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Analytical Methods

Determination of malachite green in aquatic products based on magnetic molecularly imprinted polymers

Zheng-zhong Lin [a,b,1], Hong-yuan Zhang [a,1], Ai-hong Peng [a,b], Yi-dong Lin [a], Lu Li [a], Zhi-yong Huang [a,c,*]

[a] College of Food and Biological Engineering, Jimei University, Xiamen 361021, China
[b] Fujian Provincial Key Laboratory of Food Microbiology and Enzyme Engineering, Xiamen 361021, China
[c] Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Xiamen 361102, China

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ABSTRACT

Magnetic molecularly imprinted polymers (MMIPs) were synthesized through precipitation polymerization using malachite green (MG) as template, methacrylic acid as monomer, ethylene dimethacrylate as crosslinker, and Fe_3O_4 magnetite as magnetic component. MMIPs were characterized by scanning electron microscopy, Fourier transform infrared spectrometry, and vibrating sample magnetometry. Under the optimum condition, the MMIPs obtained exhibited quick binding kinetics and high affinity to MG in the solution. Scatchard plot analysis revealed that the MMIPs contained only one type of binding site with dissociation constant of 24.0 μg mL^{-1}. The selectivity experiment confirmed that the MMIPs exhibited higher selective binding capacity for MG than its structurally related compound (e.g., crystal violet). As a sorbent for the extraction of MG in sample preparation, MMIPs together with the absorbed analytes could easily be separated from the sample matrix with an external magnet. After elution with methanol/acetic acid (9:1, v/v), MG in the eluent was determined by high-performance liquid chromatography coupled with UV detector with recoveries of 94.0–115%. Results indicated that the as-prepared MMIPs are promising materials for MG analysis in aquatic products.

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1. Introduction

Malachite green (MG) was once widely used in aquaculture because of its high efficacy for treatment of fungal and parasitic infections (Schnick, 1988). However, MG presented many adverse characteristics because of its high toxicity, as well as teratogenic, carcinogenic, and mutagenic properties (Stammati et al., 2005). Hence, MG has been restricted or banned in food production in many countries (Cha, Doerge, & Cerniglia, 2001). Nevertheless, many aquaculture farmers continue to use MG because of its low cost and high efficacy, and MG residues are still detected in fish products. Therefore, the development of detection methods for MG is still important for food safety. At present, MG residues in fish are usually determined by high-performance liquid chromatography (HPLC) (Sagar, Smyth, Wilson, & McLaughlin, 1994), HPLC–mass spectrometry (HPLC–MS) (Halme, Lindfors, & Peltonen, 2004; Scherpenisse & Bergwerff, 2005), and gas chromatography–mass spectrometry (Turnipseed, Roybal, Hurlbut, & Long, 1995). For these methods, liquid–liquid extraction or solid-phase extraction (SPE) is typically required in sample pretreatment process, which are sophisticated, tedious, and poorly selective. Thus, the discovery of separation and enrichment methods with high efficiency and selectivity is particularly important for MG measurement.

Molecularly imprinted polymers (MIPs) are synthesized polymers equipped with specific cavities designed for a target molecule (template) (Shea & Sasaki, 1991; Vlatakis, Andersson, Müller, & Mosbach, 1993). MIPs demonstrate high affinity and specificity for a given target analyte (Lavignac, Allender, & Brain, 2004; Mosbach, 1994; Wulff, 1995). Thus, MIPs have been used widely in selective separation or enrichment of many analytes (Cirillo et al., 2011; Fan et al., 2013; Gao et al., 2013; Qiu et al., 2012), including MG (Li, Yang, Qi, Qiao, & Deng, 2008; Long et al., 2009;

* Corresponding author at: College of Food and Biological Engineering, Jimei University, Xiamen 361021, China.

E-mail addresses: linzz@jmu.edu.cn (Z.-z. Lin), 1010180258@qq.com (H.-y. Zhang), ahpeng@jmu.edu.cn (A.-h. Peng), linyid@qq.com (Y.-d. Lin), 776823248@qq.com (L. Li), zhyhuang@jmu.edu.cn (Z.-y. Huang).

[1] The two authors contributed equally to this work and should be considered as co-first authors.

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Martinez Bueno et al., 2010), during sample pretreatment. However, the separation of MIPs from sample matrix requires high-speed centrifugation, which is time and labor consuming.

In recent years, the use of magnetic molecularly imprinted polymers (MMIPs) for sample pretreatment has elicited growing interest (Aguilar-Arteaga, Rodriguez, Miranda, Medina, & Barrado, 2010; Chen & Li, 2013; Men et al., 2012). Apart from the advantages of high affinity and specificity of traditional MIPs, MMIPs can be rapidly and easily separated using an external magnetic field with high efficiency and low cost.

Our work presents a novel MMIP material prepared by precipitation polymerization with MG, methacrylic acid (MAA), ethylene dimethacrylate (EDMA), and Fe_3O_4 as template, functional monomer, crosslinker, and magnetic nucleus, respectively. The MMIPs were applied to separate MG from aquatic products. The results demonstrated that MMIPs are excellent sorbents for extracting MG from fish products. Currently, only one article (Huang, Zhou, Chen, Wu, & Lu, 2015) describes the use of MMIPs for sample pretreatment, in which the MMIPs were prepared by bulk polymerization and used as SPE material for electrochemiluminescence determination of MG residue in fish samples.

2. Materials and methods

2.1. Reagents and chemicals

All reagents were analytical grade, except for acetonitrile and methanol, which were chromatographic grade pure. All chemicals were directly used without further purification. MG, MAA, polyethylene glycol (PEG), iron chloride, ferric chloride, ammonium hydroxide (25%), chloroform, dichloromethane, and acetic acid were purchased from Xilong Chemical Co., Ltd. (Shantou, China). EDMA was obtained from Aladdin Reagent (Shanghai) Co., Ltd. (Shanghai, China). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). Acetonitrile and methanol were purchased from Tedia Company Incorporation (Fairfield, USA). High-purity water was prepared using a Millipore Simplicity Ultrapure water device (>18.0 MΩ cm, Millipore, Bedford, USA).

2.2. Preparation of magnetic Fe_3O_4

PEG (0.2 g), $FeCl_2{\cdot}4H_2O$ (1.5 g), and $FeCl_3{\cdot}6H_2O$ (2 g) were dissolved in high-purity water (8 mL) in a conical flask, with vigorous stirring (1500 rpm) under N_2 atmosphere. Up to 60 mL of ammonium hydroxide was added rapidly into the solution at 60 °C. After 1 h, the black Fe_3O_4 precipitate was separated from the solution with a magnet, washed thrice with high-purity water, and then dried in vacuum.

2.3. Preparation of core–shell MMIPs and MNIPs

MG (0.25 mmol) and MAA were dispersed in chloroform under sonication, and the mixture was placed in a refrigerator at 4 °C for 12 h to obtain the pre-polymerization solution. A mixture containing the pre-prepared Fe_3O_4 and oleic acid was stirred in a round-bottom flask for 10 min, followed by the addition of the pre-polymerization solution, EDMA, and AIBN (0.1 g) in sequence. The mixture was purged with nitrogen for 20 min and stirred for 12 h at 60 °C. The resulting MMIPs were washed with acetone to remove the residual reagents. Subsequently, these MMIPs were eluted using a Soxhlet apparatus with methanol/acetic acid (9:1, v/v) until no template molecules were detected. The MMIP product was repeatedly washed with high-purity water and then dried under vacuum. Magnetic non-imprinted polymers (MNIPs) were prepared following the same procedure but in the absence of template molecules.

2.4. Characterization

The morphologies of the polymers were measured by SEM (LEO Co., LEO1530, Germany). The surface groups of the polymer were measured using an FT-IR spectrometer (JASCO, FT/IR 480 PLUS, Japan) in the range of 500–4000 cm^{-1} using the KBr pellet method. The magnetic properties of the polymers were measured by VSM (Quantum Design, MPMS XL-7, America) at room temperature.

Chromatographic separation was performed on an HPLC system (Agilent 1260 HPLC, USA) equipped with a quarternary pump and a diode array detector. An Eclipse Plus C_{18} (100 mm × 4.6 mm, 3.6 μm) column was used for analyte separation. The mobile phase was a mixture of ammonium acetate (50 mmol L^{-1}, pH 4.5) and acetonitrile (45:55, v/v) at a flow rate of 0.5 mL min^{-1}. The column temperature was 25 °C, and the injection volume was 20 μL. The detection wavelength was set at 620 nm.

2.5. Binding experiments of MMIPs and MNIPs

Up to 50 mg of MMIPs or MNIPs was mixed with 5 mL of MG standard solution in dichloromethane (20 mg L^{-1} for optimization experiments and from 10 mg L^{-1} to 200 mg L^{-1} for adsorption isotherm). The mixture was shaken for 12 h at room temperature. Afterward, the solutions and polymers were separated using a permanent magnet. The remaining MG concentrations in the solutions were measured using UV–vis (Lab Tech, UV-2100, China) at the wavelength of 620 nm. Adsorption capacity (Q) and imprinting factor were calculated using Eqs. (1) and (2), respectively.

$$Q = (C_0 - C_e) \times V/m \quad (1)$$

$$\alpha = Q_{MIP}/Q_{NIP} \quad (2)$$

where C_0 and C_e are the initial and equilibrium MG concentrations (μg L^{-1}), respectively; V is the volume of the initial solution (mL); m is the weight (g) of the polymers; and Q_{MIP} and Q_{NIP} represent the adsorption capacity (μg g^{-1}) of MMIPs and MNIPs, correspondingly.

2.6. Selectivity of MIPs

The selectivity of the polymers for MG and its structurally related compound (CV) was tested using a rebinding experiment. The experiment process and the calculation of adsorption capacity were basically the same as the above binding experiments, but MG and CV were used as adsorbates (20 mg L^{-1} in dichloromethane) alternatively.

2.7. Extraction, purification, and determination of MG in fish samples

Carps and weevers were purchased from a local supermarket in Xiamen, China. Approximately 5.0 g of the back muscle without skin and scales was treated with 1.5 mL of 20% hydroxylamine hydrochloride and 3.5 mL of 0.05 mmol L^{-1} ammonium acetate (adjusted to pH 4.5 with acetic acid). The mixture was then homogenized at 8000 rpm for 5 min. Approximately 2 g of the tissue homogenate was spiked with 0.5 mL of MG acetonitrile solution at concentrations of 2, 4, and 10 ng mL^{-1}, followed by addition of 4 mL of water. The solution was ultrasonicated for 5 min and then allowed to stand for 5 min. The mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was transferred to a centrifuge tube. The subsidence was treated following the same extraction process, and the obtained supernatant was also transferred to the centrifuge tube.

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