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Preparation of molecularly imprinted polymers using theanine as dummy template and its application as SPE sorbent for the determination of eighteen amino acids in tobacco

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ABSTRACT

In this paper, a novel dummy template molecularly imprinted polymer (DMIP) based on a vinyl-SiO_2 microspheres surface for the simultaneous selective recognition and enrichment of 18 amino acids was prepared via a surface molecular imprinting technique using theanine as a dummy template. Compared to the imprinted polymers prepared using traditional polymerization techniques, the obtained DMIPs exhibited a regular spherical shape and were relatively monodisperse. The maximal sorption capacity (Q_{max}) of the resulting DMIPs for the 18 amino acids was up to 1444.3 mg g^{-1}. A kinetic binding study showed that the sorption capacity reached 85.40% of Q_{max} in 25 min and sorption equilibrium at 30 min. The imprint factors of the sorbents ranged from 2.86 to 6.9 for the 18 amino acids, which indicated that the DMIP sorbents have high selectivity. An HPLC-UV method for the simultaneous determination of 18 amino acids in tobacco and tobacco smoke was developed using the DMIPs as sorbents for solid phase extraction (SPE) in the sample pretreatment procedure. Under the optimum experimental conditions, the materials had enrichment factors of up to 200 for the amino acids, and the recoveries of the 18 amino acids in tobacco smoke were in the range from 79% to 104% with relative standard deviations of less than 7.4%. It indicated that the obtained DMIP sorbents could specifically recognize the amino acids from complicated samples.

1. Introduction

At present, in various types of tobacco (flue-cured tobacco, burley tobacco, Maryland tobacco, etc.) and tobacco smoke, 117 amino acids in tobacco and 36 in both tobacco and tobacco smoke have been identified [1]. Amino acid is one of the most important compound in tobacco and tobacco smoke, which has important influence on the quality of tobacco and has received widespread attention [2,3]. In tobacco curing, fermentation, processing and combustion, non-enzymatic browning reactions occur between free amino acids and reducing sugars to produce varieties with the roasted popcorn smell characteristics of pyran, pyrazine, pyrrole, pyridine, etc. heterocyclic compounds. Some amino acids, such as phenylalanine, also can degrade into relish compounds, such as benzyl alcohol and phenethyl alcohol, benzaldehyde and phenylacetaldehyde, benzoic acid and phenyl acetic acid, etc. The carcinogens of cigarette smoke resulting from amino acid nitrosation are also noticeable, for the reason that cigarette smoking leads to the formation of carcinogenic N-nitrosamines, and there is a significant positive correlation between content of amino acids in tobacco and the release amount of N-nitrosamine [4]. Therefore, accurate determination of the free amino acid contents in tobacco and tobacco smoke has a vital significance.

The methods for the determination of amino acids in tobacco mainly include paper chromatography [5], thin layer chromatography [6], gas chromatography [7], high-performance liquid chromatography (HPLC) [8], ion exchange chromatography [9], capillary electrophoresis [10], etc. Among them, the HPLC method is the most classical and commonly used method. But as far as we know, the tobacco smoke and tobacco is a complex mixture of compounds, have identified the chemicals in the smoke reached 9582 kinds [1], including carbohydrates, fatty hydrocarbons, aromatic hydrocarbons, terpenoids, alcohols carbonyl compounds, phenols, esters, organic acids (including a small amount of free amino acids), heterocyclic nitrogen compounds, metallic elements, tobacco smoke (nitrogen, oxygen, carbon dioxide, carbon

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monoxide, hydrogen and other accounts for 85% of the total flue gas composition), volatile hydrocarbons, volatile esters, furan, volatile nitrile and other volatile components, etc. Therefore, it is needed to extract and enrich the amino acids in the tobacco and tobacco smoke before derivatization and HPLC. There are many methods are used to separate amino acids including precipitation [11], ion exchange [3], electromembrane extraction [12], liquid–liquid extraction [13,14], solid phase extraction [15] and electrodialysis [16], etc. In view of solid-phase extraction (SPE) has the characteristics of time-saving, separation and enrichment of analytes so that it becomes the key step in the analysis of trace compounds in samples with complex matrices. For purification of the analytes, the properties of sorbents were a key factor, while the classical sorbents ordinarily have low selectivity and low adsorption capacity. Thus, preparation of new sorbents with high selectivity, such as molecularly imprinted polymers (MIPs) has attracted the attention of research community in recent years.

In 1972, the German scientist Wulff [17] successfully synthesized amino acids and their derivatives using a highly selective carbohydrate covalently imprinted polymer, which resulted in intense interest in this technology by academics. Then, molecularly imprinted polymers of amino acids were prepared using various methods: precipitation polymerization, bulk polymerization, suspension polymerization, surface polymerization, multistep swelling suspension polymerization, magnetic polymer surface polymerization and sol–gel polymerization [18–27]. Although numerous studies were conducted to develop an effective molecular imprinting technology, the main challenges to their successful and widespread use are the tedious synthetic steps, template leakage, low binding capacity, and slow mass transfer of MIPs [28].

To overcome these deficiencies, the strategy of surface molecular imprinted polymers using dummy templates has been applied to prepare sorbents [29–36]. The molecularly imprinted polymer films were grafted on the surfaces of solid supports, such as silica gel particles, magnetite nanoparticles, quantum dots, or carbon nanotubes, to increase the accessibility of analytes to the recognizing cavities and the rate of mass transfer. Among these support particles, silica shows promising characteristics because it is a non-swelling inorganic material, stable under acidic conditions, and has high thermal resistance. Surface imprinting using silica as the support has been applied to imprint pharmaceuticals [37,38] and biological macromolecules [25,39–47]. The structural analogues of the target molecules, namely the dummy templates, can avoid the leakage of the template of the analyte [30–48]. To guarantee the selectivity and capacity of the sorbents, the selection of the dummy template was a key factor, while the available dummy template molecules are limited, especially for the simultaneous determination of homolog compounds. Therefore, the design and synthesis of the dummy template molecule is required. Unfortunately, the dummy template surface imprinting technology used to prepare amino acid MIPs has rarely been reported.

In this study, a highly binding dummy template surface of molecularly imprinted polymers (DMIPs) was synthesized on the vinyl-SiO_2 microspheres surface using theanine as the dummy template. The polymers were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The adsorption performance and selectivity of DMIPs has been systematically investigated. The novel imprinted polymer was evaluated and applied as a sorbent material for SPE combined with HPLC (DMIPs-SPE-HPLC) to simultaneously detect eighteen amino acids in tobacco and tobacco smoke. The obtained sorbent has high selectivity and sufficient capacity for the separation and enrichment of the amino acids from tobacco and tobacco smoke. The developed DMIPs-SPE-HPLC protocol significantly improved the sensitivity by loading a large volume of sample and eliminated the effect of template leakage on quantitative analysis.

2. Experimental

2.1. Materials and chemicals

L-Theanine (The, purity: 99.5%) was purchased in Sichuan Tongsheng Amino Acid Co., Ltd. China. L-Glycine (Gly, 99.5–100.5%) and L-Alanine (Ala, 99%) were purchased form Shanghai Xun Lang Industrial Co., Ltd (China). L-Histidine (His, 99%), $L_{(+)}$-Glutamic (Glu, 99%) and L-Phenylalanine (Phe, 99%) were purchased from Aladdin reagent Co. (China). L-Aspartic acid (Asp, 99.6%), L-Serine (Ser, 99%), $L_{(+)}$-Arginine (Arg, 99%), L-Threonine (Thr, 99%), L-Tyrosine (Tyr, 99%), L-Valine (Val, 99%), L-Proline (Pro, 99%), L-Methionine (Met, 99%), L-Cysteine (Cys, 99%), L-Isoleucine (Ile, 99%), L-leucine (Leu, 99%), L-Tryptophane (Trp, 99%), L-Lysine (Lys, 99%), were purchased from Beijing century biotechnology Co., Ltd. Bioko. Ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%) was purified by distillation under vacuum. Azobisisobutyronitrile (AIBN, chemical grade) was purchased from Shanghai No. 4 Reagent and H. V. Chemical Company (China) and purified through recrystallization in ethanol before use. Vinyl-trimethoxysilane (VTMS) was purchased from Nanjing Union Silicon Chemical Co. (China). Acetonitrile (purity $\geq$ 99.9%) of CHROMASOLV® gradient grade for HPLC was purchased from Sigma-Aldrich Co. Ammonium hydroxide (28%), methacrylate acid (MAA, purity $\geq$ 98%), toluene, acetic acid, sodium acetate anhydrous, phenyl isothiocyanate, triethylamine, and *n*-hexane were purchased from Shanghai Chemical Reagent Co. (China). Doubly distilled water, which was used throughout the experimental processes, was obtained from a laboratory purification system.

2.2. Instruments and operational parameters

FTIR spectra were recorded on a Tensor-27 FTIR spectrometer (Bruker, Germany) with a resolution of 2 cm^{-1} and a spectral range of 4000–400 cm^{-1}. The morphologies and structures of theanine-imprinted silica microspheres were observed using a SIRION200 SEM (FEI, Holland) and a JEM-2011 TEM (JEOL, Japan) with measurements at 5 and 200 kV, respectively. The amount of amino acids was analyzed using high-performance liquid chromatography (HPLC) system (Shimadzu, Japan). The HPLC system was composed of a LC-15C pump, an SIL-10AF injector with a 50-μL loop, and an SPD-15C dual-wavelength absorbance detector.

2.3. Procedure to prepare dummy template surfaces of molecularly imprinted polymers

Vinyl-SiO_2 microspheres were synthesized according to the literature-reported approach with some modifications [49]. VTMS (2.1 mL) was added to 50 mL of doubly distilled water under vigorous stirring until an emulsion formed. After 3 h, $NH_3 \cdot H_2O$ (1 mL) was added to the emulsion, and the mixture was continuously stirred at 25 °C for 2 h. The resulting microspheres were separated from the reaction medium by centrifugation and washed with anhydrous ethanol and doubly distilled water several times. Finally, they were dried at 60 °C for 12 h.

To prepare dummy template surface-imprinted polymers (Fig. 1.), vinyl-SiO_2 microspheres (0.5 g) were dispersed in 20 mL of toluene using an ultrasonic bath. MAA (0.3476 g, 4 mmol), EGDMA (1.132 mL, 5.5 mmol), theanine (0.1740 g, 1 mmol), doubly distilled water (15 mL) and AIBN (0.08 g) were subsequently dissolved into this solution. The mixing solution was purged with