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Graphene quantum dots as sensor for phenols in olive oil

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ABSTRACT

A new method for the determination of the phenol fraction of olive oil is reported. An optical nanosensor based on graphene quantum dots, obtained by pyrolysis of citric acid, was specifically developed for this purpose. The ensuing fluorescence sensing method, which is simple, and highly sensitive and reproducible, was used here to determine gallic acid and oleuropein as model analytes commonly found in olive oils, as well as the phenolic concentration of olive oil real samples. The detection limits were lower than 0.12 mg L^{-1} and the precision, expressed as relative standard deviation, lower than 1.7%.

1. Introduction

Graphene, a one-atom thick layer consisting of carbon atoms arranged in a honeycomb lattice with sp^2 hybridization, has attracted much attention among the scientific community in recent years by virtue of its exceptional electronic, mechanical and thermal properties [1]. Graphene is a zero-band gap nanomaterial with an infinite excitation Bohr radius – a result of the linear energy dispersion relationship of its charge carriers [2] – this conceals its luminescence. Graphene quantum dots (GQDs), which are emerging luminescent carbon-based nanomaterials, have lately aroused increasing interest in their optical and electronic properties. GQDs are graphene sheets with lateral size smaller than 100 nm in single, double and multiple layers [3], and diameters spanning the range 3–20 nm mainly. These materials possess special properties including low toxicity, high biocompatibility, high fluorescent activity, robust chemical inertness and excellent photostability [4] by effect of quantum confinement and edge (armchair or zigzag) effect. These properties confer GQDs a variety of potential uses in photovoltaic devices, bioimaging instruments, sensors and biosensors, among others [5].

So far, GQDs have been produced by using top-down or bottom-up methods. The former include electron beam lithography [6], chemical vapor deposition (CVD) [7], chemical oxidation [8,9], hydrothermal [10,11] and solvothermal treatments [12], sonication [13], hydrazine hydrate reduction [14], electrochemical preparation [15] and exfoliation, and disintegration. Most top-down methods use carbon black [3], graphene oxide (GO) [4,5,10,13,14] or carbon nanotubes (either single-wall [16] or multiwall [17]) as raw material. The bottom-up methods involve solution chemistry [18]; carbonization of organic precursors such as glucose [19], citric acid [20] or HBC (hexa-peri-hexabenzocoronene) [21]; or fragmentation of C_{60} [22]. The top-down methods have the advantage that they afford large-scale production, are simple to operate and use readily available raw materials; however, they require special equipment and typically provide low yields. By contrast, the bottom-up methods involve complex synthetic procedures and use special precursors.

Like other carbon-based materials, GQDs exhibit largely size-dependent photoluminescence (PL), which has been ascribed mainly to quantum confinement, composition, structure and shape. GDQ photoluminescence typically ranges from blue to green or, less commonly, yellow to red, with smaller GQDs having longer PL emission wavelengths than larger ones. Photoluminescence in most – but not all – GQDs is excitation wavelength-dependent; also, their PL wavelength is not pH-dependent, but its emission intensity is. Interestingly, the influence of pH varies with the synthetic method used; thus, some GQDs prepared under alkaline conditions exhibit strong PL, whereas others obtained under acid or neutral conditions exhibit maximal PL emission [23]. The presence

Abbreviations: GQDs, graphene quantum dots; EVOO, extra virgin olive oil; VOO, virgin olive oil; LOO, "lampante" olive oil; ROO, refined olive oil; FL, fluorescence.

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of oxygen-containing (carbonyl, epoxy, hydroxyl, carboxyl) functional groups at the edge of GQDs makes them very readily soluble in water and in most polar organic solvents [8]. The quantum yield (QY) of the GQDs varies with the particular synthetic method and the chemistry of their surface; thus, QY typically ranges from 2 to 22.9% for GQDs with an unpassivated surface and can easily exceed 46% for surface-passivated GQDs [6].

Graphene quantum dots have scarcely been used in analytical chemistry. The few, recent exceptions involve the determinations of glucose [24], free chlorine in drinking water [25], Cd^{2+} [26], TNT [27], Fe^{3+}[28], pyrocatechol [29], immunoglobulin G [30] and specific DNA sequences [31].

This paper reports a new application of GQDs: their use in a sensor for phenolic compounds from olive oil. The antioxidant potential of olive oil is known to be due to its containing phenols. In recent years, antioxidant properties have aroused considerable interest on account of their benefits on human health (e.g. protection against coronary heart diseases and tumors) and their impact on olive oil stability and shelf life [32].

2. Metrials and methods

2.1. Reagents and standards

All chemical reagents were analytical-grade and used without additional purification. The reagents citric acid (≥99.0%), gallic acid (≥98.0%), oleuropein (≥98.0%), sodium carbonate (≥99.5%) and folin and ciocalteu (2 N), and the solvents acetone and *n*-hexane, both in HPLC-grade, were all purchased from Sigma–Aldrich (Madrid, Spain). Sodium hydroxide and *N*,*N*-dimethylformamide were obtained from Panreac Chemical, SAU (Barcelona, Spain). HPLC-grade methanol (≥99.9%) and acetonitrile (≥99.9%) were purchased from Carlo Erba Reagents (Barcelona, Spain) and VWR Chemicals (Barcelona, Spain), respectively. The olive oil samples used in the optimization tests were supplied by Sovena España–Consumer Goods (Seville, Spain).

2.2. Instrumentation

Fourier transform mid infrared (FT-MIR) spectra were obtained on a Bruker Tensor 27FT-MIR spectrophotometer equipped with a Hyperion 2000 microscope, using KBr pellets. High-resolution transmission electron microscopy (HRTEM) images were obtained on a JEOL JEM 2010 electron microscope available at the Research Support Service (SCAI) of the University of Córdoba. The instrument had a point-to-point resolution of 0.194 nm and was operated at a medium acceleration voltage of 200 kV.

Fluorescence emission spectra were recorded on a PTI QuantaMaster™ spectrofluorometer from Photon Technology International (Barcelona, Spain) equipped with a 75 W xenon short arc lamp and an 814 PTM detection system. The software FeliX32 was used for data acquisition and instrument control. The excitation and emission slits were both 3.8 nm wide. All measurements were made at room temperature, using micro quartz cuvettes of 10 mm lightpath. UV–Vis absorption spectra were obtained on a Lambda 35 ES UV/Vis spectrophotometer from Perkin Elmer (Madrid, Spain) equipped with two radiation sources (deuterium and tungsten–halogen lamps) and photodiode detectors; measurements were made in polypropylene cuvettes at room temperature.

2.3. Synthesis of GQDs

Dots were obtained by pyrolysis of citric acid, using a slightly modified version of the procedure by Dong et al. [20]. To this end, an amount of 2 g of citric acid was placed in a vial and heated at 200 °C on a thermoblock from JP Selecta (Barcelona, Spain) until the citric acid changed from a white dust to a dark orange liquid, which took about 30 min. The resulting liquid was added dropwise to 100 mL of a 10 mg L^{-1} NaOH solution under vigorous stirring. The GQD aqueous solution thus obtained was adjusted to pH 10 with nitric acid and stored at 4 °C in an amber bottle.

2.4. Sample treatment

Refined olive oil (ROO), used as a blank in the recovery and sensitivity tests described below, was spiked with gallic acid to a final concentration in the range 0–6 mg L^{-1}. A stock standard solution containing 1 g L^{-1} gallic acid in methanol was prepared and stored at 4 °C. Working-strength solutions were obtained by dilution of the stock in methanol. Aliquots of 0.1 g L^{-1} gallic acid methanolic solution (0–120 μL) were added to a polypropylene (PP) centrifuge tube and dried at 35 °C under a nitrogen stream. Then, an amount of 2 g of ROO was placed in the tube and agitated on an MS 3 Basic vortex mixer from IKA (Staufen, Germany) at 2000 rpm for 5 min prior to liquid–liquid extraction as described below.

2.4.1. LLE extraction of phenolic compounds from olive oil

For liquid–liquid extraction, 1 mL of *n*-hexane and 2 mL of (60/40, v/v) methanol/water mixture were added to 2 g of olive oil according to Pirisi et al. [33]. The mixture was stirred in a vortex at 2500 rpm for 2 min and centrifuged at 4000 rpm for 10 min. Then, the methanol layer was separated and the extraction repeated twice. Once extraction was completed, the extracts were combined and cleaned up by (a) low-temperature fat precipitation at −20° C overnight or (b) washing with *n*-hexane (3 × 2 mL). The combined methanol extracts and the n-hexane used to clean up in the latter procedure were mixed in a vortex at 3000 rpm for 30 s and centrifuged at 4000 rpm for 10 min. Then, the *n*-hexane was discarded and the methanolic solution evaporated to dryness under a nitrogen stream at 35 °C. The ensuing residue was recovered with 200 μL of methanol (theoretical concentration factor, 10×). The low-temperature fat precipitation procedure provided poor results because fats were incompletely removed from the extracts. This led us to select cleanup with *n*-hexane.

In a typical run, 200 μL of GQD solution at pH 10 (final concentration, 1.125 mg L^{-1}) was passed through a 0.22 μm mesh nylon syringe filter and mixed with the methanolic extract (200 μL) obtained from the liquid–liquid extraction of olive oil in a fluorescence quartz microcuvette for measurement at an excitation wavelength of 380 nm.

Real samples of four different olive oil grades [viz., extra virgin olive oil (EVOO), virgin olive oil (VOO), lampante olive oil (LOO) and refined olive oil (ROO)] were analyzed by using the proposed sensing method and compared for total phenol index.

2.5. Total phenol (TP) content

The total phenol contents of the olive oils extracts was determined colorimetrically at 765 nm, using Folin–Ciocalteu (FC) reagent according to Waterhouse [34], in order to compare our sensing system with a reference method. The spectrophotometric analysis was repeated 3 times with each type of extract. The results were expressed as gallic acid equivalents (GAE, in mg L^{-1}).

We used the microscale protocol for Follin–Ciocalteu Colorimetric method as adapted for small sample volumes in order to reduce costs and waste production. The reaction was performed directly in the measuring cuvette. For total phenol determination, 20 μL of sample, 1.58 mL of ultrapure water and 100 μL of FC reagent were placed in a 10 mm, 2 mL plastic cuvette, mixed thoroughly by pipetting and incubated for 6 min. Then, 300 μL of sodium carbonate solution was added and the mixture incubated at room temperature for 2 h. The sample absorbance was measured at 765 nm. The