Layer-by-Layer Coating of Graphene Oxide on Fused Silica Fibers for Headspace Sampling of Nicotine in Hair Samples

Samira Dowlatshah\textsuperscript{a}, Alireza Ghiasvand\textsuperscript{a,b,}\textsuperscript{*}, Abdullah Barkhordari\textsuperscript{c} and Vahid Jalili\textsuperscript{d}

\textsuperscript{a}Department of Chemistry, Lorestan University, Khoramabad, Iran
\textsuperscript{b}Australian Centre for Research on Separation Science (ACROSS), School of Natural Sciences, University of Tasmania, Hobart, Tasmania 7001, Australia
\textsuperscript{c}Department of Occupational Health Engineering, School of Public Health, Shahroud University of Medical Sciences, Shahroud, Iran
\textsuperscript{d}Student Research Committee, Department of Occupational Health Engineering, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran

(Received 22 May 2020 Accepted 10 August 2020)

The surface of a fused silica fiber was activated and coated with graphene oxide (GO) film, using a facile layer-by-layer coating strategy. PTES, (3-aminopropyl) triethoxysilane, was used as a covalent cross-linker for tight-binding of the GO layers. By the combination of GO sorption capacity and APTES cross-linking ability, the developed solid-phase microextraction (SPME) fibers exhibited a very good chemical/mechanical stability and high extraction efficiency. The developed fibers were used for headspace sampling of nicotine from the hair of smokers, without any sample preparation step. The determination of the analyte was done using GC-FID technique. Despite the thin thickness of GO multilayer, it was very robust and durable and its performance was better than traditional GO coatings and polyacrylate commercial fibers. The most important experimental variables were studied. Under the optimal conditions, the limit of detection and relative standard deviation (RSD%, n = 3) was 0.02 μg g\textsuperscript{-1}, 5.6-8.9%, respectively. The calibration graph was linear over the range of 0.1-100 μg g\textsuperscript{-1}. The fiber was successfully applied for the determination of nicotine in hair samples of active and passive smokers.

Keywords: Layer-by-layer coating, Graphene oxide, Solid-phase microextraction, Nicotine, Hair

INTRODUCTION

Graphene, as an outstanding form of carbon-based nanomaterials, has been preferred as an efficient coating for separation purposes owing to its unique properties such as large active surface area and high sorption capacity. The abundant aromatic groups in its structure provide a worth bed for π-π stacking interactions. Despite poor dispersibility of graphene in solvents, graphene oxide (GO) can be dispersed in water and polar organic solvents, due to the large number of its oxygen-containing functional groups [1]. These features make GO a promising candidate sorbent for micro-sample preparation techniques [2]. Extensive efforts have been devoted recently to prepare ultrathin graphene-based films to profit the aforementioned traits [3]. Spin coating, epitaxial growth, chemical vapor deposition, and Langmuir-Blodgett technique are examples of the various methods that have been utilized for this purpose [4]. One of the simplest methods to create graphene oxide uniform films is the layer-by-layer (LBL) strategy [5], in which graphene-based films can be constructed via covalent or noncovalent interactions. In contrast to noncovalent interactions, the stability and reproducibility can be achieved through an appealing covalent interaction-based LBL approach [6].

Nicotine, as the major alkaloid, is present in leaves of the tobacco plant. During cigarette smoking, nicotine enters
into the body of smokers through inhalation, the mucosal lining of the mouth and nose, skin, and hair, etc. Nicotine passes through the lungs to the brain within a few seconds and so nicotine and its metabolites can be monitored in biological fluids, as well as in hair [7]. According to the International Agency for Research on Cancer reports, the risk of lung, esophageal, and pancreatic cancers can be increased for human exposure, due to induction atherogenic genes in human coronary artery endothelial cells [8,9]. Nicotine concentration can be monitored in biological fluids during the 3-4 days after human exposure, while hair can be assessed for nicotine concentration long-term after the exposure. It has been demonstrated that the level of drugs in hair samples is always lower than that in biological fluids [10]. Accordingly, the development of simple and efficient methods for the analysis of low levels of nicotine in biological samples is of particular interest [11-13]. Liquid-liquid extraction and solid-phase extraction are still widely utilized for analysis of drugs in hair samples [14], while using these methods are now unaffordable. These methods are usually multistep, time-consuming, and use hazardous organic solvents. In return, solid-phase microextraction (SPME) is an affordable, green and environmentally-friendly strategy with minimum consumption of hazardous solvents and reagents. This technique has been employed to determine illicit or therapeutic drugs in hair samples [15].

SPME is a well-established sample preparation technique in which analytes are distributed between the sample and extraction phase, coated on the surface of a fiber [16]. Owning to the important role of the solid phase sorbent in the extraction efficiency and selectivity various commercial and home-built coatings, with different physical, chemical, thermal, and mechanical features have been developed [17]. A typical commercial SPME fiber is a short piece of fused silica fiber that is coated with a polymeric stationary phase like polydimethylsiloxane (PDMS). In addition to being expensive, the main drawbacks of commercial SPME fiber are lack of robustness, low mechanical stability and swelling in organic solvents. Most of the commercial fibers have a short lifespan compared with home-built nanomaterial-based types. Most drawbacks of commercial SPME fibers assumed to be related to weak physical binding of the coating to the substrate, as well as the high thickness of coatings [18]. Consequently, the coating can be easily stripped or damaged when it comes into contact with the body of GC injector or sample glassware, while other parts of the fiber, such as fiber-attaching-needle, piercing needles, screw hub, fiber core, tensioning spring, sealing septa, and ferrule remain intact.

In this research, we proposed a simple solution to fabricate reliable and durable silica-based SPME fibers. For this purpose, a robust GO multilayer was coated on a fused silica fiber through a facile LBL strategy. For tight-binding of the GO layers to each other and to the silica surface, (3-aminopropyl) triethoxysilane (APTES) was used as the covalent cross-linker. By merging of GO and APTES merits, the proposed SPME fiber exhibited a very good chemical/mechanical stability and a high extraction efficiency. Furthermore, the LBL coating strategy exhibited a substantial simplicity and feasibility for renovation of worn-out silica-based SPME fibers. The prepared fibers were employed for the direct sampling of nicotine in hair samples, followed by GC-FID determination.

**MATERIALS AND METHODS**

**Reagents and Standards**

HPLC grade methanol and chloroform, nicotine (99%), hydrochloride acid (37%), hydrogen peroxide (30%), and sulfuric acid (98%) were purchased from Merck (Darmstadt, Germany). Graphite powder (45 μm, ≥99.99%), APTES, and KMnO₄ were supplied by Sigma-Aldrich (Steinheim, Germany). The stock standard solution (1000 mg/L) of nicotine was prepared in methanol. The working standard solutions were prepared by appropriate dilutions of the stock standard solution with water weekly. The stock and working standard solutions were stored at 4 °C in a fridge.

**Instrumentation**

A Shimadzu 2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector was utilized for separation and determination of nicotine. A silica capillary column of BPX-5 (25 m × 0.32 mm id × 0.25 μm film thickness) was applied. Chromatographic grade nitrogen was used as the carrier and makeup gas at the flow rates of 1 and 30 ml min⁻¹, respectively. The injector was maintained at 230 °C. All
injections were conducted in the split mode (split ratio of 1/10). The column temperature was initially set at 100 °C, increased to 260 °C at a rate of 20 °C min⁻¹, and held for 1 min. Fourier transform infrared spectra were recorded by an FT-IR 8400 spectrometer (Shimadzu, Kyoto, Japan) in the transmittance mode. A VEGA\textbackslash TESCAN CM120 (Brno, Czech Republic) field-emission scanning electron microscope (FE-SEM) was used in order to investigate the morphology of the multilayer GO.

**Preparation of the SPME Fiber**

**Preparation of GO.** GO nanosheets were prepared according to a modified Hummers’ method [6,19]. Briefly, 0.1 g graphite powder and 0.1 g NaNO₃ were added to 4.6 ml concentrated H₂SO₄. The mixture was stirred in an ice bath for 1 h. Then, 0.6 g of KMnO₄ was slowly added and after the formation of a homogenous mixture, the ice bath was removed. The mixture was stirred at room temperature for 2 h. After that, the solution was diluted with 9.2 ml of water and the resultant suspension was heated in an oil bath at 98 °C for 30 min. Afterward, 30 ml water and 2 ml H₂O₂ (30%) were added slowly and the mixture was stirred for 5 min. The mixture was centrifuged (4000 rpm) for 5 min and washed with a 10% hydrochloric acid solution six times to remove impurities. The precipitate was washed with pure water several times until the pH of the solution reached around 7. The product (GO) was then dried at 40 °C for 72 h.

**Preparation of the GO coated silica fiber.** Worn-out silica-based SPME fibers were used as the substrate to prepare new fibers. To remove the remaining polymeric coating, the fused silica fiber was immersed into THF for 1 h. After that, it was kept in 1 M NaOH for 1 h to activate the superficial hydroxyl groups. The fiber was then washed with pure water and dried at room temperature. To prepare the first cross-linker layer and enable the interaction between the substrate and APTES, the fiber was immersed into APTES for 1 min and dried at 80 °C for 10 min. For layer-by-layer deposition, 0.1 mg of GO was dispersed in 100 ml water and filtered to obtain cake-like sediment. The resultant sediment was redispersed in 2 ml of ethanol in a 5 ml plastic tube in a ultrasonic bath [20]. Then, fused silica fiber was dipped into the GO dispersion for 5 s and dried at room temperature for 30 s. The fiber was again immersed into APTES and dried similar to the previous step. These two processes were repeated until the proper thickness of the multilayer coating was obtained (Fig. 1). For conditioning, the SPME fiber was placed into the injection port of the GC instrument at 300 °C under a nitrogen flow for 1 h.

**Preparation of Real Sample, Model Matrix, and Spiked Samples**

To avoid the uncertain results caused by the matrix effect, most of extraction procedures are routinely optimized by using standard samples, which are oftentimes remarkably different from the desired real samples matrices [21]. Hence, to avoid the matrix effect and ensure the reliability and applicability of the proposed strategy for real samples, its optimization process was performed using a model matrix hair (a hair sample without nicotine). For this purpose, a blank hair sample (without nicotine) was prepared. The lack of the analyte in the model matrix was confirmed by its analysis using an ultrasonic extraction method coupled with capillary GC [22]. For this purpose, 1 g of non-colored/drug-free hair was collected from different non-smoker volunteers, in a local barbershop. The sample was cut into ~1-cm pieces and placed in a vial followed by adding 10 mL chloroform and sonicated for 20 min at 50 °C to remove its possible nicotine content. The solvent was removed, and the sample was dried at 85 °C for 1 h. The obtained model matrix was stored in a sealed plastic bag at room temperature and used for all optimization steps. Real hair samples were collected from active and passive smokers and washed with pure water and stored in sealed plastic bags at room temperature. These samples were subjected to the developed method without any sample preparation process. For preparation of the spiked sample, 10 mg of the hair model matrix was placed in 10-mL SPME vial and after closing the cap, it was spiked (via the septum of the vial) with predetermined volume of a standard nicotine solution to obtain the desired concentration. The vial was then shaken for 5 min and remained at room temperature for 24 h to reach equilibrium.

**HS-SPME Procedure**

Each hair sample (10 mg) was placed in a 10 ml SPME vial and placed in a water bath at 80 °C. The HS-SPME
Fig. 1. Schematic representation of silica surface activation and layer-by-layer coating of GO on a silica fiber by chemical bonding.

Fig. 2. FT-IR spectrum of multilayer-GO coated on the surface of a fused silica fiber.
sampling was done by GO coated fiber for 20 min (the extraction time). After that, the fiber was retracted and injected into the GC injection port for 1 min at 230 °C. Peak area was used to evaluate the extraction efficiency of the SPME procedure.

RESULTS AND DISCUSSION

Characterization of Multilayer GO

Multilayer-GO was coated on the surface of the silica fiber by using APTES as a silane coupling agent. It was used to create covalent bonds between the substrate and GO sheets. FT-IR and FE-SEM techniques were applied for the investigation of characteristic absorption bands and surface structure of multilayer-GO coating, respectively. To study the functional groups of multilayer GO, its FT-IR spectrum was recorded (Fig. 2). GO structure was characterized by the vibrational modes of 1204 and 1725 cm−1 (indicating C-O and C=O bonds, respectively), ~1620 cm−1 (relates to C=C bond), ~1405 cm−1 (indicating C-H bond), and 3388 cm−1 (indicating O-H bond). The APTES structure was also clearly observed from the peaks of 1030 cm−1 (indicating Si-O-Si stretching vibration), 472 cm−1 (correspond to Si-O-Si bending vibration), and 2921 cm−1 (indicating N-H bond). The covalent attachment of GO/Si was clearly observed by vibrational peak at 810 cm−1, indicating the Si-C bond [20]. The morphology of GO and multilayer-GO coating were evaluated by scanning electron microscope. As Fig. 3 shows, a homogeneous and dense multilayer coating has been successfully fabricated by the proposed method.

Optimization of the HS-SPME-GC-FID Procedure

The proposed HS-SPME-GC-FID method was evaluated for sampling and analysis of nicotine in solid samples. Thus, the important influential experimental factors on the extraction efficiency including desorption time, desorption temperature, sampling temperature and sampling time were optimized.

GC desorption parameters. To ensure the complete desorption of nicotine from the GO coated fiber and avoid memory effect, desorption temperature and time had to be optimized before the rest of the variables [23]. For this purpose, the effect of desorption temperature was studied over the range of 150-250 °C (Fig. 4a). The results demonstrated that 230 °C was suitable for complete desorption of the analyte from the GO fiber. Desorption time was also studied in the range of 0.5-5 min and the optimal time was found 1 min, as shown in Fig. 4b.

Effect of sampling temperature and time. Extraction temperature is one of the most important factors in SPME sampling and especially in HS mode because it directly affects the concentration of the analyte in the headspace. In this study, extraction temperature was varied from 40 to 100 °C and the peak heights were recorded against temperature. As shown in Fig. 5a, the sampling efficiency was increased up to 80 °C and then decreased slightly at higher degrees. From the thermodynamic view of point, this phenomenon can be justified by considering the bilateral effect of heating on the adsorption process [24]. Adsorption is an exothermic process and is expected to be decreased when the temperature increases. Thus, temperatures higher than 100 °C were not tested and 80 °C selected as the optimum sampling temperature.

As a significant parameter in the SPME procedure, extraction time was also evaluated by applying different times in the range of 5-25 min (Fig. 5b). The extraction efficiency enhanced by increasing time up to 15 min and then showed a constant trend. The results demonstrated that 20 min was sufficient for equilibration between the sample matrix and fiber's coating. Thus, 20 min was chosen as the optimal extraction time for further studies.

Durability and Extraction Efficiency of the Prepared Fiber

The durability of the developed fiber was evaluated by analyzing more than one hundred hair samples using a single fiber. The results indicated that the extraction efficiency did not show a significant decline (< 3%) during about three months. Additionally, the extraction efficiency of the prepared fiber was evaluated in comparison with a commercial PA fiber and a conventional GO fiber, under the same experimental conditions. The results (Fig. 6) showed that the multilayer-GO coated fiber was more efficient than the examined fibers for the extraction of nicotine.
Fig. 3. SEM images of the multilayer-GO coated fiber at different magnifications, a) 500 nm, b) 1 µm, and c) 5 µm.
Analytical Performance

Different spiked hair samples were analyzed using the developed fiber at the optimized conditions. The results indicated that the recoveries of the HS-SPME-GC method were over the range of 72.5-96.5%. The intra-fiber repeatability (n = 6) and inter-fiber reproducibility (three different fibers) were evaluated at three concentration levels (0.1, 20, and 50 μg g⁻¹). Based on the results, intra-fiber and inter-fiber RSDs were obtained over the ranges of 4.6-8.9% and 7.3-12.4%, respectively. The calibration graphs for the

**Fig. 4.** Effect of desorption temperature (a) and desorption time (b) for the analysis of nicotine in hair samples using the HS-SPME-GC method.
Fig. 5. Effect of extraction temperature (a) and extraction time (b) on the extraction efficiency of the HS-SPME-GC method for analysis of nicotine in hair samples.

Fig. 6. Comparison of the multilayer-GO coated fiber with a traditional GO coated fiber and a commercial PA fiber.
analyte was linear over the range of 0.1-100 μg g⁻¹, with determination coefficients higher than 0.99. Under the optimized conditions, the limit of detection (LOD) was obtained 0.02 μg g⁻¹ (μg nicotine per g of hair sample). To assess the applicability and reliability of the developed method, its analytical performances were compared with some similar reported methods [11,25-27], as listed in Table 1. As can be seen, the linearity range and precision of the proposed method are comparable with those of the representative methods listed, while its LOD is lower than those reports, even compared to a LC-MS/MS procedure which benefits of high-sensitivity MS/MS detection system.

### Analysis of Real Samples

To demonstrate the reliability and feasibility of the method, it was utilized for sampling and analysis of nicotine in real hair samples, obtained from a few active and passive smokers. For evaluation of the matrix-effect, the real samples were also spiked with 0.2 μg g⁻¹ of nicotine. The results (Table 2) showed that the developed HS-SPME-GC method could be successfully carried out for the determination of nicotine in real hair samples, without sample preparation and with a small impactability versus the matrix effect. As can be seen, the recovery percentages obtained for the spiked samples are acceptable. It is noteworthy that the recoveries for the solvent extraction and solid-phase extraction methods are reported in a wide range, from 7-98% [28]. It can also be concluded that the matrix-matched calibration procedure has compensated the matrix effect, to a great extent. Two sample chromatograms of a real hair sample (spiked and non-spiked) are depicted in Fig. 7.
CONCLUDING REMARKS

A facile strategy was employed to coat a multilayer-GO on the surface of a fused silica fiber, for easy preparation of new handmade SPME fibers, as well as for renovation of worn-out commercial fibers. The proposed fibers were shown to be efficient and very durable, in comparison with the traditional GO coated and PA coated commercial fibers. They were utilized for the direct analysis of nicotine in the hair of active and passive smokers through an HS-SPME-GC-FID method, without additional sample preparation step. The fabricated SPME fibers showed good stability and repeatability (4.6-8.9%). The proposed method exhibited a low limit of detection (0.02 μg g⁻¹) and a wide linear dynamic range (0.1-100 μg g⁻¹). The layer-by-layer deposition of a thin film of GO on silica fiber, by using APTS as the crosslinker reagent, made the fiber coating robust and efficient. A single fiber was used more than one

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nicotine (μg g⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
</tr>
<tr>
<td>Active smoker</td>
<td>0.2</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.087</td>
</tr>
<tr>
<td>Passive smoker</td>
<td>0.2</td>
<td>0.276</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NF</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>0.2</td>
<td>0.187</td>
</tr>
</tbody>
</table>

NF: Not found.

**Table 2.** Determination and Recovery of Nicotine in Hair Samples by the Proposed Method and GO Coated Fiber

**Fig. 7.** Sample chromatograms of a real hair sample.
hundred times during about three months without damage and decline in the extraction efficiency. The results demonstrated that the matrix-matched calibration method has had a remarkable positive effect to compensate the matrix effect.

ACKNOWLEDGMENTS

The authors are grateful for the support of Lorestan University.

REFERENCES