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Riboflavin enhanced fluorescence of highly reduced graphene oxide

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ABSTRACT

The improvement of graphene derivates' fluorescence properties is a challenging topic and very few ways were reported up to now. In this Letter we propose an easy method to enhance the fluorescence of highly reduced graphene oxide (rGO) through non-covalent binding to a molecular fluorophore, namely the riboflavin (Rb). While the fluorescence of Rb is quenched, the Rb – decorated rGO exhibits strong blue fluorescence and significantly increased fluorescence lifetime, as compared to its pristine form. The data reported here represent a promising start towards tailoring the optical properties of rGOs, having utmost importance in optical applications.

1. Introduction

Graphene, one atomically layer, attracted enormous consideration due to the unique electronic and optical properties which make it extremely promising in fields like photonics and optoelectronics, starting from transparent conductors, photovoltaic devices to optical limiters and terahertz devices [1]. Recently, chemically derived graphene, graphene oxide (GO) and reduced graphene oxide (rGO), has gained great interest due to the low cost, mass production and chemical manipulation, providing the opportunity of tailoring the optoelectronic properties. While the GO is a solution processable non-stoichiometric macromolecule with ~2–3 nm sp^2 clusters isolated by sp^3 matrix, its reduction leads to the formation of new sp^2 domains that percolate between existing sp^2 clusters [2]. The field which most benefits from the heterogenous atomic and electronic structures of chemically derived graphene is fluorescence spectroscopy. Moreover, due to its chemical processability, the broad fluorescence band arising from GO can be tuned by changing the sp^2/sp^3 ratio upon the reduction process [3–7]. Contrary, due to its gapless character, pristine graphene does not exhibit fluorescence [8]. The intrinsic and tunable fluorescence of chemically derived graphene opened up exciting ways of exploiting new applications, e.g. drug delivery, cells imaging etc. [9,10]. On the other hand, the excellent fluorescence quenching abilities of pristine and chemically derived graphene [11,12] fill out the wide field of applications (e.g. fluorescence quenching microscopy, sensing, biosensing, resonance Raman spectroscopy) [2,11].

Despite of the tunable fluorescence, the quantum yield of graphene derived species is relatively low [2] and sensitive to the surrounding environment [5]. Moreover, while the reduction of GO leads to a better conductivity, its fluorescence was reported to suffer from the increase of the reduction time [6]. Up to now, several routes were reported for improving the fluorescence of GO/rGO, such as the complexation with europium [13], silver nanoparticles [14], polyvinyl alcohol [15] or covalent surface passivation [16] etc.

Herein we report a new route towards the improvement of the fluorescence in highly reduced graphene oxide (rGO) through non-covalent interaction with a biological molecule, namely the riboflavin (Rb), a fluorescent micronutrient of the utmost importance in a variety of cellular processes, with a key role in energy metabolism [17]. On the other hand, the interaction of rGO with Rb leads to the fluorescence quenching of the latter. Therefore, rGO–Rb composite can take advantage from both: the improved optical properties of rGO (for e.g. imaging) and the quenched fluorescence of the fluorophore (for e.g. bio/sensing).

2. Experimental

2.1. Chemicals

Graphite powder, 325 mesh, 99.9995% was purchased from Alfa Aesar; potassium permanganate ($KMnO_4$) 99%, sodium nitrate ($NaNO_3$) 99%, hydrazine hydrate ($NH_2NH_2{\cdot}H2O$) 24–26% in H_2O (RT), Rb ($C_{17}H_{20}N_4O_6$, 98%) were bought from Sigma to Aldrich; hydrochloric acid (HCl) 35% was purchased from Lach-Ner; sulphuric acid (H_2SO_4) 96%, hydrogen peroxide (H_2O_2) 30% and ammonia solution (NH_3) 25% were obtained from local manufacturers.

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2.2. Methods

GO was synthesized according to Hummers and Offeman's method [18]. The detailed description of the method is given in our previous work [19]. Graphene oxide reduction was performed in Anton Paar Monowave 300 as follows: 50 μL of hydrazine hydrate (HH) and 20 μL of NH_3 solution were added to 6 mL of GO 0.05 mg/mL solution and kept in microwaves at 100 °C for 20 min. The measured pH of the resulted rGO solution was ~10.3. The solution was prepared by mixing one volume unit of Rb of a certain concentration with 1.7 volume units of rGO (0.05 mg/mL) to the final concentration of 0.5×10^{-4} M for Rb and 0.0315 mg/mL for rGO. The pH of the mixture was adjusted to the pH of the original rGO with NaOH. To avoid inner filter effect during the fluorescence measurements, the above prepared
rGO–Rb sample was filtered several days later by using a cellulose filter of 450 μm pore size and the resulted colorless and transparent supernatant was collected. For the comparison measurements the rGO blank solution was also filtered and the colorless solution was collected. The samples were kept in dark to avoid any interaction with light.

2.3. Characterization

UV–Vis absorption spectra were taken in quartz cells of 10 mm path length using Jasco V-670 UV–Vis-NIR spectrophotometer. The steady-state fluorescence spectra were recorded with Jasco LP-6500 spectrofluorimeter in quartz cell with 10 mm path length. The medium sensitivity was used for all measurements. FT-IR spectroscopic analyses were done in reflection configuration using a JASCO 6200 FT-IR spectrometer with 4 cm^{-1} resolution, in the 4000–400 cm^{-1} region. The measurements were performed on the mixture of the sample in powder state with KBr, pressed into pellets. X-ray photoelectron spectroscopy (XPS) was performed in a Specs analysis chamber, following the description from our previous work [19]. Raman measurements were performed with a Witec Alpha 300 R confocal Raman microscope system, using a 532 nm Nd-Yag laser excitation line, at the 0.6 mW laser power. AFM images were taken with a Witec Alpha 300 A atomic force microscope system, operating in non-contact mode with a standard aluminium coated silicon tip (Nanosensors). Zeta potentials were measured at 25 °C using a Malvern Instruments NanoZS90 Zetasizer instrument. Fluorescence lifetime measurements were performed on a time-resolved confocal fluorescence microscope system (MicroTime200, from PicoQuant) equipped with an inverted microscope (IX 71, from Olympus), in the liquid sample. The excitation was provided by picosecond diode laser heads (LDH-D-C-375 and LDH-D-C-485, PicoQuant) operating at 375 and 485 nm, respectively, and 40 MHz repetition rates. The signal collected through a UPLSAPO 60x/NA 1.2 water immersion objective was spatially and spectrally filtered by a 50 μm pinhole and longpass emission filters before being focused on a PDM Single Photon Avalanche Diode (SPAD) from MPD. The detector signals were processed by the PicoHarp 300 (PicoQuant) Time-Correlated Single Photon Counting (TCSPC) data acquisition unit. Data analysis and image processing were performed with the SymPhoTime software (PicoQuant). Fluorescence lifetime decay curves were tail-fitted with exponential decay curves and the quality of the fits was evaluated by analyzing the chi-square (χ^2) values and the distribution of the residuals.

3. Results and discussions

The inset of Figure 1A shows the UV–Vis absorption spectra of pristine Rb, rGO and rGO–Rb mixture before being filtered. There are four absorption bands for pristine Rb solution, in ultraviolet (UV) and visible (Vis) regions, assigned to the π–π^* transitions (223 and 267 nm) and to the combination of n–π^*, π–π^*, and π–π^* transitions (374 and 445 nm), respectively. The last of the transitions corresponds to the HOMO to LUMO transition [20,21]. The rGO presents a characteristic peak at 269 nm due to the C–C π–π^* transition discussed in the characterization section from supplementary information (ESI). In rGO–Rb mixture, the Rb bands are red shifted from 267 and 445 nm to 271 and 455 nm, respectively, while the band at 223 nm disappears. These shifts could be due to the changing in surrounding environment of Rb. After the rGO–Rb mixture was filtered (Figure 1, rGO–Rb), it is observed from the spectrum that most of the Rb was removed, since the absorption bands of Rb disappeared. This is probably due to the interaction of Rb with rGO removed by filtering, as well as intercalation of unbounded Rb between rGO sheets. The resulted solution exhibits a weak broad absorption band in UV region and a continuous absorption over the whole visible range, characteristic to rGO. These features are similar to the blank rGO (Figure 1, rGO), with specification that in the former case the overall absorption is increased and the UV band of rGO is almost vanished. A possible explanation could be the π–π stacking interaction of rGO with Rb which, on one side affects the π–π^* inter-band transition in rGO, while on the other side disturbs the intramolecular electron transitions in Rb. Figure 1B shows the photoluminescence (PL) spectra of pristine rGO and rGO–Rb solutions. The rGO–Rb aqueous suspension exhibits strong PL under UV irradiation (exc. 375 nm) which can be seen by naked eye (see inset), with emission maxima at 440 nm and a shoulder centered at ~490 nm. Unlike rGO–Rb, the pristine rGO shows no visible fluorescence when excited at the same excitation wavelength (see optical photograph from inset) and has weak fluorescence spectrum with the maximum at ~450 nm and a shoulder at ~500 nm. Additionally, the observed blue – shift (from 450 to 440 nm) of the blue band in rGO–Rb relative to rGO can be an evidence of rGO–Rb interaction. It was established [3,5] that GO possesses two emission bands, a blue band in 400–500 nm range and a second broad one in the blue to red/NIR region. After reduction, the latter band disappears [3,6], remaining only the blue band and some residues of the broad band as a shoulder, in agreement with our measurements. This is consistent with the increase in number of small sized sp^2 domains upon the reduction [3,6]. It is also noticed that no emission from Rb is detected (pristine Rb has emission at 530 nm, data is not shown), supporting the binding of Rb to the rGO sheets.

It was showed in most of graphene – related fluorescence studies [4,5,7] that the spectral position of the fluorescence emission of chemically derived graphene (λ_{em}) is dependent on the excitation wavelength (λ_{exc}), due to different possible transitions from the bottom of CB (conduction band) and nearby localized states to the wide-range VB (valence band) [4]. In order to see whether the rGO–Rb composite preserves these features, the emission spectra of rGO–Rb were recorded from 375 to 470 nm by varying the λ_{exc} with 5 nm (Figure 2B). The blue band decreases in intensity and red-shifts with increasing the λ_{exc} (from 440 to 460 nm), as in the case of pristine rGO (Figure 2A). The same red-shift (from 490 to 530 nm) and intensity decrease is observed for the band in 500–700 nm region with increasing the excitation wavelength. Such as, at λ_{exc} of 470 nm, the emission spectrum only features a broad band in 530 nm region, which might correspond to the blue band and its shoulder. While there is only a slight shift in the peak position of the PL of rGO–Rb relative to rGO, the excitation spectra of rGO–Rb taken at the three different emission wavelengths (Figure 2A and B) are very different in shape and position compared to rGO. While the pristine rGO exhibits broad excitation bands centered at ~318 nm, for all emission wavelengths, the rGO–Rb has well defined sharp excitation spectra, at 358 and 362 nm for emission at 440 and 460 nm, respectively, becoming broad with no defined maxima at λ_{em} of 530 nm.