Development and application of a quartz crystal microbalance sensor based on molecularly imprinted sol-gel polymer for rapid detection of patulin in foods

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ABSTRACT

A simple quartz crystal microbalance (QCM) sensor based on a sol-gel molecularly imprinted polymer (MIP) film as the recognition element was developed for patulin (PAT) detection. The high adsorption capacity of the proposed MIP for PAT was demonstrated by Scatchard equation analysis. Through the evaluation, based on the QCM frequency change, the developed sensor exhibited a linear concentration range of PAT from 7.5×10^{-3} $\mu g\,mL^{-1}$ to 6×10^{-2} $\mu g\,mL^{-1}$ with a lower detection limit of 3.1×10^{-3} $\mu g\,mL^{-1}$ (S/N = 3). The sensor showed good selective affinity for PAT (selectivity coefficient = 3.82) as well as good reproducibility and long-term stability. The developed sensor was tested on its ability to detect PAT in real samples through a spiking and recovery study with the recoveries ranging from 76.9% to 91.3% (RSDs < 8.4%). The good agreement results of the established MIP-QCM sensor and HPLC–MS method indicated that the developed method could accurately detect PAT in practical food samples.

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1. Introduction

Patulin (4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one, PAT) is a fungal secondary metabolite produced by a number of fungal species including *Penicillium, Aspergillus, Mucor,* and *Rhizopus* [1]. Some of these fungal species are responsible for the decay of fruits, vegetables, and grains. Therefore, PAT may exist in fruit products such as apples, pears, and hawthorns. Additionally, due to its thermal stability and acid resistance, PAT will still exist in food products after the manufacturing process [2]. As previously established, PAT exposes its toxic effects via combining with the mercapto group in proteins and amino acids due to its electrophilic ability [3] causing acute and chronic toxicity such as neurotoxicity, genotoxicity, teratogenicity, and immunotoxicity in humans, particularly in children [4]. Considering its great harm, maximum limits (MLs) of PAT have been established by the European Union (EU) as $50\,\mu g\,kg^{-1}$ in apple juice and cider, and $10\,\mu g\,kg^{-1}$ in baby food [5].

Up to now, several typical methods have been established for PAT determination, such as thin-layer chromatography (TLC) [6], high-performance liquid chromatography (HPLC) [7], and gas chromatography (GC) [8]. Piezoelectric quartz crystal microbalance (QCM) [9] is a promising candidate for PAT detection. A QCM sensor is a type of mass-sensitive chemical sensor that exhibits extreme sensitivity to the nanogram level of mass change loaded onto the surface of sensor chips [10]. The increased mass caused by the binding reaction results in a decrease of the frequency response [11,12]. Although QCM exhibits high sensitivity, the lack of selectivity limits the application of the QCM sensor [13]. Molecularly imprinted polymer (MIP) mimic the antigen-antibody binding mode by forming cavities that complement a template molecule's shape and size [14]. When the template molecule is eluted, the MIP obtain the ability to recognize the template again [15–17]. The combination of MIP and a QCM sensor may enhance the detection properties of the QCM sensor due to excellent performance qualities of MIP such as high selectivity, chemical/mechanical tolerance, low cost, ease of synthesis, and reproducibility [18]. In recent years, a few MIP-based QCM sensors have been developed to detect certain compounds, including albumin, folic acid, paraoxon and so on [19–21].

In the present research, we combined the merits of the QCM sensor with MIP to establish a highly sensitive and selective MIP-based QCM sensor for the determination of PAT. The MIP of PAT were synthesized independently by a sol-gel process and then fixed on the quartz crystal chips to develop a novel PAT-MIP QCM sensor. The developed QCM sensor was used to detect PAT in foods to

investigate the actual application ability of the prepared sensor in real samples.

2. Experimental

2.1. Instruments and reagents

The experiment data was recorded by a QCM (QCM-922, Princeton Applied Research, Princeton, NJ, USA) which was equipped with a 9 MHz AT-cut quartz crystal (Seiko EG&G Co. Ltd., Tokyo, Japan). Both sides of the quartz crystal were covered by a platinum layer (diameter 5 mm). In the process of the whole experiment, the quartz crystal was fixed in a Teflon retainer (well-type, QA-CL3, Seiko EG&G Co. Ltd., Tokyo, Japan) to ensure that only one side of the quartz crystal was in contact with the solution and that the other side was kept in the air. An ultraviolet spectrophotometer (Evolution™ 300 UV–vis, Thermo Scientific, Waltham, MA, USA) was used for evaluating the adsorption effect of the synthesized MIP. The surface morphology of the modified quartz crystal was observed by scanning electron microscopy (SEM, SU1510, Hitachi Ltd., Tokyo, Japan). HPLC–MS instrument (6410 Triple Quad LC/MS, Agilent Technologies, Santa Clara, CA, USA) was used to validate the determination results of PAT in the real samples using the PAT-MIP QCM sensor.

PAT, 2-oxindole (2-oxin), and 5-hydroxymethylfurfural (HMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-aminopropyltriethoxysilane (APTES), tetraethoxysilane (TEOS), and polyvinyl chloride (PVC, MW = 8000) were supplied by Alfa Aesar (Tianjin, China). Methyl alcohol, dichloromethane (DCM), ethyl acetate, and other chemical reagents used in the experiments were obtained from Tianjin Chemical Reagent No. 1 Plant (Tianjin, China). All of the reagents were at least analytical grade. Milli-Q purified water was used throughout the experiment. The food samples, i.e. apple juice, concentrated pear juice, and haw slices, were purchased from a local supermarket. Acetate buffer (pH = 4.5) was prepared by adding 1.8 g of sodium acetate into 0.98 mL of glacial acetic acid in a 100 mL volumetric flask. The solution was then diluted to 100 mL with deionized water.

2.2. Screening of dummy template molecules

In order to avoid the ring opening reaction of PAT, which contains a conjugated diene structure during the synthesis process, as well as the toxicity and high cost of the experiment, four structural analogues of PAT (2-oxin, 2-hydroxynicotinic acid (2-HNA), 6-hydroxynicotinic acid (6-HNA), and coumalic acid (CA)) were chosen as dummy template molecules, and then Hyperchem 8.0 software was used to select the most suitable one for template replacement.

2.3. Preparation of PAT-MIP using a dummy template

A novel type of molecular imprinted polymer (MIP) for PAT detection was prepared through a sol-gel molecularly imprinted process. In summary, 0.133 g of 2-oxin, 5 mL of methyl alcohol, and 936 μL of APTES were mixed in a 25-mL round-bottomed flask and stirred constantly. After 30 min, 1115 μL of TEOS was added into the flask, and the mixture was stirred for 50 min. Finally, 1 mL of 0.1 mol L^{-1} NH$_3$·H$_2$O was added, and the mixture was stirred for another 10 min. The mixture was incubated in a water bath at 60 °C for 10 h. The product was filtered and then aged in a vacuum oven at 60 °C for 10 h. After being soaked for 3 h in a solution contained 1.0 mol L^{-1} HCl and methanol (v:v = 1:4, 100 mL), the solid product was flushed with a copious amount of deionized water by vacuum filtration until the pH of the eluent reached neutral (pH 7). At the end of synthesis, the product was washed with methyl alcohol and

acetic acid (9:1, v:v, 300 mL) by Soxhlet extraction until no template was detected by an ultraviolet spectrophotometer. After that, the product was dried in a vacuum oven at 60 °C. As a comparison, a non-imprinted polymer (NIP) was prepared through the same process without the addition of a dummy template molecule. Fig. 1 shows the schematic representation of the PAT-MIP fabrication process.

2.4. Adsorption study of PAT-MIP

The drawing method of the adsorption isotherm curve was conducted as follows: PAT-MIP or PAT-NIP (5.0 mg) was mixed with acetate buffer (1.0 mL, pH = 4.5) containing different concentrations of PAT (10, 20, 40, 60, 80, 100, and 120 μg mL^{-1}) in centrifuge tubes. The tubes were shaken for 3 h and were then centrifuged at 5000 rpm for 15 min. The amount of unbound PAT in each supernatant was detected by the ultraviolet spectrophotometer at 276 nm. The adsorption capacity of PAT-MIP (Q, μg mg^{-1}) was calculated by Eq. (1) as follows:

$$Q = (C_0 - K \times C_f) \times V/W, \tag{1}$$

where C_0 is the initial concentration of analyte in solution (μg mL^{-1}), C_f is the final concentration of analyte after adsorption and dilution (μg mL^{-1}), K is the dilution ratio, W is the amount of polymer (mg), and V is the volume of solution (L).

2.5. Preparation of PAT-MIP-based QCM sensor chips

Before modification, the quartz crystal electrode was ultrasonically washed with ethanol and then cleaned using "piranha" solution (30% H$_2$O$_2$:98% H$_2$SO$_4$, 1:3, v/v) for 5 min. After being rinsed with acetone, ethanol, and water, the electrode was dried with nitrogen. PAT-MIP or PAT-NIP (5 mg) was dispersed in PVC-DCM (1 mg mL^{-1}) solution. After ultrasonication for 10 min, a homogeneous solution was obtained. Then, the Pt electrode layer was coated with 10.0 μL of the above resulting suspension solution, allowed to dry at room temperature, and fixed in the Teflon retainer before use.

2.6. Measurement experiments of the MIP-QCM sensor

The assembled quartz crystal resonator was connected to the QCM sensor, and the frequency response value was measured at different concentrations of PAT standard solution (0.1, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8 × 10^{-2} μg mL^{-1}) at room temperature. The operation was as follows: (1) Zero: The sensor was opened, and 50 μL of blank solution was added to the detection well. When the resonant frequency was stabilized, record the frequency value as f_0 (frequency change less than 1.0 Hz could be regarded as the stable sensor frequency); (2) Adsorption: the blank solution was extracted, and 50 μL of the test solution was added. When the frequency response value was stable again, a frequency change caused by analyte binding was obtained and recorded; (3) Elution: the test solution was extracted, and 50 μL of HCl (1.0 mol L^{-1}) was added to the detection well. After oscillation occurred for 15 min, the electrode was washed with a sufficient amount of water until the frequency response recovered to near f_0 for the removal of the bound analyte. The experiment was repeated three times for each concentration, and then the data were recorded to observe the relationship between the analyte concentration and the frequency change value of the sensor. For comparison, the NIP-based QCM sensor was also tested using the same procedure. The frequency response of the electrode that coated PVC was also measured to exclude the possible physical interference between PVC and the analyte.