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Three-dimensionally porous graphene: A high-performance adsorbent for removal of albumin-bonded bilirubin

Chun–Fang Ma[a], Qiang Gao[a,*], Kai–Sheng Xia[b,*], Zhi–Yuan Huang[b], Bo Han[b], Cheng–Gang Zhou[b]

[a] Department of Chemistry, Faculty of Material Science and Chemistry, China University of Geosciences, Wuhan, 430074, PR China
[b] Sustainable Energy Laboratory, Faculty of Material Science and Chemistry, China University of Geosciences, Wuhan, 430074, PR China

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ABSTRACT

The development of bilirubin adsorbents with high adsorption efficiencies towards albumin-bonded bilirubin is still a considerable challenge. In this work, a three-dimensionally porous graphene (3D-pGR) has been fabricated through a simple carbon dioxide (CO_2) activation of thermally exfoliated graphite oxide (EGO). Intriguingly, the resultant 3D-pGR material showed hierarchically micro-meso-macroporous structure, high specific surface area of up to 843 $m^2\,g^{-1}$, and large pore volume as high as 2.71 $cm^3\,g^{-1}$. Besides, the large planar π-configuration structure of 3D-pGR made it possible to compete effectively with albumin for bilirubin binding. Taking advantages of these fantastic characteristics, the 3D-pGR was demonstrated to be extraordinarily efficient for bilirubin removal from a bovine serum albumin (BSA)-rich solution. Under optimized conditions, the maximum adsorption capacity of 3D-pGR for BSA-bonded bilirubin was up to 126.1 mg g^{-1}, which is not only significantly higher than the adsorption capacities of currently available adsorbents towards albumin-bonded bilirubin, but also superior to those of many reported adsorbents towards free bilirubin. In addition, the hemolysis assay of 3D-pGR indicated that this material had negligible hemolysis effect. Findings from this study may open up important new possibilities for removal of protein-bonded toxins.

1. Introduction

Bilirubin, a major breakdown product of hemoglobin, is one of the sensitive indicators of liver function levels [1]. Normally, it is transported on the blood circuit to the liver as a complex with serum albumin, and excreted from hepatocytes into bile mainly as glucuronide-conjugated state; the concentration of bilirubin in the blood is almost kept at a relatively constant level [2]. However, when the liver's function is impaired (e.g., liver failure), the bilirubin cannot be adequately eliminated and thus accumulates in the blood [3]. The extra bilirubin will be deposited onto various tissues (e.g., brain), which results in a yellow discoloration of the skin (known as jaundice), brain damage, and even death in severe cases [3,4]. Therefore, removal of excess bilirubin from blood is of great significance for remission of liver disease and to gain time for patients waiting for liver transplantation.

Haemoperfusion, i.e., circulation of blood through an extracorporeal unit containing an adsorbent system for bilirubin removal, is one of the most effective and promising treatment approaches [2,5]. So far, the majority of clinic haemoperfusion systems adopt activated carbon (AC) as an adsorbent to remove bilirubin because of its low cost, abundant sources, high porosity, and good blood compatibility [6]. However, nanopores in AC are mainly less than 1 nm in diameter, which are not accessible to bilirubin molecules [7]. Not surprisingly, the reported adsorption capacities of AC materials towards bilirubin are relatively low [6]. Recently, several new types of carbon-based materials have been used as bilirubin adsorbents to improve the adsorption efficiency. [2,3,7–12] For example, the magnetic multi-wall carbon nanotubes prepared by Wei and co-workers had an increased effective surface area, which was capable of adsorbing 263.16 mg bilirubin per gram adsorbent at 30 °C. [3] The hollow mesoporous carbon spheres fabricated by Guo and co-workers exhibited large pore size (3.8 nm), high surface area (769.5 $m^2\,g^{-1}$), and large pore volume (1.0 $cm^3\,g^{-1}$), which gave an extraordinarily high bilirubin adsorption capacity of up to 304 mg g^{-1} [2]. The prominent adsorption capacities are encouraging, however, these bilirubin adsorption experiments

* Corresponding authors.
E-mail addresses: gaoqiang@cug.edu.cn (Q. Gao), xks_mail@126.com (K. Xia).

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were basically carried out in albumin-free solution, which is contrary to the facts that serum albumin is the most abundant protein in blood and bilirubin exists primarily in the form of albumin-bilirubin complex [13–15]. Despite the advantage of high effectiveness for removal of free bilirubin, the present carbon adsorbents usually performed poorly on the removal of albumin-bonded bilirubin. For example, the reported adsorption capacity of carbon nanotube sheets towards bovine serum albumin (BSA)-bonded bilirubin was 9.7 mg g^{-1} [8], which is far less than the values obtained for free bilirubin removal. Therefore, it is urgently required to find or develop new types of carbon adsorbents that have high removal efficiencies towards albumin-bonded bilirubin.

Graphene (GR), as a two-dimensional carbon nanomaterial, has attracted wide interest owing to its intriguing features, such as large theoretical specific surface area, excellent chemical stability, good electrical conductivity, and satisfying biocompatibility [16,17]. To date, GR has exhibited great potential in the fields of adsorption, catalysis, sensor, hydrogen storage, electrochemical energy storage, and drug delivery [18,19]. However, up until now, only a GR derivative (i.e., graphene oxide (GO)) has been reported as a support of chitosan (CS) to fabricate a hybrid adsorbent (GO content less than 9.09 wt%) for bilirubin removal, where the CS was found to be the major contributor to the adsorption of bilirubin and the maximum adsorption capacity for albumin-bonded bilirubin was 8.96 mg g^{-1} [4]. Given the remarkable properties of GR itself, an interesting question might be raised: Is pure GR-based material a suitable adsorbent for bilirubin removal? In fact, a remarkable characteristic of GR is its large planar π-configuration structure, which makes it more conducive to cofacial π–π stacking as compared to other carbon nanomaterials (e.g., carbon nanotubes) [20]. By virtues of this advantage, pure GR-based materials have recently been demonstrated to be superior host substrates for stable assembly of π-conjugated organic molecule on their surfaces via π–π interaction [21,22]. Significantly, Economopoulos and coworkers reported that the porphyrin, a dicarboxylic acid-functional tetrapyrrole macrocycle compound, could be well stabilized onto GR surface through strong π–π stacking interactions [23]. Taking into account the similarity of the molecular structures between porphyrin and bilirubin (tetrapyrrole dicarboxylic acid), it is naturally anticipated that the strong π–π stacking of GR might enable such material to compete effectively with albumin for bilirubin binding so as to be suited for further application in removal of albumin-bonded bilirubin.

On the other hand, in order to achieve a sufficient bilirubin adsorption capacity for GR to meet the haemoperfusion demand, the accessible surface area is the most critical concern. Unfortunately, GR nanosheets tend to restack or aggregate through self-induced π–π stacking or van der Waals attractions between nanosheets during preparation, resulting in a dramatic decrease of the surface area [24]. For example, the reported specific surface areas of GR materials derived from GR oxide are generally less than 100 m^2 g^{-1}, which is much lower than the theoretical value [25]. To address this issue, either "top-down" or "bottom-up" self-assembly of individual GR nanosheets into a macroscopic architecture has recently been investigated, including one-dimensional (1D) fiber-like structure, two-dimensional (2D) self-supporting paper, and three-dimensional (3D) network [26]. In particular, the 3D graphene (3D-GR) consisting of interconnecting network is highly advantageous in maintaining the large surface area, facilitating mass transfer, and increasing mechanical integrity. [27] Although considerable progress has been made in the fabrication of 3D-GR structure, challenges still remain in further regulation of the textural features (e.g., surface area and porosity) of 3D-GR and broadening its application scopes.

In our previous work, we developed a facile method to fabricate 3D porous GR (3D-pGR) by a simple carbon dioxide (CO_2) activation of graphite oxide (GO) [28]. Noticeably, the 3D-pGR showed hierarchically micro-meso-macroporous structure, high specific surface area of up to 532 m^2 g^{-1}, and large pore volume of up to 1.67 cm^3 g^{-1} [28]. On this basis, herein a modified CO_2-activation method using thermally exfoliated GO (EGO) as precursor was applied. Remarkably, it was found that the specific surface area (up to 843 m^2 g^{-1}) and pore volume (up to 2.71 cm^3 g^{-1}) of the EGO-derived 3D-pGR were greatly enhanced relative to those of the GO-derived analogue. Furthermore, the newly prepared 3D-pGR was employed as the adsorbent for bilirubin removal from BSA-rich solution, where an exceptionally high adsorption capability toward albumin-bonded bilirubin as well as good hemocompatibility could be implemented.

2. Experimental

2.1. Raw material and chemicals

Graphite powders were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bilirubin was obtained from Hubei Bangsheng Chemicals Co., Ltd. (Wuhan, China). The potassium permanganate ($KMnO_4$) was purchased from Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). The albumin from bovine serum (BSA, M_w = 67000), concentrated sulfuric acid (H_2SO_4, 98%), concentrated hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals and materials used in this study were commercially available and used without further purification. Distilled water was used throughout the experiments for solution preparation.

2.2. Preparation of 3D-pGR

A typical preparation process of 3D porous graphene (3D-pGR) would consist of the following steps: First, graphite oxide (GO) was synthesized by the modified Hummer's method, and more details can be found in our previous work [28]. Then, 1.0 g of dried GO was placed in a quartz tube and purged with N_2 for 30 min to remove air, and then the tube was rapidly put into a vertical furnace preheated to 800 °C. The exfoliated GO (EGO) was obtained after thermal treatment at 800 °C for 1 min followed by cooling it down to room temperature under N_2 gas flow. Subsequently, the 3D-pGR was prepared by a simple gas–solid reaction between CO_2 and EGO. Briefly, 0.2 g of dried EGO was placed in a quartz crucible and fixed in the center of tube furnace. After sweeping for 1 h using N_2 stream (50 mL min^{-1}), the furnace temperature was raised to 850 °C from ambient temperature at a rate of 10 °C min^{-1}, and then the gas stream was switched to CO_2 at the same flow rate. Finally, the black sponge-liked powders (3D-pGR) were obtained after 4 h activation.

2.3. Characterization of materials

X-ray diffraction (XRD) patterns of the as-synthesized samples were recorded on a Bruker AXS D8-FOCUS diffractometer using monochromatized Cu-Kα radiation (40 kV, 20 mA; λ = 0.1540598 nm). The microscopic features and morphologies of the samples were observed by field scanning electron microscopy (FESEM, Hitch SU8010) and high-resolution transmission electron microscopy (HRTEM, Philips CM12) operated at 120 kV. The Raman spectra were recorded using a Horiba Jobin-Yvon LabRAM HR800 Raman microspectrometer with an excitation laser of 532 nm. Specific surface area assessment and pore distribution measurements were carried out by N_2 adsorption-desorption analysis at 77 K using a Micromeritics ASAP 2020 HD88 system.