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Carbonaceous sorbents alongside an optimized magnetic solid phase extraction (MSPE) towards enrichment of crude Paclitaxel extracts from callus cultures of *Taxus baccata*

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ABSTRACT

Here, a number of synthesized and commercial nano-sorbents including graphene (G), multi-wall carbon nanotube (MWCNT), graphene oxide (GO), reduced graphene oxide (rGO) and magnetic nanoparticles anchored on GO (Fe_3O_4NPs@GO) followed by graphite (Gt) powder were employed for paclitaxel pre-purification from callus culture-derived extract of *Taxus baccata*. Based on our preliminary work, rGO and Fe_3O_4NPs@GO exhibited the maximum potential, not only to remove impurities, but also to ameliorate taxol purity. According to the results of response surface methodology (RSM) developed for the superior nano-sorbent (i.e., Fe_3O_4NPs@GO), for both proposed quadratic polynomial models, statistically substantial relationships obtained between the actual and predicted values ($p < 0.0001$). Furthermore, both agitation power and sorbent dosage compared to the sorption temperature exhibited more efficacies on the two responses of interest [i.e., efficiency of calli-derived pigments removal (ECPR) and efficiency of taxol purity (ETP)]. Based on the results of simultaneous optimization to attain the highest ratio of ECPR (96.35%) and ETP (30.60 %), the values of 29.9 g/L, 29.6 °C and 165.5 rpm were respectively predicted for sorbent dosage, sorption temperature and agitation power, and subsequently certified through experimental rechecking survey. Remarkably, the sorption capacity and magnetism feature of the magnetic nano-sorbent remained nearly constant, even upon nine consecutive treatments. Considering the results as a whole, the combination of magnetic solid-phase extraction (MSPE) using the current Fe_3O_4NPs@GO nano-sorbent and RSM can be recommended as a simple, cost-effective and fast route for sorbent-assistant pre-purification investigations of paclitaxel.

1. Introduction

Owing to its substantial mode of action to barricade the microtubule depolymerization and subsequent cell proliferation, Taxol® (generic name Paclitaxel) has been recently regarded as one of the most clinically valuable and eminent biological active compounds, worldwide [1]. This blockbuster chemotherapeutic agent is structurally clustered into a class of complex diterpenoids called “taxanes” and contains an unusual oxytane ring alongside a tricyclic core [2]. Thus far, several biological repositories have been proposed for taxol production including different tissues of the slow growing yews (*Taxus* spp.) [3], hazel tree (*Corylus avellana* cv.Gasaway), and endophytic fungi [2,4]. However, the ratio of taxol is in low quantities, environmental concerns may occur, and its

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Abbreviations: AAD, absolute average deviation; AFM, atomic force microscopy; ANOVA, analysis of variance; CCRD, central composite rotatable design; CV, coefficient of variation; DCM, dichloromethane; DI, deionized water; ECPR, efficiency of calli-derived pigments removal; ETP, efficiency of taxol purity; G, graphene; GO, graphene oxide; Gt, graphite powder; LLE, liquid–liquid extraction; MSPE, magnetic solid-phase extraction; MWCNT, multi-wall carbon nanotube; rGO, reduced graphene oxide; RSM, response surface methodology; SEM, scanning electron microscope; TEM, transmission electron microscope.

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quantity may fluctuate by various peripheral cues, particularly for yews [5]. The next candidate called "semi-synthesis", whereby the side chain is initially chemo-synthesized and subsequently conjoined to a naturally occurring precursor called baccatin III [6], but plant harvesting is yet unavoidable. As the last practical option, the biotechnological production of taxol has been recently considered as the most reliable method [4], predominantly, since the drug of choice could be stably produced in a bioreactor-based system devoid of any potential effects of peripheral elements [1].

Direct proceeding of paclitaxel isolation through plant tissue culture-derived extracts of *Taxus* spp., using HPLC alone and without obliterating the interfering biochemical compounds (e.g., tar and waxy composites plus various unfavorable plant-derived pigments), is assumed as an impractical purification approach [7]. This, in fact, goes mainly back to several challenging issues as follows: 1) unavoidable consumption of voluminous amounts of toxic organic solvents; 2) inevitable loading of the feed materials comprising dense quantities of diverse undesirable substrates on the HPLC columns; 3) lifetime decrement of the column packing material; 4) low recovery levels of the compound of interest; 5) inadequate efficiency and relatively high production costs [7]. Therefore, to cope with the above-mentioned limitations, the starting crude samples need to be primarily enriched and purified then via HPLC. In this context, a number of sorbents including activated carbon, active clay, sylopute, and mesoporous silica have been applied [1,7–9]. Regardless of some efficacies, some drawbacks have been recorded for such sorbents including lack of recoverability/reusability [10], inadequate capacity of sorption and inefficient extraction along with inevitable necessity of filtration or centrifugation [11], so its industrial/commercial scale-up may fairly hard to access, incur additional costs and/or even become failure [11].

So as to circumvent such interfering barriers, application of "magnetic solid-phase extraction" (MSPE) does appear to be a trustworthy alternative option [12], predominantly, due to some advantages as follows: First, separation of the functionalized magnetic nano-sorbents from the aqueous media could be easily taken place by means of an external magnetic field, without applying any additional steps of centrifugation or filtration, making accordingly the sorption process much faster and more facile [13,14]. Second, such methodology is believed to be economically feasible and more efficient [15]. Third, as a result of its high dispersibility nature, the sorbent is capable enough to adequately interact with the sorbate; therefore, quick mass diffusion alongside fast sorption equilibrium are expected to occur [16]. Owing to such or similar remarkable issues, MSPE technique has widely attracted lots of worldwide attentions, and some sorbents including graphene (G) has been frequently utilized as a competent SPE sorbent, as well-documented by [12]. Like graphite (Gt) powder, G possesses intrinsic hydrophobicity; hence in obtaining a more stable suspension, its oxidized version known as "graphene oxide (GO)" is alternatively utilized. In fact, thanks to some oxygenated functional groups including carboxylic acid, hydroxyl, carbonyl, and epoxide, GO is considered as a negatively charged, and hydrophilic material, so it could be uniformly dispersed in aqueous media [17]. However, GO separation from the suspension still remains a challenging issue, actually because it is naturally hydrophilic [18]. To prevail such problematic obstacles, chemical fabrication of a magnetic GO hybrid has been widely recommended, whereby both high sorption capacity of GO and the facile separation nature of magnetic constituents could be simultaneously applied [19].

Our previous works focused on the application of carbon and magnetic based nano-sorbents for pre-purification of crude paclitaxel extracts from fresh needles of *Taxus baccata* [20,21]. Besides, we started a preliminary evaluation of sorption efficacies of several carbonaceous substances to eliminate undesirable UV-active compounds existed in the plant cell culture extractions of *T. baccata* [22]. In the current work, a number of synthesized and commercial nano-sorbents including multi-wall carbon nanotube (MWCNT), G, GO, reduced graphene oxide (rGO) and magnetic nanomaterials anchored on GO (Fe_3O_4NPs@GO) followed by Gt powder were employed to inspect and compare efficiencies thereof about eradicating calli-derived impurities (i.e., dyes, waxy and tar compounds as well as other undesirable metabolites) as well as improving taxol purity from callus culture of *T. baccata*. Subsequent to selection of the best nano-sorbent, a response surface methodology (RSM)-based modeling study was also performed, actually to scrutinize possible consequences of three crucial independent criteria (i.e., sorbent dosage, temperature and shaking power) on the improvement of two responses of interest [i.e., efficiency of calli-derived pigments removal (ECPR) and efficiency of taxol purity (ETP)].

2. Experimental

2.1. Plant materials and callus preparation

Fresh stems of *T. baccata* were harvested from the Botanical Garden of the University of Tehran, Karaj, Iran. The collected stem samples were firstly sterilized via keeping in 70% ethanol for 60 s, and subsequently submerged in sodium hypochlorite (1.5%) for 20 min. For callus preparation, the treated samples were immediately washed thrice using sterilized autoclaved water and transferred then into a B5 medium [23], supplemented with 0.2 mg L^{-1} 2,4-D, 2 mg L^{-1} NAA, 0.2 mg L^{-1} Kinetin, 500 mg L^{-1} PVP (Polyvinylpyrrolidone), 25 g L^{-1} sucrose, 8 g L^{-1} agar, and an adjusted pH value of 5.8 before autoclaving [24]. Meanwhile, $AgNo_{3,}$ with the final concentration of 4 μM was also added as an exogenous elicitor. All the above-mentioned chemical substances were commercially prepared from Sigma Aldrich or Merck (Germany).

2.2. Process for crude extract material

Dichloromethane (DCM; Merck, 99.8%), *n*-hexane (Sigma-Aldrich, 95%), acetone (Merck, 99.8%), ethanol (Merck, 98%), and the other materials were purchased from the related companies. To prepare paclitaxel extract from the callus culture media, the earlier protocol of [2] was applied, with slight modifications: In brief, about 20 g of the fine powder of the calli were added to a flask containing 800 mL *n*-hexane and allowed to incubate under room temperature circumstances for 12 h. Next, mixture centrifugation was conducted for 20 min at 5000 rpm, the supernatant was removed and the pellet was collected. Then, 400 mL of MeOH:DCM (1:1) was added to the collected pellet and being sonicated for 60 min at ambient temperature. Subsequent to centrifugation of the mixture for 20 min at 5000 rpm, the supernatant was carefully transferred into a new glass flask. Under vacuum conditions, the supernatant was completely dried at 25 °C, and dissolved again in 400 mL of a solution containing DCM plus distilled water (1:1). The organic fraction was extracted through vortexing for 10 s, immediately centrifuged at 5000 rpm for 20 min, and the solvent was removed under vacuum circumstances. Finally, a "*dark brown crude extract*" was acquired and employed for the current sorption study.

2.3. Preparation of sorbents

2.3.1. Commercial sorbents

Natural flake Gt powder was provided by Qingdao Dingding Graphite Products Factory (Shandong, China), while, G and MWCNT were purchased from US Research Nanomaterials, Inc. (Houston, USA). The other nano-sorbents of GO, rGO and Fe_3O_4NPs@GO were chemically synthesized in our lab, as below.