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Development of molecularly imprinted polymers-surface-enhanced Raman spectroscopy/colorimetric dual sensor for determination of chlorpyrifos in apple juice

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ABSTRACT

Chlorpyrifos (CPF), an organophosphate insecticide, is widely identified in fruit juices. In this study, a novel molecularly imprinted polymers-surface-enhanced Raman spectroscopy (MIPs-SERS)/colorimetric dual sensor was developed to determinate CPF in apple juice. MIPs were synthesized using bulk polymerization to rapidly and selectively adsorb and separate CPF from apple juice. A colorimetric method was developed based upon color changes of synthesized silver nanoparticles (AgNPs) by interacting with CPF, while SERS spectra were directly collected by illuminating the aggregated AgNPs with Raman laser. Colorimetric method rapidly screened and semi-quantified CPF in apple juice $\geq$5 mg L^{-1} by naked eye, or accurately quantified CPF in apple juice ranging 0.1–10 mg L^{-1} by UV–vis spectroscopy. Principle component analysis and partial least-squares regression models (RMSEC = 0.0453, R^2-C = 0.9885) validated using SERS to further quantify CPF in apple juice at extremely low concentration (0.01 mg L^{-1}). This MIPs-SERS/colorimetric dual sensor can rapidly (<25 min), accurately, and cost-efficiently determine CPF in apple juice and meet the worldwide regulation of maximum residue limit.

1. Introduction

Chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate, CPF) is one of the most widely used organophosphate pesticides that can effectively inhibit acetylcholinesterase and block the signals travel between nerve cells. As a result, it causes the malfunction of central nervous system of the pests and eventually kills the pests [1]. CPF is commonly used in agriculture crops (*e.g.*, corn, soybeans, fruits, *etc.*). According to the United States Environmental Protection Agency (EPA), CPF remains the most commonly used organophosphate pesticide for crops in the United States with an estimated annual consumption of 5–10 million pounds to be applied to over 50% apple crops during 2006–2012 [2]. CPF is moderately toxic to humans and considered as a neurotoxin and endocrine disruptor, and it is especially harmful to pregnant women and infants [1,3]. A recent study also reported an association between human exposure to CPF and the incidence of lung cancer [4]. Considering its toxicity and enormous application in agriculture, effective detection of CPF residues in agri-food products is highly demanding. Traditional detection methods are based upon high cost instruments, such as high performance liquid chromatography coupled with photodiode array detector (HPLC-DAD) [5], liquid chromatography-mass spectrometry (LC–MS) [6], and gas chromatography-mass spectrometry (GC–MS) [7]. However, all these methods are laborious, time-consuming, and require large amount of organic reagents. Therefore, novel, simple, low-cost, and accurate analytical methods are highly demanded.

Silver nanoparticles exhibit intense colors due to the surface plasmon resonance (SPR) phenomenon [8,9]. Under the irradiation of electromagnetic waves, the SPR effect will produce characteristic absorption peak, which depends upon the size and shape of the particles and the dielectric properties of surrounding medium and inter-particle distances [10]. Many factors can promote the aggregation of nanoparticles and cause SPR band shift to a longer wavelength [11]. The interparticle distance changes and subsequently results in quantifiable red-shift or blue-shift of the UV–vis absorption, which is the basic principle of colorimetric sensing system [12]. Due to the rapid, sensitive, and easy-operational features, colorimetric-based detection methods have been extensively investigated as different colorimeter sensors to analyze various chemical and biological molecules [13–16]. However, the poor selectivity of colorimetric sensor has limited its application in complex sample matrices (*e.g.*, foods) because numerous interferents may cause the aggregation of nanoparticles. Many studies were

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conducted to improve the selectivity for AgNPs-based colorimetric sensors, such as the addition of different stabilizers. For example, Su and colleagues used folic acid functionalized silver nanoparticle-based colorimetric sensor to detect Hg^{2+} in a sensitive and selective manner [17]. In another study, Xiong and others modified AgNPs with calixarene to specifically detect pesticides in water [18]. However, the synthesis of these AgNPs was complicated and may affect the sensitivity of the colorimetric sensors because the stabilizers could also prohibit the aggregation of AgNPs. Therefore, cleaning up sample matrices and analyte enrichment are required before the analysis.

Surface-enhanced Raman spectroscopy (SERS) has been investigated as a rapid and reliable method for the determination of trace level chemical compounds [19–22] and microbiological agents [23,24] in agricultural and food products. When the analyte molecules are in the proximity to the surface of the noble metallic (*e.g.*, gold, silver) nanostructures, the localized SPR of the nanostructures will significantly enhance the faint Raman scattering signals derived from low concentrations of analyte molecules [25]. The enhancement factor of high quality SERS-active substrates for Raman active molecules (*e.g.*, pyridine) can reach to 10^{14}, corresponding to the detection of single molecules [26,27]. Although SERS is an ideal tool for the detection of trace level analytes, it suffers from the interference of sample matrix components whose SERS signals may overlap with the signals of analytes, resulting in the challenges in spectral deconvolution and reduced detection sensitivity and accuracy. Consequently, effective removal of sample matrix components is necessary before SERS spectral collection.

Extensive sample pre-treatments, such as extraction by organic solvents and centrifugation, are greatly involved before implementing the aforementioned colorimetric and SERS sensing techniques. However, these methods lack the selectivity and thus are not effective to fully eliminate the interferents. New approaches such as antibody-based [28] and aptamer-based [29] separation methods have been successfully developed to isolate and/or enrich chemical hazards from complicated food matrices and enable more sensitive and accurate detection using SERS. Nevertheless, high cost and laborious manufacture of antibody and aptamer for specific analytes significantly limit the application of these two methods in routine detection of chemical residues in foods. Recently, molecularly imprinted polymers (MIPs)-based SERS sensors were constructed to detect and quantify melamine [21], histamine [30], α-tocopherol [20], and Sudan I [31] in different food products. MIPs are rigid polymers developed by chemical synthesis with high affinity towards target analyte. Thus, MIPs are resistant to harsh environment and more suitable to be used as separation element for sensing systems compared to antibody and aptamer [32].

Recently, two different colorimetric-SERS dual sensors were developed to determinate mercuric ions in water samples for environmental analysis [10,12]. However, the authors failed to determine heavy metal pollution in complicated food matrices. In another study, Lang and coauthors constructed a gold nanoparticle-based colorimetric-SERS dual sensor to detect melamine in milk. However, the selectivity of this sensor was poor and the sensitivity did not meet the regulation of maximum residue limit [33]. The current study aims to develop a MIPs-SERS/colorimetric dual sensor for the determination of CPF in apple juice. MIPs are synthesized to selectively separate CPF from apple juice while AgNPs are used both as color indicator and SERS-active substrate. The colorimetric sensor is developed for initial rapid high-throughput screening and semi-quantification of CPF in apple juice, followed by SERS to further accurately quantify trace level contamination. To the best of our knowledge, this was the first study to integrate colorimetric and SERS as a single sensing system for rapid and accurate detection and quantification of trace level chemical hazards in agri-food products.

2. Experimental

2.1. Chemicals and materials

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobis(isobutyronitrile) (AIBN), chlorpyrifos (CPF), silver nitrite, sodium borohydride, hexane, ethanol, methanol, acetonitrile, acetic acid, and toluene were purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the chemical agents are of at least reagent grade. MAA and EGDMA were purified by reduced pressure distillation before use. Cellulose nitrate (CN) membrane was purchased from Sartorius Stedim Biotech S.A. (Göttingen, Germany). Apple juice samples were obtained from local grocery stores in Vancouver, Canada.

2.2. Synthesis of MIPs

The synthesis of CPF-MIPs was conducted according to the following procedure: 1 mmol of CPF was dissolved in 5 mL of toluene and mixed with 6 mmol of MAA. The mixture was stirred at room temperature for 30 min, followed by addition of 25 mmol of cross-linking agent EGDMA and 0.05 g of initiator AIBN into the mixture. The mixture was then purged with nitrogen for 10 min at room temperature, sealed, and thermal polymerized in water bath at 40 °C for 2 h, followed by adjusting the temperature to 60 °C for another 16 h. After the polymerization, the rigid polymer was crushed and passed through a 200 mesh steel sieve. Then, the template was removed by Soxhlet extraction with 200 mL of methanol/acetic acid (9:1, v/v) for 24 h, followed by extraction with 200 mL of pure methanol for another 24 h. After examining with a UV–vis spectrometer at 286 nm to validate the complete removal of CPF, the synthesized MIPs were dried overnight in a vacuum drying oven at 60 °C. In comparison, non-imprinted polymers (NIPs) were also prepared following the same procedure in the absence of CPF.

2.3. Adsorption test

The static and kinetic adsorption tests were conducted individually to determine the adsorption capacity and binding equilibrium rate of MIPs and NIPs. For static adsorption test, 15 mg of MIPs were mixed with 3 mL of CPF methanol solutions at different concentrations (*i.e.*, 5, 10, 20, 30, 40, 60, 80, and 100 mg L^{-1}). The mixtures were incubated at room temperature with shaking for 2 h, followed by centrifugation at 7500 × *g* for 10 min. The absorbance of supernatants was measured by UV–vis spectrometry at 286 nm for unbound CPF. This same procedure was also applied to test the static adsorption capacity of NIPs. The adsorption capacity was calculated using the method as reported in a previous work [34]

For kinetic adsorption tests, 15 mg of MIPs/NIPs were mixed with 3 mL of 100 mg L^{-1} CPF methanol solution. The mixtures were incubated at room temperature with shaking for various time intervals (*i.e.*, 5, 10, 20, 40, 60, 90, and 120 min), followed by centrifugation at 7500 × *g* for 10 min. The absorbance of unbound CPF in supernatants was measured by UV–vis spectrometry at 286 nm.

2.4. Pretreatment of apple juice

The apple juice samples were first determined to be free of CPF by HPLC-DAD before use. After that, 5 mL of apple juice samples were individually spiked with different amounts of CPF (*i.e.*, 0, 0.01, 0.1, 1, 5, 10, 20, and 30 mg L^{-1}). Then, 5 mL of hexane and 1.5 mL of ethanol were added and vortex-mixed for 30 s. The mixtures were centrifuged at 2000 × *g* for 1 min to form the hexane layer (top layer) and the aqueous layer (bottom layer). The top layer (5 mL) was collected and hexane was removed by reduced pressure rotary