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Hybrid-type carbon microcoil-chitosan composite for selective extraction of aristolochic acid I from *Aristolochiaceae* medicinal plants

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ABSTRACT

Aristolochic acid I is a nephrotoxic compound widely existing in many kinds of traditional Chinese medicines, especially in *Aristolochiaceae* medicinal plants. In this study, chitosan modified carbon microcoils were designed and prepared for the selective separation of aristolochic acid I from medicinal herbs. Successful modification of carbon microcoils was confirmed by scanning electron microscopy, Fourier-transfer infrared spectroscopy, elemental analysis, X-ray photoelectron spectroscopy, and thermogravimetric analyses. The effects of adsorption conditions were investigated and it was determined that the adsorption of aristolochic acid I was controlled by pH. Adsorption isotherms, kinetics, and selectivity tests were performed to evaluate the adsorption capacity and selectivity of the modified carbon microcoils. The chitosan modified carbon microcoils exhibited excellent binding ability (77.72 mg g^{-1}) and satisfactory selectivity. Finally, this material was used in solid phase extraction combined with HPLC to enrich and detect aristolochic acid I from medicinal plants. The detector response for aristolochic acid I was linear from 0.5 to 150 mg L^{-1}, and the recoveries of aristolochic acid I ranged from 73.61 to 77.73% with the relative standard deviations of less than 5%. Thus, chitosan modified carbon microcoils were ideal adsorbents for the selective extraction of aristolochic acid I from *Aristolochiaceae* plants.

1. Introduction

Aristolochic acid I (AA I) is one of the substituted 1-phaenanthrene carboxylic acids commonly found in *Aristolochia* and *Asarum* plants [1]. Previous studies have shown that *Aristolochia* and *Asarum* herbs possess various anti-inflammatory [2,3], anti-malarial [4], and anti-hyperglycemic [5] pharmacological activities. Many traditional pharmacopeias contain *Aristolochia* and related plants. It's reported that 24 kinds of medicinal *Aristolochiaceae* plants and 43 proprietary Chinese medicines that contain these herbs are recorded in the Chinese Pharmacopoeia 2015, departmental standards and local standards. Moreover, these herbs and related products have been used for weight loss [6], and are extensively used in the treatment of arthritis and rheumatism. However, these traditional Chinese medicines contain nephrotoxins and mutagens in the form of aristolochic acids and related compounds (collectively, AAs). AAs have been implicated in multiple cancer types, sometimes with very high mutational burdens, especially in upper tract urothelial cancers [7,8]. In addition, aristolochic acids have been reported to be involved in liver cancers [9]. Thus, an effective method for the extraction and analysis of aristolochic acid I in traditional Chinese medicines (TCMs) is necessary.

To date, several analytical methods have been used to determine AA I in complex samples, including thin layer chromatography, high performance liquid chromatography (HPLC) [10,11], capillary electrophoresis [12] and enhanced chemiluminescence [13]. HPLC has been widely used for the analysis of AA I, achieving a balance between cost and performance. Owing to the inherent complexity and low analyte concentrations of TCM samples, the development of an efficient method for sample pretreatment is necessary. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are common methods for analyte extraction and purification from medicinal plants. SPE has become more popular for sample preparation because it exhibits high-efficiency and consumes less organic solvent [14,15]. However, traditional SPE sorbents such as C_{18} lack selectivity. Molecularly imprinted polymers have achieved good selectivity, but often suffer from template leakage [16,17]. The design of a novel adsorbent which can specifically recognize AA I is urgently needed.

Recently, carbon coils have attracted significant attention because of their interesting helical morphology, super-elasticity,

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electromagnetic properties, hydrogen absorption, and thermal stability [18–20]. Due to these outstanding properties, carbon coils are regarded as potential materials for super-capacitors [21], wave absorbers [22], and microsensors [23]. However, carbon coils are rarely used as carriers for the recognition and extraction of analytes. Based on their unique structure and characteristics, carbon coils represent a promising carrier material, and the functionalization of carbon coils offers a strategy to obtain superior adsorbent. Liu et al. reported a method to fabricate single-helix carbon microcoils using *Cucurbita pepo L.* as a carbon precursor [24]. Their study provided a simple and versatile method to prepare carbon microcoils from bio-mass.

Chitosan, deacetylated chitin, consisting of glucosamine and *N*-acetyl- glucosamine units with the former usually exceeding 80%, is a biologically renewable, biodegradable, biocompatible, and nontoxic material [25,26]. The abundant amino and acetamino groups in chitosan imbue unique physico-chemical properties. For instance, chitosan as a primary aliphatic amine can be easily protonated and exist in its cationic form in dilute acid solution [27], indicating chitosan could bind with organic acids and form a complex through electrostatic interaction. This electrostatic interaction can be controlled by pH to achieve selective adsorption and desorption.Thus, chitosan was chosen to prepare a pH-controllable material for the selective extraction of organic acids.

Herein, we fabricated a selective adsorbent by functionalizing carbon microcoils (CMCs) with chitosan, and the material served as an SPE adsorbent to extract AA I from TCMs. According the structure of phenanthrene in AA I, the CMCs were expected to interact with AA I through π-π interactions. Because of the carboxyl group in AA I, chitosan was used to enhance the adsorption capacity and selectivity of CMCs because of its abundant amino groups. CMCs coated with chitosan (CMCs@CS) were analyzed by scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, elemental analysis, X-ray photoelectron spectroscopy (XPS), and thermogravimetric analyses (TGA). Parameters affecting adsorption efficiency such as water content, pH, and ionic strength were also investigated. Adsorption isotherms and kinetics were studied to elucidate the adsorption mechanism. Finally, specific analysis of AA I in *Aristolochiaceae* herbs was performed by HPLC combined with carbon microcoils solid phase extraction for sample pretreatment.

2. Experimental

2.1. Materials and reagents

Aristolochic acid I was purchased from Chengdu Herbpurify Co., Ltd (Chengdu, China). Chitosan (MW = 600 000 g mol^{-1}) with a 90% degree of deacetylation was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Glutaraldehyde (50% aqueous solution) was purchased from Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). HPLC-grade acetonitrile and methanol were purchased from Tianjin Kemiou Chemical Reagent Co. (Tianjin, China). Absolute acetic acid, ammonia solution (25%) and other chemicals were of analytical grade and provided by Tian in Fuyu Fine Chemical Co. Ltd (Tianjin, China). Ultrapure water was prepared using a Molement 1805b purification system (Shanghai, China).

2.2. Preparation of CMCs@CS

CMCs were synthesized using a previously reported procedure [24], and CMCs were modified as follows. Fist, 200 mg of chitosan powder was dissolved in 100 mL of acetic acid solution (0.1%) and mixed with the CMCs (200 mg). Subsequently, the mixture was sonicated for 30 min to obtain a uniform dispersion of CMCs/chitosan, and stirred for 2 h at 25 °C for preassembly. Dilute ammonium hydroxide was then added drop-wise to the pre-assembled solution to adjust the pH to 8.0. Subsequently, glutaraldehyde (50%, 10 μL) as a crosslinking agent was added and the mixture was stirred for 3 h. The obtained products were washed several times with water until the pH was neutral and dried at 50 °C.

2.3. Characterization of the CMCs@CS

SEM images were obtained using a TM-1000 scanning microscope (Hitachi, Japan). FT-IR spectra (400–4000 cm^{-1}) were recorded on a FTIR-8400S spectrometer (Shimadzu, Japan). Thermogravimetric analyses were performed on an SDT Q600 thermogravimetric analyzer (New Castle, USA) with ramp of 10 °C min^{-1} from room temperature to 800 °C. Elemental analyses (C, H, N) were performed using an ElementarVario EL III microanalyzer. X-ray photoelectron spectra were obtained using an Axis Ultra X-ray photoelectron spectrometer (Kratos Analytical Ltd).

2.4. Adsorption test

To measure the binding properties of the CMCs@CS towards aristolochic acid I, 5 mg of adsorbent was mixed with 5 mL of various working solution, and the mixture was shaken at 150 rpm and 25 °C. The concentrations of aristolochic acid I before and after absorption by the sorbent were determined by HPLC. The adsorption capacity Q (mg g^{-1}) was calculated according to the following equation:

$$Q = \frac{(C_0 - C_f)V}{m} \quad (1)$$

Where C_0 (mg L^{-1}) and C_f (mg L^{-1}) are the initial and final concentrations of AA I in solution, respectively, V (L) is the total volume of the solution, and m (mg) is the mass of CMCs@CS.

2.4.1. Optimization of adsorption conditions

To optimize the adsorption conditions and determine the binding mechanism, the effects of different water contents, pH, and ionic strengths on adsorption performance were investigated. AA I solutions (187.5 mg L^{-1}) were prepared with methanol and diluted to 150 mg L^{-1} with various solvents. First, methanol-water solutions in different proportions were used, and the final water contents of the working solution ranged from 0 to 20% due to the solubility of aristolochic acid I. The initial pH of the aqueous phase was adjusted (pH values of 1.03, 2.03, 3.04, 6.67, 8.94, and 10.77) by adding HCl or NaOH, and the ionic strength was adjusted by varying the concentration of NaCl from 0 to 40 mmol L^{-1}. These working solutions mixed with adsorbents were shaken for 30 min prior to analysis.

2.4.2. Adsorption isotherms

To accurately describe the interaction between analytes and absorbent, adsorption isotherms were obtained. Various working solutions of AA I were prepared at concentrations of 5, 10, 25, 50, 100, and 150 mg L^{-1} with 80% methanol, and shaken for 2 h after mixing with adsorbents. The adsorption process was characterized by Langmuir and Freundlich isotherm models as follows.

Langmuir equation:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{K_l Q_m} \quad (2)$$

Freundlich equation:

$$\ln Q_e = \ln K_f + \frac{1}{n}\ln C_e \quad (3)$$

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