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# Fluorescent determination of graphene quantum dots in water samples

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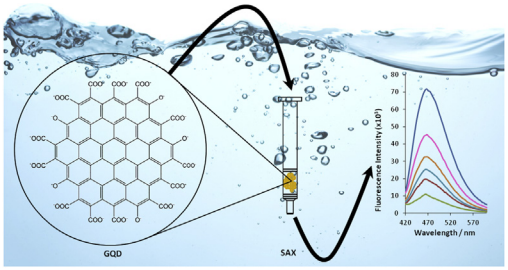
## HIGHLIGHTS

- Development of a novel and simple method for determination of graphene quantum dots.
- Preconcentration of graphene quantum dots by solid phase extraction.
- Fluorescence spectroscopy allows fast measurements.
- High sensitivity and great reproducibility are achieved.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This work presents a simple, fast and sensitive method for the preconcentration and quantification of graphene quantum dots (GQDs) in aqueous samples. GQDs are considered an object of analysis (analyte) not an analytical tool which is the most frequent situation in Analytical Nanoscience and Nanotechnology. This approach is based on the preconcentration of graphene quantum dots on an anion exchange sorbent by solid phase extraction and their subsequent elution prior fluorimetric analysis of the solution containing graphene quantum dots. Parameters of the extraction procedure such as sample volume, type of solvent, sample pH, sample flow rate and elution conditions were investigated in order to achieve extraction efficiency. The limits of detection and quantification were 7.5 μg $L^{-1}$ and 25 μg $L^{-1}$, respectively. The precision for 200 μg $L^{-1}$, expressed as %RSD, was 2.8%. Recoveries percentages between 86.9 and 103.9% were obtained for two different concentration levels. Interferences from other nanoparticles were studied and no significant changes were observed at the concentration levels tested. Consequently, the optimized procedure has great potential to be applied to the determination of graphene quantum dots at trace levels in drinking and environmental waters.



## 1. Introduction

In the last years, the advances in nanotechnology and the application of a widely variety of engineered nanoparticles in the industry and commercial products make human and environmental exposure increase every day [1]. Graphene, a two dimensional one-atom thick with $sp^2$ hybridization carbon nanoparticle, and graphene family nanomaterials have attracted much attention since the graphene first isolation in 2004 due to their exceptional electronic, mechanical, optical and thermal properties [2,3]. Recently, graphene quantum dots (GQDs), an emerging graphene based luminescent nanoparticles have become in object of interest in the scientific community due to their electronic and optical properties. GQDs are graphene nanosheets smaller than 100 nm lateral size and diameters between 3 and 20 nm. These

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nanoparticles can be as single-, double- or multi-layer [4]. Quantum confinement and edge effect make them exhibit interesting properties such as fluorescent activity, robust chemical inertness, excellent photostability, high biocompatibility and low toxicity [5]. These properties confer it a variety of potential uses in several scopes, mainly, sensors [6] and biosensors [7], bioimaging [8], drug delivery [9] and photovoltaic devices [10]. In spite of the fact that GQDs are defined as low toxic and high biocompatible nanoparticles, there are some works that put into question these features. In vivo studies in mice revealed that the GQDs were mainly accumulated in liver, spleen, lung, kidney and tumor sites [11]. Otherwise, the theory of lateral dimension explains that the in vivo biodistribution and manifestation of toxicity would be lower as smaller nanomaterial size, so GQDs smaller than 5 nm would be eliminated with urine [12]. Due to the GQDs biosafety issue still been unclear and the increasing production and use of graphene nanomaterials could increase the risk of environmental widespread, methodologies that allow the determination of these nanoparticles should be developed. Nevertheless, at present, there are only a few analytical approaches for this purpose. Some methodologies have been described for carbon nanotubes [13], gold nanoparticles [14,15] and, most recently, graphene oxide [16].

In this work, a simple, rapid and sensitive approach for the determination of GQDs in natural and tap water samples was developed. The interaction of GQDs with the charged sorbent through ionic interactions and their subsequent elution lead to the preconcentration of GQDs. The fluorescence emission of the eluted graphene nanosheets has been used as analytical signal for the quantification of the presence of GQDs. As far as we are concerned, this is the first approach to the determination of graphene quantum dots from environmental and drinking waters.

## 2. Materials and methods

### 2.1. Reagents and standards

All chemical reagents were of analytical grade and used as received without further purification. Citric acid (≥99.0%), used as precursor in the GQDs synthesis procedure, ammonium acetate (≥98.0%), nitric (≥69.0%) and sulfuric (≥95%) acids, HPLC-grade acetone (≥99.8%) acetonitrile (≥99.8%), ethyl acetate, sodium sulfate anhydrous (≥99.0%), sodium phosphate dehydrate (98.5–101.0%) and humic acid were purchased from Sigma–Aldrich (Madrid, Spain). Sodium hydroxide, hydrochloric acid, N,N-dimethylformamide (≥99.8%), dimethyl sulfoxide (≥99.5%) and hydrogen peroxide (33%) were obtained from Panreac Chemical, SAU (Barcelona, Spain). HPLC-grade methanol (≥99.9%) was purchased from Carlo Erba Reagents (Barcelona, Spain). Single Wall Carbon Nanotubes (SWCNT) were obtained from ShenzhenNanotechPortCo.Ltd (NTP) (China), with a purity over 90%, an outer diameter of <2 nm, a length of 5–15 mm and a surface area of 500–700 $m^2\ g^{-1}$. Graphene (avangraphene-2, <6 sheets) was supplied by Avanzare Innovacion Tecnologica SL (Logroño, Spain). Commercial gold nanoparticles (AuNPs), 10 nm sized, were purchased also from Sigma–Aldrich. Ultrapure water was obtained from a Milli-Q purification system (Millipore, Madrid, Spain) with a resistivity of 18.2 MΩ/cm and used for preparation of all solutions.

### 2.2. Instrumentation

Fluorescence emission spectra were recorded on a PTI QuantaMaster™ spectrofluorimeter 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 6 nm wide. All measurements were made at room temperature, using micro quartz cuvettes of 10 mm lightpath.

Fourier transform mid infrared (FT-MIR) spectra were obtained on a Bruker Tensor 27 FT-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 has a point-to-point resolution of 0.194 nm and was operated at a medium acceleration voltage of 200 kV.

### 2.3. Synthesis of GQDs

GQDs were obtained by pyrolysis of citric acid, similarly to that described in Refs. [6,16] using a slightly modify procedure similar to that described by Dong et al. [17]. To this end, an amount of 2 g of citric acid was placed in a 10 mL vial and heated at 200 °C on a thermoblock (JP Selecta, Barcelona, Spain) for 30 min. During this time the citric acid changed from a white dust to a dark orange viscous liquid. The resulting liquid was added dropwise to 100 mL of a 0.25 M NaOH solution under vigorous stirring. The GQD aqueous solution obtained was kept stirring for 10 min, adjusted to pH 7 with nitric acid and stored at 4 °C in an amber bottle.

### 2.4. Functionalization of single-walled carbon nanotubes and graphene

Carboxylated single-walled carbon nanotubes (c-SWCNTs), graphene oxide (GO) and gold nanoparticles (AuNPs) have been considered as potential interferences in this work. AuNPs were of commercial origin. C-SWCNTs were prepared by adding 100 mg of SWCNT to 20 mL of a 3:1 $H_2SO_4/HNO_3$ mixture into a glass flask similar to the procedure described by Lopez-Lorente et al. [13]. The mixture was refluxed for 1 h. After that, diluted fractions were centrifuged at 10,000 rpm for 10 min and washed with ultrapure water. The centrifugation and washing processes were repeated until the supernatant phase stopped having acidic pH. Finally, carboxylated derivates were dried at 60 °C in a heater. The same procedure was performed in order to obtain graphene oxide (GO), being diluted fractions of treated graphene centrifuged at 10,000 rpm for 20 min due to the less size of these nanoparticles. After acid treatment, SWCNTs and graphene posses carboxylic (–COOH) groups on the sidewalls and open ends (in the case of SWCNTs) being negatively charged, which make them highly water soluble.

### 2.5. Solid phase extraction procedure

Commercial SAX (Strong Anion Exchange) column with a capacity of 1 mL and 50 mg of sorbent (Biotage, Hengoed, United Kingdom) was selected for the SPE extraction of GQDs. SAX is a quaternary amine (functionalized with a chloride counter ion) silica sorbent with an average particle size of 52 μm, a pore diameter of 56 Å and a surface area of 480 $m^2\ g^{-1}$, packed in a polypropylene column (volume: 1 mL; inner diameter: 6 mm) and fitted with polyethylene frits. This SAX column was rinsed with 1 mL of ultrapure water, followed by 1 mL of 0.1 M and 15 mM ammonium acetate, respectively. Then, 20 mL of an aqueous solution containing GQDs at pH 7.0 was passed through the column at a constant flow rate of 1 mL $min^{-1}$. A daily prepared 0.5 mg/mL GQDs aqueous solution standard was adjusted to pH 7.0 with diluted $HNO_3$. After loading the sample, the sorbent was dried by passing air through the column during 3 min. The nanoparticles retained were finally