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Zinc oxide crystal whiskers as a novel sorbent for solid-phase extraction of flavonoids

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ABSTRACT

As a novel solid-phase extraction material, zinc oxide crystal whiskers were used to extract flavonoid compounds and showed good extraction abilities. X-ray diffraction, scanning electron microscopy with energy dispersive X-ray spectroscopy and surface area/pore volume characterized the sorbent. The zinc oxide was packed into a solid-phase extraction micro-column and its extraction ability was evaluated by four model flavonoid compounds. The sample loading and elution parameters were optimized and the zinc oxide based analytical method for flavonoids was established. It showed that the method has wide linearities from 1 to 150 μg/L and low limits of detection at 0.25 μg/L. The relative standard deviations of a single column repeatability and column to column reproducibility were less than 6.8% and 10.6%. Several real samples were analyzed by the established method and satisfactory results were obtained. The interactions between flavonoids and zinc oxide were calculated and proved to be from the Van der Waals' forces between the 4p and 5d orbitals from zinc atom and the neighboring π orbitals from flavonoid phenyl groups. Moreover, the zinc oxide crystal whiskers showed good stability and could be reused more than 50 times under the operation conditions. This work proves that the zinc oxide crystal whiskers are a good candidate for flavonoids enrichment.

1. Introduction

As a powerful sample pretreatment technique, solid-phase extraction (SPE) has gained more and more attentions due to its advantages such as low organic solvent consumption, high enrichment factor and simple operation. The SPE sorbents such as silica bonded C18, polymeric sorbents and polyurethane foam have been developed and used in many fields to solve practical problems [1–3]. Along with the development of material science in recent years, graphene [4,5], metal-organic frameworks [6,7], restricted access materials [8,9] and many other new materials have been investigated as the SPE sorbents and showed good extraction abilities.

Flavonoids are a kind of natural polyphenol compounds and widely exist in botanic tissues. They have shown many biological and pharmacological activities such as antioxidant, anti-inflammatory, anti-carcinogenic and anti-aging effects [10–12]. Due to the excellent activities, flavonoids are often used in the production of health foods and clinical medicines [13,14]. The contents of flavonoids are an important index to evaluate the qualities of the related flavonoid products and it is necessary to establish suitable analytical methods to detect the flavonoids in real samples. However, in many cases, the concentrations of flavonoids are very low and the matrices are complex as well [15], so, suitable SPE technique is required to assure the detection accurate and efficient. The extraction materials such as carbon nanotubes [16,17], molecularly imprinted polymers [18,19], mesoporous molecular sieves and so on [20] have been researched as the flavonoids SPE sorbents and showed good extraction abilities.

Zinc oxide (ZnO) is a promising material due to its wide bandgap, large exciton binding energy, specific electrical property, excellent thermal stability and etc. [21]. It has been researched in a wide range of high technology applications such as photodetector, light-emitting diode, gas sensor, solar cell, optical modulator waveguide, surface acoustic wave device and so on [22–26]. Recently, ZnO was also investigated in the field of solid-phase microextraction (SPME). For examples, Wang et al. fabricated the ZnO nanorods on a fused silica fiber and the prepared ZnO SPME fiber was used to extract volatile organic

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compounds (VOCs) including BTEX and 1-propanethiol by the headspace mode [27]; Alizadeh et al. also prepared the ZnO nanorods coated fused silica SPME fiber and extracted 1, 4-dichloro-nitrobenzene, biphenyl and acenaphthene in environmental water [28]; Ji et al. prepared the ZnO nanorods coated SPME fiber on a stainless steel wire and extracted aldehydes in instant noodle samples [29]. Whether ZnO has good extraction abilities on the more polar and water soluble flavonoid compounds is a scientific question that is necessary to explore. In this work, the ZnO crystal whiskers were packed into the SPE micro-column and its extraction abilities for flavonoids were studied. The results prove that the ZnO crystal whiskers are a good sorbent for extracting the flavonoid compounds.

2. Experimental

2.1. Chemicals and materials

ZnO crystal whiskers were bought from Chengdu Crystrealm Co., Ltd. (Sichuan, China). Phosphoric acid (H_3PO_4, ≥85%) and acetic acid (HAc) were purchased from Baiyin Chemical Reagent Factory (Gansu, China) and Tianjin Rionlon Pharmaceutical Science and Technology Development Co., Ltd. (Tianjin, China) respectively. Methanol was obtained from Shandong Yuwang Group (Shandong, China). Quercetin (QUE), luteolin (LUT), isorhamnetin (IRA) and kaempferol (KAE) were purchased from Energy Chemical (Shanghai, China) and their structures are shown in Fig. S1. All the above reagents were of analytical purity. Water was double-distilled. Real samples including green grape juice, tea π juice, seabuckthorn beverage and dark plum soup were purchased from the local supermarkets (Lanzhou, China). SPE cartridges (3 mL) and polyethylene sieve plates (5 μm pore size) were bought from Shenzhen Biocomma Biotech Corp. (Guangdong, China).

2.2. Instruments

The solid-phase extraction process was performed on a HOGON numerical control solid-phase extraction system (Hegong Scientific Instrument Corp., Shanghai, China). Agilent 1100 Series HPLC system with a DAD detector (Agilent Technologies, USA) was used to achieve the chromatographic separation and detection of flavonoid compounds. X-ray diffraction instrument (XRD, X'Pert PRO, Holland) and field emission scanning electron microscope (SEM) equipped with an energy dispersive X-ray spectroscopy (EDX) detector (Hitachi, Japan) were used to characterize the composition and morphology of the ZnO crystal whiskers. The BET surface area and pore volume were obtained on the nitrogen adsorption and desorption isotherms by an ASAP2010 surface analysis instrument (Micromeritics, USA).

2.3. Preparation of sample solutions

The flavonoids of QUE, LUT, IRA and KAE were dissolved in methanol with a concentration of 0.1 mg/mL respectively. The solution was used as the mother solution and stored at 4 °C in a refrigerator. Working solutions were prepared by diluting the mother solutions with distilled water.

2.4. Chromatographic conditions

The chromatographic column (250 mm × 4.6 mm I.D.) was packed by the commercial octadecylsilane bonded silica (5 μm, Fuji, Japan) in our own lab. The mobile phases of (A) and (B) were methanol and aqueous solution with 0.2% of H_3PO_4 respectively. The mobile phase composition was: 32% of A at 0–0.5 min, 62% of A at 0.51–12.5 min, 82% of A at 12.51–25 min. The □flow rate was 1.0 mL/min and the column temperature was maintained at 25 °C. 360 nm was selected as the wavelength number of the DAD detector and the injection volume was set as 20 μL.

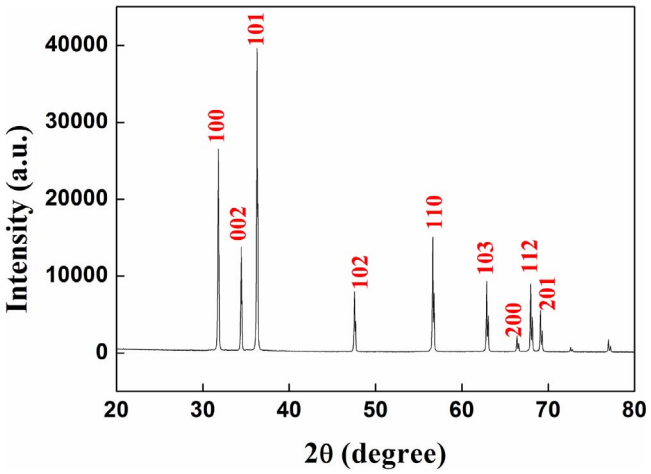

Fig. 1. XRD patterns of ZnO crystal whiskers.

2.5. Solid-phase extraction procedure

At first, 100 mg of ZnO crystal whiskers was packed into an empty SPE micro-column, then, the sorbent was flushed by 5 mL of methanol and 5 mL of water respectively. Secondly, 50 mL of the working solution with a pH value of 4.0 was loaded and the flow rate was set as 1.0 mL/min, the flavonoid compounds were captured by the ZnO sorbent. Thirdly, 1.0 mL of methanol with 1.0% of HAc was used to elute the analytes and the elution velocity was 0.3 mL/min. The flavonoids left from the ZnO sorbent and were dissolved in the eluent. At last, the desorption solution was analyzed by the HPLC-DAD. All the operations were repeated three times and the final data were their average values.

3. Results and discussion

3.1. Characterization of ZnO crystal whiskers

XRD was used to analyze the phase composition of the ZnO crystal whiskers. According to the diffraction peaks in Fig. 1, it can be deferred that the ZnO crystal whiskers has the wurtzite phase structure (JCPDS 5-664) [26]. Field emission SEM equipped with an EDX detector characterized the surface properties of the ZnO crystal whiskers. Fig. 2 shows that the ZnO crystal whiskers have the four needle-shaped structures. The needle length is about from 2 to 10 μm and the width is inhomogeneous from 0.5 μm to several micrometers. The EDX in Fig. S2 confirms that the chemical compositions are the Zn and O elements. Moreover, the BET surface area and pore volume were measured as 13.0294 m^2/g and 0.01085 cm^3/g respectively, which are very small and correspond to the smooth surface of the ZnO crystal whiskers.

3.2. Optimization of SPE parameters

3.2.1. Optimization of loading parameters (sample loading volume, sample solution pH, sample loading velocity)

Sample loading volume is an important parameter for the SPE process and it is necessary to find the maximum loading volume that reflects the maximum adsorption capacity for the extraction material. Fig. 3a gives the peak areas at different sample loading volumes. It can be seen that when the loading volumes are from 20 to 150 mL, the peak areas increase continually. When the volumes are from 150 to 250 mL, the peak areas have little change. So, it can be deferred that 150 mL was the maximum sample loading volume. According to the maximum volume, it can be calculated that the maximum adsorption capacity is (150 mL × 50 μg/L × 4 flavonoids) ÷ 100 mg ZnO = 30 μg flavonoids/100 mg ZnO = 0.3 μg flavonoids/1 mg ZnO. This result shows the ZnO sorbent has a good adsorption capacity.