

RESEARCH PAPER

## The effect of graphite sources on preparation of photoluminescent graphene nano-sheets for biomedical imaging

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### ABSTRACT

**Objective(s):** Graphene as two-dimensional (2D) materials have attracted wide attention in different fields such as biomedical imaging. Ultra-small graphene nano-sheets (UGNSs) have been designated as low dimensional graphene sheets with lateral dimensions less than few nanometres ( $\leq 500$  nm) in one, two or few layers. Several studies have proven that the process of acidic exfoliation and oxidation is one of the most effective methods to synthesize low dimensional graphene sheets. The band gap of graphene can be changed through changing the reaction temperature resulting in different photoluminescent colors. The aim of our study is synthesis of multi-color photoluminescent UGNSs for biomedical imaging.

**Materials and Methods:** Two different UGNSs were synthesized from two different graphite sources via acidic treatment with a mixture of sulfuric and nitric acids. The prepared UGNSs were characterized by UV-Vis, photoluminescent, Raman spectroscopy and scanning electron microscopy (SEM). The photoluminescence colors of the prepared UGNSs were detected under excitation wavelength of 470 nm using optical filters.

**Results:** The results showed that the graphite primary source is a determinant factor in the synthesis of different UGNSs. While altering reaction temperature didn't significantly change the emission wavelengths; however it affected their photoluminescent emission intensity.

**Conclusion:** Overall, nontoxic UGNSs synthesized by simple acidic treatment of graphite with different photoluminescent colors (green, yellow and red) can be a promising fluorescent probe for bioimaging.

**Key words:** Bioimaging; Graphite source; Photoluminescent; Ultra-small graphene nano-sheets

### How to cite this article

Moasses Ghafary S, Hatamie Sh, Nikkhah M, Hosseinkhani S. The Effect of Graphite Sources on Preparation of Photoluminescent Graphene Nano-sheets for Biomedical Imaging. *Nanomed J.* 2017; 4(3): 164-169. DOI: 10.22038/nmj.2017.8957

### INTRODUCTION

Graphene is a two-dimensional (2D) carbon structure of single or multi-layer sheets, where carbon atoms are covalently connected in a hexagonal symmetry [1-4]. Two dimension graphene sheets have zero band gap with non-fluorescent nature. Interestingly, recent studies have shown the reduction of graphene sheets to a few nanometers can lead to an increase in the band gap and emergence of photoluminescent (PL) emission feature [5, 6]. The mentioned nanostructures are called graphene quantum dots (GQDs). The reduction of graphene sheets'

size results in the disruption of the integrity and continuity of the  $\pi$  electrons in the system and emergence of the quantum confinement effect, which is responsible for creating optical properties depending on the size of graphene sheets [7, 8]. As reported, the types of edges formed upon cutting in different directions and sizes of graphene sheets are important factors in determining their different properties. The PL emission of graphene nanostructures can vary with their sizes, zig-zag edges and shapes [9].

In general, there are two strategies for the synthesis of graphene nano-sheets: top-down and bottom-up. Top-down methods involve cutting graphene-larger materials to smaller ones, while the bottom-up methods include their preparation

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Note. This manuscript was submitted on December 25, 2016; approved on February 15, 2017

from carbon precursors with conjugated carbon atoms [10, 11]. Nowadays, the top-down methods are more used by researchers due to their simple methodology, high quantum yield, low cost, and mass production [11, 12]. Acidic exfoliation and oxidation has been known as an effective approach to synthesize fluorescence structures from carbon based materials such as carbon fiber, carbon black and graphite sources [13-16].

In this study, we synthesized UGNSs with different PL emission colors from two distinct sources of graphite: synthetic (type-1) and commercial (type-2). Ultra-small graphene nano-sheets (UGNSs) and GQDs (OD) are quite similar in terms of photo-physical properties except their sizes. The results showed that primary graphite source play an important role in PL emission feature of UGNSs and reaction temperature doesn't significantly affect PL emission. According to our knowledge, there is not any report on synthesis of ultra-small graphene sheets from different graphite sources and its effect on comparison of their PL emissions. Due to the unique properties of ultra-small graphene fluorescence structures including high chemical stability, excellent optical properties, high resistance to photo-bleaching along with high biocompatibility and non-cytotoxicity in comparison to similar conventional fluorescence quantum dots (i.e. CdSe, CdS), this construct has proposed as a promising fluorescence probe for bioimaging applications [13, 17, 18].

## MATERIALS AND METHODS

### Preparation of UGNSs

The UGNSs were prepared by acidic exfoliation in a certain ratio of  $H_2SO_4$  and  $HNO_3$  (Merck-Germany) [13, 19]. For this purpose, 10 mg from each of the graphite sources was added in to mixture of  $H_2SO_4$  (50 ml) and deionized water (10 ml) and sonicated for 30 min. Then, it was injected into a three neck flask contains  $H_2SO_4$  (50 ml) and  $HNO_3$  (30 ml) and stirred overnight under nitrogen flow. Then, citric acid (Merck-Germany) was added to the solution in order to introduce carboxylic groups on graphene sheet surfaces to improve their optical properties and water solubility. Deionized water (2xvolume of the reaction) was slowly added to the flask after the completion of reaction, the color of the solution was changed to reddish brown and greenish gray in the performed reactions with type-1 graphite and type-2 graphite sources, respectively. Then, pH was adjusted to

8 using sodium hydroxide (NaOH) and sodium carbonate ( $Na_2CO_3$ ) (Merck-Germany). Eventually, the reaction mixture was filtered, desalted and freeze dried. The properties of synthesized UGNSs were characterized by UV-Vis, photoluminescent, and Raman spectroscopies.

### Characterization of UGNSs

PL spectra were recorded by Cytation™ 3 multi-mode detection system (BioTek) at room temperature. A Philips XL30 model scanning electron microscope was used to obtain the SEM images. Absorption spectra were recorded using Perkin Elmer ultraviolet-visible (UV-Vis) spectrometer. The aqueous suspension of UGNSs was used for the UV-Vis spectroscopy, with deionized water as the reference.

Raman measurements were performed using Almega ThermoNicolet Dispersive Raman spectrometer, with excitation laser wavelength of 532 nm.

In order to study of the reaction temperature and sonication time effects on PL emission of UGNSs, the exfoliation reaction was carried out in various temperatures (60, 65, 95 and 110°C) and different ultra-sonication times. To investigate the primary source effects on PL emission of GQDs, the same exfoliation reaction conditions was designed with two different graphite sources.

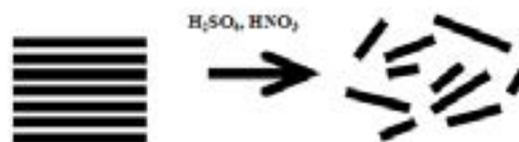


Fig. 1. Schematic image of acidic exfoliation reaction

## RESULTS AND DISCUSSION

A schematic image of acidic exfoliation process is exhibited in Fig 1. Synthesis process is based on the fact that the generated gas molecules exert a large force against the weak noncovalent forces of the graphite layers in order to expand them. Expansion and exfoliation of graphite layers results in production of graphene mono layers that are precursors of graphene nanostructures [20].

Initially, to investigate the effect of the two different graphite sources on the synthesis of UGNSs, the PL of UGNSs was compared using the variation of excitation wavelengths in the same reaction conditions.

Using two different graphite sources, two

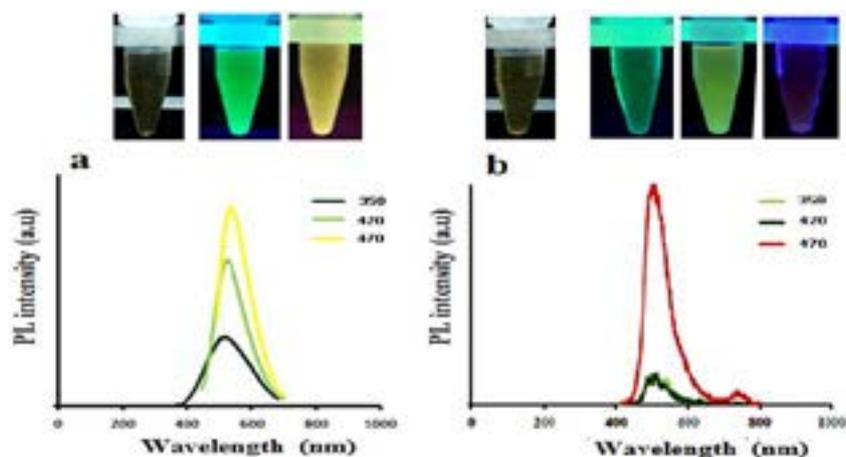


Fig. 2. The photoluminescent spectrum and digital camera photo of (a) UGNs1 under visible light (left) and excitation wavelengths at 420 nm (middle) and 470 nm (right) and (b) UGNs2 and their digital camera photo under visible light (left) and excitation wavelengths at 470 nm using optical filters (right)

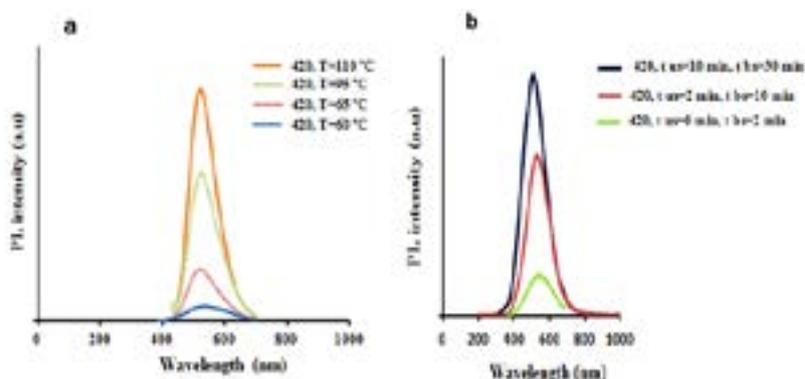


Fig. 3. The photoluminescent spectrum of synthesized UGNs (a) in various reaction temperatures (60, 65, 95, 110 ° C) and (b) in different ultra and bath-sonication times (shown by t us and t bs, respectively)

different UGNs were obtained in the same reaction conditions; UGNs 1 exhibited two different colors (green and yellow), while UGNs 2 showed multiple emission fluorescence namely green, yellow and red emission colors. The difference in PL emission colors may be due to the different lateral sizes or amounts of oxygen-containing functional groups on their surfaces [21]. Similar to our study, Ruquan et al [22] have revealed that primary source play an important role in various properties of GQDs. They obtained three different PL colors of GQDs, blue, green and yellow from three distinct types of coal as primary sources. As shown in Fig 2 a, b, by increasing the excitation wavelength from 350 to 470 nm, the emission wavelength peaks shifted towards higher wavelengths along with an increase in their intensity. The two-color fluorescence of UGNs 1 (i.e. green and yellow), produced from acidic exfoliation process of type-1 graphite, could be attributed to

the size dependent PL corresponding to quantum confinement effect [22]. While multi-color extra fluorescence UGNs (i.e. green, yellow and red) were obtained from type-2 graphite (Fig. 2a, 2b). Multi-color UGNs, when excited at a single wavelength (470 nm) the green emission peak broadens towards longer wavelengths (i.e. yellow and red regions) resulting in fluorescence at three spectral regions (green, yellow and red). The observed multi-colored emission could be attributed to the existence of differently sized graphene sheets in the aqueous solution, as a result of surface functional groups and disordered structures such as defect levels and  $sp^3$  carbons [21].

Under oxidation conditions, the newly created small traps and structural disorders may allow the excitation with a special wavelength and emission of multiple colors in a similar manner to what has been observed in UGNs 2. As mentioned above, graphene

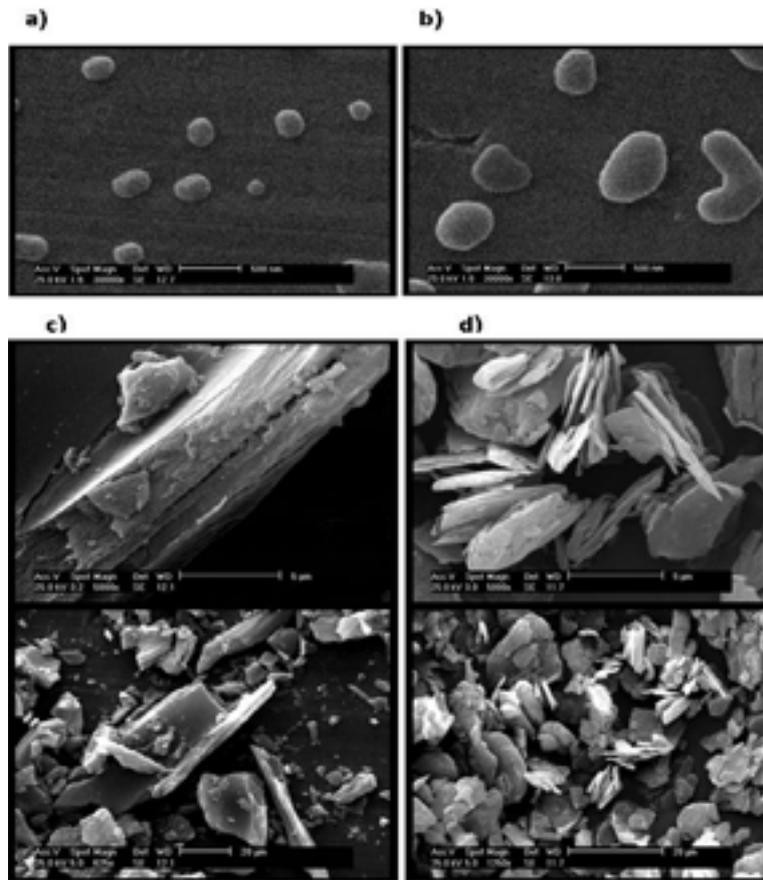


Fig. 4. SEM images of (a) UGNs1, (b) UGNs2, (c) type-1 graphite and (d) type-2 graphite

nano-sheets obtained from type-1 graphite show two different colors (green and yellow) when excited with two different wavelengths (350-420 and 470 nm). We were curious if red emitted-graphene sheets also could be obtained from type-1 graphite source. For this purpose two different parameters were considered: First, the reaction temperature and second the sonication time during the synthesis process.

As shown in Fig. 3 a, the PL intensity of UGNs showed significant changes upon altering the reaction temperature (from 60 to 110°C). Increasing temperature would accelerate the movement of molecules in the reaction mixture which may increase the oxidation rate of graphene sheets and surface defects. Therefore the PL intensity increased much more with increasing the reaction temperature. In addition, increase of the PL intensity also can be attributed to a highly mono-sized dispersion of the UGNs in higher temperatures. As reported [23-25], PL emission of QDs could be influenced by their sizes, edges, defects, and shapes. By increasing the reaction temperature from 60 °C to 65 °C, the

emission wavelength was slightly shifted to shorter wavelengths upon excitation at 420 nm. The blue shift of emission wavelength occurred probably due to the changes in the concentration of oxygen containing functional groups present on the surface of UGNs, as well as result of reducing the size of the graphite flakes upon increasing the temperature. However, when the temperature was elevated from 65 to 110 °C, with the same excitation wavelength no significant changes were observed in the emission peak and only the fluorescence intensity was

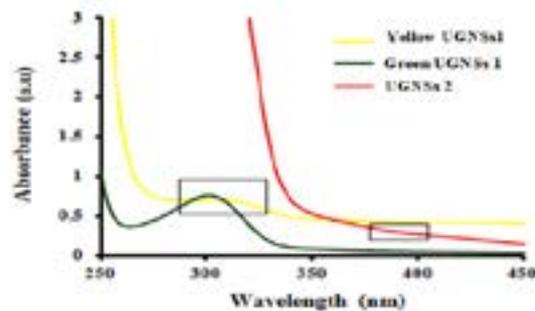


Fig. 5. The UV-Vis absorption spectrum of UGNs

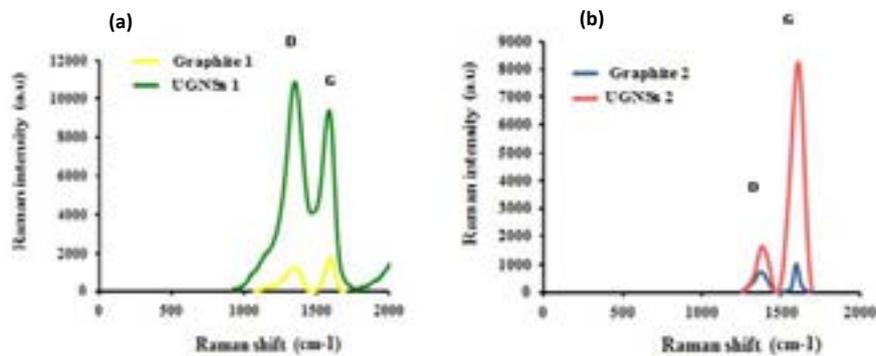


Fig. 6. The Raman spectrum of (a) UGNs1 and graphite type1 and (b) UGNs2 and graphite type2

increased; probably due to a highly mono-sized dispersion of the UGNs and the presence of small trap states related to the oxygen containing functional groups, in higher temperatures.

The PL intensity of UGNs 1 was changed and slight shift was observed in the position of the emission peak wavelength, when altering the sonication time. As shown in Fig. 3 b, UGNs obtained at 10 min ultra-sonication and 30 min bath-sonication showed green emission color. By decreasing the sonication time (2 min ultra-sonication and 30 min bath-sonication), the PL intensity was decreased and slight shift towards longer wavelengths (yellow spectrum region) was observed. It seems that in low sonication time, the possibility of breaking down graphene sheets was reduced and the larger nano-sheets with yellow emission color were synthesized. Probably, oxygen containing functional groups on the graphitic domains make them fragile; therefore red emitted-UGNs weren't obtained by further reduction of sonication time (only 2 min bath-sonication).

Scanning electron microscope (SEM) images of the two synthesized UGNs and the two graphite sources are shown in Fig 4. According to Fig 4 a, b there are major differences between the size and shape of UGNs 1 and UGNs 2. The size of the UGNs1 was in the range of 100-250 nm, with the round shapes whereas UGNs2 was between 300-500 nm without a determined shape. SEM images of type-1 graphite showed high thick planar layered structures with amorphous shapes (Fig 4 c) while type-2 graphite were thinner planar layers with crystalline structures (Fig 4 d). The UGNs 1 obtained from type-1 graphite showed different sizes that could create different emission colors and confirm the results of PL spectrums (Fig 2).

It is probably due to amorphous shapes and high thick layered structures of this source which

could break down into different sizes in oxidative process. Type-2 graphite, under the same reaction conditions, could break down into UGNs with differences in their sizes and shapes probably due to thin layer structure of graphite source which break down to different directions and make different shapes and sizes of UGNs.

UV/Vis absorption spectroscopy of the synthesized green and yellow UGNs1 exhibited a peak at 300 and 320 nm respectively, which is associated with  $n-\pi^*$  transition, and a strong absorbance at 230 nm attributed to  $\pi-\pi^*$  transition (Fig. 5), similar to previously reported studies for GQDs [26,27].

The UGNs obtained from type-2 graphite didn't reveal any absorbance behavior with possible shouldering in 350-400 nm range as seen for conventional graphene materials.

According to our knowledge, only one organized study has shown similar absorbance behavior in the lignite coal (POC)-obtained GQDs [25].

Raman spectra of the two graphite sources and UGNs are depicted in Fig 6. The two

Main peaks appeared: D-peak (related to the defects of graphene) at  $1350\text{ cm}^{-1}$  and the G-peak at  $1610\text{ cm}^{-1}$  (the excitation laser was 532 nm). The relative intensity of the D-peak to the G-peak ( $I_D/I_G$ ) for the UGNs1 is 1.16, indicating an increase of edge carbons and structural defects in these UGNs [23-25], whereas for the UGNs2 is 0.2, indicating lower disorders and better crystalline quality in these UGNs than the UGNs1 [28].

## CONCLUSION

In this study, we revealed that the graphite source plays an important role in the synthesis of UGNs and their physical properties such as size, shape and color. UGNs 1 with higher disorders were synthesized using type-1 graphite with

amorphous sheets. While UGNs 2 were obtained from type-2 graphite with crystalline structure showed lower disorders, different shapes and sizes with multi-color emission. Interestingly, in bioimaging devices like sentinel lymph node imaging system, one of the red, yellow and green fluorescence colors can be detected, separately.

These systems allow the detection of different emission colors using different excitation wavelengths and various types of optical filters. Synthesized UGNs 2 in this study can be able to emit all three colors and can be considered as a promising fluorescent tracking probe in biological imaging.

#### ACKNOWLEDGMENTS

The authors appreciate the financial support of this investigation by the research council of the Tarbiat Modares University.

#### CONFLICT OF INTEREST

The authors confirm that this article content has not any conflicts of interest.

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