Short Communication

Comparative study of adsorptive role of carbonaceous materials in removal of UV-active impurities of paclitaxel extracts

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ABSTRACT

Graphite oxide (GO) and reduced graphene oxide (rGO) nanosheets were synthesized with a low-cost manufacturing method. The morphology and structures of the synthesized samples were studied using X-ray diffraction (XRD), atomic force microscopy (AFM), Fourier-transform infrared (FTIR) and Raman spectroscopy. The efficiencies of GO and rGO as novel candidate adsorbents in the pre-purification of paclitaxel were compared and contrasted with those of commercial graphite (Gt), graphene (G) and multi-wall carbon nanotube (MWCNT). According to UV–vis and HPLC analyses, rGO was evaluated as the best absorbent for the removal of impurities in pre-purification of paclitaxel from plant cell cultures. In contrast, the GO had the poorest proficiency for paclitaxel pre-purification in comparison with the other carbonaceous adsorbents. This is attributed to the existence of many localized defects in the π-structure of GO that is related to weakness of π–π stacking interactions between crude extract impurities and GO.

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1. Introduction

Paclitaxel, as a contemporary exciting plant-derived anticancer drug, was first isolated from the inner bark of the Taxus brevifolia [1]. Using high performance liquid Chromatography (HPLC) is essential to obtain high level of purity and yield of this drug. However, purification by HPLC has no effective resolving power so long as the feed materials are not previously enriched for paclitaxel [2]. Pre-purification procedures before final HPLC runs often requires use of some adsorbents that improve purity of crude extracts. This increases the purity and yield and decreases the cost of the final paclitaxel products.

On the other hand, graphite (Gt) is one of the naturally occurring crystalline forms of carbon used as industrial absorbents. Carbon nanotubes (CNTs) are relatively new efficient carbonaceous sorbents because of their large specific surface area and hollow structure [3]. Graphene (G) is a new member of the carbon family with a one-atom-thick sheet of honeycomb carbon lattice structure [4]. Graphite oxide (GO) is a layered compound that can be synthesized by oxidizing graphite. The oxygenated functional groups on GO along with its large surface area indicate it can be used as an efficient absorbent [5]. The chemical reduction of GO is a typical method for the production of reduced graphene oxide (rGO). However, few studies have been made for exploring the possibility of using this cost effective rGO as an adsorbent.

In this investigation, efficiencies of five different carbon-based adsorbents (i.e. rGO, GO, Gt, G and CNT) were evaluated in pre-purification of paclitaxel. We focused on comparing the effectiveness and removal ability of color and impurities from crude paclitaxel extract obtained from plant cell culture, for the above mentioned adsorbents. To the best of our knowledge, this is the first report to compare the adsorptive role of carbonaceous materials in pre-purification of paclitaxel.

2. Experimental

Natural flake graphite was provided by Qingdao Dingding Graphite Products Factory (Shandong, China). Graphene and multi-wall carbon nanotube were purchased from US Research Nanomaterials, Inc. (Houston, USA). GO was prepared from natural graphite using modified Hummer’s method [6]. For preparation of rGO, 100 mg of GO in 100 mL of water was ultrasonicated for 30 min, to which ammonia solution was added to increase the pH up to 10 and then 1 mL of hydrazine hydrate was added and the solution was heated at 100 °C for 24 h. After cooling the solution, the resulting black precipitates were centrifuged and washed three times with deionized (DI) water and finally dried at 60 °C.

Fresh stems of Taxus baccata were collected from Botanical Garden of University of Tehran, Karaj, Iran. After sterilization, the
explants were cultured on a B5 medium and finally kept in a growth chamber at 24 °C under dark condition [7]. The pH of the medium was carefully adjusted to 5.8 prior to autoclaving. Calli cultures were transferred to a fresh B5 medium every 2 weeks [8]. Concisely, 20 g of dry weight freeze-dried biomass was first subjected to 800 mL of hexane at room temperature for 12 h. Then the mixture was centrifuged (5000 rpm) for 20 min and the supernatant was discarded. The pellet, in the following, was extracted with 400 mL of MeOH:CH₂Cl₂ (1:1) by sonication for 1 h at room temperature, centrifuged at 5000 rpm for 20 min and the supernatant was transferred to the next glass tubes. The extracts were dried at 25 °C under vacuum and redissolved in 200 mL of dichloromethane plus 200 mL of distilled water. Subsequent to vortexing for 10 s and centrifuging at 5000 rpm for 20 min, the organic fraction was isolated and dried under vacuum. This dark brown color dried crude extract was utilized as starting material for adsorbent treatments.

A stock solution of 1500 mg/L extract was prepared by dissolving of dried crude in dichloromethane. Since then, certain amounts of each adsorbent (i.e. rGO, GO, Gt, G and CNT) were added separately to this extract solution in 5 mL tubes. The mixture was shaken for 45 min at 40 °C and then centrifuged. During adsorption treatment, the solution turned colorless. The decolorization efficiency of adsorbents was investigated using a UV–vis spectrophotometer (Shimadzu UV-2100, Kyoto, Japan). Decolorization (%) was calculated to this extract solution in 5 mL tubes. The mixture was shaken for 45 min at 40 °C and then centrifuged. During adsorption treatment, the solution turned colorless. The decolorization efficiency of adsorbents was investigated using a UV–vis spectrophotometer (Shimadzu UV-2100, Kyoto, Japan). Decolorization (%) was calculated from the total absorbance of solution in the visible range (400–800 nm) of UV spectrum as Eq. (1):

\[
\text{Decolorization (\%) } = \left( \frac{A_0 - A_e}{A_0} \right) \times 100
\]

where \(A_0\) and \(A_e\) are the total absorbance of crude extract (from 400 to 800 nm) before and after treatment with adsorbent, respectively.

3. Results and discussion

3.1. Characterization of synthesized GO and rGO

XRD (Philips Xpert MPD, Eindhoven, Netherlands), and AFM (VEECO CP-Research, New York, USA) analyses were used to confirm the micro-structures and morphology of GO and rGO. XRD spectra obviously showed the oxidation of graphite and preparation of GO (Fig. 1A). The diffraction peak of GO appeared at 10.58° which originated from the diffraction on its (0 0 2) layer planes with the basal spacing of 0.959 nm. This was along with a broad diffraction peak at 22.29 nm which was interpreted in terms of short-range order in stacked graphene sheets [9]. The XRD pattern of rGO showed the broad peak at about 25° with interlayer spacing of approximately 0.378 nm (Fig. 1A). This was much smaller than the 0.959 nm for GO, and was closer to the (0 0 2) graphite peak of 0.334 nm. The sharp peak at \(2\theta=26.6^\circ\) indicated a highly organized crystal structure with an interlayer spacing of 0.339 nm, which was consistent with the layer spacing of normal graphite. Moreover, a weak peak at \(2\theta=8.7^\circ\), (d-spacing of 0.768 nm) appeared in the XRD pattern of rGO which was similar to the typical diffraction peak of GO.

AFM was used for quantifying the degree of exfoliation, thickness, and surface roughness of GO (Fig. 1B). The samples were prepared by drop casting onto a silicon oxide wafer (0.05 mg/mL). AFM image revealed that isolated GO nanosheets were well exfoliated and dispersed with a thickness of about 1.5 nm for each.

In the FTIR (Thermo spectrometer, Madison, USA) spectrum of GO (Fig. 2A), the peak at 1722 cm\(^{-1}\) corresponded to the stretching band of C=O in carboxylic acid or carbonyl moieties. The intense band at 3426 cm\(^{-1}\) was attributed to O–H stretching. The peak at 1638 cm\(^{-1}\) (aromatic C=C) could be assigned to the skeletal vibrations of un-oxidized graphitic domains of GO and the deformation of GO (Fig. 2B) [11]. Raman spectrum (Almega Thermo Nicolet, Madison, USA) of GO displayed two characteristic peaks, the D band around 1340 cm\(^{-1}\) and the G band at 1584 cm\(^{-1}\) (Fig. 2C). The intensity ratio of D over G band (the \(R\)-value=\(I_D/I_G\)) was usually used as a measure of the degree of disorder and the average size of the sp² domain which was calculated to be 0.91

Fig. 1. (A) XRD patterns of Gt along with synthesized GO and rGO. (B) AFM image of GO.
from the spectrum [12]. The Raman spectrum of rGO also contained both G and D bands (at 1565 and 1330 cm$^{-1}$, respectively), but the intensity ratio of D over G band (1.80) increased compared to that in GO (Fig. 2D). Maybe this was due to a decrease in the average size of the sp$^2$ domains upon GO reduction and production of new graphitic structures [12].

3.2. Selection of the best adsorbent

The removal performances of waxy compounds and plant pigments from crude paclitaxel extract, for five candidate adsorbents (i.e. rGO, GO, Gt, G and CNT) were compared and contrasted, by batch sorption treatments. Table 1 summarizes the decolorization results for these adsorbents with various amounts (20 and 30 g/L) in dichloromethane as a solvent.

The UV–vis spectra of crude extract with light brown color showed the presence of carotenoids as plant pigments which are produced from eight isoprene molecules with large delocalized $\pi$ electron system. Considering the structural features of these pigments as adsorbates and chemical structure and functionalities of carbonaceous hydrophobic adsorbents (i.e. rGO, CNT, G and Gt), the main plausible mechanism for decolorization of the crude extract was $\pi$–$\pi$ electron interactions taking place between delocalized and conjugated electrons of adsorbent and $\pi$ electron system of carotenoids [13]. However, in the case of hydrophilic GO adsorbent, oxygen functionalities in GO surfaces made many localized defects in its $\pi$-structure; So, the poorer efficiency of GO in comparison with the other adsorbents (i.e. G, rGO and CNT) may result from more weakness of $\pi$–$\pi$ stacking interactions between carotenoids and GO. In contrast, chemical reduction of graphene oxide can restore the graphitic network in the basal plane of rGO. Consequently, on the basis of chemistry involving the adsorption treatment, $\pi$–$\pi$ stacking interactions were occurred more effectively between rGO and adsorbate which was probably the reason for superiority of rGO over GO in decolorization of taxol crude extracts, particularly in dichloremethane.

On the other hand, comparison between decolorization (%) results of rGO and commercial G and CNT displayed that in higher amounts (30 g/L) of adsorbent the decolorization efficiency was almost the same for all three adsorbents, but in the lower dosages (20 g/L) the superiority of rGO over G and CNT was confirmed. This difference can be attributed to the availability of smaller surface area and fewer sorption sites in lower amounts of adsorbents. While, with the increasing adsorbent content, the number of available adsorption sites was expected to increase removal efficiency for all adsorbents. Furthermore, the purity of paclitaxel was evaluated by HPLC (Shimadzu 20AD, Kyoto, Japan), to determine the effectiveness of each adsorbent. Separation conditions involved C$_{18}$ column with mobile phase composition of water/acetonitrile (70:30, v/v) at flow rate of 1 mL/min, with an injection volume of 20 $\mu$L. UV detector at wavelength of 227 nm was used for detection. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich and used as standard. HPLC analyses confirmed the decolorization results and purity of paclitaxel decreased in the order of rGO (18.8%) > CNT (11.8%) > G (10.6%) > GO (8.7%) > Gt (8.8%) (Table 1). These results proved that adsorption treatments using these carbonaceous materials could be a suitable way to improve paclitaxel purity. Accordingly, treatment of crude with rGO resulted in more than four times of increase in the purity of paclitaxel from 3.9% to 18.8%. Therefore, from an economical perspective, rGO was eventually selected as the optimum adsorbent.

Table 1

| Adsorbent type | Adsorbent amount (g/L) | Decolorization (%) | Purity (%) |
|---------------|------------------------|--------------------|------------|
| G             | 20                     | 63.5               | 7.5        |
|               | 30                     | 85.4               | 10.6       |
| GO            | 20                     | 46.1               | 5.5        |
|               | 30                     | 62.2               | 8.8        |
| Gt            | 20                     | 48.1               | 6.0        |
|               | 30                     | 51.8               | 8.2        |
| rGO           | 20                     | 78.0               | 14.7       |
|               | 30                     | 88.1               | 18.4       |
| CNT           | 20                     | 65.1               | 8.5        |
|               | 30                     | 89.5               | 11.8       |

Fig. 2. FTIR spectra of synthesized (A) GO and (B) rGO. Raman spectra of synthesized (C) GO and (D) rGO.
adsorbent. Our findings displayed promising applications of rGO, as a cost-effective nano-adsorbent, to provide a suitable vehicle toward improvement of paclitaxel pre-purification.

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