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Production of few-layer graphene ready-to-use in aqueous suspensions

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The tremendous interest in graphene science and technology is motivated by its promising physical and chemical properties, which have just started to be engineered in electronics, photonics and composites. A main constraint related to the graphene development is that, being of strongly hydrophobic nature, the majority or totality of dispersions are prepared in poisonous organic solvents, such as N-methyl pyrrolidone or N,N'-dimethyl formamide. We detail here a protocol that describes the preparation of exfoliated graphite down to few-layer graphene by the use of ball milling and its subsequent stabilization in water. Our methodology consists of four main steps: (1) the mechanochemical intercalation of organic molecules (melamine) into the graphitic planes followed by suspension in water; (2) the washing of suspended graphene, to get rid of most of the melamine; (3) the isolation of stable graphene sheets; and (4) the drying of such aqueous media, leading to graphene in powder. In the timespan of 6-7 or 9-10 days for aqueous suspensions or dry powders, respectively, it is possible to obtain graphene for application in science and technology or for toxicological studies.

Keywords: graphene, melamine, mechanochemistry, water, powder.

Introduction

Graphene is a two-dimensional, one-atom-thick material, entirely made of carbon atoms, arranged in a honeycomb lattice. Because of their distinctive mechanical, optical, and electronic properties, graphene and its derivatives are envisioned as ideal candidates in a variety of applications.¹ However, a reliable and scalable graphene production still stands as a bottleneck for its broad commercialization. If high perfection monolayer graphene can be obtained by epitaxial growth or CVD methods, the as-obtained materials are limited to specific high-performance applications in the fields of electronics or optoelectronics.² Meanwhile, other fields such as nanocomposite materials or nanomedicine require larger quantities of graphene, which have been so far surrogated by chemical derivatives of graphene (namely its oxide, graphene oxide, GO, and its reduced counterpart, reduced graphene oxide, rGO), very interesting materials, but whose properties can be different from those of pristine graphene. There currently exist detailed preparation and functionalization methods for GO and rGO,³ but much less is available for non-oxidized graphene.

An accessible way to obtain cost-effective and suitable large amounts of graphene is the mechanical exfoliation of graphite.^{4,5,6} This can be performed in liquid media with the help of ultrasounds⁷ or high-shear forces,⁴ or else, under other conditions such as with supercritical fluids or by dry ball milling.^{5,5,6} These methods provide mostly few-layer graphene materials with reasonable sizes, acceptable level of defects and good structural characteristics to enable their exploitation in composite or medical applications.

In this context, we developed a facile and inexpensive ball milling process to exfoliate graphite into few-layer graphene (≤ 5 layers) using melamine as the exfoliating agent.⁸ This is an easily scalable and non-oxidative methodology, which allows achieving stable suspensions in polar solvents, including water, paving the way to biomedical applications such as hydrogel nanocomposites,⁹ film casting for cellular interaction studies,¹⁰ or for toxicological studies.¹¹ The presence of melamine in these graphene samples could raise safety concerns,¹² so this Protocol also describes how to efficiently remove and detect minimal amounts of melamine through a variety of techniques. Eventually, the lyophilization of these water suspensions gives rise to a light solid, which can be easily dispersed either in water or culture media.¹³

The possibility of having a few-layer graphene as a solid, dispersible in water, culture media and in principle in any organic solvent, opens new horizons in the technology of graphene, since:

- 1) this solid can be safely stored and shipped;
- 2) this solid can be dispersed in aqueous media or any organic solvent;
- 3) this solid can be used for studies in material science (inks, composites, etc.);
- 4) the safety issues of this new nanoform of carbon, which have raised substantial concern, can be addressed under the proper preparative conditions, without the use of additional potentially toxic detergents.¹⁴

The whole experimental method consists of four main steps, which are detailed as follows:

1.- Intercalation of melamine molecules within the graphitic planes. Graphite powder and melamine are forced into contact by the use of a planetary ball mill, which provides sufficient energy to overcome the π -stacking energy of the sp^2 planes and allow aromatic compounds to act as the exfoliating agent.¹⁵ Amongst the many compounds that could be used to this purpose, melamine resulted to be the most efficient, due to its ability to form extended two-dimensional networks.^{8,15} The process is carried out on a dry basis and under air atmosphere. The extraction of the solid is obtained by using water and mild ultrasonication, while the resulting heterogeneous mixture is processed in the following steps. This process is described in Step 1.

2.- Removal of melamine. Most of the melamine is taken away by washing with water at 70°C. Amongst the many methods that could be applied to such purpose, we have chosen the dialysis, due to its simplicity, cost-effectiveness and for being the least dependent on the operator. Besides, the maintenance of the colloidal stability of aqueous few-layer graphene along the washing process is crucial for a successful production, so that the dialysis is helpful in keeping graphene in the dispersion state¹⁶ and is compatible with the application of mild sonication pulses, which would be otherwise difficult. This is reported in Step 2.

3.- Isolation of stable few-layer graphene sheets in suspension. After getting rid of the majority of melamine, leaving only the minimum indispensable to keep graphene in suspension, it is necessary to separate the stable few-layer graphene sheets in suspension, from the accompanying non-exfoliated graphite. For this, we have preferred a natural sedimentation, through which most graphite leftovers deposit at the bottom. Then a critical extraction step is performed, in order to isolate the desired material. Step 3 contains full experimental description of the process. The concentration of graphene is determined by UV-Visible absorption at 660

nm with a weight extinction coefficient of $15.0184 \text{ mg mL}^{-1} \text{ cm}^{-1}$. This latter procedure is illustrated in anticipated results.

4.- Drying of the aqueous graphene suspension. Depending on the specific needs and the envisioned fate of the aqueous graphene, one might want to remove water without compacting the graphene sheets. Vacuum filtering or thermal evaporation usually cause the packing of the material along the drying process by capillary forces, often irreversibly, impairing the subsequent processability. It has been known for years that freeze-drying is a useful technique to efficiently dry carbon nanomaterials, avoiding these drawbacks and preserving the structure of the dried entities.¹⁷ Thus, we propose this methodology to obtain lyophilized few-layer graphene powders for potential use in many fields. This process is described in Step 4.

MATERIALS

REAGENTS

Preparation of few-layer Graphene through the ball milling process

- Graphite powder (Bay Carbon, Inc. SP-1 grade).
- Melamine (Sigma-Aldrich, CAS Number:108-78-1).
! CAUTION For safety reasons, a lab coat, gloves and a face mask should be worn during the manipulation of powders.
- Ultrapure water ($18.2 \text{ M}\Omega\cdot\text{cm}$; Milli-Q).
- Spectra/Por®1 Dialysis Membrane, MWCO: 6-8 kD, 20.4 mm diameter, 32 mm Nominal Flat Width (Spectrum Laboratories, Inc.).
- Millipore membrane Filter GTTP 0.2 μm pore size, 25 mm diameter (Millipore, Ref. GTTP02500).

Preparation of Gold Nanoparticles (AuNPs)

- Gold (III) chloride hydrate ($\text{HAuCl}_4 \cdot x \text{H}_2\text{O}$, Sigma-Aldrich, CAS Number 27988-77-8).
! CAUTION $\text{HAuCl}_4 \cdot x \text{H}_2\text{O}$ can cause skin corrosion and serious eye damage. For safety reasons, a lab coat, gloves, an eyeshield and a face mask should be worn during its manipulation.

- Trisodium citrate dihydrate (Sigma-Aldrich, CAS Number 6132-04-3).

! CAUTION For safety reasons, a lab coat, gloves and a face mask should be worn during the manipulation of trisodium citrate dihydrate.

Characterization

- Silicon Wafers P/Boron<100>, Resistivity: 0.01-0.02. (WRS Materials).
- Lacey Carbon film on 300 mesh nickel grids LC300-Ni. (Electron Microscopy Sciences, Ref. 215-412-8400).

EQUIPMENT

- Planetary mill (Retsch PM100), provided with a 25mL steel jar and 10 stainless steel beads of 1 cm diameter each.
- Freeze Drying System (Telstar Lyoquest).
- UV-Vis-NIR instrument (Cary 5000 UV-Vis-NIR spectrophotometer).
- Thermogravimetric instrument (TGA Q50, TA instruments), provided with Pt pans.
- Raman Spectroscopy (InVia Renishaw microspectrometer)
- High-Resolution Transmission Electron Microscopy (JEOL 2100)
- X-ray photoelectron spectroscopy (VG Escalab 200R spectrometer)
- Elemental Analysis (LECO CHNS-932 (Model NO: 601-800-500)).
- Ultrasonic cleaning bath without heating (Selecta 3000513)
- Ultrapure water system (Purelab UHQ (ELGA))

REAGENT SETUP

Ultrapure water. Prepare ultrapure water by purifying previously deionized water using the Purelab UHQ (ELGA) device according to the standard protocol.

Gold nanoparticle synthesis. Prepare AuNPs according to the procedure described in the literature.¹⁸ Briefly, AuNPs were prepared by reducing of gold(III) chloride trihydrate with trisodium citrate. In a typical procedure, add 25 ml of trisodium citrate (38.8 mM) to a boiling solution of gold (III) chloride trihydrate (500 ml, 0.5 mM) and stir the solution for 15 minutes

until a magenta suspension is obtained. The concentration of the AuNPs suspension is determined using UV-Vis spectroscopy, as has been reported in the literature.¹⁹

! CAUTION $\text{HAuCl}_4 \cdot x \text{H}_2\text{O}$ can cause skin corrosion and serious eye damage. For safety reasons, a lab coat, gloves, an eyeshield and a face mask should be worn during its manipulation.

◇ **CRITICAL** Owing to the colloidal nature of AuNPs, their stability may be highly ephemeral if proper requirements of storage and use are not met. AuNPs for graphene characterization should be freshly prepared as far as possible. However, storing a stock is possible if kept always in a fridge at 4°C, and contained in a dark bottle or other means of isolation from light and external impurities. Do not use AuNPs if their magenta color is not vivid or if they become turbid or blue. The operator must periodically register the UV-Vis spectrum of stock AuNPs and compare it with the one registered the day they were synthesized. Any deviation from such first spectrum is reason enough to waste these AuNPs and to prepare them anew, since the characterization of graphene could be seriously compromised.

EQUIPMENT SETUP

Raman Spectroscopy. Raman characterization is performed using an InVia Renishaw microspectrometer. Raman samples are prepared from stable dispersions of graphene by drop-casting over silicon oxide surfaces (small pieces of silicon wafers, WRS Materials), and left to evaporate in ambient conditions. Raman spectra are acquired with the 532 nm laser focused under the x100 objective and an incident power of 1% (keeping the energy density below $\sim 1 \text{ mW} / \mu\text{m}^2$), 10 seconds of exposure time, in the range from 1100 to 3000 cm^{-1} .

Transmission Electron Microscopy (TEM). To perform TEM analyses, stable dispersions of graphene are diluted and drop-cast on Lacey Carbon film nickel grids (3.00 mm, 200 mesh) and dried under vacuum. The samples are studied by High-Resolution Transmission Electron Microscopy (HRTEM) JEOL 2100 at an accelerating voltage of 100 kV.

Ultraviolet-visible Spectroscopy. UV-Vis spectra are recorded in 1cm quartz cuvettes (Agilent, Part no. 6610000800) on a Cary 5000 UV-Vis-NIR spectrophotometer. Dual beam mode and baseline correction are used throughout the measurements to scan the samples.

Thermogravimetric Analysis (TGA). The thermogravimetric analyses are performed with a TGA Q50 (TA Instruments) at 10 °C/min under nitrogen atmosphere, from 100°C to 800°C, to sample aliquots of 0.5-1 mg contained inside a Pt pan.

PROCEDURE

STEP 1. Intercalation of melamine molecules within graphitic planes. • **TIMING** (5 h)

The first step is the milling of the graphite with melamine in order to obtain exfoliated graphite.

- (i) Introduce 7.5 mg of graphite and 22.5 mg of melamine in a stainless steel grinding jar (25 mL) containing ten stainless steel beads (1 cm diameter).

! CAUTION For safety reasons, a lab coat, gloves and a face mask should be worn during the manipulation of powders.

- (ii) Close and place the grinding bowl in the planetary mill and tighten it with the clamping device.

! CAUTION Please ensure that the weight of the jar, filled with balls and sample, is compensated by setting the counterweight (700 g for 25 mL jar with 10 balls of 1 cm each and 30 mg of sample) and the clamping device is totally locked.

- (iii) Program the planetary mill according to the milling conditions (table 1).

Revolutions per minute	Time	Atmosphere	Interval operation
100	30 min	Air	Spinning reversal every 3 min

Table 1. Milling conditions

- (iv) Once the milling has ended, take the jar out of the planetary mill and pull the resultant solid mixture out by dispersing it in 20 ml. The suspension should be done using sonication according to the following scheme. Sonication pulses should be around 10 seconds each time.

✕ **NOTE** It is advised to use metal tweezers for the manipulation of beads.

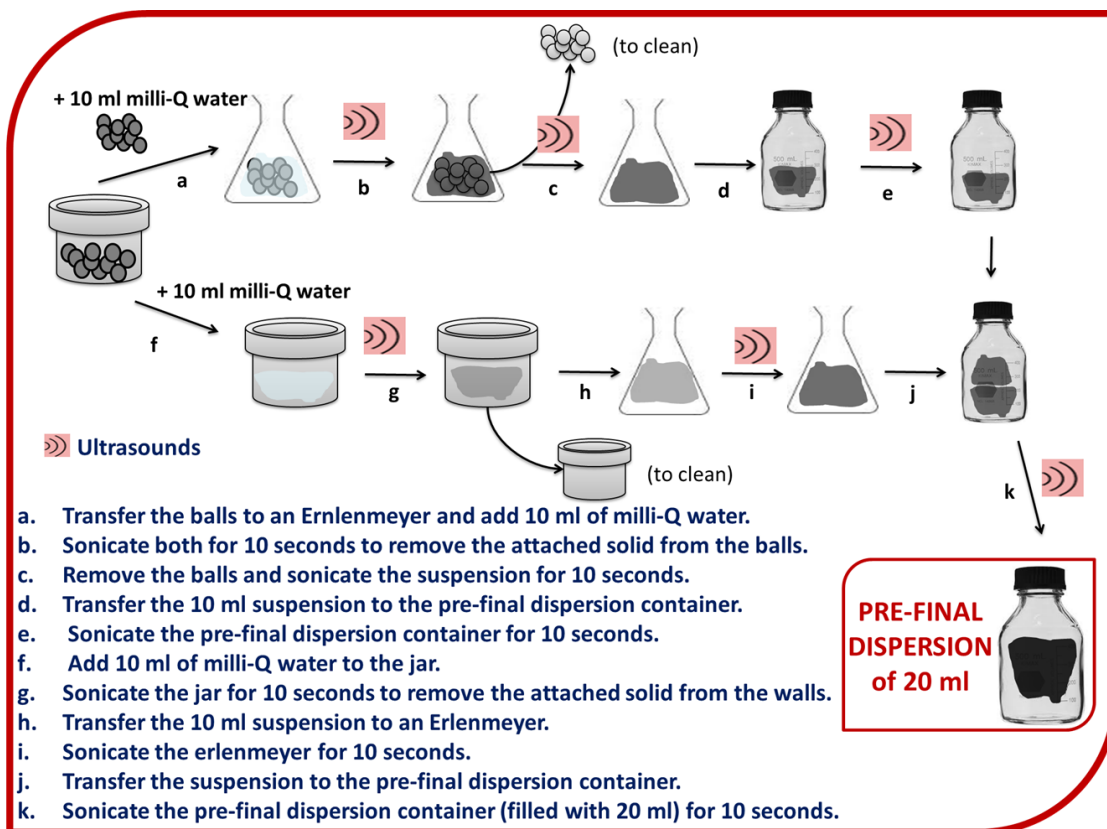


Figure 1. Pulling the solid mixture out of the jar by sonicating.

! CAUTION Keep the temperature in the sonication bath < 30°C.

! CAUTION Prolonged manipulation of steel beads inside glassware might result in glass breakage. Keep a gentle pace and do not perform abrupt movements.

(v) Repeat steps (i-iv) six times in order to obtain 2 containers of 60 ml of pre-final dispersion.

◇ **CRITICAL** The pre-final dispersion container should be sonicated upon every addition of new dispersion in order to ensure the homogenization of the sample.

STEP 2a. Removal of the melamine surplus. • **TIMING** (36 h-48 h)

The excess of melamine is removed from the pre-final dispersion by dialysis. Every container of 60 ml of pre-final dispersion will be treated separately during dialysis.

(i) Sonicate the pre-final dispersion container (60 ml) for 30 seconds.

! CAUTION Keep the temperature in the sonication bath < 30°C. Do not prolong the sonication for more than 10 seconds in each pulse.

- (ii) Transfer the pre-final dispersion (60 ml) to the previously hydrated dialysis sack (Spectra/Por®1 Dialysis Membrane, MWCO: 6-8 KD) via a 10 ml syringe with a long needle.

! CAUTION The dialysis sacks should be hydrated for at least 30 minutes before being filled to ensure their easy handling.

! CAUTION Special care must be taken to avoid piercing the sack with the needle during its filling.

! CAUTION The dialysis sack should be closed with a handmade knot and not with regular closure clamps to avoid the leakage of the sample, and to facilitate the further shaking.

- (iii) Hand-shake the dialysis sack, grabbed through the knots with a horizontal motion for a few seconds and place it in an Erlenmeyer flask filled with 500 ml of Milli-Q water, bent in a symmetrical 'U' shape. See Figures 2a-b.

- (iv) Sonicate the Erlenmeyer flask with the dialysis sack inside for no more than 1 minute to ensure the homogenization of the sample inside the membrane.

! CAUTION Keep the temperature in the sonication bath < 30°C. Do not prolong the sonication for more than 10 seconds in each pulse.

◇ **CRITICAL** When sonicating the dialysis sack, the position of the Erlenmeyer flask in the ultrasonic bath may affect the dispersion of the sample. Please ensure the waves are transmitted across the sack.

- (v) Place the Erlenmeyer flask with the dialysis sack containing the dispersion in a hot plate at 70 °C with slow stirring for at least 2 h (Figure 2a-b).

! CAUTION Please ensure that the temperature of the hot plate is well controlled and also that the magnetic stirring bar does not hit the dialysis sack.

? TROUBLESHOOTING 1

- (vi) After the elapsed time of dialysis, a qualitative control of the removed melamine in washing waters is carried out by analysis with AuNPs.²⁰ Place 2 ml of water washings and 1 ml of AuNPs in a vial and mix them by pipetting once. Check out the colour of the mixture: magenta colour indicates no melamine, purple or blue colours indicate that melamine is still being washed away from the sample (see anticipated results).

- (vii) Replace the dialysis waters with fresh Milli-Q water and repeat steps (iv)-(v)-(vi).

- (viii) Repeat step vii) until magenta colour appears twice in a row in the analysis of water washings by AuNPs.

✦ **NOTE** The dialysis usually implies one cycle overnight. In a typical experiment no melamine is usually found after 5 cycles of dialysis (see anticipated results section).

- (ix) Once the dialyses are over, transfer the dispersions from the dialysis sack (60 ml each) to a stabilization container (final volume of 120 ml), as shown in Figure 2c.

STEP 2b. Separation of few-layer graphene from non-exfoliated graphite.

● **TIMING** (120 h)

Keep the dispersion in the stabilization container (final volume of 120 ml) at room temperature for 5 days. During this time, the non-exfoliated graphite precipitates and the exfoliated graphene remains in suspension.

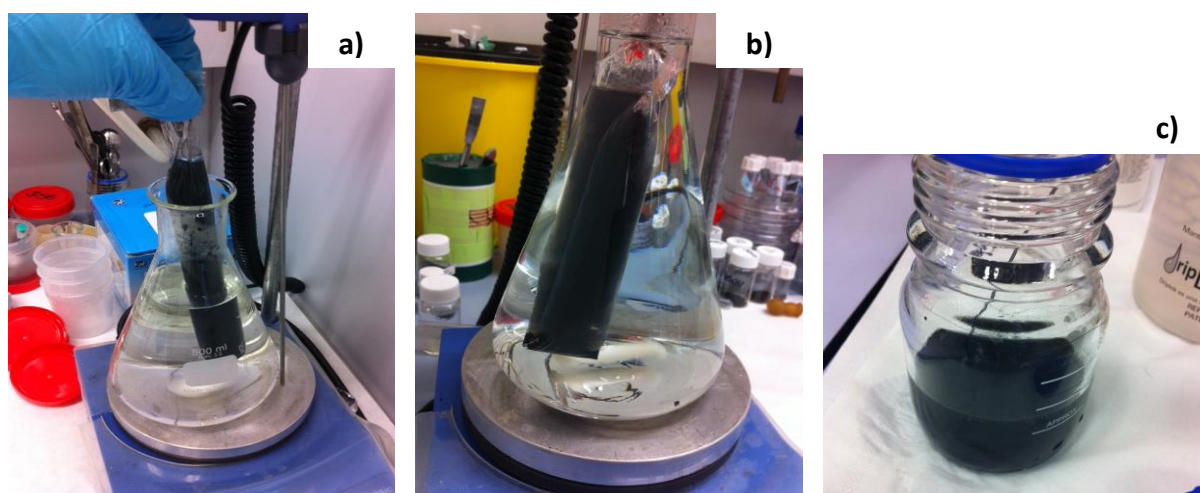


Figure 2. Images of the melamine washing process. a) and b) dialysis sack placement and handling; c) incorporation of the dialyzed suspension into a proper container for the settlement stage.

STEP 3. Isolation of stable few-layer graphene sheets in suspension. ● **TIMING** (3 h – 27 h)

The few-layer graphene dispersion should be isolated from the non-exfoliated graphite by manual extraction. The aqueous medium will contain the unwanted graphite in the bottom sediments and in some floating particles in the superficial meniscus. The liquid bulk will have evolved into a few-layer graphene gradient, with higher concentration in the vicinity of the

bottom and lower concentration in the vicinity of the surface. Thus, the extraction must be performed from bottom to top, avoiding the graphite-rich ends. The methodology is detailed as follows.

- (i) Incline the stabilization container 45°.
- (ii) Extract the dispersion with a pipette from the lowest part of the liquid without touching neither the bottom of the container nor the meniscus of the dispersion and transfer it stepwise to the final container. Sonicate the dispersion for 10 seconds to homogenize.

! CAUTION Keep the temperature in the sonication bath $< 30^{\circ}\text{C}$ and do not prolong the sonication for more than 10 seconds in a single pulse.

◇ CRITICAL STEP Avoiding the meniscus of the dispersion and the precipitated graphite is mandatory as the non-exfoliated graphite concentrates in those places. The pipetting must be done slowly and carefully in order not to disturb the state of equilibrium between the precipitate and the dispersion.

- (iii) Control the presence of melamine quantitatively within the graphene dispersion by use of AuNPs and UV-Vis at 500-650 nm (see anticipated results). For this, 1mL of AuNPs should be put in touch with 2mL of the few-layer graphene aqueous suspension.

◇ CRITICAL STEP The UV-Vis measure should be performed immediately after the AuNPs addition in order to avoid aggregation of nanoparticles.

- (iv) Control the concentration of few-layer graphene in the dispersion by UV-Vis spectroscopy at 660 nm (see anticipated results).
- (v) Verify the concentration of few-layer graphene in suspension by weighing (see anticipated results).

✕ NOTE In a typical experiment [few-layer graphene] $\sim 0.1 \text{ mg}\cdot\text{ml}^{-1}$ and [melamine] $\leq 1 \text{ ppm}$.

? TROUBLESHOOTING 2

? TROUBLESHOOTING 3

? TROUBLESHOOTING 4

|| PAUSE POINT The few-layer graphene in aqueous medium can be stored for 1 month without changes in properties.

? TROUBLESHOOTING 5

STEP 4. Drying of the aqueous graphene suspension. • **TIMING** (72 h – 96h)

- (i) Fill transparent plastic containers with ~ 60-80 ml of the few-layer graphene dispersion each.
- (ii) Freeze the dispersion by immersing the sealed container in liquid nitrogen. Take as much time as necessary to ensure the full freezing of the whole sample.
! CAUTION Avoid the direct contact of liquid nitrogen with the dispersion.
! CAUTION Wear appropriate equipment to manipulate liquid nitrogen.
- (iii) Cover the containers with a piece of aluminium foil provided with 5 equidistant 1 mm-sized holes and seal them with 2 elastic bands.
- (iv) Place the containers in the freeze-drying system and program it for 72 hours at -80°C under vacuum. The resulting powder is displayed in Fig. 3.

? TROUBLESHOOTING 6

- (v) Take carefully the plastic containers out of the freeze-drying system and extract the few-layer graphene powder from it by slowly turning the container upside down and carefully tapping it from the bottom. Aluminium foil serves as a good surface to collect and transfer these powders into their final container. Since the typical mass concentration of the aqueous few-layer graphene is ~ 0.1 mg/mL (see Anticipated Results), one can estimate the amount of solid that will come out from the freeze-drying step, keeping in mind that there will be slight deviations due to unavoidable losses.

! CAUTION For safety reasons, a lab coat, gloves and a face mask should be worn during the manipulation of powders.

◇ **CRITICAL** Keep the manipulation of few-layer graphene powder away from air streams as it is a very light and fluffy.



Figure 3. Photograph of the few-layer graphene powder after the freeze-drying step.

? TROUBLESHOOTING

Troubleshooting advice can be found in table 2.

Troubleshooting	Step	Problem	Possible reason	Solution
1	2 - (v)	The sample precipitates during dialysis	The colloidal stability of the medium has collapsed. Possibly due to sudden temperature changes or unintended hitting.	Ensure the temperature is fixed at 70°C and that the magnetic bar does not hit the sack. Sonicate the system for few seconds and control stirring.
2	3-(iv)	melamine/graphene mass ratio ≥ 0.01	Dialysis was not completed.	Repeat washing (step 2) and isolation (step 3)
3	3-(iv)	[Ball milled graphene] _{weighing} $\gg 0.1$ and \neq [Ball milling graphene] _{UV-Vis}	Part of the precipitated graphite has been swept along the final dispersion	Let the dispersion settle down for 24 h and repeat the isolation of graphene (step 3).
4	3-(iv)	The state of equilibrium between the precipitate and the dispersion is altered during the isolation	The dispersion has been accidentally shaken during the extraction step.	Sonicate the dispersion, let it settle down for 24 h and repeat the isolation of graphene (step 3).
5	3-(v)	The aqueous graphene dispersion ends up partly precipitated	The colloidal stability has collapsed, probably due to inappropriate	Sonicate the dispersion, let it settle down for 24 h and use it if no major

		within the storage time of one month.	storing conditions (temperature, external motion, etc.)	precipitation occurs. Otherwise toss it.
6	4-(iv)	The lyophilization is not completed.	The timing of the lyophilization depends on the volume to be dried.	Keep running the freeze-drying device until the powder can be perfectly seen through the walls of the plastic container.

Table 2. Troubleshooting: reasons and solutions.

• TIMING

STEP 1, Intercalation of melamine molecules within graphitic planes: 5 h

STEP 2a, Removal of the melamine surplus: 36-48 h

STEP 2b, Separation of few-layer graphene from non-exfoliated graphite 120h

STEP 3, Isolation of the stable few-layer graphene sheets in suspension: 3-27 h

STEP 4, Drying of the aqueous graphene suspension: 72-96 h

ANTICIPATED RESULTS

Few-layer Graphene in aqueous environment

In order to employ few-layer graphene, our research groups have developed a simple, reliable and sensitive colorimetric visualization of melamine in graphene dispersions through the use of AuNPs.²⁰ The resulting aggregates of AuNPs with melamine show color changes, which can be monitored using UV-Vis spectroscopy, in order to quantify the amount of melamine in graphene dispersions. The original absorbance of AuNPs at 520 nm decreases, while a new band at 640 nm appears, which it is characteristic of melamine/AuNPs aggregates. The ratio A_{640}/A_{520} for different concentrations of melamine is plotted against this concentration

exhibiting Lambert–Beer behavior ($y = 0.18x$), with excellent linearity ($r^2 = 0.99$). This methodology is used to control the amount of removed melamine during the washing process and to quantify the melamine, which could remain in the graphene dispersions. Upon the direct exposure of nanoparticles to aliquots of the washing waters, the color of the AuNPs progressively changes from magenta to blue (Figure 1a), with increasing melamine concentration. Most of the melamine is removed between the second and the third fraction, showing both blue color when exposed to nanoparticles. The fourth and the fifth fractions show magenta color as melamine is < 1 ppm (Figure 1a). The aggregation of AuNPs with melamine into the few-layer graphene is monitored using UV-Vis spectroscopy (Figure 1b). We typically obtain concentration values at safe levels of melamine at < 1 ppm (for a graphene concentration of ≥ 0.1 mg/mL).

It is worth mentioning that all the efforts invested into minimizing the amount of residual melamine are directed towards suppressing the possible toxic effect of this molecule in any applications. However, these minimum amounts still present after washing are vital for the successful re-dispersion and stabilization of the few-layer graphene in water. A recently published theoretical study has revealed that these almost negligible amounts of melamine act as anchoring points for water molecules, substantially increasing the number of stabilizing hydrogen bonds without altering the graphene-melamine interactions (mainly due to π - π stacking).²¹ The overall combination of such weak forces eventually leads to stable graphene suspension in aqueous media.

The mass concentration of graphene prepared in Step 3 is calculated by UV-Vis spectroscopy (Figure 4c,d). The absorbance at 660 nm, divided by cell length, is plotted against the concentration exhibiting Lambert-Beer behavior ($y = 15.0184x$; $r^2 = 0.9995$). We typically obtain concentration values of ~ 0.1 mg/mL few-layer graphene in Milli-Q water.

In order to verify the concentration values obtained by UV-Vis, the weighing of the solid residue coming from a known volume of graphene dispersion is carried out. For this, 10 ml of few-layer graphene dispersion is filtered through a tared membrane filter, dried under vacuum for 2 h and weighed again. The concentration calculated according to the following equation:

$$[\text{graphene (mg/mL)}] = \frac{(\text{Final weight} - \text{Tared filter weight})(\text{mg})}{10 (\text{mL})}$$

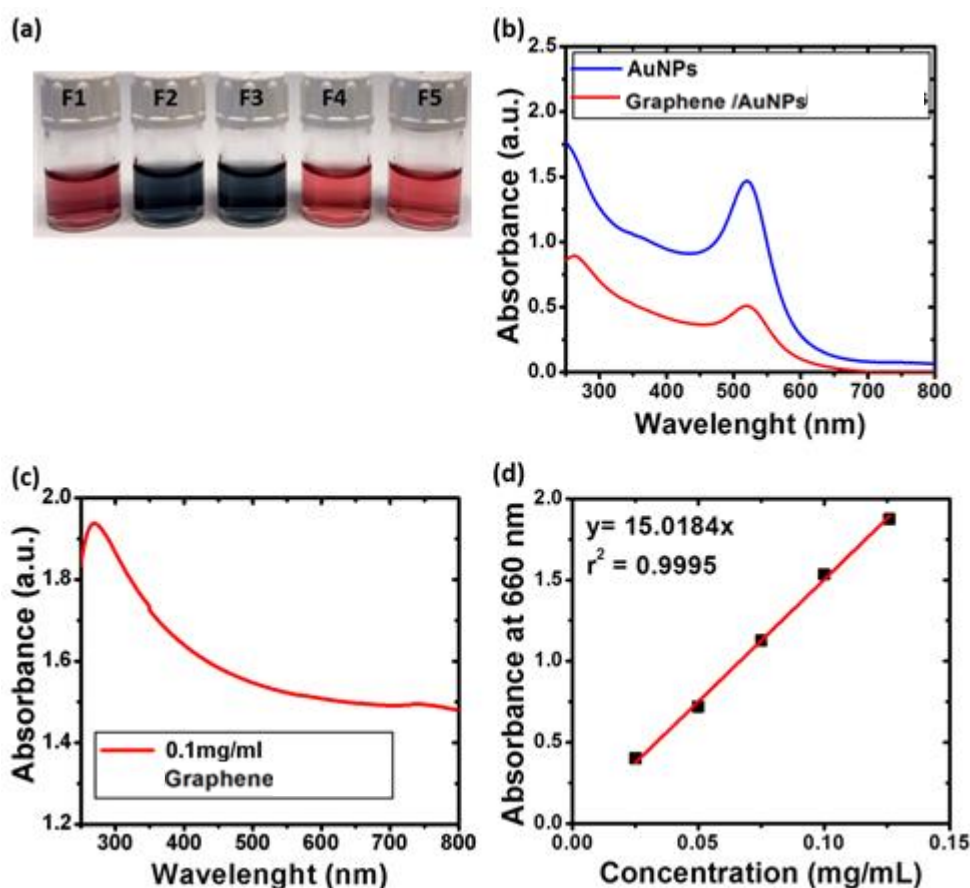


Figure 4. (a) Qualitative analysis of concentration of melamine into fractions of washing water; (b) Representative UV-Vis absorption spectrum of AuNPs and few-layer graphene / AuNPs, with < 1ppm of melamine content; (c) Representative UV-Vis absorption spectrum of 0.1 mg/ml of few-layer graphene in water; (d) Calibration plot of few-layer graphene in water.

Few-layer Graphene in powder form

Once characterized in the aqueous environment, graphene dispersion can be lyophilized in a freeze-dryer device resulting in low-density black powder. The characterization of the powder is conducted by elemental analysis, X-Ray photoelectron spectroscopy (XPS) and TGA. Elemental analysis give average values of 91.03 ± 0.51 wt% C, 0.74 ± 0.04 wt% H, 0.52 ± 0.02 wt% N and 7.71 ± 0.48 wt% O. We can estimate, from these data, traces of melamine < 0.8 wt% in the solid powder. TGA in inert atmosphere usually displays a weight loss of < 10 %, which corresponds to the melamine content and low concentration of oxygen groups, created during the ball-milling treatment (Fig. 5a).

Further proof of the non-oxidative nature of the milling treatment was achieved with the use of XPS (Fig. 5b-c). The C1s peak can be deconvoluted into three different components. The most

intense peak at 284.8 eV is assigned to sp^2 C-atoms of the graphene sheets, which is a signature of graphitic carbon. The component at 286.2 eV has usually been attributed to C–O bonds (together with C–N) and the components at 288 eV to C=O species.²² It is clear that the spectrum of powder graphene, after mechanochemical treatment, displays a similar pattern, revealing that the intrinsic structure of graphite remains largely intact after treatment and that no major oxidation occurs during milling (Figure 5b). However, the N1s spectrum (Figure 5c) exhibits a similar intensity of the components at 399.6 and 400.5 eV to C=N and C–N species respectively, due to the presence of melamine.^{8,13}

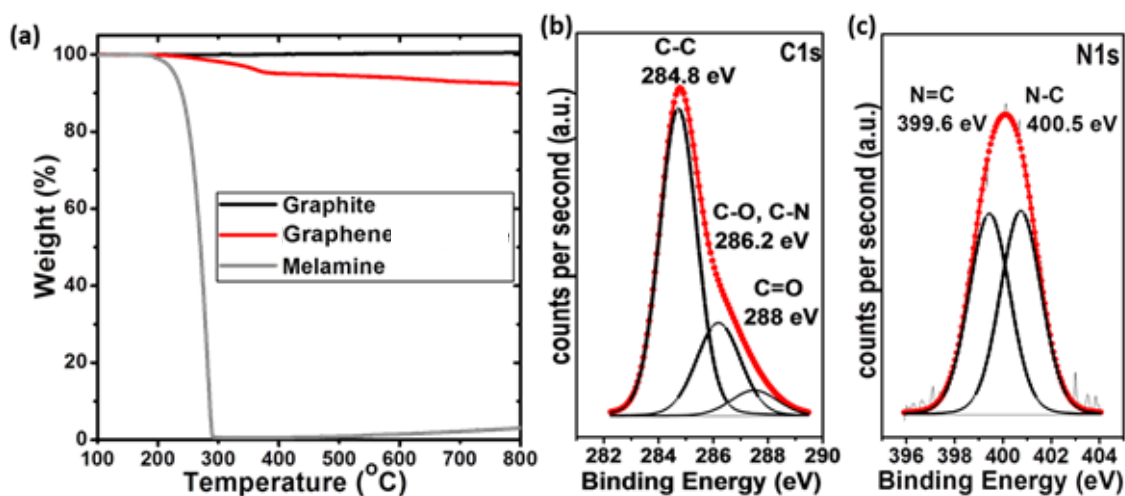


Figure 5. (a) TGA plots of graphite, melamine and few-layer graphene powder; (b) C1s and (c) N1s XPS of few-layer graphene powder.

The powder can be re-dispersed in water at a desired concentration,¹³ enabling concentrated graphene suspensions (up to 0.3 mg/mL with acceptable stability) to deposit this material onto different surfaces that can be further characterized by Raman spectroscopy and TEM as depicted in Figure 6.

✦ **NOTE** We refer to a detailed video in the supporting information of reference 13 for the readers to see how to re-disperse this graphene powder in water.

Raman spectroscopy provides essential information that can be used to characterize graphene.^{2,23} Raman spectra of a representative aliquot from few-layer graphene show the two most intense peaks of graphene, the G peak at around 1580 cm^{-1} and the 2D band at around 2700 cm^{-1} . D' appears as a shoulder at around 1600 cm^{-1} on the G band, which is characteristic of few-layer graphene. In addition, $I(2D)/I(G)$ usually falls below 1, confirming the samples to

be few-layer graphene.²⁴ This few-layer graphene typically displays full width half maximum (FWHM) of the 2D band ranging from 63 to 65 cm^{-1} .¹³ When graphene is affected by defects, the Raman feature at 1345 cm^{-1} (D band) appears in the spectrum. The evolution of the intensity ratio between the G and the D bands, $I(D)/I(G)$, has recently been used to provide a method to quantify the density of defects in graphene. $I(D)/I(G)$ ranging from 0.2 to 0.5, which is the case of this kind of samples,¹³ are attributed to defects located at the edges of our sub-micrometer flakes,²⁵ revealing that the structure of graphene basal planes are not damaged after all the processing steps.

TEM analysis is used to determine the lateral sizes of graphene samples (Figure 6b). Lateral dimension distribution is carried out using Fiji-win32 software, graphene samples have a wide size distribution around 200-2000 nm, but whose major population ($\sim 80\%$) lies in between 400 – 800 nm. Figures 6c,d show representative few-layer graphene flakes.

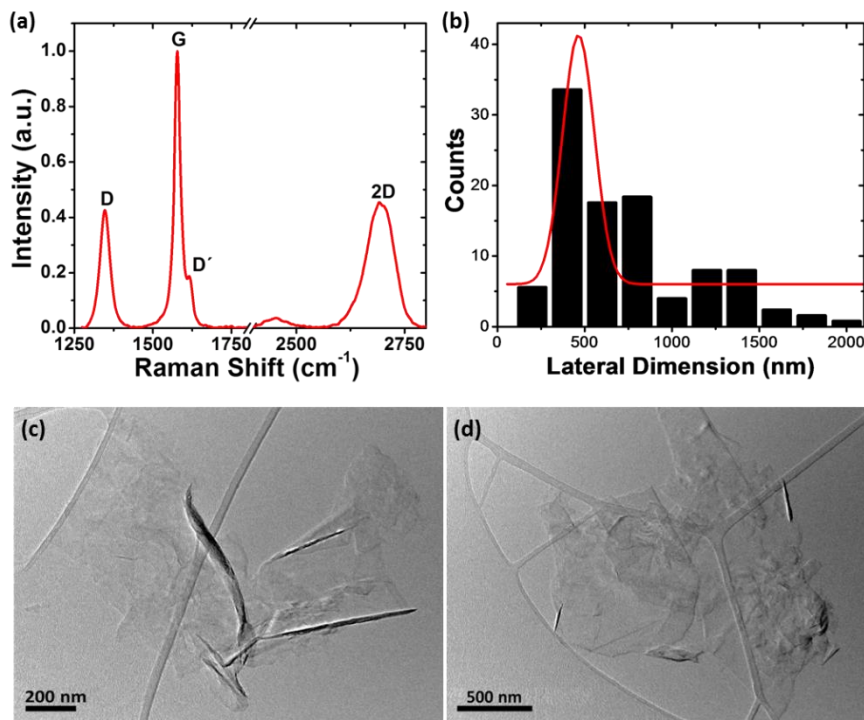


Figure 6. (a) Representative Raman spectrum of few-layer graphene. (b) Lateral size distribution of few-layer graphene from TEM images. (c)-(d) Representative TEM photos of few-layer graphene.

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AUTHORS CONTRIBUTIONS

J.M.G.-D. and V.L. contributed equally to this work. J.M.G.-D., V.L. and M.I.L. performed the experimental work and optimized the protocol. M.P. contributed to the research plan and to the writing of the manuscript. E.V. planned and supervised the research and co-wrote the paper with contributions from all the other authors. All authors discussed the results and commented the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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