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Edge-functionalization of graphene by polyglycerol; A way to change its flat topology

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ABSTRACT

This work presents a procedure for functionalization of graphene sheets from edges by polyglycerol. Hyperbranched polyglycerol with a bi-dentate aromatic segment in its focal point was synthesized and used to sandwich graphene sheets from the cut-edges. Due to the hydrophobicity of the flat surface of the edge-functionalized graphenes and hydrophilicity of their edges, they changed their conformation from the extended- to the closed-state and formed nanocapsules in aqueous solutions. Spectroscopy and microscopy evaluations showed that the average size for nanocapsules is 300 nm. They were able to encapsulate hydrophobic molecules such as doxorubicin in aqueous solutions with a high loading capacity. Aqueous solutions of nanocapsules and those with encapsulated doxorubicin were stable at room temperature for several weeks. Due to their unique properties including suitable size and shape, stability in aqueous solutions, and high loading capacity for hydrophobic anticancer drugs, graphene-based nanocapsules are promising systems in order to use in nanomedicine, especially for future cancer therapy.

1. Introduction

Since its detection in 2004, graphene has attracted significant attention due to its unrivaled electronic, thermal, physical, chemical, mechanical and optical properties [1,2]. Graphene is a flattish monolayer of carbon atoms tightly packed into a two-dimensional honeycomb lattice and is synthesized from graphite [3–5]. Due to its unique and versatile properties, graphene has been used in a wide range of applications including electronic devices, solar cells, nano-catalysts, nanocomposites, molecular sensing and chemical and biological sensors [6–15].

Recently, graphene-based nanomedicine including biological detection, drug delivery, and cancer therapies has also been investigated [16,17]. Monolayer graphene sheets with all atoms exposed on its surface show a very high surface area (theoretically 2600 m^2/g), which can be used for ultra-efficient loading of aromatic molecules such as anticancer drugs that benefit for usages in drug and gene delivery [18–22]. In addition to high loading capacity, its fluorescence-resonance-energy transfer and photothermal properties stimulate scientists to consider it as a powerful tool in nanomedicine [23–28]. Moreover, in spite of carbon nanotubes it is not carcinogen and its toxicity seems to be very low [29,30]. All above characterizes together with many other unique properties such as chemo-photothermal characteristic promise graphene as a new vector for future cancer therapy [31].

However the hydrophobicity of graphene, as well as carbon nanotubes, and probably its flat and big size restrict its application in nanomedicine. In order to improve the water solubility of graphene sheets and carbon nanotubes and to decrease their size in a suitable range for passive targeting, they should be modified by hydrophilic polymers [32,34]. A class of hydrophilic hyperbranched polymers is polyglycerol (PG) with tree-like structure and a large number of hydroxyl functional groups in its backbone and periphery [35]. Water solubility and high biocompatibility together with minimum non-specific interactions with biological systems stimulate researchers to use polyglycerol for biomedical applications [36–39].

Herein hyperbranched polyglycerol with parallel naphthol rings in its focal point has been synthesized and used to sandwich the

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cut-edges of graphene sheets. Due to the hydrophilicity of polyglycerol and hydrophobicity of graphene, edge-functionalized graphene changed its conformation from flat- to glubolar-state and created nanocapsules in aqueous solutions. Nanocapsules were able to load and transfer hydrophobic anticancer drugs efficiently.

2. Experimental

2.1. Methods and materials

2.1.1. Materials

Graphene oxide was synthesized using Hummers approach. Graphite, cyanuric chloride, H_2SO_4, H_2O_2, potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), sodium hydride, p-phenylene diamine (PPD), acetone, tetrahydrofuran, dichloromethane (CH_2Cl_2), methanol, naphthol, diethanolamine, dimethyl formamide (DMF), cation-exchange resin, and doxorubicin were purchased from Merck Chemical Company. Graphene was synthesized by reduction of graphene oxide. Glycidol was purchased from Sigma–Aldrich.

2.2. Characterization

Nuclear magnetic resonance (^{1}H NMR) spectra were recorded in D_2O, $CDCl_3$ and DMSO-d6 on a Bruker DRX 400 (400 MHz) apparatus with the solvent proton signal for reference. ^{13}C NMR spectra were recorded on the same instrument using the solvent carbon signal as a reference. Infrared (IR) experiments were performed using a Nicolet 320 FT-IR. Ultraviolet (UV) spectra were recorded on a Shimadzu (1650 PC) scanning spectrophotometer. Ultrasonic bath (Model: 5RS, 22 KHZ, Made in Italy) was used to disperse materials in solvents. The particle size and polydispersity of materials were determined using Dynamic Light Scattering (DLS) (zetasizer ZS, Malvern Instruments). Surface imaging studies were performed using atomic force microscopy (AFM) to estimate surface morphology and particle size distribution. Transmission electron microscopy (TEM) analyses were performed by PHILIPS electron Microscope. Fluorescence measurements were performed on Varian Carry Eclipse fluorescence spectrophotometer.

2.3. Synthesis of graphene oxide (GO)

GO was prepared using nature graphite powders as the raw materials by a modified Hummers method [7,8]. Briefly, graphite (0.5 g) and $NaNO_3$ (0.5 g) were put into a flask at 0 °C. Then, concentrated H_2SO_4 (23 ml) was added to the mixture and it was stirred for 1 h at 5 °C. Subsequently, $KMnO_4$ (3 g, 1.9×10^{-2} mol) was added to the reaction system step-wise over 1 h, meanwhile the temperature of the mixture was kept below 20 °C. Mixture was stirred for 2 h and afterwards, deionized distilled (DD) water (46 ml) was slowly added into the solution, and temperature of the reaction system jumped to 98 °C instantly for 30 min. 100 ml of deionized distilled (DD) water was added into the solution. Finally, 10 ml of H_2O_2 (30%) was injected into the reaction system, resulting in the formation of bright yellow suspension. The GO was separated by filtration, washed for three times with diluted HCl (3%), and then dispersed in DD water and washed by distilled (DI) water three times to remove excess acid and dried at 40 °C for 72 h.

2.4. Synthesis of graphene (G)

Graphene was synthesized by reduction of graphene oxide. In a typical reaction, PPD (1.2 g) was dissolved in DMF (100 ml) at room temperature. Then GO (1 mg/ml) was added to this solution and mixture was refluxed in an oil bath at 98 °C for 36 h to form a red solution. The reaction mixture was filtered and the product was washed with acetone and then dried to obtain pure graphene [40].

2.5. Synthesis of 2-chloro 4,6-di naphthoxy 1,3,5-triazine (CDT)

A solution of naphthol (2 g, 1.38×10^{-2} mol) and sodium hydroxide (0.55 g, 1.38×10^{-2} mol) in 10 ml water was added to a solution of cyanuric chloride (1.29 g, 6.9×10^{-3} mol) in 50 ml dichloromethane drop wise at 0 °C. Mixture was stirred at room temperature for 1 h, and then it was refluxed for additional 6 h. Mixture was cooled, filtered off and solvent was evaporated. Crude product was dissolved in 10 ml dichloromethane and a few drop of methanol was added to this solution. Pure product was separated as a light yellow solid in 68% yield.

2.6. Synthesis of 2-diethanolamine 4,6-di naphthoxy 1,3,5-triazine (DDT)

An excess of diethanolamine (0.12 ml, 2.5×10^{-3} mol) was added to a solution of CDT (0.5 g, 1.25×10^{-3} mol) in 10 ml dichloromethane drop wise at room temperature. Mixture was stirred at room temperature for 1 h, and then it was refluxed for additional 24 h at 60 °C. Solution was left at room temperature for 1 h. Then the upper phase which was the excess of diethanolamine was separated by decanter. Afterward solvent was evaporated by vacuum. In order to obtain the pure compound, crude product was dissolved in 10 ml acetone and solution was filtered and then acetone was evaporated. The purified product was separated as a light yellow solid in 70% yield.

2.7. Synthesis of 2-diethanolamine 4,6-di naphthoxy 1,3,5-triazine (DDT) graft-PG (DDTP)

The reaction was carried out in a glass reactor equipped with a mechanical stirrer and vacuum inlet. In a typical synthesis, compound DDT (0.1 g, 2.13×10^{-4} mol) was added to a tetrahydrofuran (THF) suspension of sodium hydride (0.01 g, 4.16×10^{-4} mol) and mixture was stirred for 1 h at room temperature. Then THF was vaporized using vacuum oven and glycidol (0.2 ml, 2.99×10^{-3} mol) was added to the deprotonated compound DDT gradually at 100 °C over 2 h. Mixture was stirred at this temperature for 12 h. Then it was cooled and dissolved in methanol and neutralized by filtration over cation-exchange resin. The product was twice precipitated from methanol into acetone as a viscose brown compound in 80% yield.

2.8. Edge-functionalization of graphene with DDTP (DDTP-G)

Graphene (2 mg) was dispersed in PBS pH 7.4 (10 ml) and compound DDTP (0.035 g, 2.45×10^{-5} mol) dissolved in 1 ml PBS, was added to this mixture step wise in 50 μl volumes. After addition volumes of DDTP, the resulted mixture was sonicated at room temperature for 15 min. The final mixture was stirred for 24 h at room temperature to obtain the edge-functionalized graphene. Solution was left over night and sediment as a black solid which is impurity was separated by decantation. Pure DDTP-G as a black solution was obtained.

2.9. Loading of doxorubicin on DDTP-G (DDTP-G-DOX)

Solution of DOX (5×10^{-4} mol/L in CH_2Cl_2) in 50 μl volumes up to 550 μl were added to a BPS solution (5 ml) of the edge-functionalized graphene. Mixture was sonicated at room temperature in interval times and then CH_2Cl_2 was evaporated. Mixture