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<th>Title</th>
<th>High-performance liquid chromatography separation of unsaturated organic compounds by a monolithic silica column embedded with silver nanoparticles.</th>
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<td>Author(s)</td>
<td>Zhu, Yang; Morisato, Kei; Hasegawa, George; Moitra, Nirmalya; Kiyomura, Tsutomu; Kurata, Hiroki; Kanamori, Kazuyoshi; Nakanishi, Kazuki</td>
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Kyoto University
HPLC Separation of Unsaturated Organic Compounds by Ag Nanoparticles Embedded Monolithic Silica Column

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Abbreviations: NPs, nanoparticles; PAHs, polyaromatic hydrocarbons; RP, reversed phase; PMSM, periodic mesoporous silica monolith; TEOS, tetramethoxysilane; PEO, poly(ethylene oxide); APTES, (3-aminopropyl)triethoxysilane; SEM, scanning electron microscopy; FE-SEM, field emission scanning electron microscopy; TEM, transmittance electron microscopy; XRD, X-ray diffraction; TG-DTA, thermogravimetry-differential thermal analysis; FT-IR, Fourier transform infrared spectroscopy; XPS, X-ray photoelectron spectroscopy; h, reduced plate height.

Key words: Ag nanoparticles, Cis/trans isomers, High-performance liquid chromatography, Monolithic silica column, Polyaromatic hydrocarbons
Abstract

The optimization of porous structure which ensures good separation performances is always a significant issue in HPLC column design. Recently we reported the homogeneous embedment of Ag nanoparticles in periodic mesoporous silica monolith and the application of such Ag nanoparticles embedded silica monolith for HPLC separation of polyaromatic hydrocarbons. However, the separation performance remains to be improved and the retention mechanism as compared with Ag ion HPLC technique, still needs to be clarified. In this research, Ag nanoparticles were introduced into a macro/mesoporous silica monolith with optimized pore parameters for HPLC separations. Baseline separation of benzene, naphthalene, anthracene and pyrene was achieved with the theoretical plate number for analyte naphthalene as 36000 m$^{-1}$. Its separation function was further extended to cis/trans isomers of aromatic compounds where cis/trans stilbenes were chosen as a benchmark. Good separation of cis/trans stilbene with separation factor as 7 and theoretical plate number as 76000 m$^{-1}$ for cis-stilbene was obtained. The trans isomer, however retains stronger which contradicts the long established retention rule of Ag ion chromatography. Such behavior of Ag nanoparticles embedded silica column can be attributed to the differences in the molecular geometric configuration of cis/trans stilbenes.


1 Introduction

Applicability of so-called nanomaterials in separation science has been proven and demonstrated for these decades. Silica nanoparticles (NPs) are the first nanomaterial to be utilized either as the stationary phase or a modifier supported by silica or other matrices for chromatography techniques [1-4]. In recent years, other inorganic nanomaterials (zirconia, titania, alumina, iron oxide, hydroxyapatite, carbon-based material) and some polymer nanobeads supported by silica or polymeric stationary phase were also applied to different chromatography techniques including gas chromatography [5,6], capillary liquid chromatography [7-11] and capillary electrophoresis [12-14].

Noble metal NPs, as an extended important category among all the nanomaterials, have found their applications in different fields. Among them, gold NPs, due to its well-known affinity towards amino- and thiol-containing molecules, were immobilized on stationary phase and used for chromatographic separations. Yang et al. embedded gold NPs in a fused-silica capillary column and achieved efficient separation of polyaromatic hydrocarbons (PAHs) under an open-tubular CEC condition [15]. Recently Svec et al. attached gold NPs on porous polymer monoliths in a capillary and carried out the separation of molecules such as cysteine-containing peptides in acetonitrile or water as the mobile phase [16] and nucleosides under the HILIC mode [17]. Meanwhile, Liang et al. immobilized gold NPs on a graphene oxide/silica composite stationary phase and demonstrated the separation of alkylbenzenes, benzenediol isomerides, amino acids, nucleosides and nucleobases in both reversed-phase (RP) mode and HILIC mode with good separation performance [18]. Each aforementioned research showed high potential of gold NPs for chromatographic separations of different molecules under different conditions.

Separation of lipids by silver ion chromatography has been scientifically studied and industrially applied for decades [19,20]. Only a limited number of researches, however, have been reported on the application of metallic Ag NPs for separations [21]. Recently we, for the first time, reported a method of homogeneous immobilization of Ag NPs in a macroporous silica monolith embedded with SBA-15 type periodic mesopores.
(denoted as periodic mesoporous silica monolith: PMSM), which was further used for HPLC separation of PAHs. Similar retention characteristics for unsaturated compounds were shown as compared with silver ion chromatography [20,22]. Nevertheless, the separation performance was relatively poor (no baseline separation for benzene, naphthalene and anthracene when injected together and separation factor 0.43 for anthracene) even though the Ag loading in PMSM was relatively high (32 mg per column). The inadequacy of separation efficiency is mainly attributed to characteristic pore structure of the host silica, undesirable for HPLC separation. On the macropore scale, the pressure-driven liquid flow through polygonal-shaped macroporous channels of PMSM potentially generates a more inhomogeneous flow profile compared to the Poiseuille-type one, which is undesirable for high efficiency separations [23]. Meanwhile on the mesopore scale, relatively small-sized mesopores (average 9 nm) become even smaller (average 7 nm) after the embedment of Ag NPs, which limits the accessibility of analyte molecules to the NPs.

In this research, aiming at improved separation performance of Ag NPs/silica HPLC columns, a silica column with improved porous structure for HPLC separation was chosen as a host. After the introduction of Ag NPs, the resultant monolith has been made into a HPLC column and subjected to the separation of PAHs as well as cis/trans stilbene isomers. Good baseline separation for both PAHs and cis/trans stilbene isomers were achieved with the theoretical plate number reaching 76000 m⁻¹ for the analyte cis stilbene and the separation factor as high as 7 for cis/trans stilbene isomers. The better separation performance with less Ag loading (25 mg per column) was explained by the synergic effect from host silica monolith with optimized porous structure. An opposite result of the elution order of cis and trans stilbenes as compared with that of silver ion chromatography was observed in Ag NPs embedded silica column. Such phenomenon is mainly resulted from the more planar molecular geometry of trans stilbene than its cis isomer.

2 Materials and Methods

2.1 Materials

As the silica source, tetramethoxysilane (TMOS) was purchased from Shin-Etsu Chemical Co. Ltd. (Japan). Poly(ethylene oxide) (PEO, Mw = 10000) as the phase separation inducer for the preparation of monolithic
silica, (3-aminopropyl)triethoxysilane (APTES), silver nitrate, naphthalene, anthracene, phenanthrene, 
cis-stilbene and trans-stilbene were obtained from Sigma-Aldrich Co. LLC (USA). Urea and distilled water
were purchased from Hayashi Pure Chemical Ind., Ltd. (Japan) and Wako Pure Chemical Ltd. (Japan),
respectively. All the other chemicals were from Kishida Chemical Co., Ltd. (Japan). All the chemicals were
of analytical grade and used without further purification.

2.2 Preparation of monolithic silica gel and Ag NPs embedded silica gel

Monolithic silica bearing continuous macro pores and size-expanded mesopores was prepared by our
previously reported procedure [24]. The introduction of Ag NPs homogeneously into monolithic silica
follows the method reported in one of our recent literatures with some modifications [22]. In a typical run,
0.60 g of silica monolith was immersed into 20 mL of 170 mM APTES/toluene solution in a sealed amber tube
and left for reaction at 80 °C for 24 h for the modification of silica surface with aminopropyl groups. The
modified monolith was later washed with excess amount of ethanol at 50 °C. The obtained monolith was
then immersed in 20 mL of 50 mM AgNO₃/ethanol solution at room temperature for the immobilization of Ag
ion on aminopropyl groups and in-situ reduction by ethanol, which acts both as the solvent and the reductant.
Since the silica monolith used in the present study has higher density than the one used in the previous study,
in order to achieve higher loading of Ag NPs, the reaction time of AgNO₃ solution with the
aminopropyl-modified silica was elongated to 14 d, as compared with 4 d in the previous report. The color of
the monolith turned from white to black due to the immobilization of Ag NPs on the skeleton surface. The
resultant monolith was then washed with sufficient amount of ethanol and the total removal of Ag ion in the
monolith was confirmed by the absence of formation of AgCl precipitate after adding 0.1 M HCl aqueous
solution into the ethanol obtained after washing for several times. The monolith was then dried at 60 °C for
more than 3 days and was denoted as SiO₂-NH₂-Ag. As a comparison, pure silica monolith and silica
monolith modified with aminopropyl groups were synthesized as well and denoted as SiO₂ and SiO₂-NH₂,
respectively and those derivatives of PMSM were denoted as PMSM-NH₂, PMSM-NH₂-Ag, among which, the
Ag loading in PMSM-NH₂-Ag was reached by repeated treatment for 3 times [22].
2.3 Characterizations

Macroscopic and mesoscopic morphologies of the monolithic samples were confirmed by scanning electron microscopy (SEM, JSM-6060S, JEOL, Japan) and field emission scanning electron microscopy (FE-SEM, JSM-6700F, JEOL, Japan). Meso- and microstructure of the samples were characterized by nitrogen adsorption-desorption (BELSORP-mini II, Bel Japan Inc., Japan). The size and morphology of Ag NPs embedded in the silica monolith were investigated by transmittance electron microscopy (TEM, JEM-2200FS, JEOL, Japan) equipped with a spherical aberration corrector (CETCOR, CEOS GmbH, Germany). The crystal structure was confirmed by powder X-ray diffraction (XRD, RINT Ultima III, Rigaku Co., Japan) using Cu Kα (λ = 0.154 nm) as an incident beam. Thermal properties of the samples were investigated by thermogravimetry-differential thermal analysis (TG-DTA, Thermo plus TG 8120, Rigaku Co., Japan) with a continuous air supply at 100 mL/min. Fourier transform infrared spectroscopy (FT-IR, IR Affinity-1, Shimadzu Co., Japan) and X-ray photoelectron spectroscopy (XPS, MT-5500, ULVAC-PHI Inc. Japan) were performed to confirm the molecular and atomic level information. Since nitric acid was formed as a side product and most of it further reacted with aminopropyl groups on the silica surface via acid-base reaction to form -NH₃⁺NO₃⁻ ion pairs [21], the Ag loading in the monolith was calculated from the weight of reacted AgNO₃ by weighing the dried samples before and after the reduction process.

2.4 Chromatographic measurements

Liquid chromatographic evaluations were carried out using an ordinary HPLC system with a pump (PU712, GL Sciences, Japan), a UV detector (MU701, GL Sciences, Japan), a data processor (EZ Chrom Elite Chromatography Data System, Agilent Technologies, USA), and a Rheodyne injector (8125, Rheodyne, USA). The system was operated in an isocratic mode at room temperature (~ 25 °C). For the separation of PAHs, 1.6 µL of benzene, 0.8 mg of naphthalene, 0.2 mg of anthracene, and 0.5 mg of phenanthrene were dissolved separately in 1 mL of n-hexane as the standard samples for columns made from SiO₂ and SiO₂-NH₂ monoliths. For columns made from SiO₂-NH₂-Ag monolith, all the compounds were dissolved together in n-hexane. The injection volume was 1 µL in each case.
3 Results and Discussion

3.1 Synthesis of Ag NPs embedded silica monolith

Co-continuous macropores (about 3 µm in size) together with discrete mesopores in the continuous skeletons were identified in all the samples, showing that both the macroscopic and mesoscopic morphologies did not change after surface modification with aminopropyl groups and/or the introduction of Ag NPs (Figure 1). The successful surface modification of silica surface with the aminopropyl groups is confirmed by TG-DTA (Figure S1A) with an explicit exothermic peak between 300 °C and 400 °C on the DTA curve of SiO₂-NH₂ together with the weight loss in the same temperature region on the corresponding TG curve. Such thermal behavior is attributed to the pyrolysis of the aminopropyl groups grafted on the surface of silica. Instead of one-step weight loss, two-step weight loss and their corresponding exothermic behavior, however, were observed in the TG-DTA curves of SiO₂-NH₂-Ag. The weight loss at higher temperature range (250 °C to 400 °C) corresponds to the pyrolysis of aminopropyl groups, while the one observed at lower temperature range (200 °C to 250 °C) could be attributed to decomposition of -NH₃⁺NO₃⁻ ion pairs. A similar result was also observed from the FT-IR measurement (Figure S1B). The spectrum of SiO₂-NH₂-Ag shows a pronounced IR absorption peak at 1385 cm⁻¹, which is commonly observed in the samples containing ionic nitrate [25-27]. After the reduction of Ag⁺ by ethanol, HNO₃ as a side product further reacts with the basic -NH₂ groups on the silica surface, which leads to the formation of -NH₃⁺NO₃⁻ ion pairs. The consumption of HNO₃ then further promotes the reaction to occur towards the reduction of AgNO₃ and formation of Ag NPs.

The presence of metallic silver in the monolith is confirmed by the XRD pattern of SiO₂-NH₂-Ag (Figure S2). Further atomic information on the surface of the monolith was revealed by XPS measurement. Figure S3 shows the XPS spectrum of SiO₂-NH₂-Ag in the Ag 3d binding energy region. The Ag 3d₅/₂ and Ag 3d₃/₂ peaks are centered at 367.8 eV and 373.8 eV, respectively, with a spin energy separation of 6.0 eV, which are the characteristics of metallic silver [28]. Peaks corresponding to ionic silver were undetectable in the XPS spectrum of SiO₂-NH₂-Ag. The whole XPS spectrum was shown in the supporting information (Figure S4).

The distribution and morphology of Ag NPs embedded in the monolith was observed by TEM (Figure 2). Except for the particles attached on the surface of the macropore skeletons which grows to more than 50 nm
due to the absence of spacial hinderance, most of the Ag NPs are homogeneously located in the mesopores with the average particle size as 2 nm. After surface modification, the aminopropyl groups on the silica skeletons act as the nucleation sites for the growth of Ag NPs. Since most of the surface area is contributed from mesopores and micropores, most of the aminopropyl groups are deduced to be grafted on the surface of meso or micropores. Most of the Ag NPs were therefore grown in these nanometer-sized pores. Although the diffusion-limited parameters for NPs growth became less significant when the reaction time is extended to 14 d, the extremely slow reaction kinetics due to the weak reduction activity of ethanol at room temperature during the reaction process led to the limited growth Ag NPs.

A decrease of adsorbed N₂ volume was observed from N₂ adsorption-desorption isotherms after surface modification with APTES and further immobilization of Ag NPs, giving rise to the reduction of pore volume and BET specific surface area in both the silica used in this research and PMSM (Figure 3 and Table 1). These results are consistent with other results indicating the successful surface modification with aminopropyl groups and the immobilization of Ag NPs thereafter. Average mesopore size remained similar due to big-sized mesopores (24 nm) and small-sized Ag NPs (2 nm) in the mesopores (Figure 2B, Figure 3B and Table 1), which is in accordance with the results from FE-SEM observations (Figure 1D, E, F). However, as PMSM was used as the host silica, due to its periodically aligned cylindrical mesoporous structure and smaller mesopore size, when large amount of Ag NPs was embedded into the mesopores, mesoscale space was easily blocked leading to significant decrease of BET surface area, total pore volume and mesopore size (Figure 3C, D, Table 1).

3.2 Chromatographic separation of aromatic compounds

In our previous report [22], PMSM embedded with Ag NPs showed high potential for the separation of small unsaturated molecules such as polyaromatic hydrocarbons (PAHs), though elution bands with relatively large band broadening and no baseline separation of a mixture of PAHs with different number of aromatic rings was obtained. The improvement of overall separation performance can be expected by the optimization of the pore structure of the host silica monolith as mentioned earlier. The results for the separation of PAHs are shown in Figure 4. Only small differences in retention time among four different compounds (benzene,
naphthalene, anthracene and pyrene) were observed in the cases of SiO\(_2\) and SiO\(_2\)-NH\(_2\) monolithic columns as can be seen in Figure 4A and B, possibly due to the differences in size of the compounds. The retention is greatly enhanced when Ag NPs are introduced onto the surface of silica monolith. The elution order is in accordance with the extent of unsaturation in the analyte molecules; the more unsaturated the molecule is, the stronger the interaction between the molecule and Ag NPs is, and the longer the retention time is. Baseline separation of four compounds was achieved with only 25 mg per column loading of Ag in the silica monolith (as compared with 32 mg per column in PMSM), giving the separation factor for anthracene as 0.74 (0.43 for the column of PMSM-NH\(_2\)-Ag) and theoretical plate number of 36000 m\(^{-1}\) for the retained analyte naphthalene (Table 2). This result clearly indicates that host silica plays a synergic role on the retention behavior and overall separation performance of Ag NPs embedded silica column. Generally reduced plate height is used as a term to compare the intrinsic properties of the porous system for separation and it is given as \(h = \frac{\text{theoretical plate height}}{\text{skeleton diameter}}\) \([29,30]\). Though the theoretical plate number for retained analyte anthracene is 23000 m\(^{-1}\) in SiO\(_2\)-NH\(_2\)-Ag column, smaller than that of PMSM-NH\(_2\)-Ag column (30000 m\(^{-1}\)), the h value of SiO\(_2\)-NH\(_2\)-Ag column is 21.5, much smaller than \(h = 166.7\) in the case of PMSM-NH\(_2\)-Ag (the average skeleton diameter as 2 \(\mu\)m and 0.2 \(\mu\)m, respectively for both systems, showing that the SiO\(_2\)-NH\(_2\)-Ag column is intrinsically much more superior than PMSM-NH\(_2\)-Ag for HPLC separation. The increase of mesopore size and change of mesopore shape from cylindrical periodically aligned mesopore to random cylindrical mesopores improve mesopore interconnectivity, which thus accelerates mass transfer in the porous zone making it easier for analyte molecules to access Ag NPs inside the mesopores leading to longer retention time and less band broadening (smaller h value). As a result, with lower Ag NPs loading (25 mg per column), baseline separation of PAHs with higher separation factor (0.74 for analyte anthracene as compared with 0.43 at Ag loading 32 mg per column in PMSM) was achieved. Such results demonstrate the full capability of Ag NPs embedded silica for the separation of PAHs in normal phase mode and the possibility to improve separation performance simply by optimization of the pore structure of host silica.

Stilbenes and their derivatives are commonly found in grapes and other agricultural products. In 1992, epidemiological studies revealed a paradoxical conclusion that polyphenolic components such as phytoalexin
resveratrol in red wine contributes to low incidence of coronary heart disease, known as French paradox [31,32]. Although the detailed mechanism has not yet been totally elucidated, the study of stilbene derivatives, including the effective separation of these compounds, aroused enormous interest among researchers from different research areas [33-37]. Due to the interaction between Ag NPs and unsaturated bonds as has been demonstrated above, there is a high chance that stilbenes and their derivatives can also be separated by Ag NPs embedded silica column. Comparison of separation performance of Ag NPs embedded silica column with pure silica and one modified with aminopropyl groups for cis and trans stilbene is shown Figure 5. The Ag NPs embedded silica column shows a reasonable baseline separation of cis and trans isomers of stilbene, while this could not be achieved by pure silica or aminopropyl modified silica columns, which indicates that both silanols and aminopropyl groups on silica surface have weaker interactions with unsaturated bonds than Ag NPs. Retention time for both cis and trans stilbene is extended with the presence of Ag NPs in silica monolith, presumably due to the Ag⁺-like affinity caused by the charge separation on Ag NPs when electron-rich unsaturated aromatic compounds are in contact with Ag NPs [38]. Improved mesoporous structure here ensures better access of analytes to the Ag NPs formed inside the skeletons, which leads to less band broadening and relatively high theoretical plate number. Calculated values of retention factor and theoretical plate number are shown in Table 3. The theoretical plate number for the retained analytes cis stilbene and trans stilbene are 76000 m⁻¹ and 38000 m⁻¹, respectively, and the separation factor is 7, showing much higher separation efficiency for aromatic cis and trans isomers in the normal-phase mode than that of reported columns (18000 m⁻¹ as the theoretical plate number for the retained analyte cis stilbene and 1.97 as the separation factor by the HPLC column packed with silica gel modified with poly(octadecyl acrylate) [33] and 3.2 as the separation factor for the HPLC column packed with silica gel modified with poly(acrylonitrile) [34]).

One retention rule that has been established through decades of experiences in silver ion HPLC is that cis isomers are always retained stronger than trans isomers, due to the relatively stronger charge transfer complexes formed between unsaturated bonds in cis isomers and Ag⁺ [20]. However, the contradictory result in Figure 5 shows that trans stilbene is retained stronger than cis stilbene in all the cases, including pure silica
and aminopropyl modified silica as well as Ag NPs embedded silica columns. The geometric difference between cis stilbene and trans stilbene is considered as the main contributor to the appearance of such elution order. According to the reports by Ihara et al., similar retention order of cis and trans stilbene isomers was observed from the separation results by silica gels modified with poly(octadecyl acrylate) [33] or poly(acrylonitrile) [34]. Immobilized polymer chains tend to undergo a phase transition between crystal and isotropic states at low temperature. A planar compound such as trans stilbene can be easily incorporated with the highly oriented polymer chain while the sterically more bulky cis stilbene is geometrically more difficult to interact with the polymer chain, which thus elutes earlier than trans stilbene [33]. Similarly crystalline Ag NPs embedded on silica interact with electron-rich molecules as a surface rather than a point in the case of Ag⁺. Consequently trans stilbene with geometrically more accessible π electrons interacts stronger with Ag NPs leading to a separation at the baseline with a separation factor of 7 for cis/trans stilbene isomers (Table 3). Such results indicate the high potential of Ag NPs embedded silica column for not only the enhanced separation of unsaturated organic compounds with different unsaturaties but also the recognition of geometrical isomers with the same unsaturaty.

4 Concluding remarks

Ag NPs have been successfully introduced into hierarchically porous silica monolith with optimized porous structure for HPLC separation of unsaturated organic compounds. Baseline separation of PAHs (benzene, naphthalene, anthracene and pyrene) with the theoretical plate number for naphthalene to be 36000 m⁻¹ and retention factor for anthracene to be 0.74 was achieved with Ag loading as 25 mg per column. Such enhanced separation performance with less Ag loading (32 mg per column in PMSM) was explained by the better accessibility of analytes to Ag NPs as retention sites in the improved mesopores. Outstanding separation of cis/trans stilbene isomers with theoretical plate number to be 76000 m⁻¹ and 38000 m⁻¹, respectively, and separation factor as 7 was obtained. However the retention of trans stilbene was longer than the cis isomer which was contradictory to the long established retention rules of Ag⁺ HPLC. Such separation behavior was attributed to the more planar molecular geometry of trans stilbene, which provides more insights into the detailed separation mechanism of unsaturated compounds by Ag NPs embedded silica column.
Acknowledgements

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The authors have declared no conflict of interest.

References

Table 1 Pore characteristics of the monoliths SiO\textsubscript{2}, SiO\textsubscript{2}-NH\textsubscript{2}, SiO\textsubscript{2}-NH\textsubscript{2}-Ag and PMSM, PMSM-NH\textsubscript{2}, PMSM-NH\textsubscript{2}-Ag for column preparation calculated from N\textsubscript{2} adsorption desorption isotherms.

<table>
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<th>Sample</th>
<th>S\textsubscript{BET} (m\textsuperscript{2} g\textsuperscript{-1})</th>
<th>bS\textsubscript{BET} in column (m\textsuperscript{2})</th>
<th>V\textsubscript{p} (cm\textsuperscript{3} g\textsuperscript{-1})</th>
<th>D\textsubscript{p} (nm)</th>
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<td>SiO\textsubscript{2}</td>
<td>230</td>
<td>85</td>
<td>0.94</td>
<td>24.4</td>
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<td>74</td>
<td>0.77</td>
<td>21.3</td>
</tr>
<tr>
<td>SiO\textsubscript{2}-NH\textsubscript{2}-Ag</td>
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<td>72</td>
<td>0.69</td>
<td>24.4</td>
</tr>
<tr>
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<td>0.81</td>
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<tr>
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<td>50</td>
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<td>8.1</td>
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<tr>
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<td>140</td>
<td>29</td>
<td>0.29</td>
<td>7.1</td>
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</tbody>
</table>

\(a\) PMSM was calcined at 800 °C while the rest of the monoliths were calcined at 600 °C.

\(b\) monoliths for column preparation are all in cylinder shape (83 mm in length and 4.6 mm in diameter) with their weight to be 0.37 g, 0.41 g, 0.45 g and 0.14 g, 0.16 g, 0.21 g for SiO\textsubscript{2}, SiO\textsubscript{2}-NH\textsubscript{2}, SiO\textsubscript{2}-NH\textsubscript{2}-Ag and PMSM, PMSM-NH\textsubscript{2}, PMSM-NH\textsubscript{2}-Ag, respectively.

Table 2 Calculated values of retention factor, theoretical plate number for aromatic hydrocarbons separated by SiO\textsubscript{2}-NH\textsubscript{2}-Ag monolithic column.

<table>
<thead>
<tr>
<th></th>
<th>(a k)</th>
<th>(b N (m))</th>
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<tr>
<td>Benzene</td>
<td>0</td>
<td>33000</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.20</td>
<td>36000</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.74</td>
<td>23000</td>
</tr>
<tr>
<td>Pyrene</td>
<td>1.1</td>
<td>21000</td>
</tr>
</tbody>
</table>

\(a\) Retention factor calculated as (\(t - t_0\) / \(t_0\)) where \(t\) is the elution time of naphthalene, anthracene or pyrene and \(t_0\) is the elution time of benzene (due to the lack of proper \(t_0\) sample).

\(b\) Theoretical plate number calculated as 5.54 (\(t / w_{0.5}\)), where \(t\) and \(w_{0.5}\) are the retention time and band width at half height.
Table 3 Calculated values of retention factor, theoretical plate number for cis- and trans-stilbene separated by SiO2-NH2-Ag monolithic column.

<table>
<thead>
<tr>
<th></th>
<th>$k$</th>
<th>$N$ (m)</th>
<th>$^a\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-stilbene</td>
<td>0.05</td>
<td>76000</td>
<td>7</td>
</tr>
<tr>
<td>trans-stilbene</td>
<td>0.35</td>
<td>38000</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Separation factor calculated as $k_t/k_c$, where $k_t$ and $k_c$ are the retention factors of trans-stilbene and cis-stilbene.

Figure 1 SEM and FE-SEM images of the monoliths: SiO$_2$ (A, D), SiO$_2$-NH$_2$ (B, E) and SiO$_2$-NH$_2$-Ag (C, F).
**Figure 2** TEM image of SiO$_2$-NH$_2$-Ag (A) and the magnified image at the part pointed out by the red frame (B).

**Figure 3** Nitrogen adsorption desorption isotherms and BJH pore size distributions calculated from the adsorption branch of the monoliths SiO$_2$, SiO$_2$-NH$_2$, SiO$_2$-NH$_2$-Ag (A, B) and PMSM, PMSM-NH$_2$, PMSM-NH$_2$-Ag (C, D).
Figure 4 Chromatograms of benzene (1), naphthalene (2), anthracene (3) and pyrene (4), using monolithic SiO$_2$ (A), SiO$_2$-NH$_2$ (B) and SiO$_2$-NH$_2$-Ag (C) columns. Conditions: mobile phase 0.2% acetonitrile / hexane, flow rate 1.0 mL/min, detector UV254 nm, pressure 2.1 MPa, column length 83 mm, temperature 25 °C.
**Figure 5** Chromatograms of *cis*-stilbene (1) and *trans*-stilbene (2), using monolithic SiO₂ (A), SiO₂-NH₂ (B) and SiO₂-NH₂-Ag (C) columns. Conditions: mobile phase 0.2% acetonitrile / hexane, flow rate 1.0 mL/min, detector UV254 nm, pressure 1.5 MPa, column length 83 mm, and temperature 25 °C.
HPLC Separation of Unsaturated Organic Compounds by Ag NPs embedded Silica Column

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Figure S1 TG-DTA curves (A) and FT-IR spectra (B) and of monoliths: SiO2, SiO2-NH2, SiO2-NH2-Ag.

Figure S2 XRD pattern of SiO2-NH2-Ag.
Figure S3 XPS spectrum of Ag 3d for SiO2-NH2-Ag.

Figure S4 Full XPS spectrum of monolith: SiO2-NH2-Ag.