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Microscale solid phase extraction of glyphosate and aminomethylphosphonic acid in water and guava fruit extract using alumina-coated iron oxide nanoparticles followed by capillary electrophoresis and electrochemiluminescence detection

Chia-Chen Hsu, Chen-Wen Whang*

Department of Chemistry, Tunghai University, #181, Sec. 3, Taichung Harbor Road, Taichung 40704, Taiwan

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ABSTRACT

A microscale solid-phase extraction (SPE) method using alumina-coated iron oxide nanoparticles (Fe_3O_4@Al_2O_3 NPs) as the affinity adsorbent for glyphosate (GLY) and its major metabolite aminomethylphosphonic acid (AMPA) in aqueous solution is reported. One milligram of Fe_3O_4@Al_2O_3 NPs was employed to extract both analytes in 5 ml of aqueous solution. After 5 min extraction, magnetic NPs were isolated from sample solution by employing an external magnet. Followed by rinsing the NPs with 5 μl of 20 mM $Na_4P_2O_7$ solution for 5 min, the extract was directly analyzed using the derivatization-free CE-electrochemiluminescence (CE-ECL) method. With a sample-to-extract volume ratio of 1000, the enrichment factors for GLY and AMPA were 460 and 64, respectively. The limits of detection (LODs) were 0.3 and 30 ng ml^{-1} for GLY and AMPA in water, respectively. The developed method was applied to the analysis of GLY in guava fruit. The LOD of GLY in guava was 0.01 μg g^{-1}. Total analysis time including sample pretreatment, SPE and CE-ECL was less than 1 h.

1. Introduction

Glyphosate (*N*-(phosphonomethyl)glycine; GLY) is a widely used broad-spectrum, foliar-applied herbicide for weed and vegetation control. Its degradation in the environment mainly occurs under biological conditions yielding aminomethylphosphonic acid (AMPA) as the major metabolite. Due to its relatively low mammal toxicity, GLY has become one of the most extensively used herbicides worldwide. This indiscriminate application generates some concerns regarding the possible health hazard and environmental impact caused by GLY. The US Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of GLY in drinking water at 0.7 μg ml^{-1} [1]. The maximum residual level (MRL) of GLY in most crops is set at 0.1 μg g^{-1} by the European Union [2]. In Taiwan, the MCLs of GLY in water [3] and crops [4] were set at 1.0 μg ml^{-1} and 0.1–1.0 μg g^{-1}, respectively. The monitoring of GLY at residue levels in various environmental samples and agricultural products has attracted considerable attention [5].

GLY often represents an analytical challenge because of its relatively high solubility in water, insolubility in organic solvent, high polarity and low volatility. Derivatization prior to GC analysis is necessary to lower its polarity and enhance the volatility [6,7]. Moreover, GLY possess no chromophore or fluorophore, both pre- and postcolumn derivatization have therefore been employed to HPLC [8–10] and CE [11,12] of GLY and its metabolites with UV-absorption or fluorescence detection. Non-derivatization methods, such as indirect UV detection [13], indirect laser-induced fluorescence (LIF) detection [14], electrospray ionization (ESI)-MS [15,16], flame photometric detection [17], amperometric detection [18], coulometric detection [19,20], electrochemiluminescence (ECL) detection [21] and electrospray condensation nucleation light scattering detection [22] have also been reported for HPLC and CE analyses of GLY.

Despite the high sensitivities achieved by many analytical methods, an extraction/preconcentration step is generally required for the determination of GLY and its primary metabolite AMPA in environmental matrices. Conventional organic solvent extraction cannot be employed to GLY and AMPA because of their polar nature and high water solubility. Anion-exchange solid phase extraction (SPE) using commercial SPE cartridges were the most common procedure for extraction/preconcentration of GLY and AMPA from water samples [23–27]. An ion-exchange mechanism was also used to transport GLY and AMPA through a supported-liquid membrane (SLM) containing a quaternary ammonium salt, Aliquat 336, which

* Corresponding author. Tel.: +886 4 23590248x311; fax: +886 4 23506473.
E-mail address: cwwhang@thu.edu.tw (C.-W. Whang).

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acts as an anion-exchanger carrier [8,28–30]. A matrix solid-phase dispersion extraction method has been reported for the extraction of GLY and AMPA in tomato fruit [31].

In the field of proteomic research, analytical chemists have constantly made efforts to develop suitable methods to selectively enrich phosphopeptides and phosphoproteins from complex samples. Recently, simple and rapid enrichment procedures specifically for phosphopeptides prior to matrix-assisted laser desorption/ionization (MALDI)-MS have been developed [32–35]. Alumina-coated iron oxide magnetic nanoparticles (Fe_3O_4@Al_2O_3 NPs) were used as affinity probes to selectively concentrate phosphopeptides from complex sample matrices, e.g. human serum and tryptic digests of various proteins. Alumina has been shown to have high specificity for analytes containing phosphate groups within a large range of pH [36]. One of the main advantages of using this magnetic affinity probe is that these NPs, conjugated with their target species after enrichment, can be readily isolated from sample solution by simply employing an external magnetic field. Furthermore, magnetic NPs possess large surface area which facilitates SPE. GLY is structurally similar to amino acids and both GLY and AMPA molecules contain a phosphate group. We think it might also be feasible to enrich GLY and AMPA from aqueous samples using the Fe_3O_4@Al_2O_3 NPs. Report on the use of magnetic Fe_3O_4@Al_2O_3 NPs to the SPE of small molecules is scarce in the literature. Recently, Sun et al. [37] described a mixed hemimicelles SPE of trimethoprim from environmental waters using surfactant SDS-modified Fe_3O_4@Al_2O_3 NPs.

In this paper we report a simple and rapid microscale SPE procedure for GLY and AMPA in water using magnetic Fe_3O_4@Al_2O_3 NPs as the affinity adsorbents. Following extraction/preconcentration, the analytes were readily desorbed from the NPs by rinsing with $Na_4P_2O_7$ solution and directly analyzed with the derivatization-free CE-ECL method [21]. The applicability of this method was demonstrated by analyzing GLY in water extract of guava fruit.

2. Experimental

2.1. Apparatus

The laboratory-assembled CE-ECL detection system was similar to that described previously [21]. A 0–30 kV power supply (Glassman High Voltage, Whitehouse Station, NJ, USA) provided the separation voltage. Fused-silica capillaries (50 μm i.d. × 360 μm o.d. × 55 cm total length, Polymicro Technologies, Phoenix, AZ, USA) were used for separation. A ITO electrode (Delta Technologies, Stillwater, MN, USA), situated at the capillary outlet and biased at 1.6 V (versus a Pt-wire reference), was used for in situ generation of the active $Ru(bpy)_3^{3+}$. ECL signals were captured using a Hamamatsu R928 photomultiplier tube (PMT; Hamamatsu city, Japan), positioned under the ITO electrode. Optimal conditions for the CE-ECL detection have been described previously [21].

2.2. Chemicals

Glyphosate (GLY), aminomethylphosphonic acid (AMPA), aluminum isopropoxide and tris(2,2′-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3Cl_2$) were purchased from Aldrich (Milwaukee, WI, USA). Tetrasodium diphosphate decahydrate ($Na_4P_2O_7{\cdot}10H_2O$) was obtained from E. Merck (Darmstadt, Germany). Iron oxide (Fe_3O_4, particle size 20–30 nm) and sodium silicate were bought from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were of analytical-reagent grade. All solutions were filtered through a 0.45-μm pore-size membrane filter before use.

2.3. Preparation of alumina-coated iron oxide (Fe_3O_4@Al_2O_3) magnetic nanoparticles

Fe_3O_4@Al_2O_3 NPs were prepared following the procedure described by Chen et al. [32] with some modifications. Instead of preparing Fe_3O_4 magnetic NPs by ourselves, we used the commercially available Fe_3O_4 powder as the initial substrate. Iron oxide in deionized water (5 mg ml^{-1}, 40 ml) was suspended through sonication under nitrogen gas. Aqueous sodium silicate solution (0.6%, pH 9, 40 ml) was added into the above solution and vortex-mixed for 24 h at 35 °C to coat a thin film of SiO_2 onto the surfaces of magnetic particles. The particles were triply rinsed with 40 ml of deionized water, and then resuspended in 40 ml of deionized water. Aluminum isopropoxide (20 mg) was added to the nanoparticle solution followed by sonication for 30 min at room temperature. The mixture in a reaction vial was heated at 80 °C in an oil bath with vigorous stirring for 1 h, and then the vial cap was opened to release the generated gas (2-propanol). The mixture was continually heated at 90 °C for 30 min, followed by reflux at 90 °C for another 2 h. After the mixture was cooled to room temperature, the Fe_3O_4@Al_2O_3 NPs were isolated, triply rinsed with 40 ml of water, and dried at 80 °C for 1 h.

2.4. Procedure for SPE of GLY and AMPA

Scheme 1 displays the steps followed to extract GLY and AMPA from aqueous samples. In a 25-ml polypropylene vial, 5 ml of sample solution was ultrasonically mixed with 1 mg of Fe_3O_4@Al_2O_3 NPs for 5 min. The NPs which conjugated with GLY and AMPA were aggregated by an external magnet. About 4 ml of the supernatant solution was removed using a pipet. The residual solution and NPs were totally transferred to a 1.5-ml microcentrifuge tube. The particles were aggregated again by positioning a magnet to the outside of tube wall so that the residual solution could be completely removed by pipet. The isolated particles were then ultrasonically rinsed with 5 μl of $Na_4P_2O_7$ solution for 5 min to desorb the analytes. After positioning a magnet to the outside of the microcentrifuge tube, the 5-μl supernatant solution was collected using a micropipette, followed by direct CE-ECL analysis.

2.5. Guava sample preparation

Three grams of homogenized guava peel (~1 mm thick) were mixed with 10 ml of water and 0.1 ml of 10 mM EDTA solution in a 25-ml polypropylene vial. The mixture was ultrasonically shaken for 10 min. After centrifuging at 2000 rpm for 5 min, 7 ml of supernatant was taken and mixed with 7 ml of acetonitrile to precipitate the proteins. The mixture was centrifuged at 3000 rpm for 10 min. Five milliliters of the supernatant was taken and was subjected to SPE using Fe_3O_4@Al_2O_3 NPs described above.

3. Results and discussion

3.1. Calibration of CE-ECL analysis

Direct analysis of GLY and AMPA was performed using the nonderivative CE-ECL detection method. A typical electropherogram of GLY and AMPA is shown in Fig. 1. Calibration curves for GLY and AMPA were found linear ($r \geq 0.99$) in the concentration ranges 0.17–85 and 5.55–111 μg ml^{-1}, respectively. The limits of detection (LODs) for GLY and AMPA in water, calculated as three times signal-to-noise (S/N) ratio, were 0.06 and 3.65 μg ml^{-1}, respectively. These LODs were barely adequate for the screening of residual GLY in some crops and fruits. In order to further lower the LODs, SPE with magnetic NPs was employed to enrich the analytes before CE-ECL