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## Selective separation of fullerenes on hydroxyphenyl-triphenylporphyrin–silica stationary phases

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### Abstract

A novel immobilization method for the preparation of tetraphenylporphyrin–silica stationary phases is investigated. Stationary phases consisting of immobilized hydroxyphenyl-triphenylporphyrin (HPTPP) on silica are shown to exhibit unmatched selectivity ( $\alpha = k'_{C_{70}}/k'_{C_{60}}$ ) and improved efficiency for the separation of fullerenes, with  $\alpha = 7$  using 100% toluene as the mobile phase. The HPTPP species are immobilized via reaction with glycidoxypopyltrimethoxysilane. The high shape selectivity of columns packed with HPTPP–silicas enables the single step separation of higher molecular mass fullerenes and higher fullerene isomers using strong fullerene solvents as mobile phases. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Stationary phases, LC;  $\pi$ – $\pi$  Interaction; Fullerenes; Hydroxyphenyl-triphenylporphyrin; Silica

### 1. Introduction

An efficient method for separating individual fullerenes on a preparative scale is still required. Commonly, fullerene syntheses [1] yield a variety of fullerene molecules whose structures are determined by Euler's theorem and the isolated pentagon rule. The structural similarity of these fullerenes has proven a formidable obstacle in fullerene separations. Further, the poor solubility of fullerenes in most organic solvents limits the choice of mobile and stationary phases that can be used. As shown by Ruoff et al. [2], the solubility of  $C_{60}$  is only 0.043 mg/ml in hexane, 0.25 mg/ml in dichloromethane, and 2.8 mg/ml in toluene. Commonly used reversed-phase liquid chromatographic (LC) stationary phases (e.g.,  $C_{18}$ –silica) exhibit reasonable fullerene selec-

tivity, but only when using mobile phases in which the fullerenes are poorly soluble (hexane, dichloromethane, acetonitrile). Such columns do not possess the retentive power required to separate large quantities of fullerenes in strong mobile phase solvents in which the fullerenes are most soluble (i.e., 1,2-dichlorobenzene and 1-methylnaphthalene have  $C_{60}$  solubilities  $\approx 30$  mg/ml). The inability to prepare and separate large (gram) amounts of fullerenes in a cost-effective manner has hampered the study of higher molecular mass fullerenes and endohedral metallofullerenes. It has also limited the development of fullerene-based commercial products (e.g., products using fullerenes such as catalysts, superconducting materials, or polymer additives [3]).

Several types of HPLC stationary phases have been studied [4–18] for their potential as an effective means to separate fullerenes. LC supports with  $\pi$ -acidic character, in particular, have been shown to be

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well suited for the separation of fullerenes. The tri(dinitrophenyl)–silica or “Buckyclutcher” phase suggested by Welch and Pirkle [4] was the first  $\pi$ -acidic phase to become commercially available for the separation of fullerenes. It exhibits moderate fullerene selectivity using a mobile phase of 100% toluene ( $\alpha_{C_{70}/C_{60}} = 1.5$ ). A more effective  $\pi$ -acidic LC stationary phase is the popular 2-(1-pyrenyl)ethyl–silica phase [19], or “Buckyprep” column, which exhibits a selectivity of  $\alpha_{C_{70}/C_{60}} = 1.8$  using a 100% toluene mobile phase. More recently, Kimata et al. [20] prepared an even more selective pentabromobenzyl–silica phase which exhibits  $\alpha_{C_{70}/C_{60}} = 2.5$  using a mobile phase of 100% toluene.

Tetraphenylporphyrin (TPP)–silica phases were first prepared in our laboratories in 1993 primarily for the purpose of separating aromatic anions [21] and polycyclic aromatic hydrocarbons [22]. Columns packed with TPP–silica have also been found to separate  $C_{60}$  and  $C_{70}$  and higher molecular mass fullerenes [23–30] with extraordinary selectivity (e.g.,  $\alpha_{C_{70}/C_{60}} = 4–7$  in a 100% toluene mobile phase). This paper describes a new method for immobilizing TPPs on silica supports. This method involves the addition of [5-(*p*-hydroxyphenyl)-10,15,20-triphenyl]porphyrin (HPTPP) to glycidoxypropyltrimethoxysilane (GPTS) followed by coupling of this reaction mixture with the surface silanols of the silica gel packing material. The characterization of the resulting stationary phase by chromatographic and mass spectrometric (MS) techniques is included. This method results in a stationary phase that demonstrates improved selectivity and efficiency in fullerene separations, beyond that achieved on previous TPP–silica phases. The interaction strength between fullerene solutes and this new HPTPP–silica stationary phase is great enough to permit the isomeric separation of higher fullerenes in a single column pass using strong mobile phase solvents.

## 2. Experimental

### 2.1. Chemicals

Chlorobenzene and 1,2-dichlorobenzene were ob-

tained from Burdick and Jackson (Muskegan, MI, USA). Analytical grade 1,2-methylnaphthalene was obtained from Aldrich (Milwaukee, WI, USA) and filtered through a 0.2  $\mu\text{m}$  TEFLON® membrane filter prior to use. TPP–silica phases were prepared from 10  $\mu\text{m}$ , 100 Å pore size Nucleosil silica obtained from Machery–Nagel (Düren, Germany). All other reagents were purchased from Aldrich and used without further modification.

A carbon arc synthesis similar to that used by Parker et al. [1] was employed to produce the fullerenes. The reactor had water-cooled collection surfaces, a variable arc gap, and forced helium flow across the gap which decreased slag formation. The collected soot was Soxhlet extracted for 24 h with toluene. This resulted in a deep red solution, which was filtered through a 0.45  $\mu\text{m}$  cellulose nitrate filter (Millipore, Bedford, MA, USA) prior to drying at 130°C under vacuum.

### 2.2. Porphyrin synthesis

The HPTPP derivative was synthesized using an adaptation of the method created by Rothemund [31] to synthesize *meso*-TPPs. To 2 l of refluxing propionic acid were added 23 g of *p*-hydroxybenzaldehyde (1 equiv.) and 57 ml (3 equiv.) of benzaldehyde. After complete dissolution of the aldehydes, 52 ml (4 equiv.) of freshly distilled pyrrole was added and refluxing was continued for 1 h. After cooling slightly, 300 ml of 95% ethanol was added to precipitate the crude porphyrin product. The crude porphyrin was collected by vacuum filtration, washed with several portions of 95% ethanol and allowed to dry overnight at room temperature. The crude product was purified on silica gel (70–230 mesh). TPP was eluted first with 3 l of chloroform. The desired HPTPP was then eluted using 1.5 l of 10:90 (v/v) glacial acetic acid–chloroform solution. The collected HPTPP solution was then evaporated to dryness using a rotoevaporator and oven dried overnight at 110°C, yielding approximately 5.3 g (4.5%) of HPTPP.

The purity of the isolated HPTPP was analyzed using fast atom bombardment (FAB)-MS, thin layer chromatography, and UV/Visible spectroscopy before immobilization on the silica support. FAB-MS of the porphyrin in 3-nitrobenzyl alcohol showed the

parent ion at 631  $m/z$  (mass/charge) for the HPTPP species, matching the expected molecular mass of 630.75 g/mol for HPTPP. The UV/Visible spectrum of the porphyrin in dichloromethane exhibited a Soret band at 419 nm, a  $Q_y(0,1)$  band at 515 nm, a  $Q_y(0,0)$  band at 550 nm, a  $Q_x(0,1)$  band at 589 nm, and a  $Q_x(0,0)$  band at 646 nm.

### 2.3. Preparation of TPP–silica phases

Two methods of porphyrin immobilization were studied involving the reaction of 3-GPTS and HPTPP (Fig. 1). The first method (route I of Fig. 1) involved coupling HPTPP to the silica gel substrate via an ether linkage between HPTPP and the epoxide terminus of 3-glycidoxypropyl–silica gel. The 3-glycidoxypropyl–silica gel was prepared by refluxing 1 ml of GPTS for 2 h with 1.8 g of dried silica gel in 30 ml of toluene under a controlled nitrogen atmosphere. The resulting GPTS–silica was then filtered through a sintered glass funnel and washed with several aliquots (totaling 75 ml each) of dry toluene, dichloromethane, and acetone. After drying overnight at 125°C, the GPTS–silica was refluxed for 4 h

with 1.8 g of HPTPP and 50  $\mu$ l of tributylamine in 50 ml of *N,N*-dimethylformamide (DMF). The resulting HPTPP–silica phase was filtered and washed again to remove any noncovalently bound species from the silica surface.

The primary immobilization method (route II of Fig. 1) for preparing porphyrin–silica phases described in this paper involved the reaction of HPTPP with GPTS in DMF first, followed by the coupling of this reaction mixture to the silica substrate. Typically, approximately 1.8 g of HPTPP was added to 50 ml of anhydrous DMF. The phenol group of HPTPP was reacted with 500  $\mu$ l of GPTS and 50  $\mu$ l of the catalyst tributylamine by refluxing while stirring under a controlled nitrogen atmosphere. Porphyrin immobilization was carried out by adding 1.8 g of dried silica and refluxing for 4 h, followed by shaking for 16 h. The HPTPP–silica was then filtered through a sintered glass funnel and washed with several aliquots (totaling 50 ml each) of dry dichloromethane, acetone, 10% acetic acid in acetone, and acetone.

Several “blank” phases were prepared and compared to HPTPP–silicas to better understand the

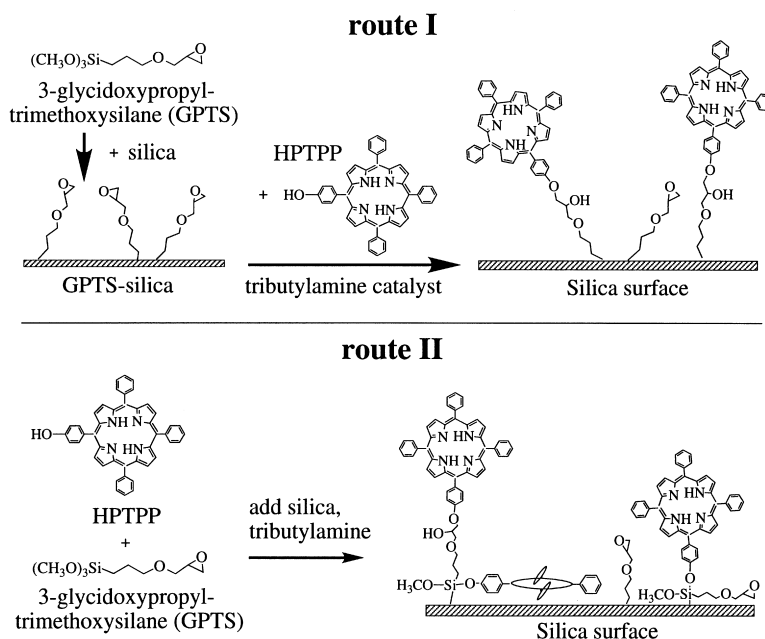


Fig. 1. Primary synthesis and immobilization schemes used to prepare [5-(*p*-hydroxyphenyl)10,15,20-triphenyl]porphyrin–silica (HPTPP) phases described in this paper.

structural nature of the HPTPP–silica stationary phase. One stationary phase was prepared according to the procedure illustrated in route II of Fig. 1, with the alteration that 1.75 g of TPP was used in place of HPTPP. A second “blank” phase was prepared using the procedure shown in route II of Fig. 1, but without GPTS. The third and final phase for comparison was made by substituting 370  $\mu\text{l}$  of ethyltrimethoxysilane (ETMS) for the GPTS used in the procedure shown in route II of Fig. 1.

The [5-(*p*-carboxyphenyl)-10,15,20-triphenyl]porphyrin (CPTPP)–silica phases were prepared by the method described previously [21]. Surface coverages of the bonded porphyrin phases were obtained via calculations based on the nitrogen content of the silica phase obtained by elemental analysis [21]. Typical HPTPP–silica and CPTPP–silica surface coverages ranged from 0.2 to 0.5  $\mu\text{mol}/\text{m}^2$ .

#### 2.4. Column packing

Approximately 1.5 g of TPP–silica was passed through a 15  $\mu\text{m}$  sieve using a Gilson (Washington, OH, USA) SS-5 vibratory sieve shaker and slurried in 25 ml of 10:63:27 (v/v/v) toluene–2-propanol–methanol. The slurry mixture was sonicated for 15 min and transferred into the slurry chamber of an Alltech HPLC column slurry packing system. All porphyrin–silica phases were packed into either 250 $\times$ 4.6 mm or 100 $\times$ 4.6 mm stainless steel columns using the downfill slurry method [32] under 6000 p.s.i. pressure and using 70:30 (v/v) 2-propanol–methanol as a packing solvent. The void volume of the column was determined by injection of a 0.05 mM naphthalene solution prepared in the appropriate mobile phase.

#### 2.5. Instrumentation

The HPLC system used in these studies consisted of a Scientific Systems (State College, PA, USA) model 200 solvent delivery system and model 210 pressure regulator, a Rheodyne model 7125 injection valve with either a 20  $\mu\text{l}$  or 500  $\mu\text{l}$  sample loop, an Anspec (Ann Arbor, MI, USA) SM95 UV–Vis detector, and a Hewlett Packard (Avondale, PA, USA) 3396A computing integrator. Columns were thermostated using a Fisher (Pittsburgh, PA, USA)

water jacket connected to a Fisher Model 80 Isotemp constant temperature circulator.

#### 2.6. Matrix-assisted laser desorption time-of-flight MS (MALDI-TOF-MS)

MALDI-TOF-MS was carried out on a Vestec-2000 instrument (Houston, TX, USA). The laser wavelength was 337 nm. A saturated (50 mM) 2,5-dihydrobenzoic acid solution with 50% aqueous acetonitrile containing 0.1% trifluoroacetic acid was used as the matrix. One  $\mu\text{l}$  of fullerene solution in  $\text{CS}_2$  was analyzed on the flat surface of the probe tip (2 mm diameter) with the matrix. The insulin A chain (formula weight = 2531.6 g/mol) was used as the internal standard to calibrate the molecular mass of the detected species.

#### 2.7. Electrospray ionization MS (ESI-MS)

The ESI-MS instrument consisted of a Vestec electrospray source and a Vestec model 201 VG Fisons single quadrupole mass spectrometer. Porphyrin samples were delivered to the source in a 10  $\mu\text{l}$  injection loop at 5  $\mu\text{l}/\text{min}$  in 4:50:46 (v/v/v) acetic acid–acetonitrile–water.

### 3. Results and discussion

#### 3.1. Separation of $C_{60}$ and $C_{70}$

Columns packed with CPTPP–silica have previously demonstrated [23,26,27] strong retention and unmatched selectivity in the separation of  $C_{60}$  and  $C_{70}$ . Fig. 2a illustrates a typical chromatogram for the separation of  $C_{60}$  and  $C_{70}$  in 100% chlorobenzene on a column packed with CPTPP–silica. Some peak asymmetry is evident, but the selectivity observed on CPTPP–silica is great enough to compensate for the low efficiency and provides excellent solute resolution ( $R_s > 3$ ). Fig. 2b shows the same separation using the new HPTPP–silica stationary phase (made via Fig. 1, route II immobilization procedure). It can be seen that the resolution of  $C_{60}$  vs.  $C_{70}$  on this phase is twice that of  $C_{60}$  vs.  $C_{70}$  on a CPTPP–silica phase, and that peak efficiency ( $N$ ) for the fullerenes has improved significantly, especially

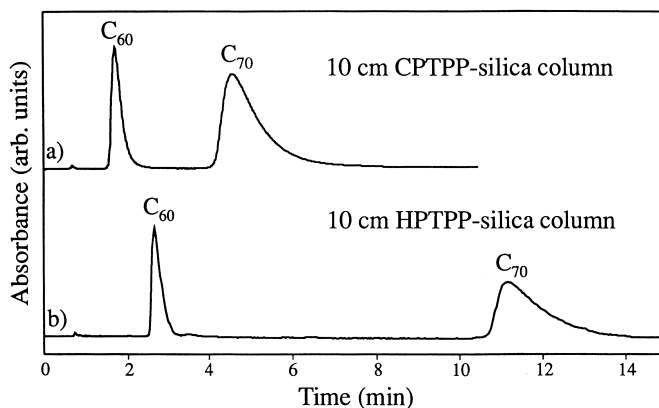


Fig. 2. Comparison of 100×4.6 mm columns packed with CPTPP(1)- and HPTPP(1)-silicas. Injection of 0.01 mg of  $C_{60}$  and  $C_{70}$  each; mobile phase=100% 1-chlorobenzene; flow-rate=1.7 ml/min; detection wavelength=410 nm; ambient column temperature.

for  $C_{70}$ . The efficiency improves from  $N=1400$  plates/m for  $C_{70}$  on the CPTPP-silica phase to  $N=4900$  plates/m on the HPTPP-silica phase.

The reproducibility of these results was examined by preparing a number of columns packed with different HPTPP- and CPTPP-silicas and testing them under similar conditions (Table 1). Using the same silica substrate, it was shown that batch-to-batch variability in terms of  $C_{70}/C_{60}$  selectivity is less than 5%. Chlorobenzene, a strong mobile phase for fullerenes, was chosen because fullerene elution times for columns packed with HPTPP-silica were prohibitively long ( $t_{C_{70}} > 1$  h at 1.0 ml/min) using a toluene mobile phase. It is shown that both columns packed with CPTPP-silicas exhibit  $\alpha_{C_{70}/C_{60}} = 4.5$  in a chlorobenzene mobile phase, with  $N=2200$  plates/m for  $C_{60}$  and 1500 plates/m for  $C_{70}$ . In contrast, all columns packed with HPTPP-silica had  $\alpha_{C_{70}/C_{60}} = 5.7$ , with similar efficiencies for both  $C_{60}$  and  $C_{70}$  (>4000 plates/m each).

TPP-silica phases exhibit high fullerene selectivity in a number of strong mobile phase solvents; i.e., solvents in which the fullerenes are most soluble (see Table 2). For example, the CPTPP-silica phase has high fullerene selectivity in carbon disulfide ( $\alpha_{C_{70}/C_{60}} = 3.4$ ) and 1,2 dichlorobenzene ( $\alpha_{C_{70}/C_{60}} = 2.4$ ). In contrast, the pentabromobenzyl-silica phase reported by Kimata et al. [20] has an estimated selectivity of only  $\alpha_{C_{70}/C_{60}} = 1.9$  in a mobile phase of 100% carbon disulfide.

The new HPTPP-silica phase reported here is even more selective, with  $\alpha_{C_{70}/C_{60}} = 7.0$  using a mobile phase of 100% toluene,  $\alpha_{C_{70}/C_{60}} = 4.5$  in carbon disulfide, and  $\alpha_{C_{70}/C_{60}} = 3.5$  using 100% 1,2 dichlorobenzene as the mobile phase. The ability to use strong mobile phase solvents allows for improved peak efficiencies, higher throughput, and increases the potential of columns packed with HPTPP-silica to perform preparative scale separations.

Table 1

Capacity and selectivity factors for  $C_{60}$  and  $C_{70}$  on several columns packed with CPTPP- and HPTPP-silicas<sup>a</sup>

TPP-silica column type	Coverage ( $\mu\text{mol}/\text{m}^2$ )	$k'_{C_{60}}$	$k'_{C_{70}}$	$\alpha_{C_{70}/C_{60}}$	$R_s$	$N(C_{60})$ (plates/m)	$N(C_{70})$ (plates/m)
10 cm CPTPP(1)	0.19	1.32	5.81	4.4	3.1	$2.2 \cdot 10^3$	$1.4 \cdot 10^3$
10 cm CPTPP(2)	0.14	1.52	6.94	4.6	3.4	$2.3 \cdot 10^3$	$1.6 \cdot 10^3$
10 cm HPTPP(1)	0.16	1.75	9.96	5.7	6.6	$4.2 \cdot 10^3$	$4.9 \cdot 10^3$
10 cm HPTPP(2)	0.41	5.36	31.9	5.7	7.6	$4.9 \cdot 10^3$	$5.1 \cdot 10^3$
10 cm HPTPP(3)	0.22	1.78	10.9	5.7	6.1	$4.2 \cdot 10^3$	$4.0 \cdot 10^3$
25 cm HPTPP(4)	0.52	2.20	12.6	5.7	10	$4.7 \cdot 10^3$	$4.1 \cdot 10^3$

<sup>a</sup>Conditions as reported in Fig. 2 legend.

Table 2

213 Influence of mobile phase on the fullerene selectivity of columns packed with CPTPP- or HPTPP-silicas<sup>a</sup>

Mobile phase	Solubility of C <sub>60</sub> (mg/ml) <sup>b</sup>	$\alpha_{C_{70}/C_{60}}$ on CPTPP-silica	$\alpha_{C_{70}/C_{60}}$ on HPTPP-silica
Toluene	2.8	5.7	7.0
<i>p</i> -Xylene	5.2	5.1	6.3
Chlorobenzene	7.0	4.5	5.7
Carbon disulfide	7.9	3.4	4.5
1,2-Dichlorobenzene	33	2.4	3.5

<sup>a</sup> HPLC conditions: injection of 2  $\mu$ g C<sub>60</sub> and C<sub>70</sub> each; flow-rate = 1.0 ml/min; ambient column temperature.<sup>b</sup> From Ref. [2].

### 3.2. Separation of higher fullerenes

While C<sub>60</sub> and C<sub>70</sub> are the primary fullerenes of interest, the number of investigations into the nature and properties of the higher fullerenes (primarily C<sub>76</sub> to C<sub>96</sub>) has grown in conjunction with the ability to separate these species. However, the percentage of higher fullerenes in a fullerene reactor is only 1–10 weight% of the total amount of fullerenes formed. The low percentage of higher fullerenes present in a fullerene mixture makes their efficient separation extremely difficult on a preparative scale. Often, the higher fullerenes are not completely resolved from one another in a single pass through an HPLC column even when using weak mobile phases. Reinjections must be made, greatly increasing the overall purification time. When working in weak

mobile phase solvents, fractions of higher fullerenes that are collected are very dilute. This is costly in terms of the amount of solvent used, as well as in the operator time required to collect and concentrate the higher fullerene samples.

To demonstrate the selectivity of the new HPTPP-silica phases and the effect of a higher operating temperature on the quality of fullerene separations on this material, an injection of toluene fullerene extract was made on a 250 $\times$ 4.6 mm column packed with HPTPP-silica at 70°C (Fig. 3). This separation of 0.1 mg of fullerene extract was carried out at a flow-rate of 1.0 ml/min with a mobile phase of 1:1 (v/v) methylnaphthalene-toluene. Not only is partial isomeric separation of the higher fullerenes possible in a single pass through a HPTPP-silica column, but column selectivity is great enough to also partially

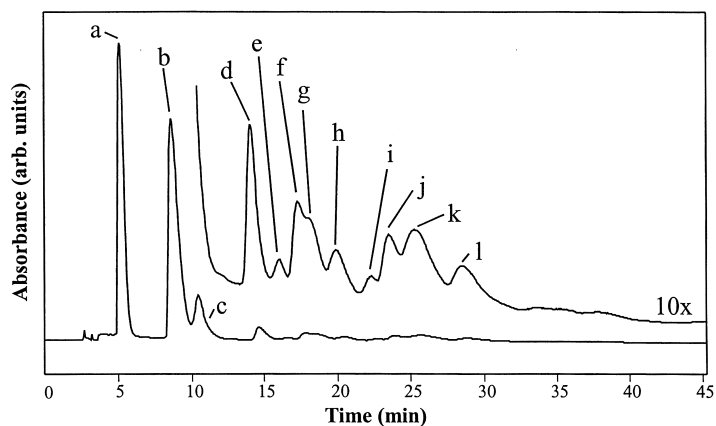


Fig. 3. Separation of higher molecular mass fullerenes on a 250 $\times$ 4.6 mm column packed with HPTPP(4)-silica. Injection of 0.1 mg of fullerene soot; mobile phase = 1:1 (v/v) 1-methylnaphthalene-toluene; flow-rate = 1.0 ml/min; detection wavelength = 410 nm; temperature = 70°C. Identity of peaks: C<sub>60</sub> (a); C<sub>70</sub> (b); C<sub>70</sub>O (c); C<sub>76</sub> (d); C<sub>76</sub>O (e); C<sub>78</sub>-D<sub>3</sub> (f); C<sub>78</sub>-C<sub>2v</sub> (g); C<sub>78</sub>O (h); C<sub>82</sub> (i); C<sub>84</sub>-I (j); C<sub>84</sub>-II (k); C<sub>84</sub>O (l).

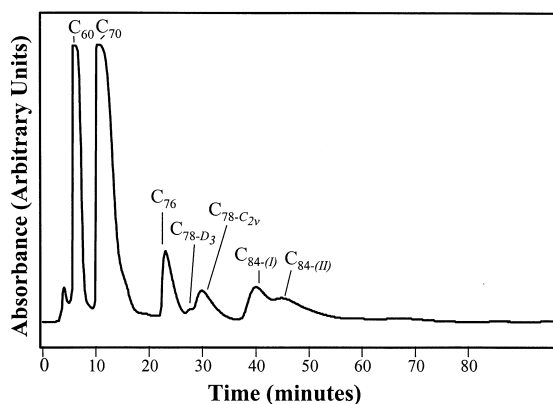


Fig. 4. Separation of higher molecular mass fullerenes on a  $250 \times 4.6$  mm column packed with HPTPP(4)-silica. Injection of 12 mg of fullerene soot; mobile phase=100% 1,2 dichlorobenzene; flow-rate=0.85 ml/min; detection wavelength=410 nm; ambient column temperature.

resolve the corresponding oxide peaks of the higher fullerenes.

The greatest advantage of columns packed with HPTPP-silica is their potential for preparative scale fullerene separations. Twelve mg of a higher fullerene extract was injected on a  $250 \times 4.6$  mm column packed with HPTPP-silica using a mobile phase of 100% 1,2-dichlorobenzene. The high selectivity of HPTPP-silica resulted in excellent resolution of the higher molecular mass fullerenes, including the partial isomeric separation ( $R_s = 0.7$ ) of the two major  $C_{84}$  isomers (see Fig. 4).

Peak assignments were based on mass spectrometric and visible spectroscopic data for each band, and by comparison of the data to published results [33]. Fullerene isomers larger than  $C_{84}$  (i.e.,  $C_{90}$ ,  $C_{94}$ ,  $C_{96}$ ) were not isolated, due to the fact that these

fullerenes are present at very low levels (less than 1% each of the total amount of fullerenes formed) and their retention is so long that band dispersion makes it nearly impossible to observe such small amounts of solutes.

Currently, no other stationary phase has demonstrated the ability to separate higher fullerene isomers with such resolution in a single column pass using such strong mobile phases as 1,2-dichlorobenzene. In comparison, as reported by Kimata et al. [20], even when using a weaker mobile phase (carbon disulfide), only four peaks were resolved ( $C_{60}$ ,  $C_{70}$ ,  $C_{76}$ , and  $C_{84}$ ) from the injection of a higher fullerene extract on a pentabromobenzyl-silica phase.

### 3.3. Characterization of porphyrin-silica phases

The proposed HPTPP-silica stationary phases were first prepared using the route I immobilization procedure shown in Fig. 1. This involved coupling HPTPP to the silica gel substrate via an ether linkage between HPTPP and the epoxide terminus of 3-glycidoxypropyl-silica gel (see Section 2.3). It was hoped that using this immobilization method, TPP-silica phases could be prepared with greater ease (compared to the method employed for linking CPTPP to silica [21]). Also, it was expected that the ether linkage between HPTPP and the silica gel would be more hydrolytically stable than the amide linkage traditionally used to couple CPTPP to silica gel. The change in the length and hydrophobicity of the TPP-silica tether arm (from CPTPP- to HPTPP-silica) was not expected to significantly affect the selectivity of the stationary phase for fullerenes. As shown in Table 3, an HPTPP-silica

Table 3  
Comparison of HPTPP-silica with various blank porphyrin-silica phases<sup>a</sup>

TPP-silica column type	Coverage ( $\mu\text{mol}/\text{m}^2$ )	$k'_{C_{60}}$	$k'_{C_{70}}$	$\alpha_{C_{70}/C_{60}}$	$R_s$	$N(C_{60})$ (plates/m)	$N(C_{70})$ (plates/m)
HPTPP-silica(GPTS to silica first)	0.08	0.87	5.61	6.4	7.7	8 500	7 300
CPTPP-silica	0.19	4.41	21.2	4.8	3.2	3 300	1 000
HPTPP-silica	0.16	7.85	54.8	7.0	11.4	8 300	11 000
TPP-silica(with GPTS)	<0.02	Negligible	Negligible	Negligible	Negligible	—	—
HPTPP on silica(adsorbed—no GPTS)	<0.02	Negligible	0.07	Negligible	0.4	20 000	2 900
HPTPP-silica(using ETMS, not GPTS)	0.11	1.98	11.1	5.6	7.2	7 900	5 200

<sup>a</sup> HPLC conditions: injection of 2  $\mu\text{g}$   $C_{60}$  and  $C_{70}$  each; mobile phase=100% toluene; flow-rate=1.0 ml/min; ambient column temperature.

phase prepared by route I immobilization did demonstrate reasonable fullerene selectivity, with  $\alpha_{C_{70}/C_{60}} = 6.4$  and  $R_s = 7.7$  using 100% toluene as the mobile phase. However, HPTPP–silica phases immobilized via route I typically had very low surface coverages ( $< 0.1 \mu\text{mol}/\text{m}^2$ ) and demonstrated low capacity factors for  $C_{60}$  and  $C_{70}$ . This limited the use of these phases with stronger mobile phases for preparative scale fullerene separations.

In contrast, it was found that immobilization via route II resulted in an HPTPP–silica phase with improved fullerene selectivity and efficiency, and high capacity factors for  $C_{60}$  and  $C_{70}$  (Table 3). To better understand the nature of this HPTPP–silica phase, MS and chromatographic characterization was carried out.

After refluxing the HPTPP with GPTS in DMF for 4 h (see Fig. 1, route II), an aliquot of the porphyrin–silane mixture was removed via syringe and frozen in a dry ice–acetone mixture to stop the reaction process. ESI-MS of this mixture was carried out according to the conditions listed earlier (see Section 2.7). The major peaks in the mass spectrum have  $m/z$  ratios of 631, 647, 835, 1261, and 1466 units.

Proposed structures for several of these species are illustrated in Fig. 5. The 631  $m/z$  (Fig. 5a) and 647  $m/z$  (Fig. 5b) peaks are attributed to starting reagents (monohydroxyTPP, dihydroxyTPP) left over from

the coupling reaction. The 1261  $m/z$  peak is attributed to the associated, but noncovalently bound TPP dimer that is formed in solution. The expected peak ( $m/z$  ratio=867) corresponding to the molecule formed by the reaction of the epoxide group of GPTS with the phenol group of HPTPP was not seen via ESI-MS. Instead the primary products ( $\sim 80$  weight% of total products formed) of the GPTS and HPTPP reaction were found to have molecular masses corresponding to 835  $m/z$  and 1466  $m/z$  units. The 835  $m/z$  peak is proposed to be a product of the reaction of a methoxy group of GPTS with the phenol group of HPTPP (Fig. 5c). The 1466  $m/z$  peak is attributed to the reaction of the epoxide terminus of the 835  $m/z$  species with the phenol group of another HPTPP molecule forming a diporphyrin species (Fig. 5d).

The ESI-MS results were unexpected for two reasons: (1) it was assumed that HPTPP would be more reactive towards the epoxide terminus of GPTS than with the methoxy silane terminus; and (2) the phenol–Si bond has been reported in the literature to have limited hydrolytic stability [34]. Instead, the HPTPP–silica phase prepared by the route II immobilization method was found [35] to be only slightly less hydrolytically stable than the previously reported CPTPP–silica phase. Indeed, HPTPP–silica phases prepared by this technique have been used for

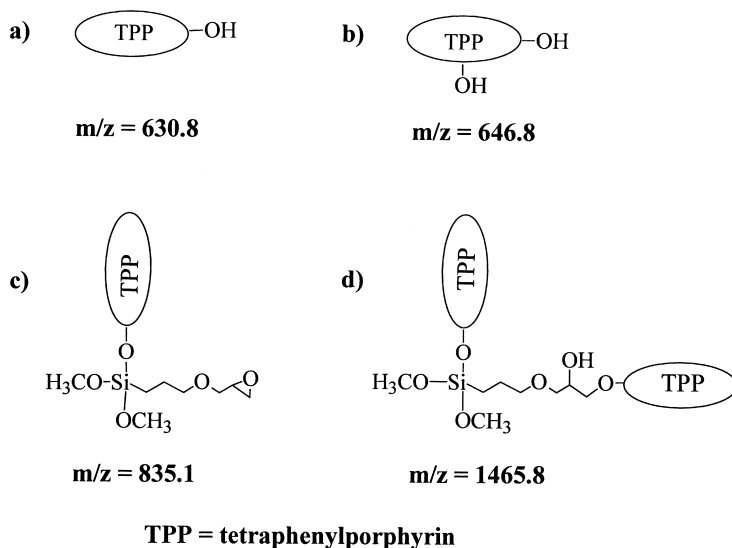


Fig. 5. Proposed structures of HPTPP derivatives present in GPTS–HPTPP reaction mixture used to prepare HPTPP–silica phases.



several hundred hours in this laboratory without significant (i.e., less than 5%) change in fullerene retention times. Although the phenol–Si bond may be chemically labile, steric hindrance via the immobilized TPP is likely aiding in the stability of these phases.

Several analogous HPTPP–silica phases were characterized chromatographically (Table 3) to substantiate the ESI-MS results. First, the immobilization of TPP was attempted via route II (Fig. 1), with the alteration that TPP was used in place of HPTPP. No measurable surface coverage of bonded TPP was achieved as determined by elemental analysis. No peaks with  $m/z$  greater than 615  $m/z$  were found by mass spectrometric analysis of the reaction mixture. These results indicate that the hydroxyl group is vital to the preparation of the HPTPP–silica phase. It also indicates that TPPs are not being covalently bound to the surface via reaction of the epoxide terminus of GPTS with the pyrrolic nitrogens of TPP or HPTPP.

As another “blank”, the route II immobilization procedure was again followed, but without GPTS. After washing, this HPTPP–silica phase was found to have a surface coverage of less than 0.02  $\mu\text{mol}/\text{m}^2$ . Chromatographic characterization of this phase (injection of  $C_{60}$  and  $C_{70}$  in a 100% toluene mobile phase) showed no measurable retention of  $C_{60}$ , and minimal retention of  $C_{70}$ , resulting in  $R_s=0.4$ . It is suspected that the weak retention of  $C_{70}$  is due to noncovalently adsorbed HPTPP on the silica surface.

Another HPTPP–silica “blank” phase was prepared according to the route II immobilization technique, but ETMS was used in place of GPTS. A bonded porphyrin phase with a surface coverage of 0.11  $\mu\text{mol}/\text{m}^2$  resulted. Although slightly lower than the surface coverages found for HPTPP–silica phases, this experiment supports the theory that coupling is occurring via reaction of the phenol group of HPTPP with the methoxy silane terminus of GPTS.

As indicated in Table 3, HPTPP–silica phases made with either GPTS or ETMS demonstrate improved fullerene selectivity over CPTPP–silica phases. Clearly, the method of immobilization plays an important role in achieving high column selectivity. It is proposed that the short tether arm length of HPTPP–silica (for porphyrins immobilized via the phenol–Si bond) is aiding the shape selectivity of

these phases. As the tether arm of HPTPP–silica is four atoms shorter than the tether arm of CPTPP–silica, HPTPP–silica phases should exhibit decreased lateral mobility with respect to CPTPP–silica phases. We propose that solute shape selectivity is increasing with increasing rigidity of the stationary phase, as previously shown by  $C_{18}$ –silica shape selectivity trends for polycyclic aromatic hydrocarbons [36–40]. However, the selectivity and overall retention of HPTPP–silica phases made with ETMS ( $\alpha_{C_{70}/C_{60}} = 5.8$  in toluene) does not match the selectivity of HPTPP–silica phases immobilized via route II ( $\alpha_{C_{70}/C_{60}} = 7.0$  in toluene). The epoxide arm is thus clearly responsible for some of the increased fullerene selectivity. It is possible that the presence of immobilized diporphyrin species (see Fig. 5d) results in the increased ability of the fullerene solute to participate in simultaneous interactions with two bonded TPP molecules. This is quite similar to the previously observed effect of silica pore size on fullerene retention (with smaller pore diameters allowing increased cooperative binding of fullerene solutes by multiple immobilized porphyrins [25]).

The increased efficiency of the HPTPP–silica phase is attributed to improved mass transfer of fullerenes between the mobile phase and the HPTPP–silica stationary phase. This hypothesis is drawn from Knox plots (Fig. 6) of reduced plate height versus reduced velocity for  $C_{60}$  in a mobile phase of 100% toluene on columns packed with CPTPP– or HPTPP–silica. The calculation for reduced velocity requires an estimate of the molecular diffusivity of  $C_{60}$  in toluene. A molecular diffusivity,  $D_m$ , of  $1.95 \cdot 10^{-5} \text{ cm}^2/\text{s}$  for  $C_{60}$  in toluene was calculated as previously described in the literature [29]. As both columns were packed and tested under identical conditions, packing considerations cannot account for the significant difference in the slope of the HPTPP–silica and CPTPP–silica plots. The steeper slope of the column packed with CPTPP–silica indicates a phase that exhibits poorer mass transfer of fullerenes. It appears, therefore, that fullerene mass transfer is strongly dependent on the overall thickness of the TPP–silica phase. With the shorter tether arm of HPTPP–silica, it is likely that the HPTPP–silica stationary phase has a smaller mean thickness compared to the CPTPP–silica phase. This reduces the partitioning distance that is

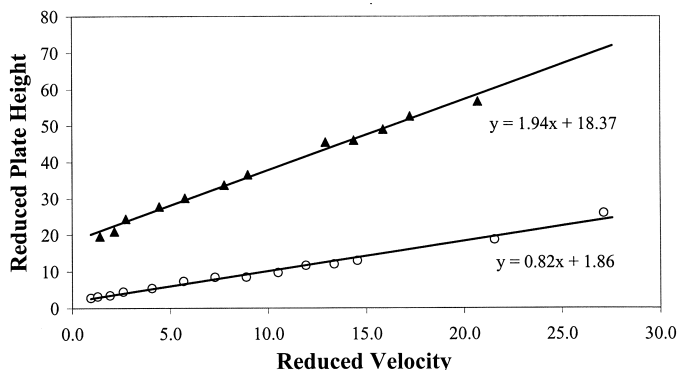


Fig. 6. Knox plot for CPTPP–silica (▲) and HPTPP–silica (○). Results were obtained by the injection of 0.4  $\mu\text{g}$  of  $\text{C}_{60}$  on a  $100 \times 4.6$  mm column packed with TPP–silica; mobile phase = 100% toluene; detection wavelength = 410 nm; ambient column temperature.

required for fullerene interaction with HPTPP–silica. This, in turn, improves mass transfer and yields improved peak efficiencies for HPTPP–silica phases.

#### 4. Conclusions

TPP–silica phases prepared by the new immobilization technique have demonstrated unmatched selectivity for the separation of fullerenes in a variety of strong solvents, with  $\alpha_{\text{C}_{70}/\text{C}_{60}} = 7.0$  on HPTPP–silica using 100% toluene as the mobile phase. The high selectivity of HPTPP–silica phases and the ability to use columns packed with HPTPP–silica with mobile phase solvents in which the fullerenes are most soluble should make the use of larger columns containing HPTPP–silica in the preparative scale of fullerenes very attractive.

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