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Graphene Oxide-Ionic Liquid Used as Solid-Phase Microextraction Coating for Polyphenolic Compounds' Extraction and Determination with GC-MS After On-Fiber Derivatization in Wine

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Abstract: We describe the use of a home-made fiber coated by graphene oxide modified by an ionic liquid having methylimidazolium cation with an amino-functional group for the extraction of polyphenolic compounds (P.C.s). We then performed the determination by gas chromatography coupled with mass spectrometry after on-fiber derivatization. The authors optimized the main parameters influencing the extraction and derivatization processes. The on-fiber derivatization was employed within 15 min at 60 °C using 20 µL of trimethylsilyl reagents. Under the optimized conditions, the calibration curves for 12 P.C.s were linear from 0.1 to 1000 µg/L, and the detection limits were between 0.02 and 0.1 µg/L. We determined the single fiber repeatability obtained for all calibration points and the fiber to fiber reproducibility for 100 µg/L to be < 14.82% and < 5.87%, respectively. The extraction efficacy of the home-made fiber due to high intermolecular and electrostatic attractions was much better than the commercial fibers. We successfully applied the method to the analysis of P.C.s in wine samples with the recoveries from 72.8 to 99.9%.

Keywords: GO-[APMIM][NTf2] coated fiber, SPME-GC-MS, polyphenolic compounds, derivatization on-fiber, wine samples.

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INTRODUCTION

Polyphenolic compounds (P.C.s) are involved in color formation in plants, and plants can also synthesize some new polyphenolic compounds to protect against pathogens in stress situations (1). Polyphenolic compounds are essential in terms of their positive effects on human health. Their antimutagenic, anticarcinogenic, antioxidant, anti-inflammatory, antiallergic, and antipathogenic properties are reported (2, 3). Wine is a significant natural antioxidant source when compared to other alcoholic beverages (4).

Because of the complexity of the sample matrix, literature mentions of sample pretreatment techniques, including liquid-liquid extraction (LLE), solid-phase extraction (SPE), stir bar sorptive extraction (SBSE), and solid-phase microextraction (SPME) before their chromatographic detection for extraction and preconcentration of polyphenolic compounds in wine samples (5-8). SPME, an equilibrium-based sample pretreatment technique, provides a single-step analysis by integrating sampling, extraction, preconcentration, and sample introduction for the target analytes. The main advantages of SPME are fast mass transfer, its solvent-free, and simple nature, and lower sample volume (9). Enrichment of organic compounds from

different kinds of sample matrices is essential, and so new coatings must be developed on fibers for SPME. Literature suggests the orientation of the preparation of new coating materials to the selectivity performance to target analytes as well as the development of more durable, firmer, and highly consistent supports (10).

Graphene oxide (G.O.) obtained by oxidation of epoxy (C-O-C), hydroxyl (C-OH), carbonyl (C=O) and carboxylic acid (COOH) groups from basal and surface corners of a single graphite layer has excellent properties such as large surface area, excellent structure, and good chemical stability (11). Recently, graphene oxide-based materials have been progressed and used as coating materials in the sample preparation technique such as SPME, SPE because of its facile functionalization (12, 13). Ionic liquids (I.L.s), being potential environmentally friendly solvents, have a great attraction in separation science due to low volatility, high viscosity, excellent thermal stability, and high polarities (14). Besides, its use as stationary phase in liquid chromatography, gas chromatography, and capillary chromatography, and extraction material in micro liquid extraction techniques, I.L.s have been preferred in SPE and SPME as sorbent and coating material both alone and grafted on different supports such as graphene oxide and carbon nanotubes to improve extraction efficiency and stability (15-18).

So far, liquid chromatography-diode array detector (LC-DAD) and liquid chromatography-mass spectrometry (LC-MS) techniques have been reliable solutions for the determination of P.C.s. However, rarely has gas chromatography-mass spectrometry (GC-MS) been preferred due to the need for derivatization for polar P.C.s. In the literature, direct methods (19,20), solid supported-LLE (SS-LLE) (5), SPE method using molecularly imprinted polymers (6), and SPME method using commercial polyalcoholic fiber (P.A.) (8) are available. Along with them, application of carbowax-templated resin (CW/TPR) (21), polystyrene-divinylbenzene-polyacrylonitrile (PS-DVB-PAN) coated fiber (22), and poly(ionic liquid)-based molecularly imprinted polymer (PIL-MIP)-coated fiber (23) before L.C. to wine, fruit juices, and beer samples. Many extraction techniques including LLE (24), liquid-liquid microextraction (LLME) (25), SPE using Oasis MAX cartridges (26), SPSE using polydimethylsiloxane (PDMS) coated stir bar (27), SPME with P.A. fiber (28- 30) are present for the determination of P.C.s in wine, fruit juice, and medicinal plant samples before GC-MS/FID or multidimensional GC-MS techniques. Despite the most probable preference of L.C. methods, G.C. can also be a choice for the determination of P.C.s in a complex sample matrix. GC-MS technique has some advantages such as better chromatographic separation using a capillary column, improved detectability, lower matrix effects, more accurate result, cost-effectiveness, and a more straightforward interface concerning LC-MS.

References concerning the SPME-GC-MS method using only commercial fibers are available for some polyphenolic compounds such as resveratrol, gallic acid, caffeic acid, p-coumaric acid, ferulic acid, syringic acid, and protocatechuic acid (28-30). SPME fibers coated with graphene oxide modified with ionic liquids having vinyl- and benzylimidazolium by sol-gel technique have been performed and used for extraction and determination of phthalate esters and polyaromatic hydrocarbons in water and coffee samples in our previous studies (18, 32). In this work, the extraction performance of coating possessing graphene oxide modified with amino-terminated methylimidazolium cation was evaluated for the analysis of twelve P.C.s (syringic acid, protocatechuic acid, cinnamic acid, p-coumaric acid, sinapinic acid, ferulic acid, caffeic acid, quercetin, kaempferol, chlorogenic acid, resveratrol, and gallic acid) in wine using direct immersion-SPME coupled with GC-MS. The graphene oxide-(1-(3-aminopropyl)-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) (GO-[APMIM][NTf_2]) coated fiber was prepared with layer by layer coating technique onto the surface of stainless steel wire using a crosslinker agent. We performed the derivatization of P.C.s using trimethylsilyl (TMS) reagents on the fiber. The authors compared the efficiency of GO-[APMIM][NTf_2] coated fiber with commercial fibers and G.O. coated fiber. Finally, we applied the optimized SPME method for the determination of P.C.s in real white, red, and fruit wine samples.

MATERIALS AND METHODS

Chemicals and Materials

We procured the P.C. standards (caffeic acid, gallic acid, quercetin, kaempferol, chlorogenic acid, resveratrol, syringic acid, protocatechuic acid, cinnamic acid, p-coumaric acid, sinapinic acid, and ferulic acid) from Alfa Aesar (Karlsruhe, Germany). We also purchased ethyltrimethylsilane (ETMS), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) as derivatization compounds from the same vendor. 1-Methylimidazole, 3-bromopropylamine hydrobromide, bis(trifluoromethane) sulfonimide lithium (LiNTf2), and graphene oxide (2 mg/ mL, dispersion in water) were obtained from Sigma-Aldrich (St. Louis,USA). We purchased the modified dihydroxyethylene urea as a cross-linking from Hunstman (Utah, USA). All other used reagents were of analytical reagent grade. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, USA). We procured a manual SPME holder, amber glass vials (20 mL) with screw caps and polytetrafluoroethylene/ silicone septa, and polyacrylate (PA, 85 µm), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm),

carboxen/polydimethylsiloxane (CAR/PDMS, 85 µm) and carbowax/ polyethylene glycol (CW/PEG, 60 µm) fibers from Supelco (Bellefonte, USA). Using a 5 µL microsyringe from Hamilton (Reno, USA), we produced the SMPE fibers and obtained a stainless steel wire having O.D. 150 µm from a local market (Istanbul, Turkey).

Stock standard solution of 1000 mg/L of each P.C.s was prepared using methanol and stored at -18 ºC. The intermediate solution of the mixture standard of P.C.s was prepared at 200 mg/L in methanol and stored at 4 ºC. We prepared the pH 8 buffer solution using 1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl).

The analyzed samples, including red wine, white wine, and fruit wines, were purchased from local supermarkets in Izmir. The alcohol content was in the range of 12%-13.8% for the studied wines. The bottles of wine were stored at 4 ºC and protected from light before analysis. The authors prepared synthetic wine solutions including 12% (v/v) of ethanol with pH adjusted to 3.5 by tartaric acid for optimization and performance evaluation of the DI-SPME method.

Instrumentation

We performed the G.C. analysis on a Trace 1300 gas chromatograph QP2010 equipped with an ISQ QD single quadrupole mass spectrometer and split/splitless injector (Thermo Scientific, USA). A TG-5MS fused silica capillary column (30.0 m × 0.25 mm I.D., 0.25 µm film thickness) supplied from Thermo Scientific (West Palm Beach, FL, USA) was used. High purity helium was employed as the carrier gas, at a flow rate of 1.2 mL min-1. We employed the following separation temperature program in G.C. for TMS derivatives: initially oven temperature held at 80 ºC for 3 min, then programmed at 10 ◦C min-1 to 220 ◦C (held for 4 min), finally increased to 280 ◦C at 20 ºC min-1 (held for 2 min). The injection port temperature was at 250 °C for GO-[APMIM][NTf2] coated SPME fiber. We carried out all injections on the splitless mode for 5 min. After each analysis, we heated all fibers at a desorption temperature for 5 min in the extra G.C. injection port to prevent carry-over effects. The authors operated the MS in the electron impact (E.I.) at 70 eV under selected ion monitoring mode (SIM) by monitoring two relevant m/z fragments for TMS derivatives given in Table S1. The GC-MS ion source and interface were set at 250 ºC and 280 ºC, respectively. Figure 1 gives the chromatogram of P.C.s and their M.S. spectra.

The Fourier transform infrared (FTIR) spectra of G.O. and GO-[APMIM][NTf2] were obtained by using a Thermo Fisher Scientific Nicolet iS10 model FTIR Spectrometer. We employed thermogravimetric analysis (TGA) for investigation of their thermal properties. The TGA was performed between 30 and 450 °C at a rate of 10°C/min under a nitrogen atmosphere with a Perkin–Elmer Diamond TG/DTA instrument. X-ray Diffractometer measurements were made by a Philips X'Pert PROBE model with a monochromatic Cu-Kα X-ray source at 2θ = 10-60°. The authors evaluated the surface morphology GO-[APMIM][NTf2] coated fiber with scanning electron microscopy (SEM, Carl Zeiss 300VP, Jena, Germany).

Derivatization Procedure

By on-fiber derivatization process, the fibers after SPME firstly were inserted into a flask, dried under nitrogen atmosphere for 5 min and then transferred into the headspace of a 1.5 mL glass vial containing 20 µL of BSTFA: TMCS (9:1, v:v) solution and held at 60 °C for 15 min.

Preparation of the Fiber

After etching of stainless steel wire as previously described (18), the tip of wire was dipped into dihydroxyethylene urea cross-linking reagent for 1 h, then inserted into G.O. dispersion in water for 30 min. These steps were repeated for three times for efficient coating. We dipped the G.O. coated fiber vertically into the 0.02 g [APMIM][NTf2], which was synthesized according to the previous methods given in the literature (18, 33), in 5 mL of methanol for 2 h at room temperature. This step was repeated in sextuplicate to increase the thickness of the fiber coating. Then, the home-made fiber prepared was dried at room temperature overnight and conditioned in sextuplicate in the injection port of G.C. at 250 °C for 5 min under nitrogen atmosphere.

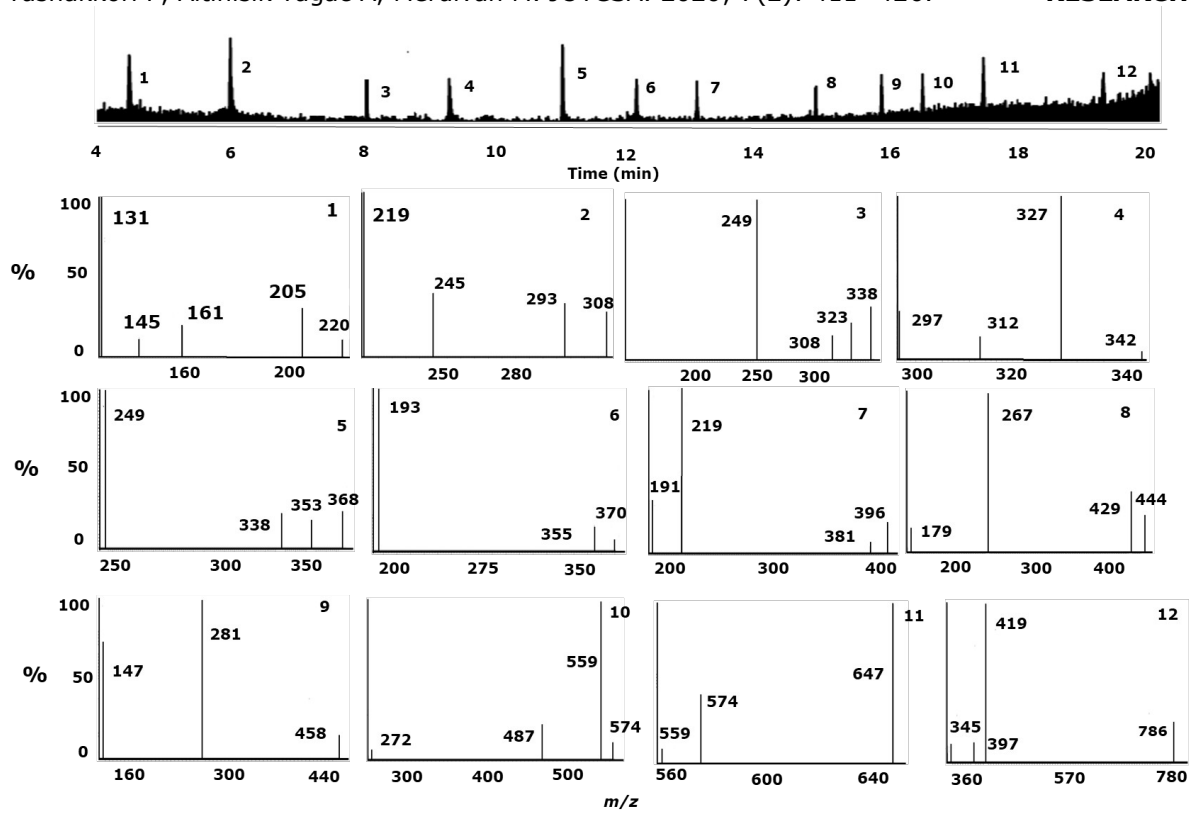

Figure 1. Chromatogram of P.C.s standards and M.S. spectra of P.C.s by the proposed method. Peak identification: (1) cinnamic acid, (2) p-coumaric acid, (3) ferulic acid, (4) syringic acid, (5) sinapinic acid, (6) protocatechuic acid, (7) caffeic acid, (8) resveratrol, (9) gallic acid, (10) kaempferol, (11) quercetin, (12) chlorogenic acid.

The characterization results of GO-[APMIM][NTf2] coating material by FTIR, XRD and T.G. were shown in Figure S1. FTIR (cm-1): 2914, 1847, 1578, and 1456. XRD (2θ) = 23.67°, 38.17° and 40.51°. TG (25-450°C): from 100% to 70% weight loss.

DI-SPME Procedure for Analysis of P.C.s

Firstly, synthetic wine or wine sample solution was prepared by adjusting pH to 8 and placed into a 20-mL amber glass vial. The vial was placed in a metallic block on a magnetic heater. After equilibration for 5 min, the 1 cm tip of the GO-[APMIM][NTf2] coated SPME fiber was exposed to the test solution at adjusted temperature for a particular time while stirring at 400 rpm using a stirring bar. Extractions were performed using a metallic block and a heater with a magnetic stirrer. After extraction, the fiber was dried under a nitrogen stream for 5 min, derivatized by BSTFA:TMCS mixture, and inserted into GC-MS for analyzing P.C.s. Because of the complex composition of wine, we have used two home-made fibers prepared in the extraction experiments. Using one prepared GO-[APMIM][NTf2] coated fiber, we were successful to perform 150 injections with no remarkable decrease in recovery and repeatability.

RESULTS AND DISCUSSION

Optimization of the Derivatization Process

G.C. analysis needs a derivatization procedure for hydroxyl functional groups. The most commonly used derivatization reagents are TMS reagents containing N-methyl-N-(trimethylsilyl)trifluoroacetamide), N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide, BSTFA and TMCS for derivatization of polyphenolic compounds and their metabolites (25, 28, 34, 35). TMS reagents are hydrolyzed in aqueous solutions. Because of this, the SPME fiber after extraction was dried under nitrogen atmosphere. Therefore, drying time for fiber after extraction, suitable derivatizing reagent and volume, and time and temperature of derivatization were investigated. The optimal conditions to get the highest signal came to an agreement at 60 ºC for 15 min using 20 µL of BSTFA: TMCS (9:1) after drying the fiber for 5 min under nitrogen gas for all P.C.s studied following the extraction.

Optimization of the SPME Conditions

SPME experimental conditions such as sample pH, ionic strength, extraction temperature, and extraction time were investigated to obtain reproducible results with high extraction performance of the GO-[APMIM][NTf2] coated SPME

fiber for P.C.s by using DI-SPME method. We carried out all the experiments in triplicate using synthetic wine sample solutions containing 100 µg/L P.C.s standard and reported the mean values.

Sample pH and Ionic Strength

The pH of a sample solution affects the retention of analytes on the surface of the coating material of SPME fiber. It is possible to observe an interaction between the analyte and the sorbent when the polarities of them are close to each other. The pH of the sample is adjusted to obtain reproducible extraction efficiency, taking into account the pKa of analytes. Depending on the pKa values of P.C.s, we performed the extraction experiments to control the effect of pH at pH 4 and 8. The authors saw that the extraction efficiencies of P.C.s studied were higher at pH 8 except syringic acid and gallic acid (Figure 2A). The reason is that the P.C.s studied were ionized mainly at pH 8 and the sorption between the ionized P.C.s and imidazolium cation took place by electrostatic interaction. Besides, the aromatic ring and –O.H., -OCH3 and -COOH groups in P.C.s caused dipole-dipole and π-π interactions during the sorption to the surface of GO-[APMIM][NTf2] coated fiber. The time of analysis is optimal at pH 8 and selected for further studies because the peak areas of most P.C.s studied were high at pH 8.

In HS-SPME and rarely in DI-SPME methods, increasing the ionic strength of the aqueous solution improved the peak areas, favoring the extraction of analytes into the fiber and solubility of the extracted compounds. Thus, we investigated the impact of ionic strength on the uptake of P.C.s after exposure to the fiber at three different NaCl concentrations (0%, 10%, and 25%, w/v). As seen in Figure 2B, the addition of NaCl caused a slight increase in the peak areas of P.C.s (10%, w/v) initially and then led to a more decrease in the extraction efficiency. The presence of a high amount of salt may cause an increase in the viscosity of the solution, which decreases the mass transfer rate and affects the peak areas of P.C.s negatively. So, we used 10% NaCl solution as a test for further experiments.

Extraction Temperature and Extraction Time

Extraction temperature is an essential factor for the extraction efficiency. It influences the mass transfer and affects the extraction time in the SPME method. Therefore, we investigated the effect of extraction temperature on the extraction efficiency of P.C.s by exposing the fiber to the sample solution at 30 and 70 °C for 30 min. Figure 2C shows the temperature profiles obtained. The authors achieved the highest extraction efficiency at 30 °C for all P.C.s. Increasing temperature caused a decline in the peak areas of P.C.s. This event may be explained by that the high temperature causes a rapid motion of neutral or ionized P.C.s but decrease the diffusion of P.C.s on fiber coating due to the exothermic extraction process (36). The researchers performed subsequent experiments at 30 °C for the home-made fiber because of the maximum extraction efficiency at this temperature.

The extraction time deals with the interaction of analytes in solution and fiber coating in SPME. The extraction time was investigated from 15 to 90 min at 30 °C by stirring at 400 rpm to acquire the adsorption equilibrium for P.C.s on the fiber surface. As shown in Figure 2D, the peak areas of all P.C.s reached the maxima in 30 min. Thus, considering the extraction efficacy and the analytical time, 30 min was chosen as the optimized fiber exposure time.

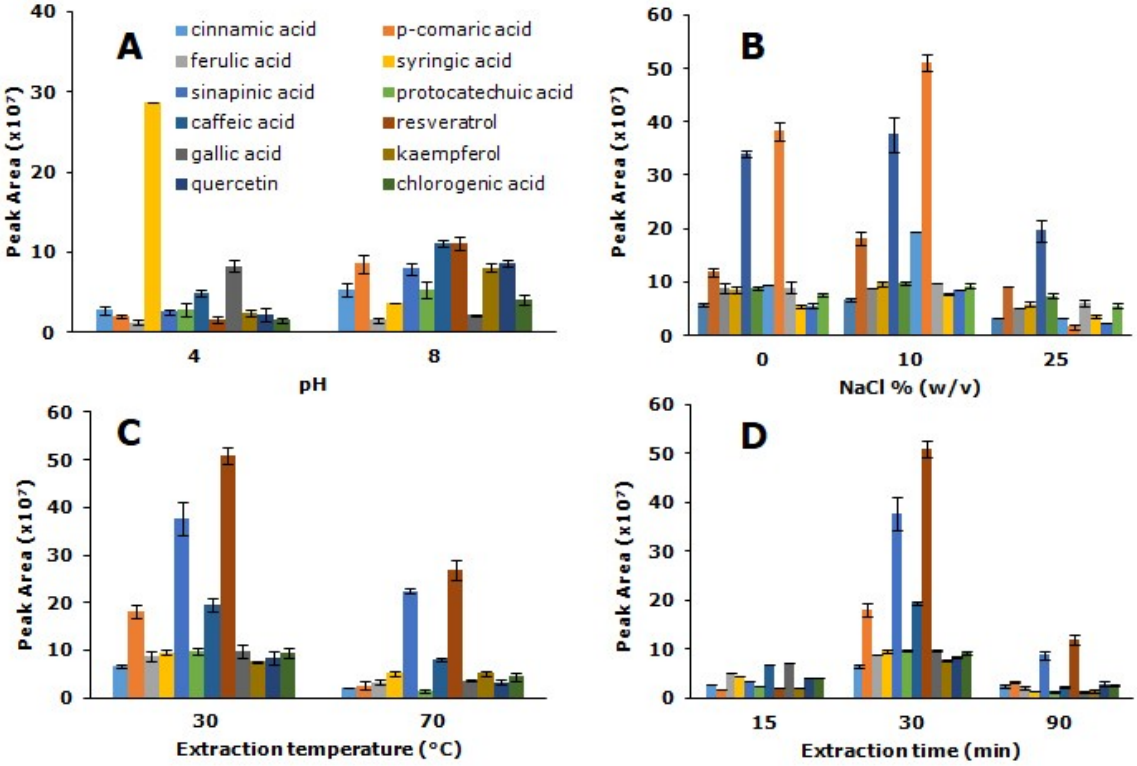

Figure 2. Effects of pH of sample solution (A), ionic strength (B), extraction temperature (C), and extraction time (D) of GO-[APMIM][NTf2] fiber on the DI-SPME method.

Comparison of Extraction Efficiency of GO-[APMIM][NTf$_2$] Coated Fiber with Commercial Fibers and G.O. Coated Fiber

DI-SPME method was also optimized for P.A., DVB/CAR/PDMS, CAR/PDMS, and CW/ PEG commercial SPME fibers to compare their extraction efficiency with the GO-[APMIM][NTf$_2$] coated fiber. These fibers were conditioned on the injection port of G.C. before the extraction experiments at 280 °C, 270, 300, and 240 °C for P.A., DVB/CAR/PDMS, CAR/PDMS, and CW/ PEG for 30 min according to the manufacturer recommendation, respectively. We carried out the SPME extraction experiments in the range of 15- 60 min with 100 µg/L P.C.s solution at pH 8 and 400 rpm to obtain the optimum extraction time. The authors obtained the highest extraction efficiency as 30 min for all commercial SPME fibers. We studied the extraction temperature at 30 and 70 °C at the optimized extraction time. The experimental results indicated that the optimum extraction temperature was 30 °C for all commercial fibers. Although the optimal extraction parameters of commercial fibers are the same as the GO-[APMIM][NTf$_2$] coated fiber, the extraction efficiency of the home-made coated fiber was much higher than those commercial fibers (Figure 3). Within the commercial SPME fibers, the peak areas of cinnamic acid and kaempferol with P.A. fiber and the peak area of cinnamic acid with CW/PEG fiber were only higher than that of the home-made fiber. As seen in Figure 2, the presence of [APMIM][NTf$_2$] caused much increase in the extraction efficacy of P.C.s with respect to only G.O. coated fiber.

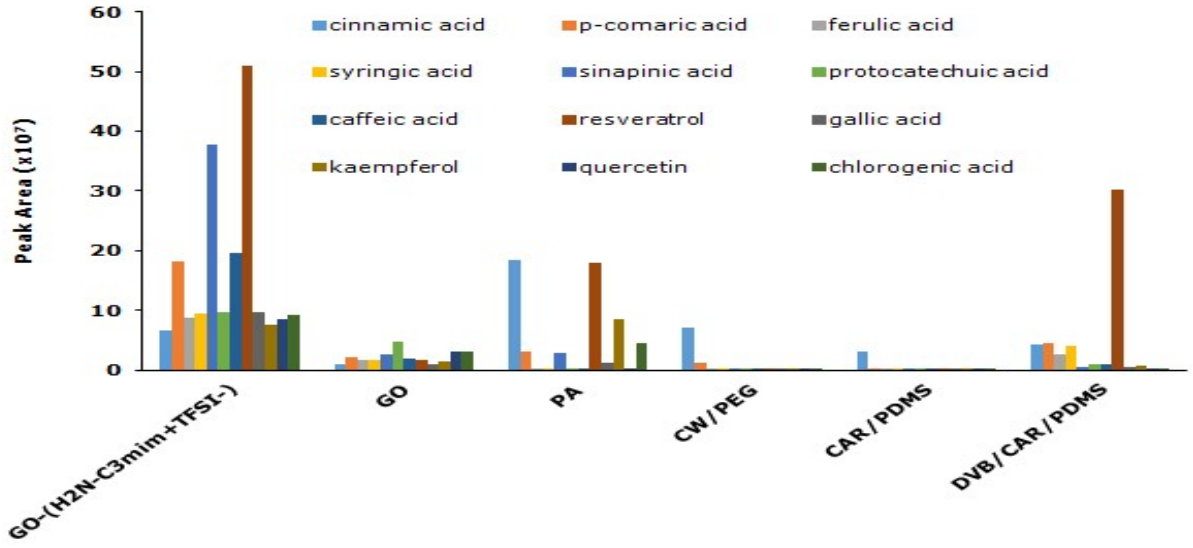

Figure 3. Comparison of the extraction efficiencies of GO-[APMIM][NTf_2] coated fiber with G.O. coated and P.A., CAR/PDMS, DVB/CAR/PDMS, and CW/ PEG commercial fibers. Conditions: C_{PCs} = 100 µg/L; sample pH = 8; stirring rate = 400 rpm; extraction temperature = 30 °C; extraction time = 30 min.

Table 1. Analytical figures of merit for GO-[APMIM][NTf2] coated fiber in DI-SPME-GC-MS method.

PCs	Linear range (µg/L)	R2	LOD (µg/L)	Precision (RSD, %)	Fiber-to-Fiber (RSD, %)
Cinnamic acid	0.1-1000	0.998	0.05	2.48-11.37	2.39
p-Coumaric acid	0.25-1000	0.998	0.1	2.22-9.23	2.79
Ferulic acid	0.25-1000	0.998	0.1	2.57-9.68	2.18
Syringic acid	0.1-1000	0.999	0.02	1.32-14.82	4.68
Sinapinic acid	0.1-1000	0.997	0.02	0.71-13.29	3.04
Protocatechuic acid	0.1-1000	0.997	0.02	0.92-8.42	5.71
Caffeic acid	0.25-1000	0.997	0.05	3.48-14.74	1.64
Resveratrol	0.1-1000	0.997	0.02	0.93-10.53	4.79
Gallic acid	0.25-1000	0.997	0.02	0.90-9.85	2.33
Kaempferol	0.1-1000	0.997	0.02	0.78-9.85	5.87
Quercetin	0.1-1000	0.998	0.02	3.14-9.24	4.06
Chlorogenic acid	0.1-1000	0.997	0.02	2.73-11.92	2.25

Table 2. Comparison of the proposed method with previous SPME and other extraction methods for determination of P.C.s studied.

	Coating Material/ Sorbent	**Extraction type**	**Sample**	**PCs**	**LR (µg/L)**	**LOD (µg/L)**	**RSD (%)**	**Recovery (%)**	**Refs**
LC-DAD	MIP	SPE	wine	GA PrA	10-70* 0.1-4.5	0.4* 0.1*	6.4-8.0 7.0-8.1	89.1-98.3 95-100	(6)
GC-MS		LLME	plasma	Phenolic acids	10-5000	0.5-16.9	3.8-18.4	80-110	(25)
LC-FLD	PDMS CW/TPR	SBSE SPME	Wine, must, fruit juice	trans-res trans-res/ cis-res	0.5-50 5-150/ 2-150	0.1 2/0.5	6.9 5.3/4.8	82-105	(21)
LC-DAD	PA	SPME	Wine, spirit, grape juice	res	0.1-500	0.4	6.5-12.6	92.2-99.4	(8)
GC-MS	PDMS stir bar	SBSE	Wine	res, picetannol	0.2-1	0.004-0.015	5-9	79-109	(27)
GC-MS	PA	SPME	Wine and grape	res	1-150	0.09	2.4	85-116	(28)
LC-DAD	PIL/MIP	SPME	Fruit juice and beer	CA FA	0.1-200 0.05-200	0.019-0.024 0.011-0.042	2.3-8.2 4.6-8.0	80.1-111 72.1-109	(23)
LC-MS/MS	PS-DVB-PAN	SPME	Wine, berry, grape	CA res	1.5-500 5-500	0.5 1.5	5 4	82 77	(22)
Multidimensional GC-MS	PA	SPME	Wine	res	10-5000	7.08	3.0-9.2	72.7-94.7	(29)
GC-MS	Oasis MAX	SPE	Wine	trans-res	up to 2500	0.24	8	92.5-108.2	(26)
GC-FID	PA	SPME	Synthetic solution	pCuA, SyA, PrA, FA, CA, GA	2.2-354.4	0.01-1.77	9.78-17.89	-	(30)
GC-MS	GO-[APMIM][NTf2]	SPME	Wine	CnA, SyA, SnA, PrA, Res, Kfl, Qcn, ChA pCuA, FA, CA, GA	0.1-1000 0.25-1000	0.02-0.05 0.02-0.1	0.78-11.92 0.90-14.74	72.8-99.9 80.7-99.8	This study

LC-FLD: Liquid chromatography-fluorescence detector MEPS: Microextraction packed sorbent,
CnA: Cinnamic acid, SyA: Syringic acid, SnA: Sinapinic acid, PrA: Protocatechuic acid, Res: resveratrol, Kfl: Kaempferol, Qcn: Quercetin, ChA: Choloregenic acid, pCuA: p-Coumaric acid, F.A.: Ferulic acid, CA: Caffeic acid, GA: Gallic acid. *** µg/mL**

Surface Morphology of GO-[APMIM][NTf2] Coated Fiber

We characterized the surface morphology of the G.O. and GO-[APMIM][NTf2] material by SEM (Figure 4). In Figure 4a, it is evident G.O. sheets seem the sheet-like structure with a smooth surface and wrinkled edge. After a combination with I.L. (Figure 4b), the coating material had a rougher surface, which pointed out that I.L. stacks assembled on the surface of the G.O. layers. The GO-[APMIM][NTf2] is porous with a much rougher surface, which indicates that the coating material has a large surface area. Figure 4c-d shows that the coating possessed a homogeneous and porous structure. The porous structure of the coating could have increased the available surface area of the fiber, as well as its extraction ability. From the SEM images in Figure 4c-d, the coating thickness was determined as 27.5 µm for the GO-[APMIM][NTf2] coating material.

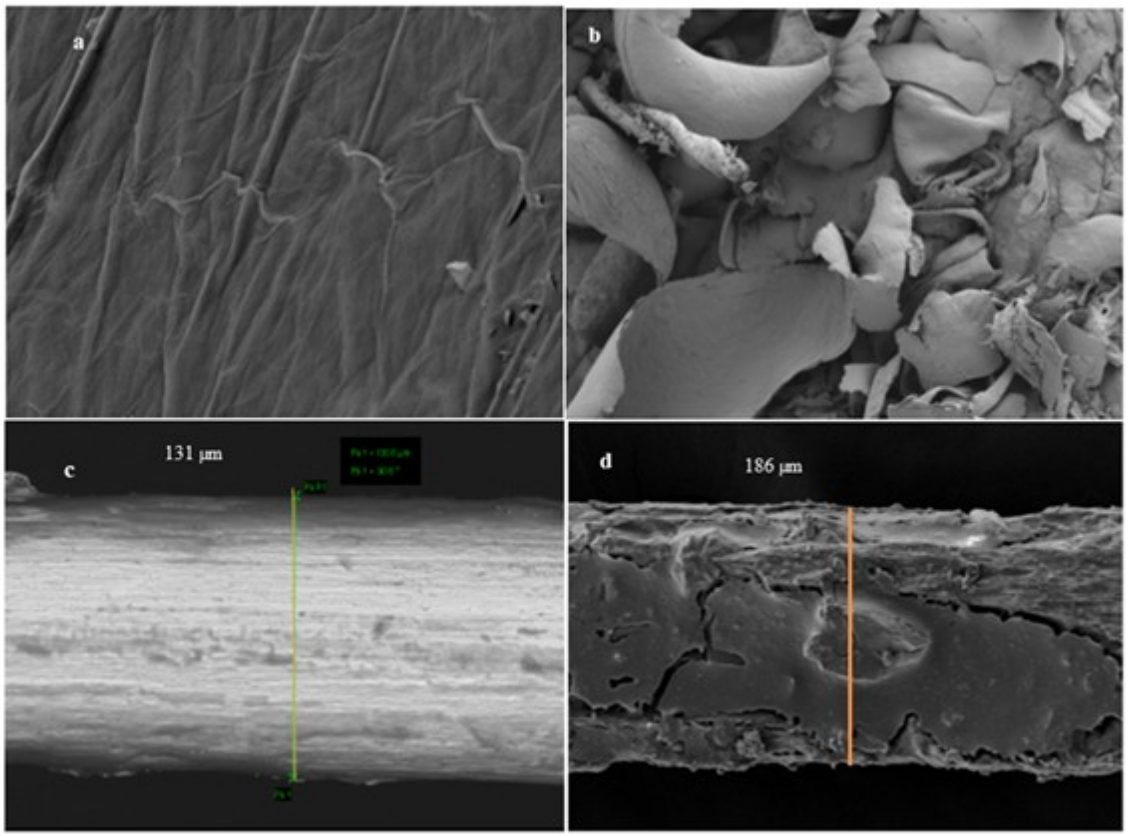

Figure 4. SEM images of GO, 2 µm, 10000 X (a); GO-[APMIM][NTf2] 20 µm, 500 X (b); etched stainless steel fiber, 20 µm, 1000 X (c); GO-[APMIM][NTf2] fiber, 20 µm, 1000 X (d).

Application to Real Sample Analysis

The proposed GO-[APMIM][NTf2] coated fiber was used for the analysis of twelve P.C.s in red wine, white wine, and fruit wine samples by DI-SPME-GC-MS method (Table 3). By SPME, known as a non-exhaustive method, the amount of analytes found in the sample represents the free concentration of analytes (20). We examined the recoveries of 12 P.C.s by spiking 5 and 200 µg L-1 concentration of P.C.s to the wine samples to evaluate the accuracy of the proposed DI-SPME-GC-MS method with the home-made coated fiber. As shown in Table 3, we acquired the recoveries of P.C.s in the range of 75.4- 99.8 % for red wine, 75.2- 99.9 % for white wine, and 71.2- 99.7 % for fruit wines with the RSDs less than 13.71% depending on the P.C.s and samples. Figure 5 shows the typical chromatograms of fruit wines, white wine, and red wine samples as blank and red wine spiked of P.C.s standard.

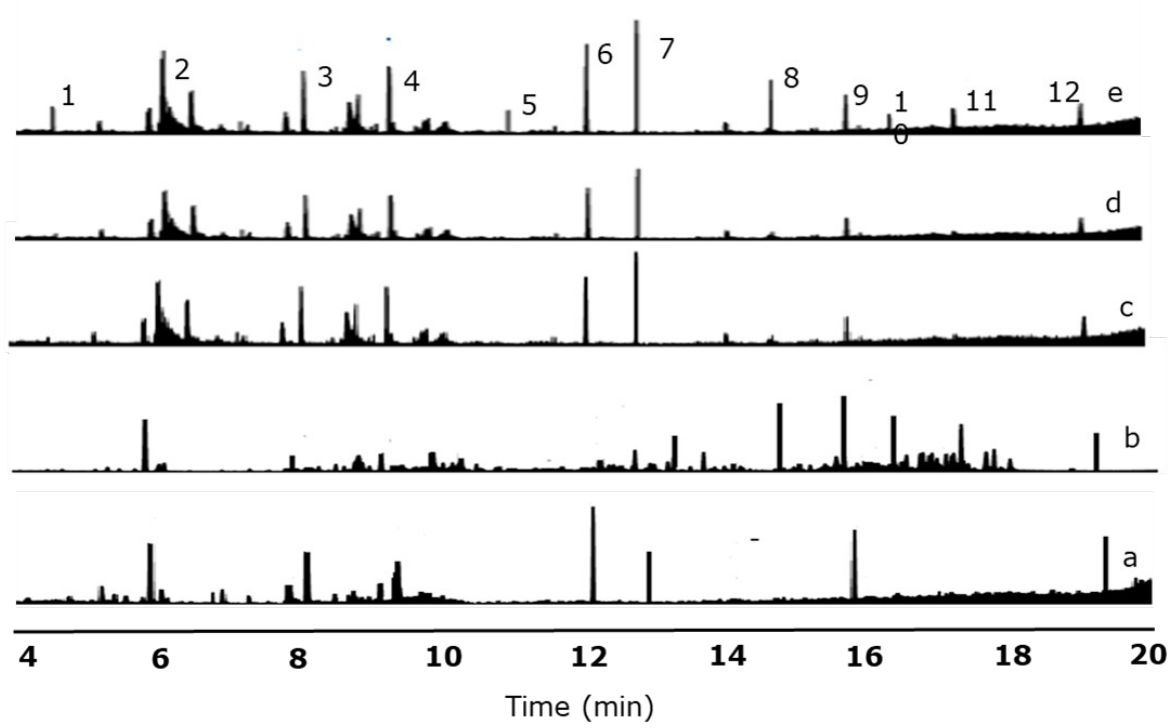

Figure 5. Chromatograms of P.C.s for wine samples by the proposed method. (a) blank melon wine, (b) blank blueberry wine, (c) blank white wine, (d) blank red wine, and (e) blank red wine spiked with P.C.s at 20 µg L-1. Peak identification: (1) cinnamic acid, (2) p-coumaric acid, (3) ferulic acid, (4) syringic acid, (5) sinapinic acid, (6) protocatechuic acid, (7) caffeic acid, (8) resveratrol, (9) gallic acid, (10) kaempferol, (11) quercetin, (12) chlorogenic acid.

CONCLUSION

The GO-[APMIM][NTf2] coated fiber was prepared and successfully applied for the determination of P.C.s in wine samples by the proposed DI-SPME under optimized conditions (30 °C, 30 min, 5 min desorption) and on-fiber derivatization (20 min) combined to GC-MS method. The developed home-made fiber exhibits high durability, excellent thermal behavior, high fiber-to-fiber reproducibility, and long term stability without a reduction in the extraction performance after more than 150 extraction cycles. Also, the extraction efficiency of the GO-[APMIM][NTf2] coated fiber was much better than the studied commercial fibers (P.A., CAR/PDMS, DVB/CAR/PDMS, and CW/PEG). The prepared fiber presents a wide linear range, low LODs, and excellent repeatability and reproducibility in the determination of the P.C.s in different kinds of wine samples. Besides, by using the home-made SPME fiber, good recoveries for the analysis of P.C.s were succeeded in the wine samples.

The performance of the present coating material could be due to electrostatic interactions between imidazolium cation and the P.C.s, as well as the π-π and dipole-dipole interactions with G.O. and I.L., and the P.C.s. Thus, GO-[APMIM][NTf2] coated fiber can be taken into account as SPME fiber for the extraction of P.C.s in various kinds of food samples.

Table 3. Analytical results of P.C.s* in wine samples (n= 3).

Wine samples	Added (µg/ L)		CnA	pCuA	FA	SnA	CA	ChA	SyA	PrA	GA	Kfl	Qcn	Res
Red	0	Found	58.3±7.1	255.7±0.3	382.0±0.3	ND	2.8±0.2	108.0±0.6	270.0±0.6	1.4±0.8	194.0±3.2	233.012.3	103.7±0.9	81.5±0.6
	5	RR,RSD(%)	96.7±6.13	97.6±9.7	97.5±8.4	75.4±7.2	84.0±8.9	98.9±2.4	99.7±3.9	91.6±7.1	99.8±6.3	99.5±10.6	99.2±6.8	98.9±0.2
	200	RR,RSD(%)	95.2±5.2	96.8±7.2	96.4±3.3	79.2±4.9	87.5±1.5	89.9±2.5	96.8±1.0	92.5±6.6	90.1±5.6	98.1±6.2	94.3±5.4	97.6±1.0
White	0	Found	2.9±0.7	69.6±0.9	85.1±0.4	ND	308.0±1.4	7.8±2.7	54.8±0.2	496.0±0.1	491.0±4.9	ND	ND	ND
	5	RR,RSD(%)	89.4±5.3	95.18±.3	99.0±12.4	75.22±.8	99.6±1.8	86.0±9.4	98.1±6.6	99.9±4.0	99.9±7.8	83.5±10.1	80.5±8.3	80.0±4.9
	200	RR,RSD(%)	91.8±2.5	93.1±5.8	91.9±1.5	78.4±2.4	97.4±3.8	92.9±6.5	93.4±1.3	97.3±0.9	97.3±4.0	88.9±8.1	88.7±5.8	84.7±0.6
Black mulberry	0	Found	ND	238.0±0.1	301.0±0.2	ND	415.5±2.7	447.5±0.1	280±0.5	1.6±0.4	407.0±0.8	ND	338.0±1.8	ND
	5	RR,RSD(%)	86.4±,5.0	99.4±6.8	99.6±3.1	76.6±9.2	96.5±5.6	99.6±5.7	99.5±6.6	81.6±5.4	99.6±2.4	78.2±4.4	99.5±1.1	86.2±10.2
	200	RR,RSD(%)	92.60±0.8	93.6±0.2	93.8±3.5	80.9±0.9	93.3±3.6	95.1±1.2	93.4±0.3	83.7±3.2	92.9±2.9	89.9±2.1	94.7±0.3	88.8±5.4
Blueberry	0	Found	ND	107.0±0.8	ND	ND	415.5±4.5	3.7±0.5	ND	1.7±0.2	416.0±2.1	448.5±7.9	469.0±6.7	482.0±0.2
	5	RR,RSD(%)	82.2±8.3	88.7±8.3	80.7±8.1	77.6±3.1	99.5±5.2	82.2±6.3	80.0±10.5	79.6±8.4	99.6±8.2	99.6±6.9	99.7±7.8	99.7±4.5
	200	RR,RSD(%)	88.5±4.6	86.1±5.1	82.4±4.1	85.1±2.3	96.8±6.5	91.0±4.7	83.8±4.3	83.2±5.2	86.1±0.9	86.2±5.0	87.1±1.9	83.0±3.5
Melon	0	Found	ND	371.0±1.8	311.0±2.0	ND	453.0±0.1	294.0±0.2	272.0±4.5	441.0±7.9	312.0±0.8	ND	ND	ND
	5	RR,RSD(%)	81.8±4.63	99.7±1.3	99.8±1.5	73.4±7.7	99.8±0.9	99.6±1.8	99.6±3.9	99.9±0.6	99.8±1.8	84.2±4.8	84.4±5.1	81.6±1.4
	200	RR,RSD(%)	93.7±1.3	95.7±0.5	95.9±0.8	89.9±0.1	96.4±0.7	95.1±0.8	92.4±0.7	96.0±0.6	91.4±0.9	93.5±1.1	88.5±1.2	96.5±2.8
Red plum	0	Found	ND	448.0±8.5	364.0±4.2	ND	957.0±6.6	314.0±4.2	ND	ND	ND	398.0±8.5	975.0±10.6	ND
	5	RR %,RSD	84.6±4.8	99.7±1.3	99.5±6.0	75.2±1.4	99.8±6.8	99.4±3.96	78.8±8.4	72.8±7.2	77±.64.1	99. ±51.9	99±.89.2	76.2±6.6
	200	RR %,RSD	91.0±4.4	98.3±1.4	93.1±3.5	85.1±4.4	95.1±0.8	97.4±3.0	89.5±7.9	83.0 ±3.2	81.7±2.7	96.6±2.2	97.0±0.5	93.3±4.3

[a]Values are mean ± standard deviation

[b]RR : Relative recovery; ND: not detected

* P.C.s; CnA: Cinnamic acid, SyA: Syringic acid, SnA: Sinapinic acid, PrA: Protocatechuic acid, Res: t-resveratrol, Kfl: Kaempferol, Qcn: Quercetin, ChA: Chlorogenic acid, pCuA: p-Coumaric acid, F.A.: Ferulic acid, CA: Caffeic acid, GA: Gallic acid

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SUPPORTING INFORMATION SUMMARY

Details of parameters of SIM mode for P.C.s after derivatization and characterization studies of GO-[APMIM][NTf2] coating material are given in the supporting information.

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Graphene Oxide-Ionic Liquid Used as Solid-Phase Microextraction Coating for Polyphenolic Compounds' Extraction and Determination with GC-MS After On-Fiber Derivatization in Wine

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Supporting Information

Table S1. Parameters of SIM mode for PCs after derivatization.

Compound	Molecular weight	TMS groups	TMS derivatized molecular weight	t_R (min)	Characteristic fragments[a]
Cinnamic acid	148	1	220	4.48	131, 161, 205, 145, 220
p-Coumaric acid	164	2	308	6.01	219,293,245,308
Ferulic acid	194	2	338	8.07	249,323,338,219
Syringic acid	198	2	342	9.30	327,342,312,297
Sinapinic acid	224	2	368	11.01	353,368,338,249
Protocatechuic acid	154	3	370	12.13	193,370,355,73
Caffeic acid	180	3	396	13.04	291,396,381,73
Resveratrol	228	3	444	14.71	267,179,429,444
Gallic acid	170	4	458	15.82	281,147,179,458
Kaempferol	286	4	574	16.44	487,574,559,272
Quercetin	302	5	662	17.35	647,574,559,662
Chlorogenic acid	354	6	786	19.16	419,786,397,345

[a] Quantitations are underlined.

Characterization of GO-[APMIM][NTf ₂] coating material

In the FTIR study (Figure S1(A)), as well as the main peaks of GO, the presence of IL was verified by the peaks at 2914, 2847, 1578 and 1456 cm^{-1} corresponding to the stretching vibrations of C-H in imidazole ring, aliphatic groups, C-N and C=N groups in imidazole ring, respectively. In thermal gravimetric analysis curves (Figure S1(B)), GO-[APMIM][NTf_2] coating with fewer thermally labile oxygen functional groups has a mass loss in the range of 200-450°C at the low slope (from 95% to 70% weight loss) beside the moisture loss at 150 °C. In XRD powder patterns (Figure S1(C)), the appearance of new peaks at 2θ = 23.67°, 38.17° and 40.51° explain the exfoliation of GO due to the removing of water molecules and the oxide groups and interaction with IL.

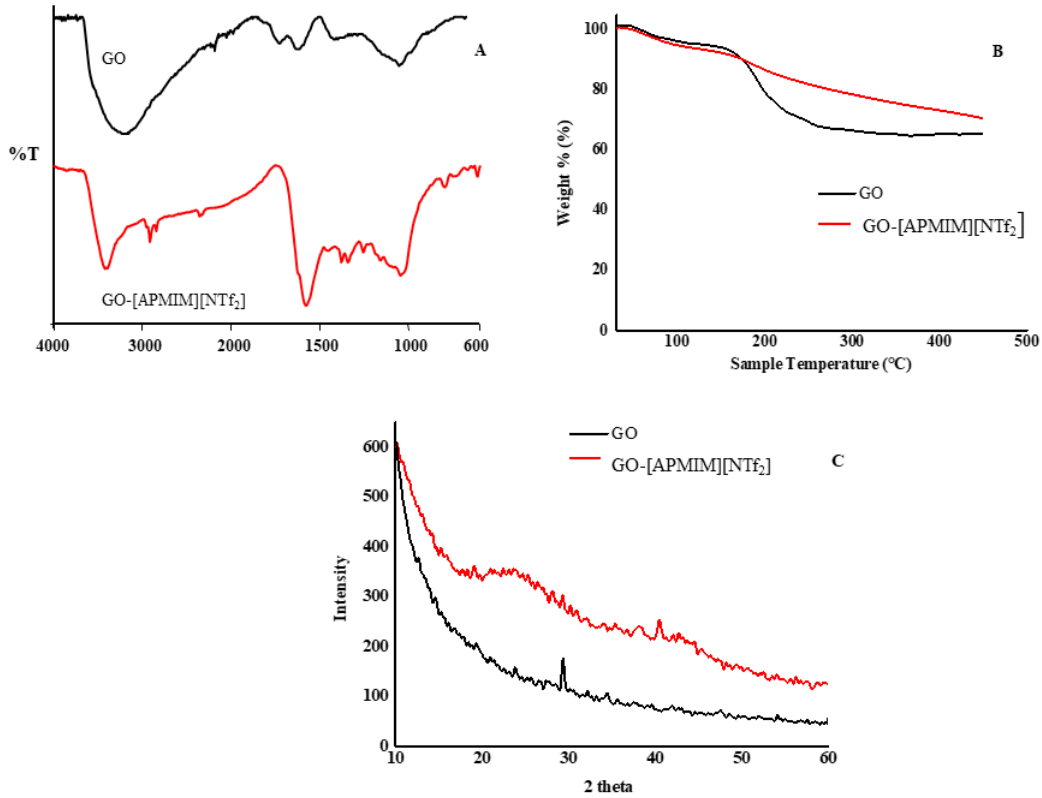

Figure S1: FTIR spectra (A), thermogravimetric analysis (B), and XRD patterns of GO and GO-[APMIM][NTf $_2$] (C).