

Synthesis and characterization of stable polymer-coated C₈ stationary phase with high durability under extreme pH conditions for high-performance liquid chromatography

Atsushi Kobayashi*, Kazuko Takezawa, Hideki Takasaki,
Taketoshi Kanda, Hiroshi Kutsuna

Shiseido Material Science Research Center, 2-2-1 Hayabuchi, Tsuzuki, Yokohama 224-8558, Japan

Abstract

A highly chemically stable polymer-coated silica-based C₈ stationary phase was developed by combining modification with octyl groups and a polymer coating technology. The stationary phase was prepared by the following procedure: (1) introduction of octyl groups to the silica surface; (2) coating the C₈ silica with a silicone polymer. ²⁹Si solid-state NMR spectra indicated that a silicone polymer reacted not only with residual silanol groups on the silica surface, but with those generated from silanes used for the introduction of octyl groups. Column durability was evaluated with an acidic mobile phase (60 °C, pH 1) and a basic mobile phase (50 °C, pH 10) in accelerated damaging conditions. The C₈ phase showed a high durability under both conditions.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Stationary phases, LC; Polymer coating; Alkylated silica; Pyridine; Acidic durability; Basic durability

1. Introduction

Stationary phases for reversed-phase liquid chromatography (RPLC) have been studied widely [1], and a huge number of brands of columns are commercially available in the market [2]. Silica-based reversed-phases are still the most commonly used in RPLC [3], although other supports such as titania [4] and zirconia [5] have been developed. Silica is advantageous, because it can be easily chemically modified and the ease to design particle diameter, pore size and specific surface area makes it possible to prepare high-efficiency packed columns.

Some limitations, however, apply to silica. Silica by nature dissolves in an alkaline solution. Therefore, mobile phases are, in general, limited to acidic and neutral pH conditions [6]. Also, undesirable secondary effects originated from silica

surface are often discussed, such as peak tailing for basic or chelating compounds.

Several attempts to improve the performance of silica-based phases have been studied. Alkylated silica is end-capped by trimethylsilane and hexamethyldisilazane to reduce the effects caused by residual silanol groups [7,8]. As another unique attempt, the authors' group has been reporting a polymer coating technology for silica surface, based on vapor-phase chemical deposition of silicon monomer molecules [9–15]. In these attempts, alkyl chains and other ligands were introduced to the polymer-coated silica through hydrosilylation. The resultant polymer-coated silica phases possessed advantages of both silica- and polymer-based phases, and showed several improvements such as a minimized peak tailing of basic compounds and chemical stability against basic mobile phases.

Nowadays, mass spectrometry (MS) has become the most powerful detection method for liquid chromatography (LC), which provides high sensitivity, and high selectivity to a wide range of compounds. Mobile phases optimized for drug

* Corresponding author. Fax: +81 45 590 6099.

E-mail address: atsushi.kobayashi@to.shiseido.co.jp (A. Kobayashi).

discovery research are often designed at either high or low pH [16]. Stable stationary phases under both acidic and basic mobile phases are demanded.

In terms of the total consumption in the analytical market, C₁₈ columns dominates in number, mainly because of their high capability to retain substances, and the circulation of C₈ columns lags behind. However, C₈ columns are sometimes preferred when a relatively large difference in selectivity, from those obtained with C₁₈ columns, or a large reduction in run time are needed.

As a continuing study of the polymer coating technology, this paper will describe the synthesis and characterization of a polymer-coated C₈ silica phase, designed for improved durability under both acidic and basic conditions. Also, other additional advantages will be explored.

2. Experimental

2.1. Materials

High-purity silica (Shiseido, Tokyo, Japan) (particle size: 5 and 3 μm, pore diameter: 8 nm, surface area: 300 m²/g, metal impurity: <5 ppm) was used as starting materials for polymer-coated C₈ stationary phase. 1,3,5,7-Tetramethylcyclotetrasiloxane was purchased from Toshiba Silicone (Tokyo, Japan). Octyltrichlorosilane was purchased from Shin-Etsu Chemical (Tokyo, Japan). All the solvents and reagents used to synthesize packing materials were purchased from Nacalai Tesque (Kyoto, Japan), and used without further purification. Acetonitrile and methanol of HPLC grade (Nacalai Tesque) were used for preparing mobile phases. Water was purified with a Mill-Q system (Nihon Millipore, Tokyo, Japan).

2.2. Synthesis of polymer-coated C₈ stationary phase

The polymer-coated C₈ stationary phase was synthesized according to the following synthetic method. A suspension of 20 g of silica in 80 mL of toluene and 9.5 g of pyridine was refluxed for 2 h. A 9.9 g of octyltrichlorosilane was added to the stirred suspension, and refluxed for 16 h. The silylated silica was washed with toluene and acetonitrile, and stirred with acetonitrile–water (60:40) for 3 h, and then washed with methanol. The silylated silica was dried under vacuum at 120 °C for 6 h. The C₈ modified silica and 1,3,5,7-tetramethylcyclotetrasiloxane were reacted according to the method of Fukui et al. [17]. The product was washed with chloroform and methanol and dried under vacuum at 60 °C for 4 h. The silicone-polymer-coated silica was placed in a desiccator, the bottom of which was filled with aqueous ammonia. The desiccator was heated at 60 °C for 16 h and the product was washed with chloroform and methanol and dried vacuum at 60 °C for 4 h. A suspension containing 20 g of the alkali-treated silica, 0.9 mL of trimethylchlorosilane, 1.6 mL of hexamethyldisilazane, 1.5 mL of pyridine and 15 mL of

toluene was refluxed for 5 h. The product was washed with toluene, chloroform and methanol, and dried vacuum at 60 °C for 4 h.

2.3. Instrumentation

Pore diameter and surface area of the starting silica were measured with an Omnisorp 100 CX (Beckman Coulter, Tokyo, Japan) by the nitrogen adsorption method. Particle size distribution was measured with a Multisizer II (Beckman Coulter, Tokyo, Japan). Metal impurity was measured with a Jobin Yvon 70 Plus Combined ICP Spectrometer (Rigaku, Tokyo, Japan). Elemental analysis was carried out with an Organic Elemental Analyzer 2400CHN (Perkin-Elmer Japan, Yokohama, Japan). ²⁹Si solid-state nuclear magnetic resonance (NMR) was measured with a JNM ECA400 (JEOL, Tokyo, Japan).

2.4. Alkyl density

Alkyl density (μmol/m²) was defined as a value obtained by dividing a carbon content increase after the introduction of octadecyl groups by their molecular mass and the surface area of the starting silica.

2.5. Chromatography

The polymer-coated C₈ stationary phase was packed into stainless steel columns (150 mm × 4.6 mm i.d., 100 mm × 4.6 mm i.d.) by a high-pressure slurry packing procedure. Measurements were performed using a NANOSPACE, a high-performance liquid chromatography (HPLC) system (Shiseido). Data acquisition and integration were performed with an SMC data system (Shiseido).

Several test solutions were prepared. A test mixture solution for general characterization contained uracil, caffeine (C), phenol (P), 2-ethylpyridine (EP), methyl benzoate (MB), benzene (B), dimethylaniline, toluene (T), phenylacetylacetone, and naphthalene. A general characterization test was carried out under the following HPLC conditions; column size: 150 mm × 4.6 mm i.d., mobile phase: methanol–water (50:50), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 254 nm.

The column durability tests in a basic side were carried out under the following HPLC conditions; column size: 150 mm × 4.6 mm i.d., mobile phase: 4 mmol/L sodium tetraborate (pH 10) in methanol–water (10:90), flow rate: 1.0 mL/min, detection: UV at 254 nm, column oven temperature: 50 °C, sample: uracil and benzyl alcohol. A percentage of retention factor at 20 h over the initial one at each test was used as a figure of merit for column durability. The column durability tests in an acidic side were carried out under the following HPLC conditions; column size: 150 mm × 4.6 mm i.d., mobile phase: (A) 2% trifluoroacetic acid (pH 1) in water, (B) 2% trifluoroacetic acid in ace-

tonitrile, ratio of B: 65% (0–20 min)–0% (20–80 min)–98% (80–85 min)–65% (85–90 min), flow rate: 1.0 mL/min, detection: UV at 254 nm, column oven temperature: 60 °C, sample: uracil, propylbenzene, butylbenzene, propyl benzoate, and amylbenzene. The 90-min process was repeated 15 times. A percentage of retention factor of amylbenzene at the 15th run over the initial one was used as a figure of merit for column durability. The total column durability tests under both acidic and basic conditions were designed to take 20 h, approximately.

A tailing factor was obtained at 5% of the peak height, under the following HPLC conditions; column size: 150 mm × 4.6 mm i.d., mobile phase: acetonitrile–water (30:70), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 254 nm, sample: pyridine.

Measurements of number of theoretical plates and column pressure were carried out under the following HPLC conditions; column size: 150 mm × 4.6 mm i.d., mobile phase: methanol–water (50:50), temperature: 40 °C, detection: UV at 254 nm, sample: naphthalene.

Analysis of a standard mixture was carried out under the following HPLC conditions; column size: 100 mm × 4.6 mm i.d., mobile phase: 50 mmol/L phosphate buffer–methanol (45:55), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 220 nm. The mixture contained ethenzamide, phenytoin, ibuprofen, lidocaine, progesterone, verapamil, and dibucaine.

3. Results and discussion

C₈ columns, in general, are less stable under acidic and basic conditions, in comparison with C₁₈ columns. To increase chemical stability of a C₈ phase, the polymer-coating process was carried out on a C₈ phase synthesized with the multifunctional C₈ reagent. It is well known that trifunctional silylating agents result in more stable phases in acidic conditions than monofunctional ones [18]. On the other hand, the excellent durability of polymer-coated stationary phases under basic conditions have been reported [14,15]. It seems interesting to see what happens by combining these two processes.

To evaluate the improvement in durability brought by the polymer coating process, C₈ stationary phases with and without the polymer coating were synthesized. Acidic durability values of non-polymer-coated C₈ phase and polymer-coated one were 74 and 86%, respectively. Basic durability values of these two are 85 and 96%, respectively. These results clearly indicate an advantage of the polymer coating process in improving its chemical stability at both acidic and basic conditions.

The surface structures before and after polymer coating process were observed with ²⁹Si solid-state NMR. The Fig. 1 shows an NMR spectrum at each synthetic step of the polymer-coated C₈ phase. The largest signal in each spectrum comes from silica, the starting material. Signals A, B, and C come from the attached C₈ groups. The signal A cor-

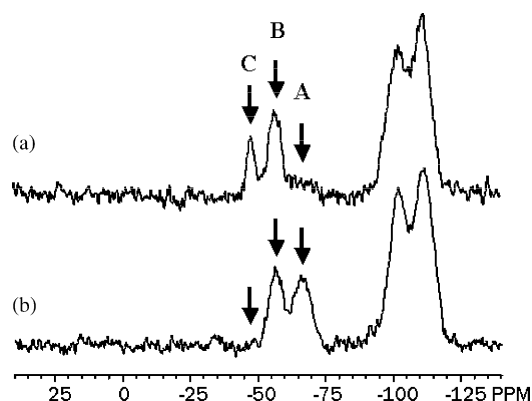


Fig. 1. ²⁹Si solid-state NMR spectrum at each synthetic step of polymer-coated C₈. (a) Before polymer coating and (b) after polymer coating.

responds to a silicon atom surrounded by three oxygen atoms (no adjacent hydroxyl group), each of which is linked to another silicon atom. The bonding structure is expected to show a very strong structure against acidic hydrolysis. The signal B corresponds to a silicon atom bonded to two oxygen atoms and one hydroxyl group. The signal C corresponds to a silicon atom bonded to an oxygen atom, and two hydroxyl groups. The structure of the signal C seems relatively weak against acidic hydrolysis. The spectral change after the polymer coating process indicates that these hydroxyl groups are involved in cross-linking reaction of the polymer, and therefore, the C₈ groups must be firmly attached to the silica surface with additional covalent bonds. As shown in previous reports [14,15], the silica covered with the silicone polymer makes it to prevent the dissolution of silica under basic mobile phase. Therefore, the polymer-coated C₈ phase showed high durability under basic condition. The final polymer-coated phase showed an alkyl density of 3.8 μmol/m², and a total carbon content of 11%.

Separation characteristics of the phase was examined with various standard compounds according to the method of Kobayashi et al. [14]. The definitions of chromatographic parameters are listed in Table 1. $\alpha(T/B)$, or hydrophobicity, represents an extent of retention increase caused by one additional methyl group on a sample molecule. $\alpha(MB/T)$, or surface polarity, represents an extent of dipole moment interaction between carbonyl ester moiety and silica surface. $\alpha(EP/B)$, or ion exchange ability, represents an extent of interaction between cationic compounds and acidic silanol groups. $\alpha(C/P)$, or hydrogen bonding capacity, represents an amount of neutral silanol groups left on the silica surface.

Results obtained with various silica-based C₈ packing materials are listed in Table 2. The polymer-coated C₈ phase

Table 1
Definition of chromatographic parameters

| |
|---|
| Hydrophobicity, $\alpha(T/B) = k(\text{toluene})/k(\text{benzene})$ |
| Surface polarity, $\alpha(MB/T) = k(\text{methyl benzoate})/k(\text{toluene})$ |
| Ion-exchangeability, $\alpha(EP/B) = k(2\text{-ethylpyridine})/k(\text{benzene})$ |
| Hydrogen bonding capacity, $\alpha(C/P) = k(\text{caffeine})/k(\text{phenol})$ |

Table 2
Characterization of various silica-based C₈ packing materials

| Column | $\alpha(T/B)$ | $\alpha(MB/T)$ | $\alpha(EP/B)$ | $\alpha(C/P)$ |
|-------------------------------|---------------|----------------|----------------|---------------|
| Polymer-coated C ₈ | 1.90 | 0.53 | 0.45 | 0.24 |
| A | 1.95 | 0.51 | 0.48 | 0.28 |
| B | 2.00 | 0.50 | 0.30 | 0.36 |
| C | 1.92 | 0.54 | 0.50 | 0.28 |
| D | 1.93 | 0.67 | 0.91 | 0.85 |
| E | 1.97 | 0.51 | 0.54 | 0.25 |

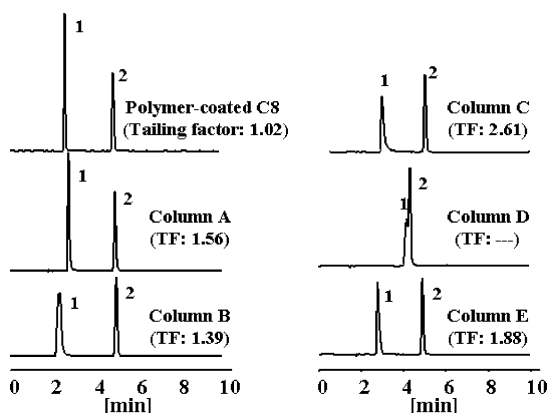


Fig. 2. Peak profiles of pyridine (peak 1) and phenol (peak 2). Column size: 150 mm \times 4.6 mm i.d., mobile phase: acetonitrile–water (30:70), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 254 nm.

showed small values for $\alpha(EP/B)$ and $\alpha(C/P)$. These parameters can be explained by the existence of the polymer layer covering the silica surface and cross-linking structure between the ligands and silica surface.

The residual silanol groups sometimes cause negative effects in separations. The peak profiles of pyridine and phenol are shown in Fig. 2. Pyridine is one of the typical compounds that sensitively responds to residual silanol groups. The polymer-coated C₈ column showed excellent peak shapes for basic compounds, which seems to agree with the above results of the $\alpha(EP/B)$ and $\alpha(C/P)$ values.

The durability under acidic and basic mobile phases of various silica-based C₈ columns are shown in Fig. 3. Although column D showed the strongest acidic durability, it could not be used at pH 8 and above. Column E could not be

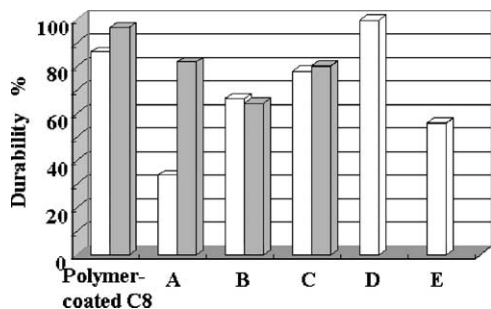


Fig. 3. Durability under acidic and basic mobile phase of various silica-based C₈ columns. Open square: acidic durability, filled square: basic durability.

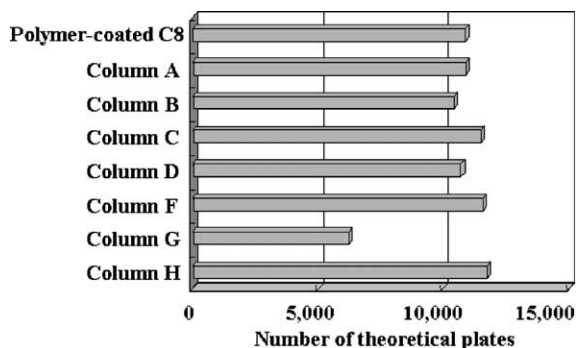


Fig. 4. Number of theoretical plates of various silica-based C₈ columns. Column size: 150 mm \times 4.6 mm i.d., mobile phase: methanol–water (50:50), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 254 nm, sample: naphthalene.

used at pH 8, neither. The polymer-coated C₈ phase showed excellent durability under both acidic and basic conditions.

The column efficiency and column pressure were examined for the polymer-coated phase and other conventional C₈ columns (particle size: 3 μ m). The column efficiency was evaluated by a number of theoretical plates of the naphthalene peak. The column pressure was measured under methanol–water (50:50), which is known to generate a high viscosity. The column efficiency and the column pressure are shown in Figs. 4 and 5, respectively. The polymer-coated C₈ phase showed a high column efficiency and, at the same time, the lowest column pressure among the tested. Although information about detailed surface structure and geometrical parameters of silica used for other columns is not available, it seems that the polymer-coating phase showed the best results among the tested phases, in terms of separation impedance.

Chromatograms of the mixture of various compounds were obtained with the polymer-coated phase and other conventional C₈ phases (Fig. 6). Some of the conventional columns showed peak tailing and overlapping of peaks. As far as the test mixture is concerned, the polymer-coated C₈ column showed a preferable separation profile within relatively short time.

Separation of the test mixture was compared between the polymer-coated C₈ and a conventional C₁₈ column (Fig. 7).

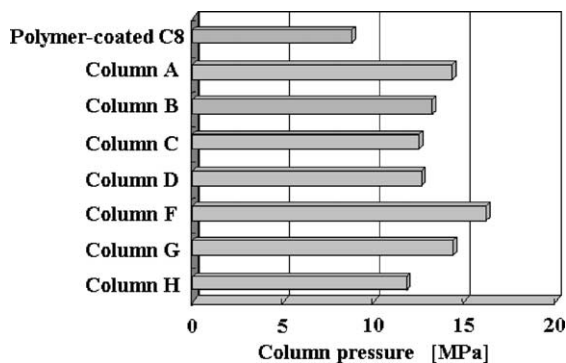


Fig. 5. Column pressure of various silica-based C₈ columns. Column size: 150 mm \times 4.6 mm i.d., mobile phase: methanol–water (50:50), flow rate: 1.0 mL/min, temperature: 40 °C.

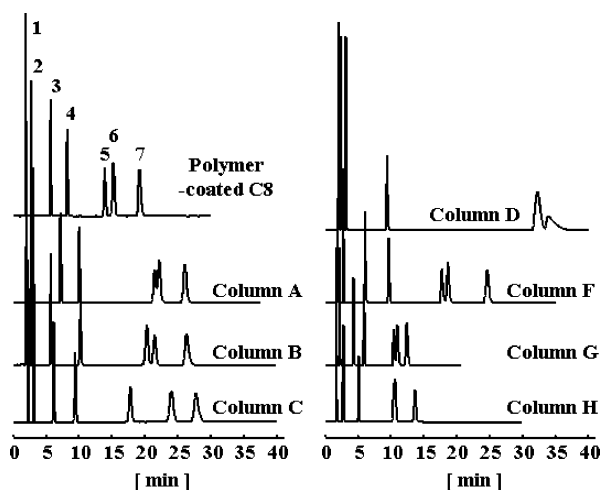


Fig. 6. Chromatograms of standard sample obtained with conventional C_8 columns. Column size: 100 mm \times 4.6 mm i.d., mobile phase: 50 mmol/L phosphate buffer–methanol (45:55), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 220 nm, sample: ethenzamide, phenytoin, ibuprofen, lidocaine, progesterone, verapamil, and dibucaine.

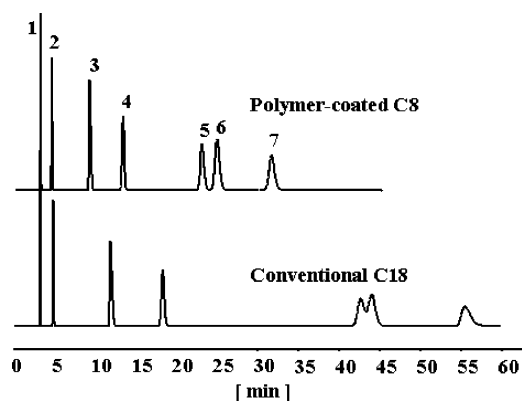


Fig. 7. Chromatograms of standard sample obtained with polymer-coated C_8 column and conventional C_{18} column. Column size: 150 mm \times 4.6 mm i.d., mobile phase: 50 mmol/L phosphate buffer–methanol (45:55), flow rate: 1.0 mL/min, temperature: 40 °C, detection: UV at 220 nm, sample: ethenzamide, phenytoin, ibuprofen, lidocaine, progesterone, verapamil, and dibucaine.

This can be one of the examples where a large overall retention of the C_{18} column may not provide the best separation for samples. The polymer-coated C_8 column adequately retained a relatively polar group of compounds in the mixture, and still finished the separation of all the compounds in a shorter time.

4. Conclusions

The polymer-coated C_8 stationary phase was developed by combing the polymer coating technology and the modification with octyl groups. The polymer-coated C_8 phase showed an improvement in durability under both acidic and basic conditions. Its packed column showed a high separation efficiency with a relatively low pressure drop. The characterization study with various standard compounds found that polymer-coated C_8 showed typical retention characteristics of C_8 phases.

Acknowledgement

We thank Dr. Osamu Shirota for his proofreading of this manuscript.

References

- [1] U.D. Neue, B.A. Alden, T.H. Walrer, *J. Chromatogr. A* 849 (1999) 101.
- [2] J. Zhao, P.W. Carr, *Anal. Chem.* 72 (2000) 302.
- [3] R. Majors, *LC–GC* 21 (2003) 332.
- [4] J. Winkler, S. Marmé, *J. Chromatogr. A* 888 (2000) 51.
- [5] R.A. Henry, *Am. Lab.* 34 (22) (2002) 18.
- [6] J. Kohler, P.D. Chase, R.D. Farlee, J. Vega, J.J. Kirkland, *J. Chromatogr.* 352 (1986) 275.
- [7] A. Pryde, *J. Chromatogr. Sci.* 12 (1974) 486.
- [8] R.E. Majors, M.J. Hopper, *J. Chromatogr.* 12 (1974) 767.
- [9] O. Shirota, Y. Ohtsu, O. Nakata, *J. Chromatogr. Sci.* 28 (1990) 553.
- [10] H. Kutsuna, Y. Ohtsu, M. Yamaguchi, *J. Chromatogr.* 635 (1993) 187.
- [11] T. Kanda, H. Kutsuna, Y. Ohtsu, M. Yamaguchi, *J. Chromatogr. A* 672 (1994) 51.
- [12] T. Kanda, O. Shirota, Y. Ohtsu, M. Yamaguchi, *J. Chromatogr. A* 722 (1996) 115.
- [13] A. Ohkubo, T. Kanda, Y. Ohtsu, M. Yamaguchi, *J. Chromatogr. A* 779 (1997) 113.
- [14] S. Kobayashi, I. Tanaka, O. Shirota, T. Kanda, Y. Ohtsu, *J. Chromatogr. A* 828 (1998) 75.
- [15] A. Kobayashi, T. Kanda, Y. Ohtsu, O. Shirota, *Am. Lab.* (2002) 58.
- [16] A. Espada, A. Rivera-Sagredo, *J. Chromatogr. A* 987 (2003) 211.
- [17] H. Fukui, T. Ogawa, M. Nakano, M. Yamaguchi, Y. Kanda, *Controlled Interphases in Composite Materials*, Elsevier, New York, 1990.
- [18] Y. Sudo, *J. Chromatogr. A* 737 (1996) 139.