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Analytical Methods

Rapid detection and quantification of 2,4-dichlorophenoxyacetic acid in milk using molecularly imprinted polymers–surface-enhanced Raman spectroscopy

Marti Z. Hua[a], Shaolong Feng[a], Shuo Wang[b], Xiaonan Lu[a,*]

[a] Food, Nutrition and Health Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada
[b] Tianjin Key Laboratory of Food Science and Health, School of Medicine, Nankai University, Tianjin 300071, China

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ABSTRACT

We report the development of a molecularly imprinted polymers–surface-enhanced Raman spectroscopy (MIPs–SERS) method for rapid detection and quantification of a herbicide residue 2,4-dichlorophenoxyacetic acid (2,4-D) in milk. MIPs were synthesized via bulk polymerization and utilized as solid phase extraction sorbent to selectively extract and enrich 2,4-D from milk. Silver nanoparticles were synthesized to facilitate the collection of SERS spectra of the extracts. Based on the characteristic band intensity of 2,4-D (391 cm^{-1}), the limit of detection was 0.006 ppm and the limit of quantification was 0.008 ppm. A simple logarithmic working range (0.01–1 ppm) was established, satisfying the sensitivity requirement referring to the maximum residue level of 2,4-D in milk in both Europe and North America. The overall test of 2,4-D for each milk sample required only 20 min including sample preparation. This MIPs-SERS method has potential for practical applications in detecting 2,4-D in agri-foods.

1. Introduction

Since the commercialization of 2,4-dichlorophenoxyacetic acid (2,4-D) after World War II, 2,4-D and its derivatives have been widely used to control broadleaf weeds in multiple settings, including agriculture, aquatic areas, landscape, and turf (Walters, 1999). With the extensive use, the residue of 2,4-D has been detected in the environment water (Yang, Jiao, Zhou, Chen, & Jiang, 2013), fresh produce (Ting & Kho, 1998), dairy products (Bogialli et al., 2006), and commonly in human urine (Ye, Wong, Zhou, & Calafat, 2014). The toxicity of 2,4-D has been debated for decades both in academia and government, including the frequent reviews and evaluations focusing on the carcinogenicity to humans (Health Canada, 2016; Smith, Smith, La Merrill, Liaw, & Steinmaus, 2017; von Stackelberg, 2013; World Health Organization, 2015). Regardless, maximum residue levels (MRLs) of 2,4-D in various food products are strictly regulated worldwide. In particular, the MRLs of 2,4-D in milk are set as 0.01 mg/kg (ppm) (European Commission, 2005), 0.03 ppm (Health Canada, 2013), and 0.05 ppm (2,4-D; Tolerances for Residues, 2012).

Currently, liquid chromatography–mass spectrometry (LC–MS) is still the gold standard for the detection of 2,4-D in foods with the limit of quantification (LOQ) at sub-ppb level (Bogialli et al., 2006; Xiong et al., 2014). However, LC–MS is well known as high-cost, time-consuming, and requiring expertise in operation. Moreover, complicated pre-treatment and clean-up procedures are usually required in prior to a 0.5–1 h of sample running (Bogialli et al., 2006). Besides chromatographic methods, immunoassays have also been reported with the working ranges at sub-ppm level in water (Vinayaka, Basheer, & Thakur, 2009) and orange peel (Vdovenko et al., 2013), but a long incubation time of 2–3 h was required. Rapid detection methods for 2,4-D include ratiometric fluorescence sensor (Wang et al., 2016), carbon nanotube liquid gated transistor (Wijaya et al., 2010), and others. Yet, their applications were limited to samples requiring no or little matrix effect, such as water and soil extracts. Therefore, a rapid and relatively cost-effective detection method that can be applied to real food samples in a high-throughput manner is highly required, especially for perishable foods, such as milk.

Molecularly imprinted polymers (MIPs) are polymers that are chemically synthesized, forming cavities with high affinity to a selected "template" molecule. Briefly, the template (*i.e.*, targeted analyte) and selected functional monomers assemble spontaneously when they are mixed, followed by co-polymerization with the cross-linkers. After that, the template molecules are removed from the complex, leaving the MIPs with cavities that are complementary to the template (Fig. 1).

* Corresponding author at: Food, Nutrition, and Health Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.
E-mail address: xiaonan.lu@ubc.ca (X. Lu).

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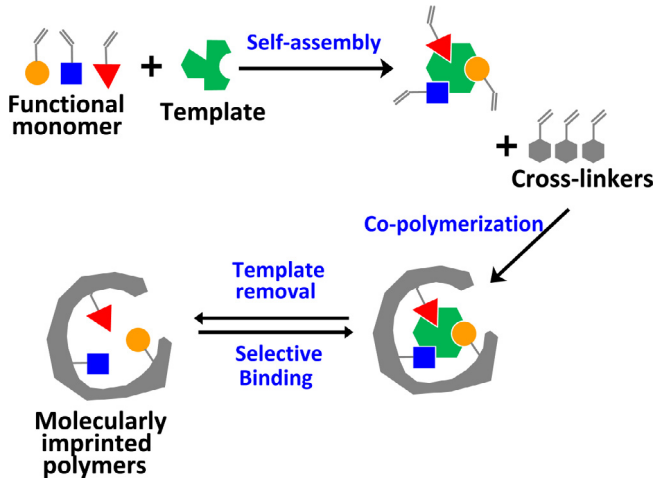

Fig. 1. Synthesis of molecularly imprinted polymers.

MIPs are referred as "artificial antibodies" for their unique binding ability to the targeted molecules. A variety of applications have been reported with the utilization of this reversible binding characteristic (Chianella et al., 2013; Vasapollo et al., 2011; Wackerlig & Schirhagl, 2016). MIPs are chemically and thermally stable, easy to prepare, and cost-effective (Haupt, Dzgoev, & Mosbach, 1998; Wackerlig & Schirhagl, 2016). Different forms of MIPs synthesized using 2,4-D as the template were reported to successfully extract 2,4-D residue from the environmental water (Yang et al., 2013), soil (Peng, Li, Zhang, & Gong, 2014), and foods (Herrero-Hernández, Carabias-Martínez, & Rodríguez-Gonzalo, 2009).

Surface-enhanced Raman spectroscopy (SERS) is a rapid, sensitive, and label-free fingerprinting technique that has been widely applied to the detection of environmental pollutants, biological imaging, and biomolecule detection (Stiles, Dieringer, Shah, & van Duyne, 2008). The inherently weak Raman scattering signal can be significantly enhanced by depositing the targeted molecules onto the surface of noble metal nanoparticles, such as gold and silver (Stiles et al., 2008). The nanoparticles serve as the substrates and can magnify the intensity of Raman scattering signals via electromagnetic and/or chemical mechanisms (Procházka, 2016). SERS has been reported for detecting trace amount of chemical and biological substances in different sample matrices. Several studies reported the application of SERS to the detection of 2,4-D in the environmental water with a limit of detection (LOD) of roughly 0.2 ppm (Jia, Xu, Zhang, Hu, & Xu, 2012) and 0.02 ppm (Costa et al., 2009). Particularly, Zhu et al. (2016) achieved a LOD of 2,4-D at sub-ppb level in water samples using nano-silver bundle array as the substrate, but preparing this unique nanostructure required complicated fabrication steps. To the best of our knowledge, using SERS to detect 2,4-D in food samples have not been reported yet, possibly due to the interference in SERS spectrum as a result of an insufficient sample clean-up procedure.

MIPs and SERS were recently integrated for the detection of chemical hazards in agri-food products, such as chloramphenicol in milk and honey (Gao et al., 2014), melamine in milk (Hu et al., 2015; Hu & Lu, 2016), and chlorpyrifos in apple juice (Feng, Hu, Ma, & Lu, 2017). The reported overall turnaround for each test was only about 15–20 min, which was much less than the time required in LC-MS or some immunoassay methods. However, establishing complicated chemometric analysis models (*e.g.*, partial least squares regression and principal component analysis) was required for further qualitative and quantitative analysis (Feng et al., 2017; Gao et al., 2014; Hu et al., 2015). In the current study, a novel MIPs-SERS method was developed to quantify 2,4-D in milk in a rapid and high-throughput manner, and a simple logarithmic working range that covered the MRL concentrations was successfully constructed.

2. Materials and methods

2.1. Chemicals and reagents

Ethyleneglycol dimethacrylate (EDMA), 4-vinylpyridine (4-VP), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,2′-Azobis (2-methylpropionitrile) (AIBN), silver nitrate ($AgNO_3$), sodium citrate, sodium chloride (NaCl), and magnesium sulphate dehydrate ($MgSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the other chemicals were of analytical grade and all of the solvents were of HPLC quality. Skimmed milk was purchased from a local grocery store in Vancouver, BC, Canada and stored at 4 °C until usage.

2.2. Synthesis of molecularly imprinted polymers (MIPS) and non-imprinted polymers (NIPs)

The procedure of polymer synthesis was modified from a previous study (Haupt et al., 1998). For MIPs, 20 mmol of EDMA, 4 mmol of 4-VP, 1 mmol of 2,4-D, and 0.3 mmol of AIBN were dissolved into 4 mL of methanol and 1 mL of deionized water in a 20-mL glass bottle. The solution was then stirred and purged with nitrogen for 2 min. The bottle was then capped and heated at 45 °C for 4 h and then at 60 °C for 2 h in a water bath. The resultant polymers were crushed into a powder with a pestle and mortar, and the powder was passed through a 200-mesh (74 μm) steel sieve. Then, the template was removed from MIPs by Soxhlet extraction with 200 mL of methanol–acetic acid (85:15, v/v) for 24 h, followed by extraction with 200 mL of methanol for another 24 h. Last, the MIPs were dried in a vacuum oven at 60 °C for 4 h. NIPs were prepared in the same pattern but without the addition of 2,4-D as the template.

2.3. Adsorption test of MIPs and NIPs

The kinetic and static adsorption tests were conducted to determine the binding equilibrium time and the adsorption capacities of both polymers (*i.e.*, MIPs and NIPs), separately (Feng et al., 2017).

For kinetic adsorption tests, 15 mg of polymers were mixed with 3 mL of 2,4-D methanol–water (50:50, v/v) solution (100 ppm). The mixtures were shaken for various time intervals (*i.e.*, 5, 10, 20, 40, 60, 90, and 120 min) at 22 °C, followed by centrifugation at 7500*g* for 10 min. The UV absorbance of the supernatant at 283 nm was measured to monitor the change in 2,4-D concentration.

For static adsorption tests, 15 mg of polymers were mixed with 3 mL of 2,4-D methanol–water (50:50, v/v) solution at different concentrations (*i.e.*, 5, 10, 20, 30, 40, 60, 80, and 100 ppm). The mixtures were shaken for 2 h at 22 °C, followed by centrifugation at 7500*g* for 10 min. The UV absorbance of the supernatant at 283 nm was measured. The adsorption capacity and selectivity were then assessed (Hu et al., 2015).

2.4. Pre-treatment of milk

The pre-treatment of skimmed milk was adapted from a modified QuEChERS method (Sack, Vonderbrink, Smoker, & Smith, 2015). In each 50-mL centrifuge tube, 5 mL of skimmed milk was added and then spiked with 2,4-D stock solutions to different concentration levels (*i.e.*, 10 ppm, 1 ppm, and 0.05 ppm). Each sample was incubated at 22 °C for 20 min to allow 2,4-D to be fully mixed. Non-spiked milk samples followed the same procedure started from here. After that, 10 mL of deionized water was added and mixed. Then, 25 mL of acetonitrile containing 1% formic acid was quickly added and the tube was shaken for 10 s immediately. A mixture of $MgSO_4$ (6 g) and NaCl (1.5 g) was added and the tube was again shaken for 10 s immediately, followed by centrifugation at 5000*g* for 4 min. The supernatant was collected and evaporated to almost dryness. The residue was re-dissolved with 2 mL of methanol–water (50:50, v/v) solution, labelled as "pre-treated sample", and stored at 4 °C for further test. The recovery of pre-