

Preparation of pyrenebutyric acid bonded silica stationary phases for the application to the separation of fullerenes

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Abstract

A novel immobilization method was proposed for the preparation of pyrenebutyric acid-bonded silica (PYB-silica) stationary phases. The pyrene moiety was grafted to silica gel through spacers of aminoalkyl silanes. The HPLC separation of C₆₀, C₇₀ and higher fullerenes on the new pyrenebutyric acid-bonded silica stationary phases was also studied. Based on the temperature effect, the intermolecular interaction between stationary phases and solutes and the retention mechanism were discussed. The results of column loading capacity test demonstrated the potential for the separation of fullerenes in large amounts on the PYB-silica stationary phases.

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Keywords: Fullerenes; Pyrenebutyric acid; Separation; Stationary phases

1. Introduction

Since fullerenes were first discovered [1] and found to be widely applied in various fields [2–4], an efficient method for separating individual fullerenes was required. Liquid chromatography (LC) is the best choice for this application. In the past decade, the efficiency of several LC stationary phases has been tested [5–20], including conventional octadecylsilica (ODS) phases [8–10] as well as charge-transfer chromatography phases [11–20]. The stationary phases most frequently employed for fullerenes separation consisted of organic groups covalently bonded onto the surface of silica gel [11–17] or those modified on the metal oxide supports [18,19]. The interactions of fullerenes with the immobilized organic moieties should play a major role in determining the retention properties of such a stationary phase. Reversed-phase LC stationary phases exhibited reasonable fullerene selectivity, which probably resulted from the accessibility of these solutes to the bonded C₁₈ ligands [13]. But the retention

decreases with increasing solubility of the target compounds in the mobile phase. Hence, when strong solvents such as toluene or 1-methyl-naphthalene are used, these compounds cannot be separated. The poor solubility of fullerenes in the mobile phases used prevented the separation of large amounts of fullerenes, which hampered other studies of fullerenes.

Compared with the conventional method using ODS phases, the method based on charge-transfer interaction between immobilized selectant acceptors and a solute donor or vice versa had been found to exhibit more efficient separation of fullerenes [11–19]. The first commercially available phase was known as “Buckyclutcher” and comprised immobilized 3,3,3-tri-dinitrophenyl bonded silica [11]. Based on its π -acidic character, it could separate C₆₀ and C₇₀ using toluene as mobile phase, through a charge-transfer mechanism; however, the retention factors and selectivity were low, and their use was feasible only with weak solvents. A more effective retention was achieved by using other packing materials with large ligands such as 2-(1-pyrenyl)ethyl [20,21] or 3-(1-pyrenyl)propyl [22] (PYE) and tetraphenylporphyrin (TPP) [17,23], which could interact with a large fraction of the surface of the fullerene molecule. The PYE and TPP bonded silica stationary phases

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provided high selectivity and efficiency for preparative applications of fullerenes among the currently available stationary phases. Furthermore, the PYE-bonded silica stationary phases had been commercially available because of its good chromatographic performance. Tanaka et al. [20,21] and Lochmüller et al. [22] prepared PYE phases in a similar method in which the pyrenyl unit was first converted into a silanization reagent for which chemical coupling on silanol groups of silica was achieved. Briefly, the PYE phases in [22] were synthesized via reaction of 3-lithiopyrene obtained by *n*-butyl lithium lithiating 3-bromopyrene with alkenyl bromides leading to 3-alkenylpyrene followed by hydrosilation reactions with dimethylmonochlorosilane. The resulting functionalized silylalkyl pyrenes reacted with surface silanol groups of silica gel to generate the PYE phases. Although the PYE stationary phases were excellent in the separation of fullerenes, the preparation thereof required troublesome procedures that increase the cost of preparation. Therefore, an easy and economic method for the preparation of stationary phase with pyrene ligand is necessary.

In this paper we described a new and simple method for immobilizing PYB on silica supports. In the proposed method, the pyrene ligands were immobilized to the silica gel with two spacers different in length by an amide bond. The stationary phases were synthesized via amide reaction of pyrenebutyryl chloride with different aminosilanes-bonded silica in which aminosilanes had been grafted to the silica surface or first with aminosilanes, and then reacted with surface silanol groups of silica gel. The resulting stationary phases were successfully applied to the separation of fullerenes. The retention mechanism of fullerenes on the resulting stationary phases and their temperature effect were discussed. The potential application of the resulting stationary phase to the preparative separation of fullerenes was demonstrated.

2. Experimental

2.1. Chemicals

All reagents were obtained from commercial sources and were of analytical-reagent grade. Toluene and 1-chlorobenzene were all purchased from the Shanghai General Chemical Reagent Factory (Shanghai, China). 3-Aminopropyltrimethoxysilane (APTS) and *N*-(2-amethyl)-3-aminopropyltrimethoxysilane (NAPTS) were purchased from the Chemical Plant of Wuhan University (Wuhan, China). Pyrenebutyric acid (PYB, content $\geq 99\%$) was purchased from Aldrich (New Jersey, America). The samples of fullerenes were purchased from Fullerenes Institute of Wuhan University (Wuhan, China). Spherical silica gel was made in our laboratory by oil-emulsion method [24]. The mean particle diameter of the silica gel was 5–7 μm . Toluene as mobile phase was filtered through a G-4 fritted glass

funnel and degassed in an ultrasonic bath for 5 min under reduced pressure before use.

2.2. Pyrenebutyryl chloride (PYBC) synthesis

Pyrenebutyryl chloride was prepared by adding 0.50 g of dried pyrenebutyric acid to an ice-cold solution of 10.0 mL anhydrous thionyl chloride with stirring. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. Then the resulting mixture was evaporated to dryness under reduced pressure for the following reaction.

2.3. Preparation of PYB-bonded silica phases

Two methods of pyrenebutyric acid immobilization were studied involving the reaction of APTS (or NAPTS) and pyrenebutyryl chloride (PYBC) (Fig. 1). The first method (route I of Fig. 1) involved coupling PYBC to the silica gel substrate via an amide linkage between PYBC and the amido terminus of APTS (NAPTS)-silica gel. The APTS (NAPTS)-silica gel was prepared by refluxing 1.8 g of APTS (NAPTS) for 24 h with 4.00 g of dried silica gel in 30 mL of anhydrous toluene under a controlled nitrogen atmosphere. The resulting APTS (NAPTS)-silica was then filtered through a sintered glass funnel and washed with several aliquots (totaling 75 mL each) of anhydrous toluene, dichloromethane and acetone. After drying overnight at 100 °C under vacuum condition, 2.0 g of the APTS (NAPTS)-silica was refluxed for 24 h with PYBC and 1.0 mL of triethylamine in 50 mL of anhydrous toluene. The resulting PYB-bonded silica phase was filtered and washed again to remove any unreacted species from the silica surface. The resultant stationary phases were termed as PYB-APTS-silica I and PYB-NAPTS-silica I, respectively.

The second immobilization method (route II of Fig. 1) for preparing PYB-bonded silica phases described in this paper involved the reaction of PYBC with APTS in toluene first, followed by the reacting with the silica. Briefly, triethylamine (0.5 mL) and the APTS (1.00 g) were dissolved in 40 mL anhydrous toluene and cooled in an ice bath. Then the toluene solution of PYBC was added dropwise over several minutes with stirring. The reaction mixture was gradually allowed to warm to room temperature and stirred under a nitrogen atmosphere. After 24 h, 2.00 g of dried silica was added to the above reaction mixture and heated to reflux for 24 h with stirring under a controlled nitrogen atmosphere. The PYBC-silica was then fitted through a sintered glass funnel and washed with several aliquots (totaling 50 mL) of dry toluene, dichloromethane and acetone. The resultant stationary phase was termed as PYB-APTS-silica II.

Two “blank” phases were prepared according to the procedure illustrated in route I and were termed as APTS-silica and NAPTS-silica, respectively. They were compared to PYB-silicas to better understand the structural nature of the PYB-bonded silica stationary phases.

2.4. Column packing

Approximately 2.0 g of each of five stationary phases (PYB-silicas and NH₂-silicas) was slurried in 25 mL of 2-propanol. The slurry mixture was stirred for 10 min and ultrasonicated for 3 min, and then transferred into the slurry chamber. The packing solvent was methanol. All the stationary phases were packed into 150 mm × 4.6 mm stainless steel columns using the downfill slurry method under 6000 p.s.i. pressure allowing 200 mL of solvent to be flushed through the columns.

2.5. Instrumentation

The HPLC system used in these studies was an Elite III high-performance liquid chromatography system (Dalian, China), consisting in a P230 liquid chromatographic pump, UV228 UV–vis spectrophotometric detector, Rheodyne 7125 injector system (sample loop 20 μL for analytical separation, 500 μL for preparative separation), the data were processed by using Echrom 2000 ChemStation software (Elite company, Dalian, China). Columns were thermostated using a ZW stable temperature controller column oven. Elemental

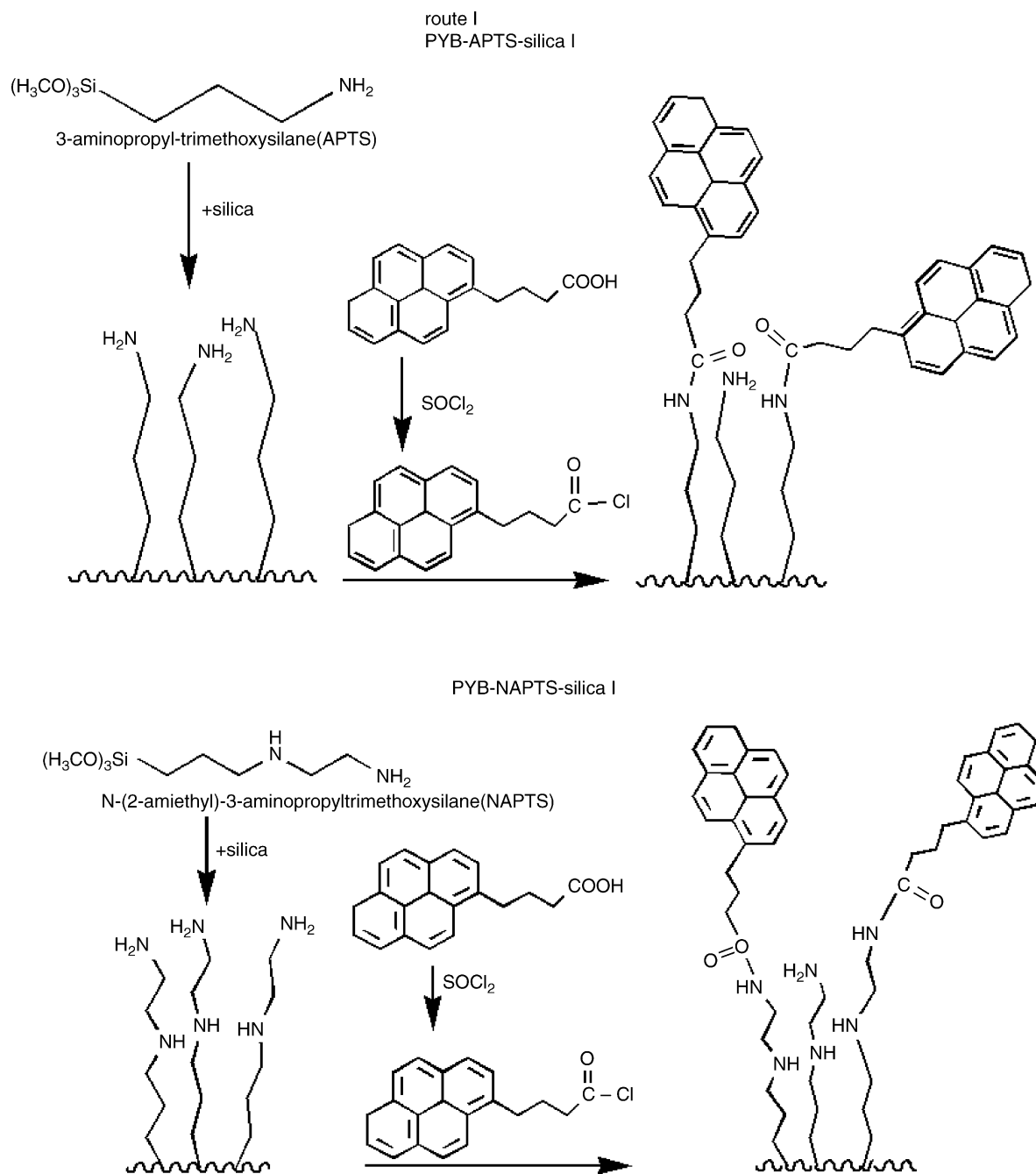


Fig. 1. Scheme illustrating preparation of pyrenebutyric acid bonded-silica by two different routes (note: only the primary reactions between silica and ligands are presented).

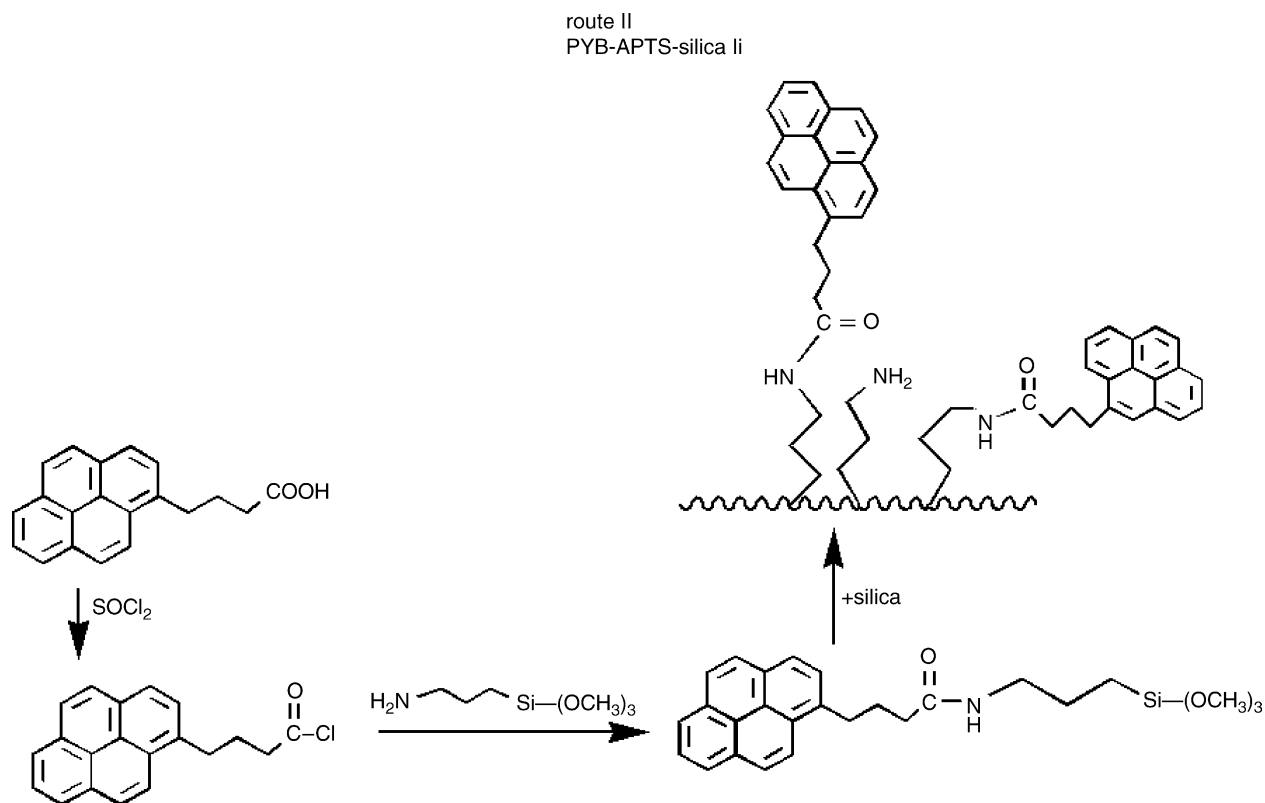


Fig. 1. (Continued).

analysis was performed with a MOD-1106 elemental analyzer (Carlo Erba, Italy). The surface area, average pore size and the pore volume were obtained by the results of nitrogen adsorption analysis with a Coulter SA 3100 plus surface area and pore size analyzer (Beckman, America).

2.6. Chromatographic measurements

The column efficiencies were measured at 10 °C, using a standard solution of C₆₀. The temperature dependence of C₆₀ and C₇₀ was determined by measuring the capacity factors of the fullerenes over the temperature range 30–70 °C using toluene as the mobile phase. The saturated solutions of C₆₀ and C₇₀ were injected in duplicate at a given temperature. When experiments were completed at a given temperature, the column was allowed to reach equilibrium at the next temperature by flushing it with a constant stream of mobile phase for 35 min prior to making an injection. The column void times (*t*₀) were determined by injecting a small plug of cyclohexane and recording the perturbation signal. Under these conditions only a slight decrease in *t*₀ was observed at higher temperature.

3. Results and discussion

It has been reported previously that pyrenyl group is an electron donor and possesses a special planar structure, which

can strongly interact with fullerenes [19–21]. Based on the consideration, we prepared new PYB-bonded silica stationary phases for the separation of fullerenes. Some of the physicochemical characteristics of the PYB-bonded silica stationary phases were shown in Tables 1 and 2. Elemental analysis results provided evidence of the successful immobilization of the PYB onto the silica. According to the carbon contents, the surface coverage [25] of PYB on the silica was 1.10, 1.91 and 1.16 μmol m⁻², respectively, as shown in Table 2.

In order to compare the properties of the three PYB-bonded silica stationary phases, we measured the capacity factors of C₆₀ and C₇₀, their separation factors, resolution and their efficiency using toluene as mobile phase. The results were listed in Table 3. It can be seen that the PYB-silicas

Table 1
Pore characteristic of the stationary phases^a

Stationary phase	Average pore size (nm)	Specific surface area (m ² g ⁻¹)	Specific pore volume (cm ³ g ⁻¹)
Silica	16.01	254	0.99
PYB-APTS-silica I	13.93	220	0.74
PYB-APTS-silica II	13.93	160	0.46
PYB-NAPTS-silica I	15.30	190	0.52

^a Data obtained by the nitrogen adsorption analysis. The pore size and the pore volume were calculated from desorption data using the Barrett–Joyner–Halenda (BJH) method. The specific surface area was calculated using the BET equation.

Table 2
The characteristics of bonded phases

Bonded phase	Carbon content ^a (%)	Surface coverage ^b ($\mu\text{mol m}^{-2}$)	Ligand interval ^c (\AA)
APTS-silica I	4.01	1.09	12.3
NAPTS-silica I	5.45	1.14	12.1
PYB-APTS-silica I	11.44	1.10	12.3
PYB-APTS-silica II	19.92	1.91	9.3
PYB-NAPTS-silica I	13.36	1.16	12.0

^a Data obtained by elemental analysis.

^b Surface coverages of the bonded PYB phases were obtained via calculations based on the carbon content of the bonded silica phase obtained by elemental analysis [25].

^c The average ligand interval on the stationary phases was calculated from the surface coverage according to the general equation proposed by Lochmüller et al. [28]. Ligand interval (\AA) = $(1/(C \times N))^{1/2}$, where C is the surface coverage of bonded species on the silica (mol/m^2) and N is the constant ($N = 6.022 \times 10^3$ molecules $\text{m}^2/(\text{mol } \text{\AA}^2)$).

showed high retentivity for fullerenes using toluene as the mobile phase. To assess the role of the immobilized pyrenyl group on the fullerene retention, an aliquot of the fullerenes was also injected into two “blank” columns packed only with aminopropylsilica (APTS-silica) and *N*-(2-amiethyl)-3-aminopropylsilica (NAPTS-silica), respectively. Both of the C_{60} and C_{70} eluted in the void volume, indicating that the retention of fullerenes on the PYB-silicas was based on the specific interaction with immobilized pyrenyl group on the stationary phases, not with surface silanols or aminoalkyl groups present on the support.

As shown in Table 3, the three PYB-bonded silica stationary phases exhibited substantial differences in the retention selectivity toward C_{60} and C_{70} . The differences in retentions and selectivity of C_{60} and C_{70} on the PYB-APTS-silica I and PYB-APTS-silica II stationary phases could be explained by the difference of the surface coverage on these two stationary phases in Table 2. Although the retentions and selectivity of C_{60} and C_{70} on PYB-APTS-silica I were slightly less than those on PYB-APTS-silica II under the same condition, it gave a higher efficiency for fullerenes in terms of the number of theoretical plates. In addition, it was also shown in Table 3 that the retentions of C_{60} and C_{70} and their selectivity on the PYB-NAPTS-silica I phase were larger than those on the PYB-APTS-silica I phase. This suggested the contribution of the long amide arm in the bonded phase to the fullerenes retention because the tether arm of PYB-NAPTS-silica I was three atoms longer than that of PYB-APTS-silica I when both phases had similar surface coverage values shown in Table 2. It was assumed that solute retentions and selec-

tivity increased with increasing the mobility of the stationary phase, as previously shown by C_{18} - and C_{30} -silica selectivity for fullerenes [26]. With the longer tether arm of PYB-silica, the PYB-NAPTS-silica I stationary phase, which was more easily relaxed and movable, could interact with fullerenes with less steric restriction.

Besides in toluene, PYB-bonded silica phases exhibited high selectivity for separation of fullerenes in a number of other strong mobile phase solvents in which the fullerenes were most soluble [17]. For example, the PYB-APTS-silica I phase has a fullerenes selectivity of $\alpha = 1.71$ while the PYB-NAPTS-silica I has a selectivity of $\alpha = 2.13$ in a mobile phase of chlorobenzene. The baseline separation of C_{60} and C_{70} could be achieved in the mobile phase of chlorobenzene on both columns, suggesting that the fullerenes interaction with the immobilized PYB is quite strong. The ability to use strong mobile phase solvents increased the potential of columns packed with PYB-bonded silica phases to perform preparative scale separations. Considering the poison of the solvents, toluene was chosen in the experiment as the mobile phase for other strong solvents were more unsafe than toluene.

In order to acquire more information regarding the nature of the interactions between the immobilized PYB and fullerenes, the separation of fullerenes was examined by using toluene as mobile phase at various column temperatures. Typical chromatograms for C_{60} and C_{70} separations using toluene as mobile phase at various column temperatures are shown in Fig. 2. These chromatograms showed the small temperature effect of this separation with the PYB-bonded silica phases. Meanwhile, the temperature dependence of the capacity factors of C_{60} and C_{70} , and of their separation factors on the PYB-bonded silica phases using toluene as the mobile phase were illustrated in Figs. 3 and 4, respectively. Fig. 3 clearly demonstrated that an increase in column temperature resulted in a slight change in the capacity factors of C_{60} and C_{70} on all of the PYB-bonded silica phases. Moreover, it showed that the capacity factors of C_{60} and C_{70} exhibited different temperature effect on the PYB-bonded silica stationary phases. On the column PYB-APTS-silica I, the capacity factors of C_{60} and C_{70} decreased slightly with increasing column temperature, which had good agreement with that in the previous investigation [15,20,27]; whereas increased a little on PYB-NAPTS-silica I and PYB-APTS-silica II. Even so, the selectivity factor for C_{70} and C_{60} , as shown in Fig. 4, decreased on all of the PYB-bonded silica stationary phases with increasing column temperature.

Table 3
Capacity and selectivity factors for C_{60} and C_{70} on columns packed with PYB-silicas^a

Stationary phases	$k'_{C_{60}}$	$k'_{C_{70}}$	$\alpha_{C_{70}/C_{60}}$	R_s	$N(C_{60})$ (plates/m)	$N(C_{70})$ (plates/m)
PYB-APTS-silica I	0.90	2.00	2.23	4.29	12281	7839
PYB-APTS-silica II	1.06	2.50	2.35	2.77	4639	2440
PYB-NAPTS-silica I	1.22	3.38	2.76	3.12	4532	1895

^a HPLC conditions: injection amount: 1.0 μL saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; flow rate: 1.0 mL/min; detection wavelength: 380 nm; column temperature: 25 $^\circ\text{C}$.

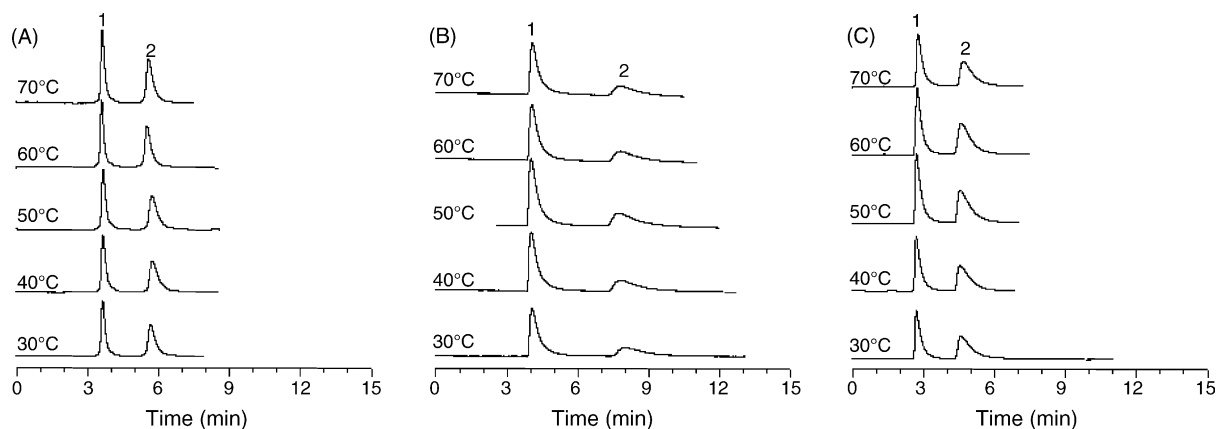


Fig. 2. Chromatograms for the separation of C_{60} and C_{70} with columns packed with (A) PYB-APTS-silica I, (B) PYB-NAPTS-silica I and (C) PYB-APTS-silica II. Peak 1: C_{60} ; peak 2: C_{70} . Injection amount: 1.0 μ L saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; flow rate: 1.0 mL/min; detection wavelength: 380 nm; column temperature: 30–70 $^{\circ}$ C.

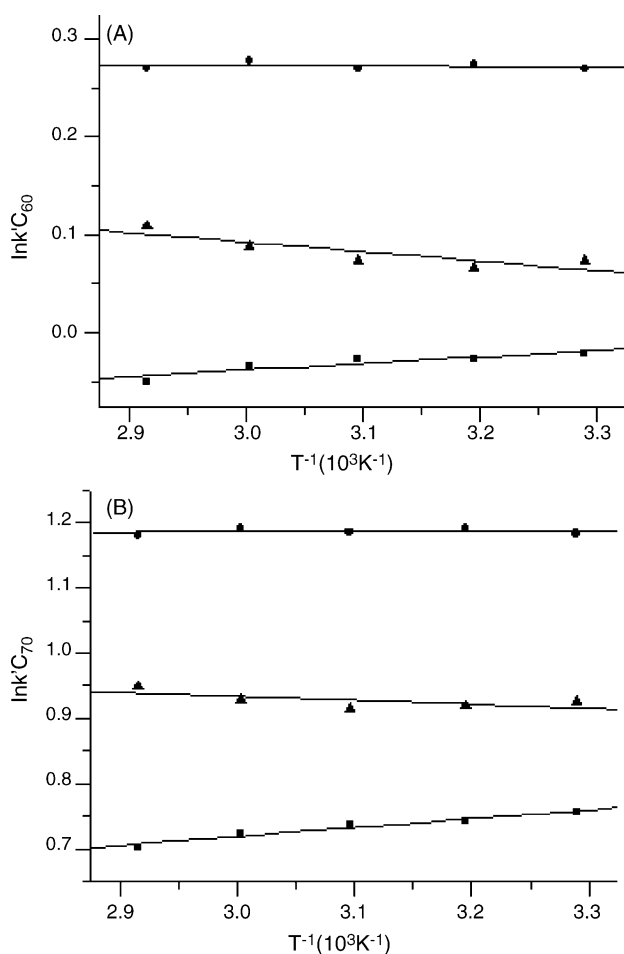


Fig. 3. Plot of $\ln k'$ vs. $1/T$ for C_{60} (A) and C_{70} (B) on the PYB-APTS-silica I (■), PYB-NAPTS-silica I (▲) and PYB-APTS-silica II (●). Injection amount: 1.0 μ L saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; flow rate: 1.0 mL/min; detection wavelength: 380 nm; column temperature: 30–70 $^{\circ}$ C.

Jinno et al. [15] had reported that the molecular sizes of C_{60} and C_{70} were approximately $8 \text{ \AA} \times 8 \text{ \AA}$ with the carbon atoms arranged like a soccer ball and $10 \text{ \AA} \times 7 \text{ \AA}$ like a rugby ball, respectively. Therefore, comparing with the ligand interval on the stationary phases in Table 2, which was calculated by the assumption of the uniform distribution for both the aminopropyl-groups on the silica surface and the pyrenyl-groups attached to those aminopropyl-groups [28], the molecular sizes of C_{60} and C_{70} makes them small enough either to adsorb on a single PYB ligand or to be adsorbed between two neighboring PYB groups, interacting with both. The observed differences in temperature effect on fullerene retention on the PYB-silica phases were primarily caused by the difference of the surface ligands arranged on stationary phases. It was assumed that the conformational change of stationary phase [27,29–30] and the dissociation of fullerenes [31,32] at higher temperatures can both influence fullerene

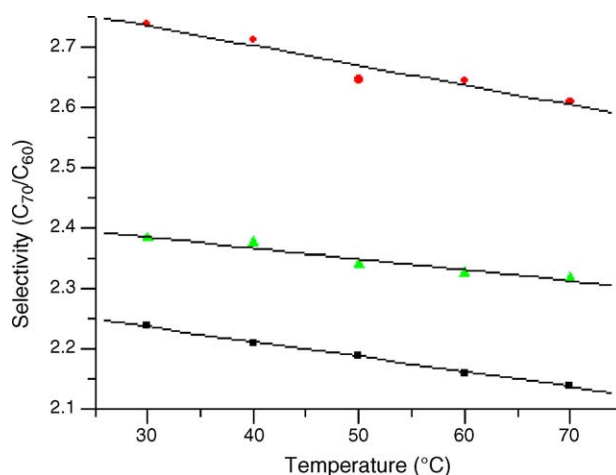


Fig. 4. Plot of $\alpha_{C_{70}/C_{60}}$ vs. temperature on the on the PYB-APTS-silica I (■), PYB-NAPTS-silica I (▲) and PYB-APTS-silica II (●). Injection amount: 1.0 μ L saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; flow rate: 1.0 mL/min; detection wavelength: 380 nm; column temperature: 30–70 $^{\circ}$ C.

retention yet in contrary direction. On the PYB-APTS-silica I phase, the conformational change of stationary phase worked toward the usual temperature effect on fullerene retention because pyrene ligands would interact with increasing temperature, which led to less interaction between stationary phase and solutes. Combined with the effect of fullerenes dissociating, the retentions of fullerenes decrease slightly at higher temperatures. On the other two stationary phases, because of higher surface coverage and longer tether arms, respectively, the self-association of pyrene ligands could take place under room temperature, so the surface of the stationary phase would change little with increasing temperature and the dissociation of fullerenes would increase the interaction between stationary phase and solutes, leading to the unusual temperature effect of fullerenes retention. A clearer understanding of the thermodynamic behavior would require a more detailed investigation.

Knox plots of reduced plate height versus reduced velocity for C_{60} in a mobile phase of toluene on the three PYB-bonded silica columns were used to evaluate the column efficiency. Since calculation of reduced velocity required an estimate of the molecular diffusivity of C_{60} in toluene, the value of C_{60} molecular diffusivity ($D_m = 1.95 \times 10^{-5} \text{ cm}^2/\text{s}$) reported in literature [33] was used for the calculation. Knox plots were illustrated in Fig. 5. And the fitting coefficients were shown in Table 4. As all columns were packed and tested under identical conditions, packing considerations cannot account for the significant difference in the three plots. It could be seen that the A term values for all of the phases were very high. This may be caused by either bad packing or wide particle size distribution. It was evident that the B term value in PYB-APTS-silica I was lower than that in PYB-APTS-silica II and PYB-NAPTS-silica I, which meant that the intraparticle pore diffusion and fluid flow of solutes in the pore structure of the PYB-APTS-silica I packing was smaller than that

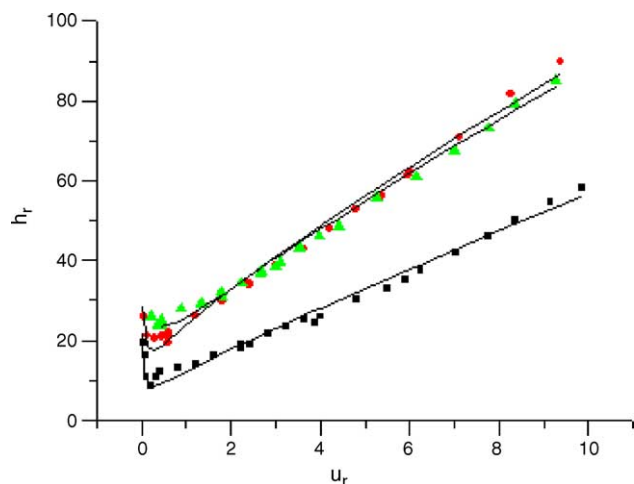


Fig. 5. Knox plot for PYB-APTS-silica I (■), PYB-APTS-silica II (▲) and PYB-NAPTS-silica I (●) columns. Injection amount: 1.0 μL saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; detection wavelength: 380 nm; column temperature: 10 $^{\circ}\text{C}$.

Table 4

Comparison of the fitting coefficients of Knox plots ($h_r = B/u_r + Au_r^{0.33} + Cu_r$) for the three PYB-bonded silica stationary phases^a

Stationary phase	A	B	C
PYB-APTS-silica I	6.60 \pm 0.76	0.81 \pm 0.07	4.21 \pm 0.23
PYB-APTS-silica II	11.17 \pm 1.13	1.59 \pm 0.21	7.84 \pm 0.24
PYB-NAPTS-silica I	14.10 \pm 0.71	4.24 \pm 0.36	5.98 \pm 0.18

^a HPLC conditions: injection amount: 1.0 μL saturated solution of C_{60} and C_{70} mixture in toluene; mobile phase: toluene; detection wavelength: 380 nm; column temperature: 10 $^{\circ}\text{C}$.

of the PYB-APTS-silica II and the PYB-NAPTS-silica I. Meanwhile, the value of C reflects the efficiency of mass transfer. So the mass transfer of fullerenes on PYB-APTS-silica I ($C = 4.21$) was less than that of PYB-APTS-silica II ($C = 7.84$) and PYB-NAPTS-silica I ($C = 5.98$). This can be ascribed to the high surface coverage on PYB-APTS-silica II and the longer tether arm on PYB-NAPTS-silica I, respectively. On PYB-APTS-silica II, the surface of the silica gel was covered by a sufficiently dense layer of pyrene ligands, and their self-association took place. Thus, the penetration of solute molecules into the layer was restricted. Similarly, for PYB-NAPTS-silica I with longer tether arm, it was likely that PYB-NAPTS-silica I had a larger thickness compared to the PYB-APTS-silica I. This increased the partitioning distance that was required for fullerene interaction with PYB-silica. This, in turn, decreased the mass transfer.

Most of the available stationary phases gave a more than adequate separation of C_{60} and C_{70} fullerenes using the optimum mobile phase in each case [34]. However, problems would arise in such practical applications as separating higher fullerenes or separating fullerenes in preparative scale. In terms of these two problems, the prepared PYB-silica phases could attain excellent results. Table 5 shows the results of loading studies of the separation of fullerenes for the columns packed with PYB-APTS-silica I and PYB-NAPTS-silica I. The retentions and separation factor of C_{60} and C_{70} decreased

Table 5

Preparative separation of C_{60} and C_{70} on the PYB-APTS-silica I and PYB-NAPTS-silica I columns with different injecting amounts^a

Amount injected (mg)	k'_1	α	R_s	N (plates/m)
Column: PYB-APTS-silica I				
0.01	1.03	1.99	3.29	8500
0.05	0.95	1.95	2.78	6506
0.1	0.93	1.94	2.61	6722
0.6	0.86	1.87	1.84	2585
2	1.01	1.54	0.70	500
Column: PYB-NAPTS-silica I				
0.01	0.94	2.64	3.00	5572
0.05	0.78	2.61	2.56	4523
0.1	0.76	2.58	2.45	4256
0.6	0.64	2.44	1.80	2083
2	0.76	2.11	0.81	577

^a HPLC conditions: sample: 2.8 mg/mL of C_{60} and C_{70} mixture in toluene; mobile phase condition: toluene; flow rate: 1.0 mL/min; detection wavelength: 380 nm; column temperature: 25 $^{\circ}\text{C}$.

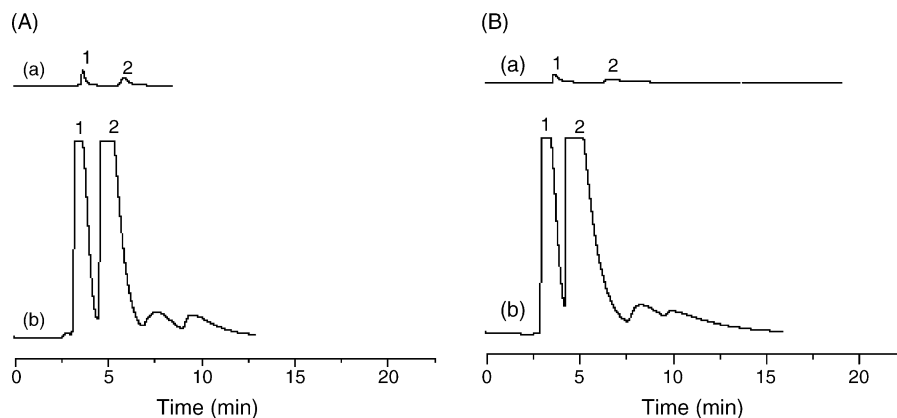


Fig. 6. Separation of C_{60} and C_{70} on the PYB-APTS-silica I (A) and PYB-NAPTS-silica I (B). Peak 1: C_{60} ; peak 2: C_{70} . Mobile phase: toluene. Sample: 2.80 mg/mL of C_{60} and C_{70} mixture in toluene. Injection amount: 0.01 mg for (a) and 2.00 mg for (b). Flow rate: 1.0 mL/min. Detection wavelength: 380 nm; Column temperature: 25 °C.

somewhat when the injection amount increased. The chromatogram in Fig. 6 shows the analytical separation with the injection of 0.01 mg of a mixture of fullerenes (a) and the preparative separation with the injection of 2.00 mg of the same solution (b) on these two columns. Approximately 2.00 mg of C_{60} and C_{70} was baseline resolved. Furthermore, partial higher fullerenes in the sample could be separated from C_{60} and C_{70} in a single injection of fullerenes mixture. All of above demonstrated that the PYB-bonded silica stationary phases had the ability to separate fullerenes in large amounts and even separate higher fullerenes partially.

4. Conclusion

The stationary phases with amino-linked and immobilized pyrenebutyric acid onto silica gel via silylation has been conveniently prepared. On these columns C_{60} and C_{70} can be successfully separated. The interaction between pyrene ligand and fullerenes was assumed to be the governing retention mechanism. Meanwhile, the long amide arm in the bonded phase also contributed to the retention of fullerenes. Furthermore, a certain amounts of C_{60} and C_{70} could be separated on the columns packed with PYB-APTS-silica I and PYB-NAPTS-silica I stationary phases. And higher fullerenes were separated partially from the large amounts of C_{60} and C_{70} on them. Comparing to some commercially available charge-transfer stationary phases, the simplicity for preparation of stationary phase and excellent performance made PYB-bonded silica stationary phases commercial potential for the separation of fullerenes.

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References

- [1] H.W. Kroto, J.R. Heath, S.C. O'Brien, R.F. Curl, R.E. Smalley, *Nature* 318 (1985) 162.
- [2] T. Tsang, *Solid State Commun.* 80 (1991) 623.
- [3] R. Baum, *Chem. Eng. News* (1993) 3.
- [4] H. van de Waterbeemd, M. Kansy, B. Wagner, H. Fisher, in: V. Pliska, B. Testa, H. Van deWaterbeemd (Eds.), *Methods and Principles in Medicinal Chemistry-Lipophilicity in Drug Action and Toxicology*, VCH, Weinheim, 1996, and reference cited therein.
- [5] K. Jinno (Ed.), *Separation of Fullerenes by Liquid Chromatography*, The Royal Society of Chemistry, Cambridge, UK, 1999.
- [6] J.M. Hawkins, T.A. Lewis, S.D. Loren, A. Meyer, J.R. Heath, Y. Shibato, R.J. Saykally, *J. Org. Chem.* 55 (1990) 6250.
- [7] D.M. Cox, S. Behal, M. Disko, S.M. Gorun, M. Greaney, C.S. Hsu, E.B. Kollin, J. Millar, J. Robbins, W. Robbins, R.D. Sherwood, P. Tindall, *J. Am. Chem. Soc.* 113 (1991) 2940.
- [8] K. Jinno, T. Uemura, H. Ohta, H. Nagashima, K. Itoh, *Anal. Chem.* 65 (1993) 2650.
- [9] A. Gügel, K. Müllen, *J. Chromatogr.* 628 (1993) 23.
- [10] D.L. Stalling, K.C. Kuo, C.Y. Guo, S. Saim, *J. Liq. Chromatogr.* 16 (1993) 699.
- [11] C.J. Welch, W.H. Pirkle, *J. Chromatogr.* 609 (1992) 89.
- [12] K. Kimata, T. Hirose, K. Moriuchi, K. Hosoya, T. Araki, N. Tanaka, *Anal. Chem.* 67 (1995) 2556.
- [13] Y. Saito, H. Ohta, K. Jinno, *Anal. Chem.* (2004) 267A.
- [14] C.E. Kibbey, M.R. Savina, B.K. Parseghian, A.H. Francis, M.E. Meyerhoff, *Anal. Chem.* 65 (1993) 3717.
- [15] K. Jinno, K. Yamamoto, T. Ueda, H. Nagashima, K. Itoh, J.C. Fetzer, W.R. Biggs, *J. Chromatogr.* 594 (1992) 105.
- [16] K. Jinno, K. Nakagawa, Y. Saito, H. Ohta, H. Nagashima, K. Itoh, J. Archer, Y.L. Chen, *J. Chromatogr. A* 691 (1995) 91.
- [17] D.E. Coutant, S.A. Clarke, A.H. Francis, M.E. Meyerhoff, *J. Chromatogr. A* 824 (1998) 147.
- [18] Y.L. Hu, Y.Q. Feng, J.D. Wan, S.L. Da, *Anal. Sci.* 17 (Suppl.) (2000) a321.
- [19] Q.W. Yu, Z.G. Shi, Y.Q. Feng, J. Yang, *Anal. Chim. Acta* 536 (2005) 39.
- [20] N. Tanaka, Y. Tokuda, K. Iwaguchi, M. Araki, *J. Chromatogr.* 239 (1982) 761.
- [21] K. Kimata, K. Hosoya, T. Araki, N. Tanaka, *J. Org. Chem.* 58 (1993) 282.
- [22] C.H. Lochmüller, M.L. Hunnclutt, R.W. Beaver, *J. Chromatogr. Sci.* 21 (1983) 444.
- [23] J. Xiao, M.E. Meyerhoff, *J. Chromatogr. A* 715 (1995) 19.

- [24] X.Z. Xiao, Y.Q. Feng, S.L. Da, Y. Zhang, *Anal. Lett.* 33 (2000) 3355.
- [25] C.E. Kibbey, M.E. Meyerhoff, *Anal. Chem.* 65 (1993) 2189.
- [26] H. Ohta, Y. Saito, N. Nagae, J.J. Pesek, M.T. Matyska, K. Jinno, *J. Chromatogr. A* 883 (2000) 55.
- [27] K. Jinno, H. Ohta, Y. Saito, T. Uemura, H. Nagashima, K. Itoh, Y.L. Chen, G. Luehr, J. Archer, J.C. Fetzer, W.R. Biggs, *J. Chromatogr.* 648 (1993) 71.
- [28] C.H. Lochmüller, A.S. Colborn, M.L. Hunnicutt, *Anal. Chem.* 55 (1983) 1344.
- [29] H. Ohta, Y. Saito, K. Jinno, J.J. Pesek, M.T. Matyska, Y.L. Chen, J. Archer, J.C. Fetzer, W.R. Biggs, *Chromatographia* 40 (1995) 507.
- [30] H. Ohta, K. Jinno, Y. Saito, J.C. Fetzer, W.R. Biggs, J.J. Pesek, M.T. Matyska, Y.L. Chen, *Chromatographia* 42 (1996) 56.
- [31] W.H. Pirkle, C.J. Welch, *J. Org. Chem.* 56 (1991) 6973.
- [32] M. Diack, R.N. Compton, G. Guiochon, *J. Chromatogr.* 639 (1993) 129.
- [33] M. Kele, R.N. Compton, G. Guiochon, *J. Chromatogr. A* 786 (1997) 31.
- [34] M.P. Gasper, D.W. Armstrong, *J. Liq. Chromatogr.* 18 (1995) 1047.