Extraction Methods

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Graphene and Graphene Oxide Sheets Supported on Silica as Versatile and High-Performance Adsorbents for Solid-Phase Extraction**

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Graphene (G), a monolayer of carbon atoms densely packed into a two-dimensional honeycomb crystal lattice, has recently sparked much research interest.[1] It combines unique electronic properties and intriguing quantum effects with exceptional thermal and mechanical properties.[2] Notably, G is a double-sided polyaromatic scaffold with an ultrahigh specific surface area (theoretical value 2630 $m^2 g^{-1}$ [3]), making it a promising candidate for sorption material with high loading capacity. Its large delocalized π-electron system also endows G a strong affinity for carbon-based ring structures, which are widely present in drugs, pollutants, and biomolecules.[4]

Solid-phase extraction (SPE) is a powerful tool to preconcentrate and purify analytes of interest from a great variety of sample matrices.[5] Considering the superior properties and high chemical stability of G, it thus may serve as a good adsorbent for SPE. Notably, G is usually considered to be non-polar and hydrophobic. Graphene oxide (GO), in contrast, contains much more polar moieties, such as hydroxy, expoxy, and carboxy groups,[1c] and thus has a more polar and hydrophilic character than G (as depicted in Figure 1 A). The intrinsic properties of G and GO therefore might render them superior qualities as adsorbents for reversed-phase (RP) and normal-phase (NP) SPE, respectively. The extraction can be carried out with a SPE cartridge/column,[6] or simply by dispersing G or GO sheets in sample solution followed by collecting the analyte-adsorbed G or GO sheets by centrifugation.[7] The extraction can also be assisted by aptamer or magnetic particles.[8] However, the direct use of G or GO as SPE adsorbents has several problems. Firstly, irreversible aggregation of G or GO sheets may occur during isolation from a homogeneous solution, such as filtration and centrifugation. The aggregation may reduce the sorption capacity of the adsorbent and hinder effective adsorption and elution of analytes. Secondly, G and GO sheets are polydisperse in their thickness, lateral size, and shape.[9] Thus, there still is a concern that miniscule G or GO sheets may escape from the SPE cartridge/column, especially under high pressure in on-line SPE systems. For GO, its solubility in many solvents may aggravate the adsorbent loss in cartridge/column format. Furthermore, it is also difficult to completely collect the miniscule G or GO sheets from a well-dispersed solution even by high-speed centrifugation.

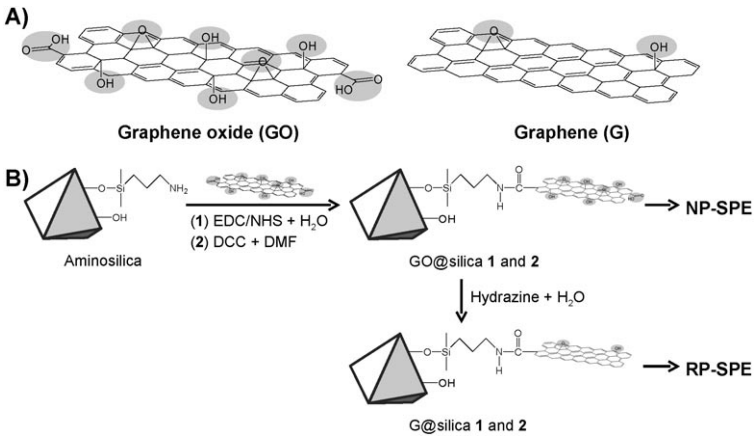

Figure 1. A) Models of GO and G sheets. The shadowed sections indicate the polar groups in the GO and G sheets. B) Chemical routes to the synthesis of GO@silica and G@silica. NP-SPE = normal-phase SPE, RP-SPE = reversed-phase SPE

To avoid the above-mentioned problems and still maintain the advantageous properties, we developed new SPE adsorbents by covalently binding G and GO sheets to silica. GO was synthesized by a modified Hummers method.[10] To prepare GO bound silica (GO@silica), the carboxy groups of GO were linked to the amino groups of an amino-terminated silica adsorbent (average particle size 45 μm). Then, the G bound silica (G@silica) was obtained by hydrazine reduction of GO@silica. The overall procedure is shown in Figure 1 B. Two methods were compared to immobilize GO sheets on the aminosilica. Considering that GO can be well dispersed in water, we tried an aqueous synthesis method with EDC/NHS (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride/*N*-hydroxysuccinimide) as a coupling agent (approach 1). The products were denoted as GO@silica **1** and G@silica **1**. However, we found that the concentration of EDC must be kept below 0.1 mM in the aqueous phase; otherwise aggregation of GO sheets will be observed. A similar phenomenon was also observed elsewhere.[11] Therefore, we also attempted an organic phase synthesis method with *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling agent in dimethylformamide (DMF) (approach 2). In this

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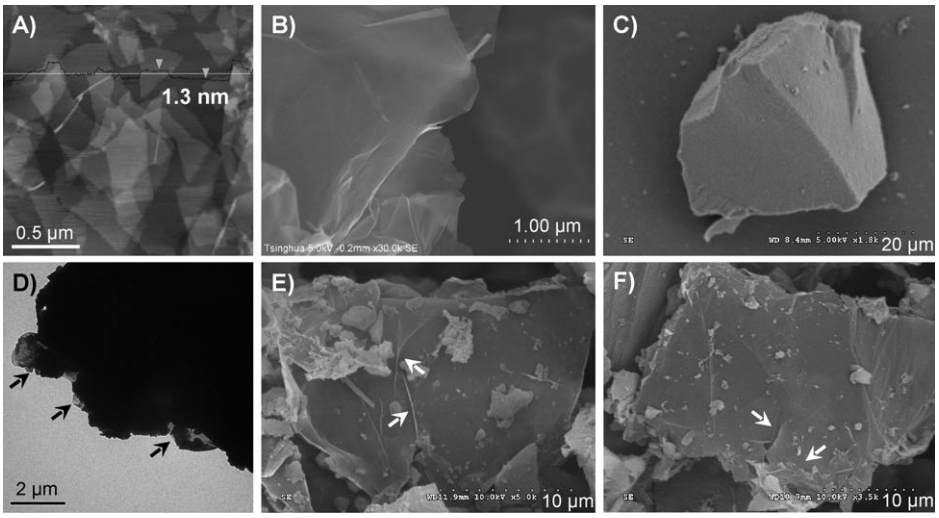

Figure 2. A) AFM image of GO sheets with a height profile. The two arrows in the height profile indicate a thickness of 1.3 nm for a GO sheet. B) High-resolution SEM image of GO sheets. C) SEM image of a bare silica particle. D) TEM image of a GO@silica particle. E,F) SEM images of GO@silica **1** (E) and G@silica **1** (F).

approach, a higher concentration of coupling agent could be employed, and the products are denoted as GO@silica **2** and G@silica **2**.

The newly synthesized adsorbents were characterized by different techniques. As shown in Figure 2A, the AFM image indicates that the prepared GO sheets were single-layered with the lateral size ranging from dozens of nanometers to several micrometers. Figure 2B shows an SEM image of a semitransparent GO sheet, also suggesting its single-layer nature. Figure 2C shows an SEM image of a bare aminosilica particle. It can be seen that the bare aminosilica particle has an irregular shape and a clear and smooth surface. Figure 2D is a typical TEM image of GO@silica **1**. After immobilization of GO, the aminosilica particles were encapsulated by GO flakes, as indicated by the arrows in Figure 2D. It can also be clearly observed in the SEM image (Figure 2E) that GO@silica **1** is tightly covered by the corrugated GO flakes. The arrows indicate the wrinkles formed by the GO flakes on the silica surface. After chemical reduction by hydrazine, no significant change in SEM image was observed for G@silica **1** (Figure 2F). The G maintains its morphology of nanosheet as indicated by the arrows. The SEM images of GO@silica **2** and G@silica **2** are given in the Supporting Information Figure S1, which are similar to Figure 2E,F, indicating that both synthesis methods can successfully graft GO and G onto the silica surface. The immobilization of GO and G on the silica was also confirmed and characterized by FTIR spectroscopy, EDX spectroscopy, XPS, and XRD analysis (Figures S2–S5 in the Supporting Information). The BET measurements revealed high specific surface areas for GO@silica and G@silica (292.2–326.4 $m^2 g^{-1}$, see Table S1 in the Supporting Information). These values are also higher than that previously reported for fullerene bound silica.[12] Specifically, the specific surface areas of adsorbents **2** are slightly higher than those of adsorbents **1**, suggesting that the coupling reaction was more efficient in the organic phase than in the aqueous phase. This result was also supported by elemental analysis. From the carbon content of the adsorbents, the amounts of immobilized GO or G on aminosilica can be estimated to be 0.98–5.00% (Table S1). The carbon content of the adsorbents synthesized by approach 2 is evidently higher than that of those synthesized by approach 1, showing that more GO or G sheets were immobilized onto the aminosilica surface in organic media.

We first tested the analytical performance of G@silica in RP-SPE. RP-SPE involves a polar sample matrix (usually water) and a nonpolar stationary phase. Four chlorophenols were selected as model analytes (see Figure S6 for chemical structures). These highly toxic compounds are widespread in the environment and are regarded as potential precursors of dioxins.[13] RP-SPE is usually used to extract chlorophenols from environmental water samples. For comparison, the experiments were also performed with several other commonly used RP adsorbents (C18, HLB, and CNTs) with the same adsorbent amount (20 mg).

The results of RP-SPE are shown in Figure 3A. It can be seen that G@silica **2** yielded excellent performance with recoveries of all the chlorophenols approaching 100%. The recoveries obtained with G@silica **1** were lower than those with G@silica **2**, because more G sheets were bound to silica in organic phase, thus providing greater adsorption capacity. C18 yielded inferior recoveries to G@silica **2** because of insufficient adsorption capacity, as the analytes could be detected in the flow-through and washing solution. Especially for 2-CP, which is more polar than the other chlorophenols, its lower recovery indicates that C18 shows a poor sorption capacity for polar compounds. To obtain acceptable recoveries with C18, more adsorbent must be used to enhance the sorption capacity. However, this would increase the solvent consumption and is unfavorable for miniaturization. HLB represents a hydrophilic–lipophilic balance RP adsorbent with enhanced retention for polar analytes, and thus also gave a comparable performance to G@silica **2**. CNTs showed sufficient adsorption of the analytes, but the elution was incomplete because of strong adsorption. Thus, CNTs yielded low recoveries especially for the highly hydrophobic PCP. To obtain adequate recoveries with CNTs, more eluent solvent is needed, but this would compromise the preconcentration factor. Compared with the previous reports regarding CPs,[14] G@silica also demonstrates better recoveries with a less adsorbent. Overall, our results demonstrate that G@silica **2** can be regarded as a high-performance adsorbent for RP-SPE. Its superiority over C18 and CNTs may be ascribed to two aspects: on the one hand, the hydrophobic polyaromatic basal plane and large surface area of G offer a high adsorption

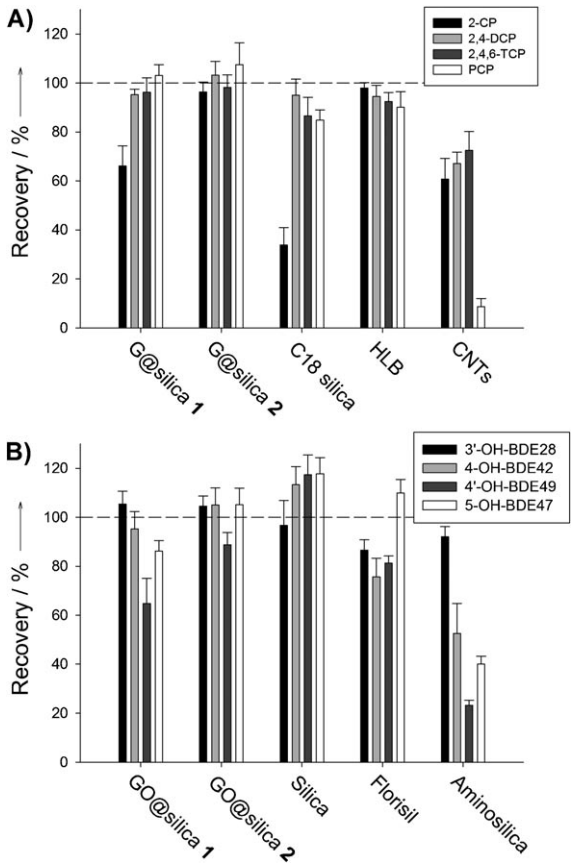

Figure 3. A) Comparison of the analytical performance of G@silica with other adsorbents for the RP-SPE of chlorophenols. The cartridges were loaded with 0.5 μg of each chlorophenol in aqueous solution and eluted with 1 mL of alkaline methanol or pure methanol. 2-CP: 2-chlorophenol; 2,4-DCP: 2,4-dichlorophenol; 2,4,6-TCP: 2,4,6-trichlorophenol; PCP: pentachlorophenol; HLB: Oasis HLB adsorbent (*m*-divinylbenzene and *N*-vinylpyrrolidone copolymer); CNTs: multiwalled carbon nanotubes. B) Comparison of the analytical performance of GO@silica with other adsorbents for the NP-SPE of OH-PBDEs. The cartridges were loaded with 2.5 ng of each OH-PBDE in hexane solution and eluted with 1 mL of methanol or 1:1 dichloromethane/acetone. In all cases, the SPE cartridges were packed with 20 mg of adsorbents.

capacity toward aromatic compounds; on the other hand, the hydrazine reduced G still contains some hydrophilic groups such as hydroxy groups on its plane (as indicated by XPS measurements in Figure S4). These hydrophilic groups can enhance the water wettability of the adsorbent, improving the adsorption of polar analytes, and facilitating the desorption of nonpolar analytes. This feature makes the G@silica behave like a hydrophilic–lipophilic balance adsorbent and is thus favorable in the analysis of groups of analytes with a wide range of polarity.

Despite the polydispersity of G sheets in nanoscale, the bulk properties of graphene can be uniform. Thus, the reproducibility of G@silica RP-SPE was satisfactory (see Table S2). Notably, good run-to-run RSDs (RSD = relative standard deviation; $<8.5\%$) were obtained based on six successive extractions on a single SPE cartridge. This result indicates good reusability of the SPE cartridges. In addition, we also determined the adsorption capacity of G@silica. Using PCP as target molecule, the maximum adsorption capacity reached 156.8 $\mu g\,g^{-1}$ on G@silica **2** (Figure S7).

We then tested the analytical performance of GO@silica in NP-SPE. NP-SPE involves a mid-to-non-polar sample matrix and a polar stationary phase. Four hydroxylated polybrominated diphenyl ethers (OH-PBDEs) were selected as model analytes (Figure S6). These compounds can pose health risks, such as thyroid disruption and cytotoxicity,[15] and have been found as natural products or metabolites of PBDEs in human and other biological species.[16] Analysis of OH-PBDEs in environmental samples often involves a step of extracting the samples with nonpolar solvents followed by NP-SPE as an additional purification step. Thus, we herein tested a GO@silica-packed SPE cartridge to extract OH-PBDEs from hexane solution. Satisfactory elution was achieved with methanol as eluent (Figure S8). We also compared the performance of GO@silica with several other commonly used NP adsorbents, including silica, aminosilica, and Florisil. As shown in Figure 3B, GO@silica **2** yielded the best performance with recoveries 88.8–105.1%. The recoveries on GO@silica **1** were inferior to those of GO@silica **2**, as GO@silica **2** would carry more polar groups available for adsorption than GO@silica **1**. The performance of silica was also acceptable, but aminosilica and Florisil yielded evidently poorer recoveries than that of GO@silica **2**, mainly because of insufficient elution and retention. The reproducibility test of GO@silica NP-SPE was also satisfactory (see Table S3). Good reusability of GO@silica-packed SPE cartridges has also been demonstrated in the run-to-run assays (RSDs $<6.5\%$). Accordingly, GO@silica **2** was shown to be a good adsorbent for NP-SPE.

Another important application of SPE is the use as desalting step for MALDI-TOF MS analysis. Salts in biological samples have a strong negative effect on the quality of MALDI MS signals and can even completely suppress the signals.[17] Therefore, a desalting step, such as SPE, must be applied prior to the analysis by MALDI-TOF MS. Currently, one of the most commonly used SPE adsorbent for protein desalination is C18 silica.[18] However, C18 is not suitable for detection of small or hydrophilic peptides such as phosphopeptides. The previous reports have revealed the potential of using G as an adsorbent for desalting and subsequent MALDI-TOF MS.[7] In the present study, we also explored the power of G@silica in RP-SPE desalting of biosamples for MALDI-TOF MS. The SPE experiments were firstly carried out with a mixture of three proteins prepared in phosphate-buffered saline, including an acidic (bovine serum albumin (BSA), pI 4.7), a basic (RNase A, pI 9.6), and a neutral protein (myoglobin, pI 7.1). Considering that G@silica **2** is more efficient than G@silica **1**, the following experiments were only conducted with G@silica **2**. As shown in Figure 4A, without SPE, the protein signals were rather low. After SPE with G@silica, the signals were dramatically enhanced, and all the three protein could be easily identified: RNase A (13668.68 $[M+H]^+$), myoglobin (16947.73 $[M+H]^+$), BSA (22155.45 $[M+3H]^{3+}$, 33257.11 $[M+2H]^{2+}$, 66494.55 $[M+H]^+$). No peaks were observed in the flow-through, indicating that all proteins had been adsorbed on the

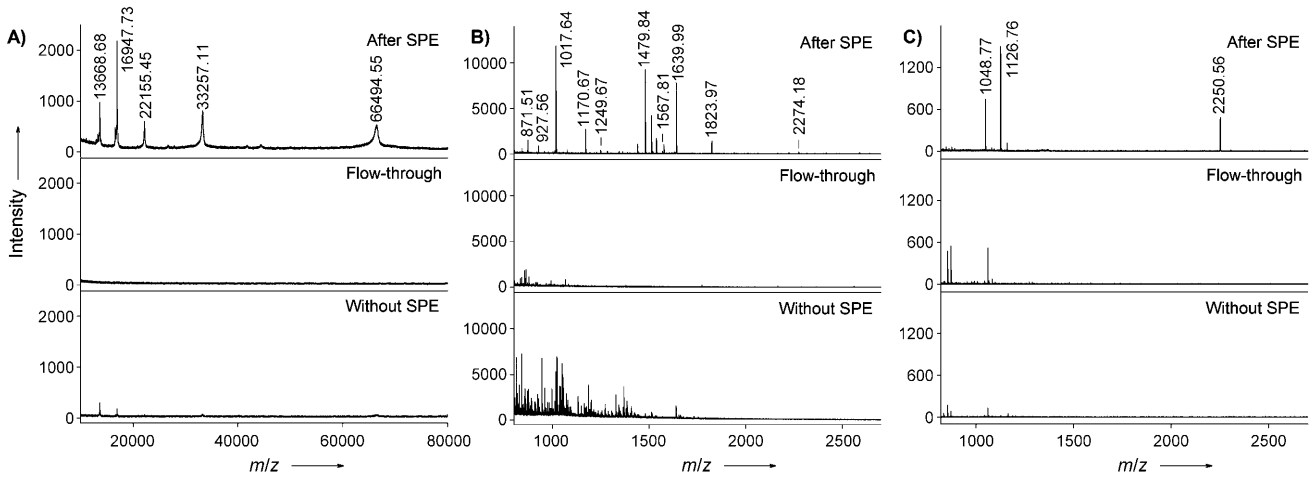

Figure 4. A) MALDI-TOF MS analysis of a protein mixture (50 μg mL^{-1} RNase A + 50 μg mL^{-1} myoglobin + 500 μg mL^{-1} BSA). B) MALDI-TOF MS analysis of a peptide mixture derived from tryptic digest of BSA. C) MALDI-TOF MS analysis of a synthetic phosphopeptide (LRRApSLGGGGC, 40 μM). The samples were loaded on 20 mg of G@silica, rinsed with 2 mL of water as a desalting step, and eluted with 80% acetonitrile/0.1% trifluoroacetic acid. Matrix: α-cyano-4-hydroxycinnamic acid.

G@silica. The protein recoveries on G@silica ranged from 82.4% to 94.7% (Table S3). Notably, BSA is known as a very sticky protein, which yielded poor recoveries on commonly used C18 and HLB, but still showed a good recovery on G@silica (Table S4). The cartridge-to-cartridge RSDs of proteins were also satisfactory (< 12%, Table S5). Compared with the previous report using fullerene bound silica,[12] G@silica-based SPE yielded a more significant enhancement in MS signals.

We then examined the SPE of peptides with tryptic digest of BSA. As shown in Figure 4B, without SPE, the peptide signals were greatly suppressed. No peptide peaks were observed in the flow-through, indicating a complete adsorption of peptides. After SPE with G@silica, a very clear peptide fingerprinting was obtained with highly enhanced signals. This demonstrates the reversible adsorption of peptides on G@silica.

As known, hydrophilic and phosphorylated peptides can usually be lost during desalting with C18 SPE. Therefore, we also tested the G@silica SPE cartridge with a synthetic phosphopeptide (LRRApSLGGGGC) sample prepared in Tris-HCl (Tris = tris(hydroxymethyl)aminomethane) buffer. It can be seen from Figure 4C that, without SPE, no peaks corresponding to the phosphopeptide were observed. There were also no phosphopeptide peaks present in the flow-through, indicating that G@silica has a specific affinity toward the hydrophilic phosphopeptide. After SPE, strong signals of the phosphopeptide and its dimer (1126.76 $[M+H]^+$, 2250.56 $[2M-H]^+$) were detected. Similar results were also obtained at a lower concentration (1 μM) of phosphopeptide (Figure S9). To summarize, the distinct mass spectra before and after G@silica SPE in Figure 4 strongly demonstrate the merit of our adsorbents for SPE of biomolecules for MALDI-TOF MS analysis.

In conclusion, we have demonstrated that G and GO supported on silica provide a versatile and high-performance platform for SPE towards various analytes ranging from small molecules of pollutants to biomolecules such as proteins and peptides. The different polarity of G and GO makes them useful and versatile adsorbents for RP- and NP-SPE. Superior or comparable performance was achieved compared with commercially available adsorbents. Notably, G bound silica is capable of extracting sticky proteins with large molecular weight and phosphorylated peptides, making them particularly suitable for handling biological samples for MALDI-TOF MS analysis. Our results further reveal the remarkable potential of G-based materials for sorption applications.

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