



ELSEVIER

Journal of Chromatography A, 869 (2000) 137–141

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Effects in high-performance liquid chromatography of a high pH in the mobile phase on poly(methyloctylsiloxane) immobilized by $\gamma$ -radiation on titanium-grafted silica

Rosely Barbosa Silva, Kenneth E. Collins, Carol H. Collins\*

*Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13083-970 Campinas (S.P.), Brazil*

## Abstract

Effects of high-pH environments on a stationary phase prepared by  $\gamma$ -radiation immobilization of poly(methyloctylsiloxane) on titanium-grafted silica were investigated by HPLC testing with standard sample mixtures. The HPLC parameters indicate good stationary phase stability to 10 000 column volumes each of mobile phases with pH of 7, 9 and 12. At pH 13, the efficiency decreases slowly, although reasonably good separations are still possible until increasing flow resistance no longer allows easy passage of the mobile phase. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** pH effects; Titanium-grafted silica; Silica, titanium grafted; Poly(methyloctylsiloxane) stationary phases; Stationary phases, LC

## 1. Introduction

Various approaches have been used to maintain the chemical integrity of silica-based bonded-phase columns in different pH environments [1–6]. Factors, such as bonding chemistry and properties of the silica support material, including pore size and pore size distribution, are involved in determining the resistance of a packing material to acid hydrolysis [6]. Similar factors also determine the rate of degradation of  $C_{18}$  columns at intermediate pH, where the type and purity of the silica support, temperature and nature of the silane stationary phase play a role [1]. At higher pH, other factors, such as type and concentration of buffer, and even the nature of the buffer cation, influence bonded-phase stability [5].

The rate of bonded-phase degradation at pH 7–10 increases with higher concentrations of many buffers [3]. The use of alkali metal-based buffers may reduce the dissolution of silica supports and thus enhance column stability at high pH [2].

Attempts to overcome the limitations of silica supports in reversed-phase high-performance liquid chromatography (HPLC) have involved use of support materials more resistant to pH, such as zirconia [7–24], titania [17,19,25–34] and alumina [7,17,19,25,30,35–43], usually coated with polymers made in situ from substituted ethylenes, butadienes and styrenes. Porous  $TiO_2$  has been characterized and tested for direct use in normal-phase HPLC [26,32], reversed-phase HPLC [27–33] and ion-exchange HPLC [25,34].

More recently, chemical incorporation of a metal oxide onto the silica surface has been investigated, as this may minimize the limitations of silica by

\*Corresponding author. Fax: +55-19-7883-023.

E-mail address: chc@iqm.unicamp.br (C.H. Collins)

modifying the silica surface through chemical incorporation of a metal oxide material, known for its stability in high-pH environments, onto the silica surface. Zirconium grafted onto the silica surface has been used to produce packing materials [44] which give good results at least up to pH 10, using an ammonium buffer [45]. The methodology for preparing titania grafted onto silica [46] and for immobilization of poly(methyloctylsiloxane) in the titanium-grafted silica pores using  $\gamma$ -irradiation [47] has been reported and shows that titanium-grafted silica is suitable for use as a chromatographic support for applications in reversed-phase HPLC.

In this report we demonstrate that a polymeric stationary phase immobilized on titanium-modified silica provides good stability for columns used with high-pH aqueous mobile phases.

## 2. Experimental

### 2.1. Reagents and materials

Titanium-modified silica was prepared as previously described [46,47]. Poly(methyloctylsiloxane) (PMOS, product PS-140 from Petrarch Systems/Hüls America) has a mean molar mass of 6200. Analytical-reagent grade or HPLC-grade solvents were obtained from Merck (dichloromethane, carbon tetrachloride, acetone, benzonitrile, benzene, toluene and naphthalene) or Mallinckrodt (methanol). The aqueous solutions were prepared with distilled, deionized water (Milli-Q, Millipore). Aqueous sodium hydroxide of appropriate concentrations to give pH 7, 9, 12 or 13 was mixed with methanol in the ratio 30:70 (v/v) for stability testing.

### 2.2. Preparation of the stationary phase

Equal masses of titanium-modified silica (Ti–Si) and PMOS were added to dichloromethane by first dissolving the PMOS in the solvent, then stirring in the dried support. This mixture was stirred for 3 h at room temperature and the dichloromethane was then removed by evaporation to obtain the stationary phase (Si–Ti–PMOS) with a 50% poly(methyloctylsiloxane) nominal loading. Once the Si–Ti–PMOS stationary phase had been prepared, samples were

sealed in glass ampoules, under air, and irradiated to 120 kGy of absorbed dose by means of an industrial  $^{60}\text{Co}$   $\gamma$ -ray source (IBRAS-CBO, Campinas, Brazil) at a dose rate of  $\sim 40 \text{ kGy h}^{-1}$ . Portions of irradiated packing material were extracted for 6 h in a Soxhlet apparatus with  $\text{CH}_2\text{Cl}_2$ , then allowed to dry at ambient temperature prior to heating for 24 h at  $110^\circ\text{C}$ . The columns,  $150 \times 3.8 \text{ mm}$ , made from locally purchased type 316 stainless steel tubing, had highly polished internal surfaces and were downward packed at 42 MPa with a Haskel packing pump using 10% slurries of the packing material in  $\text{CCl}_4$  with methanol as the propulsion solvent.

### 2.3. Instrumentation

Quantification of titanium in the packing material before and after the stability test at pH 12 was obtained by X-ray fluorescence, using a Tracor Model Spectrace 5000 instrument. The calibration curve was prepared using mixtures of purified silica and  $\text{TiO}_2$  (Riedel-de Haën), containing from 1 to 10% (w/w) Ti.

Elemental analyses for carbon (% C) were obtained on a Perkin-Elmer Model 2400 CHN analyzer. The quantity of PMOS (%) was then calculated using the equation:

$$\% \text{ PMOS} = (w_{\text{PMOS}}/w_{\text{sample}}) \cdot 100$$

$$\text{where } w_{\text{sample}} = w_{\text{support}} + w_{\text{PMOS}}$$

Since 62% of the polymer mass refers to carbon,  $w_{\text{PMOS}} = (w_{\text{C}}/0.62)$  and  $w_{\text{C}} = \text{carbon mass}$ , then

$$\% \text{ C} = (w_{\text{C}}/w_{\text{sample}}) \cdot 100$$

$$\% \text{ C} = 0.62 \cdot \frac{w_{\text{PMOS}}}{w_{\text{sample}}} \cdot 100$$

### 2.4. Column testing

Reversed-phase column testing was done with a system consisting of a Shimadzu LC-10AD pump, a Shimadzu Model SPD-10AV UV–Vis absorbance detector (at 254 nm), a Rheodyne Model 7125 injector (5- $\mu\text{l}$  loop) and a Hewlett-Packard Model 3396 integrator. The test mixture contained appropriate quantities of acetone, benzonitrile, benzene, toluene and naphthalene.

### 2.5. Stability testing

The evaluation of chemical stability was carried out at ambient temperature (25°C) by passing MeOH–NaOH<sub>(aq)</sub> (70:30, v/v) mobile phase through each column at 2.0 ml min<sup>-1</sup> to a total of 10 000 column volumes ( $V_c$ ) for each pH tested. The empty column volume,  $V_c$ , was 1.7 ml and was calculated as  $V_c = \pi r^2 L$ , where  $r$  = column radius and  $L$  = column length. Chromatograms of a mixture of solutes of different polarities were obtained periodically with the mobile phase methanol–water (70:30, v/v), at a flow-rate of 0.3 ml min<sup>-1</sup>, near the optimal flow-rate as determined by a Van Deemter plot. Prior to each test the column was conditioned with this mobile phase at the same flow-rate for 30 min. Chromatographic performance was evaluated by means of efficiency (plates/m), retention factor ( $k$ ), resolution ( $R_s$ ), separation factor ( $\alpha$ ) and asymmetry ( $A_s$ ) at 10% of peak high, manually determined from the chromatograms (paper velocity at 2.0 cm min<sup>-1</sup>) for naphthalene or the toluene–naphthalene pair, as appropriate. The hold-up time,  $t_M$ , was determined from the refractive index deviation obtained by injection of pure methanol.

### 3. Results and discussion

Table 1 shows the amount of titanium, determined by X-ray fluorescence spectroscopy, and the amount of poly(methyloctylsiloxane) present on the titanized silica support before and after the sequence of pH tests. The  $\gamma$ -irradiation (120 kGy) has immobilized the PMOS on the titanium-modified silica so that even after prolonged contact with a high pH mobile phase, the amounts of both titanium and PMOS are only slightly reduced.

Table 1

Amounts of titanium and PMOS in the packing material before and after passage of 10 000 column volumes each of mobile phases with different pH values

Mobile phase pH	Titanium (%)		PMOS (%)	
	Before	After	Before	After
7	5.1	4.2	30	29
12	5.1	3.9	27	27

Table 2

Chromatographic parameters for PMOS immobilized onto titanized-silica vs. volume of mobile phase through the columns

pH	Timing	Plates/m <sup>a</sup>	$k^a$	$R_s^b$	$\alpha^b$	$A_s^a$
7	Start	24 960	4.6	2.9	1.2	1.7
7	Finish <sup>c</sup>	23 640	4.4	2.9	1.2	1.7
9	Start	28 000	4.7	2.8	1.2	1.3
9	Finish <sup>c</sup>	28 320	4.8	2.8	1.2	1.5
12	Start	25 150	3.8	2.4	1.2	1.2
12	Finish <sup>c</sup>	24 840	3.6	2.3	1.2	1.2
13	Start	24 770	4.6	2.8	1.3	1.5
13	Finish <sup>d</sup>	20 450	5.0	1.8	1.2	1.6

<sup>a</sup> Calculated for naphthalene.

<sup>b</sup> Calculated for toluene–naphthalene.

<sup>c</sup> After 10 000 column volumes.

<sup>d</sup> After 5000 column volumes.

The chromatographic parameters presented in Table 2 show the stability of the packing material at different pH values after extended washing with MeOH–NaOH<sub>(aq)</sub> (70:30, v/v) at pH 7, 9, 12 and 13. Except for pH 13, the chromatographic parameters, calculated for naphthalene and the toluene–naphthalene pair, remain essentially constant throughout the washing test. The changes in the efficiency, retention factor and resolution at pH 13 are accompanied by settling of the bed and increased resistance to the passage of pH 13 mobile phase at the 2.0 ml min<sup>-1</sup> flow-rate.

Fig. 1 shows chromatograms made before the washing test and after washing with pH 12 and with pH 13 mobile phases. The separations have remained essentially the same throughout the period of contact with the basic environments. Note that, even though increased resistance caused the pH 13 test to be terminated after only 5000 column volumes, the standard mixture is still well separated at a lower flow-rate (Fig. 1C).

### 4. Conclusions

The results show good chemical stability to 10 000 column volumes each at pH 7, 9 and 12. When the pH is increased to 13, a change in the column bed no longer allows the facile passage of this mobile phase at 2 ml min<sup>-1</sup> after 5000 column volumes. Thus,

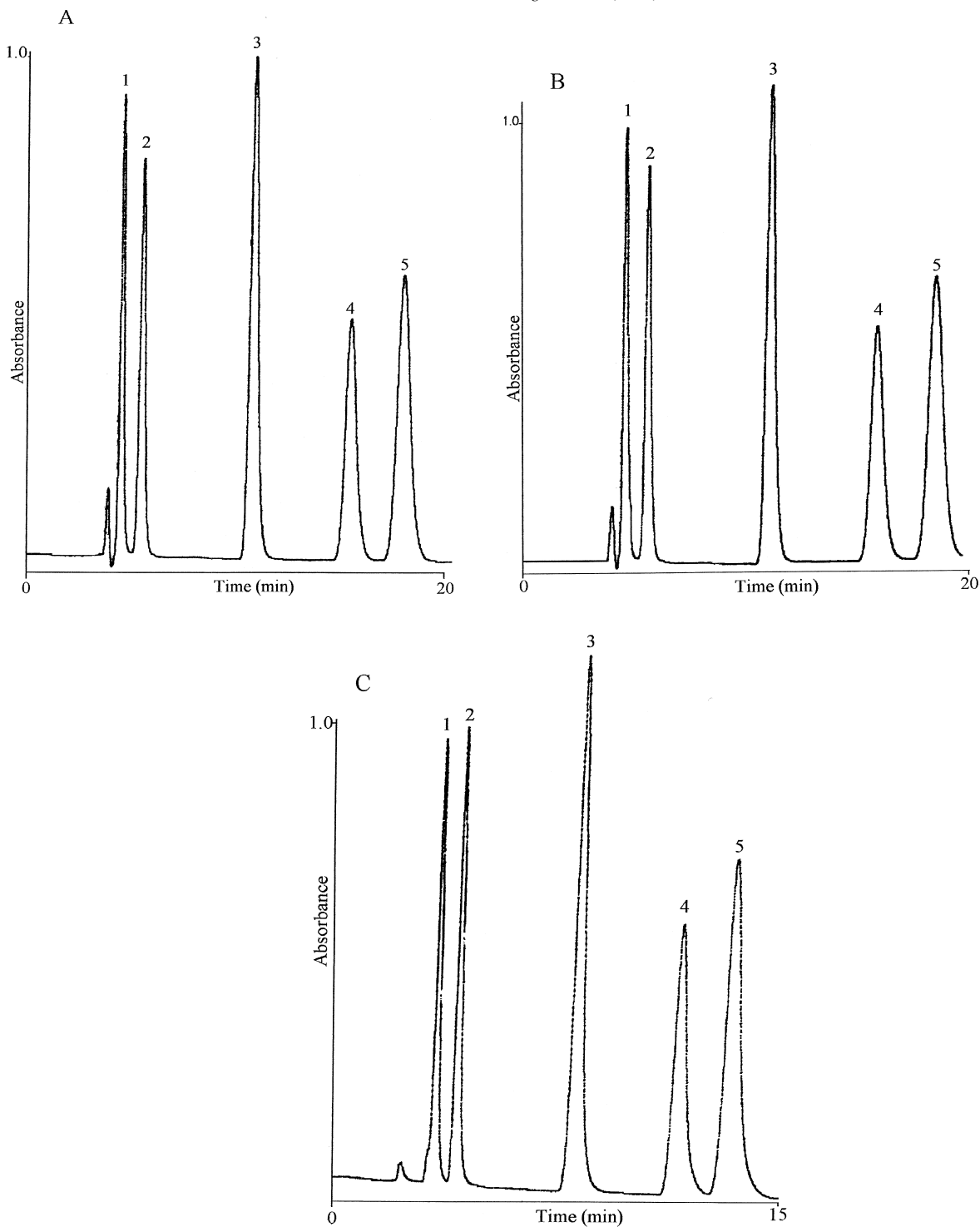


Fig. 1. Chromatograms (A) before testing; (B) after 10 000 column volumes at pH 12 and (C) after 5000 column volumes at pH 13. Conditions: mobile phase: MeOH–water (70:30, v/v); flow-rate:  $0.3 \text{ ml min}^{-1}$ ; detection: UV at 254 nm; test solutes: (1) acetone, (2) benzonitrile, (3) benzene, (4) toluene and (5) naphthalene.

titanium modified silica shows significant promise as a support when high pH mobile phases are required.

## Acknowledgements

The authors acknowledge the financial support of the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), grants 95/3834-0 and 94/3580-5. We also gratefully acknowledge Rosângela Bassi and IBRAS-CBO S.A. for carrying out the  $^{60}\text{Co}$  irradiations.

## References

- [1] J.J. Kirkland, M.A. van Straten, H.A. Claessens, *J. Chromatogr. A* 691 (1995) 3.
- [2] J.J. Kirkland, *J. Chromatogr. Sci.* 34 (1996) 309.
- [3] H.A. Claessens, M.A. van Straten, J.J. Kirkland, *J. Chromatogr. A* 728 (1996) 259.
- [4] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, *J. Chromatogr. A* 762 (1997) 97.
- [5] J.J. Kirkland, *LC·GC* 5 (1997) S46.
- [6] R.C. Ludwig, T.S. Reid, R.A. Henry, K.J. Duff, *LC·GC* 16 (1998) 158.
- [7] M.P. Rigney, T.P. Weber, P.W. Carr, *J. Chromatogr.* 484 (1989) 273.
- [8] M.P. Rigney, E.F. Funkenbusch, P.W. Carr, *J. Chromatogr.* 499 (1990) 291.
- [9] T.P. Weber, P.W. Carr, E. F Funkenbusch, *J. Chromatogr.* 519 (1990) 31.
- [10] J.A. Blackwell, P.W. Carr, *J. Chromatogr.* 549 (1991) 43.
- [11] J.A. Blackwell, P.W. Carr, *J. Chromatogr.* 549 (1991) 59.
- [12] W.A. Schafer, P.W. Carr, E.F. Funkenbusch, K.A. Parson, *J. Chromatogr.* 587 (1991) 137.
- [13] D.A. Hanggi, N.R. Marks, *LC·GC* 11 (1993) 131.
- [14] J. Yu, Z. El Rassi, *J. Chromatogr.* 631 (1993) 91.
- [15] J. Yu, Z. El Rassi, *J. Liq. Chromatogr.* 16 (1993) 2931.
- [16] H.J. Wirth, K.O. Eriksson, P. Holt, M. Aguilar, M.T.W. Hearn, *J. Chromatogr.* 646 (1993) 129.
- [17] J.J. Pesek, V.H. Tang, *Chromatographia* 39 (1994) 649.
- [18] A. Kurganov, V. Davankov, T. Isaeva, K.K. Unger, F. Eisenbeiss, *J. Chromatogr. A* 660 (1994) 97.
- [19] A. Kurganov, U. Trüding, T. Isaeva, K.K. Unger, *Chromatographia* 42 (1996) 217.
- [20] J. Li, P.W. Carr, *Anal. Chem.* 68 (1996) 2857.
- [21] J. Li, P.W. Carr, *Anal. Chem.* 69 (1997) 837.
- [22] J. Li, P.W. Carr, *Anal. Chem.* 69 (1997) 2193.
- [23] J. Li, P.W. Carr, *Anal. Chem.* 69 (1997) 2202.
- [24] J. Li, P.W. Carr, *Anal. Chem.* 70 (1998) 1934.
- [25] R.M. Chicz, Z. Shi, F.E. Regnier, *J. Chromatogr.* 359 (1986) 121.
- [26] M. Kawahara, H. Nakamura, T. Nakajima, *J. Chromatogr.* 515 (1990) 149.
- [27] U. Trüding, G. Müller, K.K. Unger, *J. Chromatogr.* 535 (1990) 111.
- [28] K. Murayama, H. Nakamura, K. Takahashi, A. Yoshida, *Anal. Sci.* 10 (1994) 815.
- [29] K. Murayama, H. Nakamura, T. Nakajima, K. Takahashi, A. Yoshida, *Microchem. J.* 49 (1994) 363.
- [30] J.J. Pesek, M.T. Matyska, J. Ramakrishnan, *Chromatographia* 44 (1997) 538.
- [31] M. Grün, A.A. Kurganov, S. Schacht, F. Schith, K.K. Unger, *J. Chromatogr. A* 740 (1996) 1.
- [32] K. Tani, Y. Suzuki, *J. Chromatogr. A* 722 (1996) 129.
- [33] M. Zaharescu, T. Cserhádi, E. Forgács, *J. Liq. Chromatogr. Rel. Technol.* 20 (1997) 2997.
- [34] K. Tani, H. Kubojima, *Chromatographia* 47 (1998) 655.
- [35] G. Heinemann, J. Kohler, G. Schomburg, *Chromatographia* 23 (1987) 435.
- [36] R.V. Arenas, J.P. Foley, *Anal. Chim. Acta* 246 (1991) 113.
- [37] J.E. Haley, A. Ragharu, B.M. Dunn, *J. Chromatogr.* 541 (1991) 303.
- [38] J.R. Garbow, J. Asrar, C.J. Hardiman, *Chem. Mater.* 5 (1993) 869.
- [39] R.V. Arenas, J.P. Foley, *Analyst* 119 (1994) 1303.
- [40] R.V. Arenas, J.P. Foley, *Am. Lab.* 26 (1994) 32CC.
- [41] Y. Mao, B.M. Fung, *J. Chromatogr. A* 790 (1997) 9.
- [42] D.H. Reeder, J. Li, P.W. Carr, M.C. Flickinger, A.V. McCormick, *J. Chromatogr. A* 760 (1997) 71.
- [43] T. Cserhádi, E. Forgács, K. Payer, P. Haber, R. Kaliszan, A. Nasal, *LC·GC Int.* 11 (1998) 240.
- [44] L.F.C. Melo, I.C.S.F. Jardim, *J. Chromatogr. A* 845 (1999) 423.
- [45] L.F.C. Melo, C.H. Collins, K.E. Collins, I.C.S.F. Jardim, *J. Chromatogr.* (1999) in press.
- [46] Y. Gushikem, C.R.M. Peixoto, L.T. Kubota, in: M. Abe, T. Kataoka, T. Suzuki (Eds.), *New Developments in Ion Exchange Materials, Fundamentals and Applications*, Kodansha–Elsevier, Tokyo, 1991, p. 607.
- [47] R.B. Silva, C.H. Collins, *J. Chromatogr. A* 845 (1999) 417.