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Detection of theophylline using molecularly imprinted mesoporous silica spheres

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1-Dodecanethiol (PubChem CID: 8195)
Tetraethoxysilane (PubChem CID: 6517)
Phenyltriethoxysilane (PubChem CID: 13075)
Cetyltrimethylammonium bromide (PubChem CID: 5974)
Sodium hydroxide (PubChem CID: 14798)
Acetic acid (PubChem CID: 176)
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ABSTRACT

Here, we report a three-dimensional (3D) network molecularly imprinted polymer (MIP) on electrode surface to achieve an efficient and specific detection of theophylline in foodstuffs, using theophylline as the template molecule, mesoporous silica nanospheres (MSNs) as the signal transducer to shuttle electrons, and both phenyltriethoxysilane and pyrrole as functional monomers. The electron microscope images reveal the presence of well-distributed hierarchically MSNs with a pomegranate-like morphology, topped with MIP uniform layer. Electrochemical characterizations were carried out to monitor the properties of the resulting sensing platform based on the MIP/gate effect employing hexacyanoferrate molecules as the electrochemical probe. The data show that due to the high conductivity and electron transfer ability of the prepared theophylline-imprinted membrane, this method exhibits excellent sensitivity and binding affinity with a linear dynamic concentration range in excess of six orders of magnitude and low detection limit (0.66 nM), meeting the requirements of theophylline trace analysis.

1. Introduction

In last several years, there has been a growing interest in natural phytotoxins, owing to their presence as contaminants in feed and food which bring about great potential hazard for animal and human health (Egan et al., 2016). Nevertheless, the occurrence of phytotoxins at subchronic levels is probably underestimated. Alkaloids are a class of attractive secondary plant metabolites serving as cornerstone of the modern pharmaceutical industry due to their broad spectrum of biological activities (Liu, Li, & Guo, 2017). Though alkaloids exhibit impressive pharmacological activation, the major hurdle in their clinical application arises from the remarkable toxicity in central nervous system (Machado et al., 2018). Theophylline is one such natural alkaloid that has shown to own variety of bioactivities at low-dose, but frequent adverse reactions including anxiety, restlessness, diarrhoea, nausea and heart palpitations may occur with high serum levels (Hopkins & MacKenzie-Ross, 2016; Yaman et al., 2016). A thin line of safety exists between the therapeutic and toxic dose thereby illustrating that a reliable and sensitive method for the detection of trace levels of theophylline is required for clinical study, toxicological research and forensic analysis. Electrochemical techniques coupled with convenient procedure and portable instrumentation provides an advantage to detect theophylline. Literature survey unveils that diverse chemically modified electrodes have been explored earlier (Wang et al., 2015; Wang, Ding, Li, & Hu, 2018; Yin, Meng, Su, Xu, & Ai, 2012; Zhuang, Chen, Wang, Liu, & Chen, 2017), however, the interference from structurally related substances is inevitable. Some other research groups determined theophylline using aptamer (Chen, Guo, Tang, Shen, & Miao, 2018), antibody (Barlag et al., 2013) and DNA (Ahn et al., 2015) as electrochemical recognition element, but these methods suffered limitations such as complex process and consumption of costly reagents in each assay. In this regard, the exploration for other

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inexpensive sensor receptors which possess improved selectivity and simplified operation is urgent.

Originated from the intention to form binding cavities in bottom-up synthetic polymer structures, molecular imprinting strategy has acted as a powerful technique for preparing tailor-made materials that can bind to specific molecules selectively and reversibly in the presence of structurally related compounds (Liu, Zhu, Hu, Peng, & Du, 2017), and has provided a favorable and promising alternative to meet the need of analytical approaches with high sensitivity, good stability and applicable reliability for food safety inspection. As the new type of biomimetic receptors, molecularly imprinted polymers (MIPs) exhibit typically three-dimensional (3D) polymeric networks prepared by polymerizing selected functional monomers around a target substance (template) in presence of a cross-linking agent, which possess tailored binding cavities for selective and specific recognition of appointed substance complementary in size, shape, electrostatic environment and functional groups (Wan, Chen, Huang, & Shen, 2017). Traditional MIPs fabricated using acrylic polymers or organic acrylates by radical polymerization have the inherent problems of non-specific adsorption, template leaching and failure to realize specific binding in aqueous system. However, inorganic matrices, such as siloxane-based MIPs produced through hydrolysis and condensation of single or multiple silane monomers in acidic or basic condition using the sol-gel process (Lofgreen & Ozin, 2014), are a hopeful manner to overcome some of these drawbacks. MIPs via sol-gel process present significant advantage as they are allowed to be gelated facilely and easily at room temperature, which is especially important for maintaining the weak interactions with the template molecules. Additionally, the sol-gel derived siloxane networks display remarkably higher porosity, surface area and chemical inertness, as well as negligible swelling and change upon exposure to organic solvents (Rezaei et al., 2014; Xu, Chi, Li, Liu, & Kan, 2015). But on the other hand, the sol-gel based MIPs is lacking of electrocatalytical activity and conductivity which result in low sensitivity of the sensor.

To overcome this situation, electrosynthesis of conductive MIPs-based sensors enables the in-situ generation of a highly stable, reproducible, compact and uniform molecularly imprinted polymeric film with good adherence to an electrode surface of any shape and size (Erdossy, Horvath, Yarman, Scheller, & Gyurcsanyi, 2016). Moreover, the thickness, density and morphology of the MIP layer can also be effectively controlled by the electropolymerization conditions including applied potential and the number of cyclic voltammetric cycles, etc. This characteristic gives the possibility of generating a direct communication between the electrode surface and coating in a very simple mean for the development of electrochemical sensors. With regard to the choice of polymer materials, polypyrrole (Ppy) has been known as one of the most favorable members, particularly for the development of excellent sensor devices, due to its ease of preparation, good biocompatibility, outstanding conductivity and electrochemical redox activity even in neutral solutions (Ceto et al., 2016). Moreover, Ppy can be easily imprinted with target molecules because of the ability to entrap anionic counter ions during its process of electropolymerization (Tonelli, Ballarin, Guadagnini, Mignani, & Scavetta, 2011).

In this work, combining the merits of sol-gel approach and electropolymeric Ppy, a new kind of theophylline imprinting materials using pomegranate-like mesoporous silica nanospheres (MSNs) as Supporting materials was synthesized and applied for the recognition and ultrasensitive detection of theophylline based on electrochemical technique. As far as we are aware, the usage of sol-gel, conducting polymer and mesoporous silica hybrid nanocomposite as a MIP for theophylline determination has not been reported. The resulting MSNs based molecularly imprinted electrochemical sensor not only exhibits excellent applicability in theophylline determination with broadened linear detection range, high sensitivity, and low detection limit, but also presents eminent selectivity when used in coexisting of other structurally related substances.

2. Experimental section

2.1. Reagents and materials

Sodium dodecyl sulfate (SDS, 99%), 1-dodecanethiol (C_{12}-SH, 98%), tetraethoxysilane (TEOS, 99%) and phenyltriethoxysilane (PTEOS, 99%) were obtained from Aladdin Chemistry Co. Ltd. Cetyltrimethylammonium bromide (CTAB, > 99%), sodium hydroxide (NaOH, > 99%), pyrrole (99%), acetic acid (> 99.5%) and ethanol (> 99.7%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Trifluoroacetic acid (TFA, 99%) and theophylline (98%) were supplied by Macklin Biochemical Co., Ltd. Unless otherwise specified, the reagents were used directly without purification and ultrapure water (≥18.2 MΩ) was used throughout all experiment.

2.2. Characterizations

Morphological and structural investigations were obtained by scanning electron microscopy (SEM, Hitachi S-4800, 5 kV) and transmission electron microscopy (TEM, Tecnai G220, 200 kV). The SEM images of MSNs were collected by using the solid powder, and the SEM pictures of MIP were taken on electrode surface. Chemical information about MSNs@Ppy-sol-gel-theophylline and MIP samples was conducted via Fourier transform infrared spectroscopy (FTIR, Nicolet-740). All electrochemical experiments were performed on a CHI 660E electrochemical workstation (Chenhua Instrument Co. Ltd., Shanghai, China) using a MIP modified glassy carbon electrode (GCE, ϕ = 3.0 mm) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode, respectively.

Chromatographic experiments were performed with an Agilent 1200 high performance liquid chromatography (HPLC) system and separations were achieved on an Agilent XDB-C18 column (1.8 μm, 50 mm × 4.6 mm) with column oven temperature of 40 °C (Al-Jenoobi et al., 2015). With an injection of 10 μL, the analytes were separated with mobile phase comprised of 30% methanol in water. The flow rate and UV detection wavelength were 0.8 mL min^{-1} and 280 nm, respectively.

2.3. Synthesis of MSNs

MSNs were synthesized using CTAB as the surfactant in basic C_{12}-SH aqueous solution (Chen et al., 2010). Typically, 0.2 g of CTAB was added to an emulsion composed of 0.7 mL of 2 M NaOH, 0.02 mL of C_{12}-SH and 96 mL of water under agitation. The resulting mixture was continuously agitated at 80 °C for 30 min. Subsequently, 1.5 mL of TEOS was added quickly, and stirred vigorously at the same temperature for 2 h. After the reaction, the white precipitate was filtered and washed three times with water before drying at 60 °C for 24 h. Finally, the organic and other constituents in the products were removed by calcination at 550 °C for 5 h in air.

2.4. Fabrication of MIP-based sensor

Prior to use, the GCE was pretreated to a mirror-like surface by polishing in Al_2O_3 pastes with particle sizes of 1.0 and 0.5 μm on microcloth pads, and then thoroughly rinsed with ethanol/water (1:1, *v/v*) and water, respectively.

In a typical procedure for preparing polymerization solution, MSNs (60 mg) was mixed with water (2.1 mL) and ethanol (3.3 mL) under vigorous stirring. After its complete dispersion, theophylline (27 mg) was dissolved in this mixture and maintained for 5 min. Next, the PTEOS (225 μL), TEOS (225 μL) and TFA (30 μL) were further added by constant stirring. After the resulting mixture was stirred for 2 h at room temperature, pyrrole (15 μL) and SDS (15 mg) were then placed in the solution, which was continually sonicated for 10 min. To obtain the MIP nanocomposite modified electrode, the pretreated GCE was immersed