Chemical Engineering Journal 255 (2014) 577–584

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Functional two-dimensional nanoarchitectures based on chemically converted graphene oxide and hematoporphyrin under the sulfuric acid treatment

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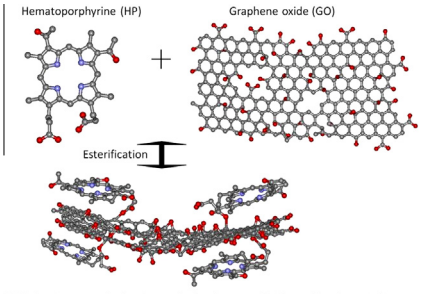
HIGHLIGHTS

- A covalent functionalization of graphene oxide is proposed.
- A novel graphene oxide hybrid material was created.
- A stable pH HP/GO nanocomposite has been obtained.
- An esterification reaction was catalyzed by sulfuric acid.

GRAPHICAL ABSTRACT



HP/GO nanocomposite has been obtained as a result of esterification reaction.

ARTICLE INFO

Article history:
Received 1 April 2014
Received in revised form 26 May 2014
Accepted 20 June 2014
Available online 27 June 2014

Keywords:
Raman scattering
Graphene oxide
Esterification reaction
Hematoporphyrine
Nanocomposite
Drug delivery carrier

ABSTRACT

A model photosensitizer, hematoporphyrin (HP), captured by graphene oxide (GO) nanocarrier with and without the treatment by sulfuric acid was investigated for UV–VIS absorption, luminescence properties and Raman scattering. We demonstrate that combination of optical techniques can be efficiently used to detect an interfacial structure of hematoporphyrine/graphene oxide (HP/GO) nanoensembles. Finally, stable in aqueous media at neutral pH HP/GO nanocomposite were obtained as a result of esterification reaction catalyzed by sulfuric acid. The nanocomposite with stronger absorption and emission represents a layered structure composed of hematoporphyrine oligomers adsorbed on the surface of GO in monolayer through electrostatic and π-stacking interactions. The formation of such hybrid structures, where graphene oxide plays a role of a non-toxic sheet-like nanocarrier, open the way to create a functional nanocomposites with a high level of selective emission and efficient photosensitizing ability utilizing unique graphene properties as singlet oxygen generation enhancer.

1. Introduction

Drug delivery refers to all approaches and systems for transporting pharmaceutical compounds in the body as needed to safely achieve its desired therapeutic effect [1]. Those technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving drug efficacy, as well as patient convenience and compliance [2]. However, commonly used synthetic carriers (such as polymer and lipid particles) and natural particulates (that range from pathogens to mammalian cells) in many cases are "low-skilled" to meet clinical expectations. One of the reasons is that many physiological processes are really driven at the nanoscale where basic mechanisms of molecular recognition, signals transduction, energy conversation and biological transformation take place. Since both analytical techniques and

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http://dx.doi.org/10.1016/j.cej.2014.06.081
1385-8947/

capabilities to produce and manipulate object sizes in nanometer ranges were available only since last decade, there has been tremendous interest in the use of nanoobjects for more efficient methods of drug delivery. It has been reported that nanostructures have the ability to protect drugs from degradation, allow their target delivery, increase bioavailability etc. [3]. The key to understanding the potential of nanoobjects as drug delivery carriers is that their minute size, smaller than cells and cellular organelles, allows them to penetrate basic biological structures, disrupting their abnormal functions.

In the midst of a wide diversity of nanostructured drug delivery systems [4], graphene based architectures initially were envisaged as abnormal case keeping in mind their different nature in respect to biomolecules or cellular substructures. However, it was found that graphene and especially its oxidized modification, graphene oxide, can be highly optimized for their specific functions *in vivo* and possess features that are often desired in drug delivery carriers [5]. Graphene oxide (GO) is a flexible two-dimensional carbon layer with oxygen-containing functional groups (epoxy, hydroxyl and carboxyl) – on the basal plane and at the edges (Fig. 1) [6–10]. For drug delivery system GO branched by appropriate side substitutes represent a set of features like good solubility and stability in physiological solutions, also a unique ability of graphene structure to attach and deliver aromatic, water insoluble drugs [11]. GO's use as a drug carrier was initiated in 2008 by the Hongjie Dai group at Stanford University (CA, USA) [12]. Now, the most important examples of GO surface modifications are with precursors such as polyethylene glycol, folic acid, chitosan, polyethilenimine, polyacrylic acid, amphiphilic copolymers, sulfonic acid and amino group, dextran, gelatin, nanoparticles etc. [13–16].

The first step to use graphene oxide as a drug delivery carrier is a functionalization of GO with functional molecule, particle etc. GO can be successfully functionalized through its functional groups (Fig. 1) using both covalent bonding [11,13–16]) or via weak, non-covalent π–π stacking [17]. In particular, GO was effectively functionalized with anticancer drugs like doxorubicin [17–20]. Porphyrins, as well, were successfully linked with GO *via* both π–π stacking and covalent bonding between amino and carboxyl groups [21,22].

Porphyrins are heterocyclic macrocycles and as highly conjugated planar systems demonstrate high level of both light absorption and emission, which can be modulated by peripheral substitutes. It was precisely those optical properties of porphyrins which stimulated their use as photosensitizers (PHS) in Photo Dynamic Therapy (PDT) and Fluorescence Diagnosis (FD) [23]. Many PHS used clinically in PDT such as Photofrin®, Visudine®, and Foscan® are based on the porphyrin structure [24]. However, porphyrins are usually unstable during light exposure [25–27]. Last studies show that the use of nanocarriers significantly improve the photo-physical properties of porphyrins in respect to their free form in aqueous solutions, – porphyrin based PHS encapsulated in various nanocarriers (liposomes, micelles, nanoparticles) demonstrate stronger absorption, high level of singlet oxygen generation without damage of PHS etc. [28]. However, up to now there is a quite limited available information on the effect of GO on the optical properties of porphyrins, – in particular on ones of natural origin like hematoporphyrin (HP), – an endogenous porphyrin formed by the acid hydrolysis of hemoglobin [29] that has been used as an antidepressant and antipsychotic as well as in phototherapy of cancer since the 1920s [30–32]. At the same time, HP ∼ GO aggregate, as well, can be used in a new drug for PDT/FD if HP ∼ GO based formulations will demonstrate high luminescence intensity and increased protection against singlet oxygen damage.

As revealed by published information, the optical properties of HP strongly depend on its aggregation state, – position of the bands, their intensity, luminescence efficiency and singlet oxygen production rate drastically decreased under the aggregation of HP [33]. The main approach to increase the efficiency of HP as PDP/FD specific drug is to prevent the formation of aggregates using (i) oligomerization, (ii) changing the equilibrium to the monomeric forms (encapsulation) or (iii) by inducing formation of monomer friendly organized architectures. The first approach (i) was successfully realized by S. Schwartz in 1960 for the detection of tumors by treating HP with concentrated sulfuric acid [34,35]. The more effective bioactive products of this reaction are porphyrin oligomers containing of 2–8 the pyrrole rings connected by ester bonds. The possibility of the oligomerization process is the result of the presence within of HP molecule two hydroxyethyl groups (secondary alcohols) and two propionic acid (carboxylic) groups attached to the pyrrole rings. As dihydroxy and dicarboxylic acid derivative HP can be involved in Fischer/Fischer–Speier esterification reaction with refluxing a carboxylic acid and alcohol in the presence of an acid catalyst. Sulfuric acid is a catalyst in esterification reaction as it is well known for many years [36,37]. The actual challenge is to extend the use the "oligomerization approach" in a new functional supramolecular architecture through combination of HP with nanostructured carriers.

In the present work we study influences of nanosize GO on the spectral properties of HP in the presence of sulfuric acid with the aim to develop less-costly and more efficient approach for formation a functional HP ∼ GO architectures through self-assembling

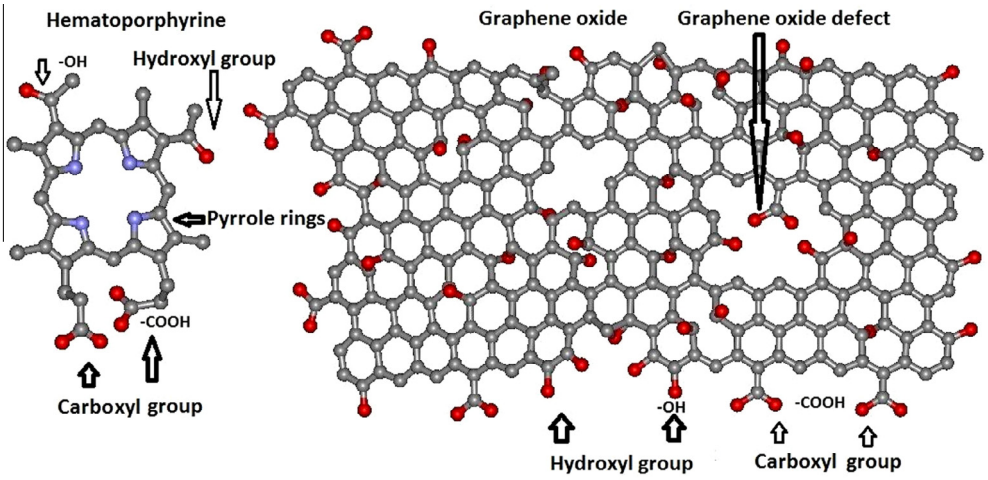

Fig. 1. Computer-drawn structure of Hematoporphyrine molecule and Graphene oxide flake (HyperChem 5.02, HyperCube, Inc.).