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Graphene oxide membrane as an efficient extraction and ionization substrate for spray-mass spectrometric analysis of malachite green and its metabolite in fish samples

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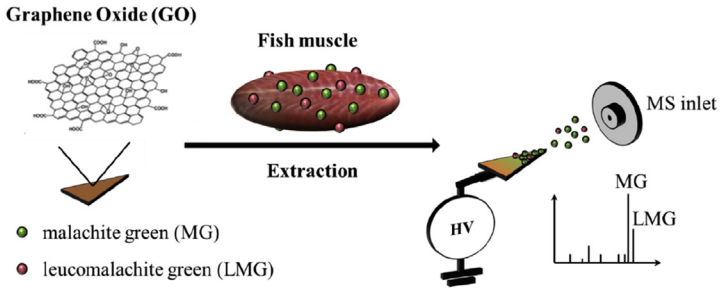
HIGHLIGHTS

- GOM used for efficient extraction of malachite green.
- Coupling of the GOM-spray with MS allows for the detection of malachite green.
- GOM-MS employed for analysis of malachite green in aquaculture freshwater and seawater.
- GOM-MS can quantitate malachite green and its metabolite in fish muscle samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A graphene oxide (GO) nanosheet-modified N^+-nylon membrane (GOM) has been prepared and used as an extraction and spray-ionization substrate for robust mass spectrometric detection of malachite green (MG), a highly toxic disinfectant in liquid samples and fish meat. The GOM is prepared by self-deposition of GO thin film onto an N^+-nylon membrane, which has been used for efficient extraction of MG in aquaculture water samples or homogenized fish meat samples. Having a dissociation constant of 2.17×10^{-9} M^{-1}, the GOM allows extraction of approximately 98% of 100 nM MG. Coupling of the GOM-spray with an ion-trap mass spectrometer allows quantitation of MG in aquaculture freshwater and seawater samples down to nanomolar levels. Furthermore, the system possesses high selectivity and sensitivity for the quantitation of MG and its metabolite (leucomalachite green) in fish meat samples. With easy extraction and efficient spray ionization properties of GOM, this membrane spray-mass spectrometry technique is relatively simple and fast in comparison to the traditional LC-MS/MS methods for the quantitation of MG and its metabolite in aquaculture products.

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1. Introduction

Various ambient ionization methods have been developed for mass spectrometry (MS), such as desorption electrospray ionization (DESI), desorption atmospheric pressure photoionization (DAPPI), laser ablation electrospray ionization (LAESI), dielectric barrier discharge ionization (DBDI), and low-temperature plasma ionization (LTP) [1]. Paper spray ionization mass spectrometry (PSI-MS) is a recently developed ambient ionization MS technique for a simple, fast, and no carry-over effect analysis [2]. The technique operates by directly identifying analytes with several microliters of sample on a paper or porous substrate [3]. When a high DC voltage (~1–5 kV) is applied, the sprayed, charged microdroplets containing analytes are transported to the mass spectrometer for analysis. The ionization mechanism of paper spray ionization (PSI) is similar to that of electrospray ionization (ESI) [4]. Taylor cones of spray solvent are observed when applying the two techniques, but PSI may have greater spray efficiency as a result of formation of more than one Taylor cone along the paper edge. The expanded PSI-MS technique has been applied to various studies, including the detection of drugs and their metabolites in biofluids, the analysis of food contaminants, profiling lipids in bacteria and microalgae for identification of biodiesel production, and monitoring biomacromolecule interactions [5–9]. However, PSI-MS analyses of complex biological samples such as blood and urine sometimes suffer from substantial matrix interference. Therefore, recent efforts have been made to prepare new solid-phase microextraction (SPME) substrates, such as C18-polyacrylonitrile-coated metal blades, carbon nanotube-modified paper, silanized paper, and Delrin plastic cartridges, to extract and preconcentrate analytes and minimize matrix effects [10–14]. The binding affinity of the extraction substrate toward the analytes affect the analysis sensitivity.

Malachite green (MG) is a triarylmethane dye that has been controversially applied in the aquaculture industry to treat and prevent protozoal and fungal infections [15]. MG can potentially cause carcinogenesis, mutagenesis, teratogenicity, and toxicities and thus it is classified as a class II health hazardous material by Occupational Safety and Health Administration (OSHA, USA) [16,17]. The acute oral LD_{50} (Lethal Dose, 50%) values of MG in rats have been reported to be ~275–520 mg kg^{-1} body weight [16,17]. Although MG has been banned in most countries, it is still used in many parts of the world because of its high efficacy, low cost, and ready availability, as well as the existence of less restrictive laws for nonaquaculture purposes. The standard procedure to examine the illegal use of MG is to extract the remaining MG and its metabolite leucomalachite green (LMG) in aquaculture products prior to quantitation by liquid chromatography tandem-mass spectrometry (LC-MS/MS) [18–20]. LC-MS/MS method is sensitive and accurate; however, it requires a labor-intensive sample preparation process and a time-consuming LC separation before MS detection [21].

We prepared a simple graphene oxide (GO) nanosheet-modified N^+-nylon membrane (GOM) as an extraction and spray substrate for MG, coupled with an ion-trap mass spectrometer for rapid detection (Scheme 1). GO nanosheets having single-layer two-dimensional networks of sp^2-and sp^3-hybridized carbon atoms possess a high specific surface area (~800 $m^2\,g^{-1}$) for MG adsorption, mainly through $\pi-\pi$ stacking coupled with electrostatic interactions [22,23]. This novel GOM-spray MS detection method is a new type of PSI-MS technique for fast quantitation of MG and LMG in either liquid type samples or aquaculture product samples. We further demonstrated that MG and LMG in fish meat samples can be enriched by GOM followed by the GOM-spray MS for the direct analysis.

2. Materials and methods

2.1. Chemicals

Malachite green (MG), leucomalachite green (LMG), basic fuchsin, ammonium acetate, hydroxylamine hydrochloride, *p*-toluenesulfonic acid, potassium permanganate, 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA) and 3-hydroxypicolinic acid (3-HPA) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Acetonitrile, formic acid, phosphoric acid, and sulfuric acid were purchased from J. T. Baker (Phillipsburg, NJ, USA). Graphite powder was purchased from Alfa Aesar (Ward Hill, MA, USA). Nylon paper (Hybond–N^+) was purchased from GE Healthcare Bioscience (Buckinghamshire, UK).

2.2. Synthesis and characterization of graphene oxide

GO was synthesized using a modified Hummers' method [24]. Briefly, a mixture of graphite flakes (0.75 g) and $KMnO_4$ (4.5 g) was added to a 9:1 mixture of concentrated H_2SO_4 and H_3PO_4 (100 mL). The mixture was then heated to 50 °C and stirred for 12 h. The mixture was cooled to room temperature and then poured slowly into 100 mL of deionized (DI) water containing 9% H_2O_2. The aqueous mixture was then centrifuged at a relative centrifugal force (RCF) of 35,000 g for 1 h, and the supernatant was discarded. The residue was repeatedly washed with 200 mL of DI water until the solution reached a pH value of 6.0. The aqueous solution was then sonicated for 1 h and centrifuged at a RCF of 25,000 g for 0.5 h. The GO solution was collected, and the remaining pellet was discarded. The GO concentration in the supernatant was determined to be 1.60 g L^{-1} by using a freeze–drying method. Zeta potential experiment was conducted using a Zetasizer 3000HS analyzer (Malvern Instruments, Malvern, UK). Transmission electron microscopy (TEM) was performed using an HT-7700 system (Hitachi, Tokyo, Japan) operated at 75 kV. The average size of GO was determined using an atomic force microscope (Shimadzu SPM-9600 AFM, Shimadzu Co, Kyoto, Japan). The Raman spectra of GO was recorded by using a DXR Raman microscope (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a 50× objective, a Nd:YAG laser (532 nm) and a charge-coupled detector. The signal collection time for each sample was 30 s.

2.3. Preparation and characterization of graphene oxide modified membranes

10 μL sodium phosphate buffer solution (5 mM, pH 7) containing GO (0–5.6 mg mL^{-1}) was dropped onto an isosceles triangle shape of hybond–N^+ membrane (N^+M; positively charged nylon transfer membranes, pore size: 0.45 μm), with a size of approximately 25 mm^2 (base width of 5 mm, height of 10 mm). The membrane was allowed to stand for 2 h and the weakly bound GOs were then removed from the GOM surface by gently washing with ultrapure water (20 mL). GOMs were then dried in air for 2 h at room temperature and characterized by field emission scanning electron microscopy (SEM, JEOL JSM-6500F, Hitachi, Tokyo, Japan).

2.4. Dissociation constant of MG and GOM

The dissociation constant (K_d) for the GOM and MG was determined using a Scatchard equation. GOMs were separately equilibrated with aliquots (1 mL) of sodium phosphate buffer (5 mM, pH 7.0) containing different concentrations (0–200 nM) of MG for 1 h. The unbound MG was quantified by Acquity TQD LC/MS/MS System (Waters, Massachusetts, USA). Chromatographic separation was performed using a reversed-phase Waters Symmetry C18 column

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