $t_2 = 2.18$  nm. Thus, it appears that there are two layers of nearly equal thickness, 1.04 nm and 1.14 nm ( $t_2 - t_1$ ), respectively.

It is tempting to conclude that PMOS immobilizes as distinct mono-layers of equal thickness on the pore walls and that perhaps additional mono-layers might be added in silica of larger pore size. Indeed, it is so tempting that we conclude – tentatively – that this is so, even though we are aware of the assump-

tions that contribute to this conclusion. Various tests of this model with different silicas and different polymers will be reported in future communications.

#### 4. Conclusions

Gamma irradiation contributes importantly to the immobilization of PMOS sorbed into the pores of HPLC silica. Absorbed doses of 100–120 kGy serve to maximize the reversed-phase separation efficiency of columns packed with  $\text{SiO}_2$ (PMOS) particles. Peak symmetry of columns packed with  $\text{SiO}_2$ (PMOS) particles is improved by radiation doses in the range 0–40 kGy, suggesting that subjecting HPLC phases to ionizing radiation may, in favorable cases, be useful in decreasing the effects of surface silanols and impurities. Determinations of the quantities of PMOS self-immobilized and radiation immobilized are consistent with a model having two immobilized layers of thickness  $\sim 1.1$  nm each.



## Acknowledgements

The authors acknowledge financial support from FAPESP, FAEP, CNPq and CAPES and thank IB-RAS-CBO (Campinas, SP, Brazil) for performing the irradiations of the stationary phases and Prof. C. H. Collins for helpful discussions and suggestions.

## References

- [1] C.F. Poole, S.K. Poole, *Chromatography Today*, Elsevier Science Publishing, New York, 1991, Chap. 4.
- [2] U.D. Neue, *HPLC Columns: Theory, Technology and Practice*, Wiley-VCH, New York, 1997.
- [3] M. Petro, D. Berek, *Chromatographia* 37 (1993) 549.
- [4] J.J. Kirkland, *LC·GC (Current Issues in HPLC Technology)*, (May, 1997) S46.
- [5] L.S. Sander, S.A. Wise, *CRC Crit. Rev., Anal. Chem.* 18 (1987) 298.
- [6] Y. Ohtsu, H. Fukui, T. Kanda, K. Nakamura, M. Nakano, O. Nakata, Y. Fujiyama, *Chromatographia* 24 (1987) 380.
- [7] H. Engelhardt, H. Low, W. Beck, W. Gotzinger, *Polymer encapsulated stationary phases*, in: H.A. Mottola, J.R. Steinmetz (Eds.), *Chemically Modified Surfaces*, Elsevier, Amsterdam, 1992, p. 225.
- [8] G. Schomburg, *Trends Anal. Chem.* 10 (1991) 163.
- [9] M. Hanson, A. Kurganov, K.K. Unger, V.A. Davankov, *J. Chromatogr. A* 656 (1993) 369.
- [10] K.D. Bartle, M. Novotny, *J. Chromatogr.* 94 (1974) 35.
- [11] G. Schomburg, H. Husmann, S. Ruthe, M. Harraiz, *Chromatographia* 15 (1982) 599.
- [12] U. Bien-Vogelsang, A. Deege, H. Figge, J. Köhler, G. Schomburg, *Chromatographia* 19 (1984) 170.
- [13] H. Figge, A. Deege, J. Köhler, G. Schomburg, *J. Chromatogr.* 351 (1986) 393.
- [14] G. Schomburg, A. Deege, J. Köhler, U. Bien-Vogelsang, *J. Chromatogr.* 282 (1983) 27.
- [15] M.J.J. Hetem, J.W. de Haan, H.A. Claessens, C.A. Cramers, *J. Chromatogr.* 540 (1991) 53.
- [16] J.J. Kirkland, M.A. van Straten, H.A. Claessens, *J. Chromatogr. A* 691 (1995) 3.
- [17] M.J. Wirth, R.W.P. Fairbank, H.O. Fatunmbi, *Science* 275 (1997) 44.
- [18] R.E. Majors, *LC·GC* 16 (1998) 228.
- [19] A. Charlesby, *Radiat. Phys. Chem.* 18 (1981) 59.
- [20] J.W.T. Spinks, R.J. Woods, *Introduction To Radiation Chemistry*, 3rd ed, Wiley-Interscience, New York, 1990.
- [21] W. Schnabel, *Degradation by high energy radiation*, in: H.H.G. Jellinck (Ed.), *Aspects of Degradation and Stabilization of Polymers*, Elsevier, Amsterdam, 1978, p. 149.
- [22] G. Schomburg, J. Köhler, H. Figge, A. Deege, V. Bien-Vogelsang, *Chromatographia* 18 (1984) 265.
- [23] E.F. Sanchez, J.A. Dominguez, J.E. Munoz, M.J. Molera, *J. Chromatogr.* 299 (1984) 151.
- [24] T.A. Anazawa, I.C.S.F. Jardim, *J. Liq. Chromatogr.* 17 (1994) 1265.
- [25] R.P.W. Scott, C.F. Simpson, *J. Chromatogr. Sci.* 30 (1992) 59.
- [26] K.E. Collins, M.L.M.M. Granja, R.G. Pereira Filho, T.A. Anazawa, I.C.S.F. Jardim, *Chromatographia* 45 (1997) 99.
- [27] G.J. Eleer, J. Lyklema, *Adsorption of polymers*, in: G.D. Parfitt, C.H. Rochester (Eds.), *Adsorption From Solution at the Solid/liquid Interface*, Academic Press, London, 1983.