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Ultra-performance liquid chromatography coupled with graphene/polyaniline nanocomposite modified electrode for the determination of sulfonamide residues

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ABSTRACT

An ultra-performance liquid chromatography (UPLC) coupled with graphene/polyaniline (G/PANI)-modified screen-printed carbon electrode was developed for separation and sensitive determination of eight sulfonamides (SAs) in shrimp. Electrospraying was selected for electrode modification because it can generate the well dispersion of G/PANI nanocomposites on the electrode surface. Prior to electrochemical detection, eight SAs were completely separated within 7 min by using reversed phase UPLC (C4) with mobile phase containing 70:25:5 (v/v/v) of potassium hydrogen phosphate (pH 3): acetonitrile:ethanol. For amperometric detection, the detection potential acquired from hydrodynamic voltammetry was found to be +1.4 V. Under optimal conditions, a wide linearity and low limit of detection were obtained for eight SAs in the range of 0.01–10 μg mL^{-1} and 1.162–6.127 ng mL^{-1}, respectively. Compared to boron-doped diamond (BDD) electrode, a G/PANI-modified screen-printed carbon electrode offered higher sensitivity with lower operating cost. To determine SAs in shrimp samples, solid-phase extraction was used to clean up and preconcentrate the samples prior to UPLC separation. To validate this developed method, a highly quantitative agreement was accomplished with UPLC–UV system. Thus, this proposed system might be an alternative approach for rapid, inexpensive, and sensitive determination of SAs in shrimps.

1. Introduction

Sulfonamides (SAs) are a group of sulfur-containing medicines. Their structures consist of benzene ring with amine group ($-NH_2$) at a C4 position and sulfonic acid with different alkyl groups (Fig. S1). SAs are widely used to prevent the growth of bacteria and treat the infections from certain microorganisms and protozoa [1,2]. Nowadays, SAs are used as an additive in animal feed to prevent bacterial contamination [3,4]; however, using excess amount of SA in animal feed can affect the consumer health. SA residues can cause severe allergy, carcinogenic disorder and antibiotic resistance in human. The European Union (EU) has established the maximum residue limits (MRLs) for SAs in animal meat at 0.1 μg mL^{-1} [5–7]. Therefore, development of rapid and accurate method for sensitive determination of SAs in animal meat is greatly required for food safety and food quality control.

Various analytical approaches have been developed for the determination of SA residues in real samples, such as biological fluids, animal feeds, drugs, foods, and animal meats. Thin-layer chromatography (TLC) [8,9], enzyme-linked immunosorbent assay (ELISA) [7,10,11], and gas chromatography (GC) [12–14] have been applied for SAs determination; nonetheless, these analytical techniques are tedious and time-consuming. Especially, the immunoassay technique, which requires time-consuming analysis and high operating cost. Recently, high performance liquid chromatography (HPLC) [15–17] coupled with photodiode array detector and mass spectrometry was also developed for the determination of SA residues; however, the HPLC separation time was still longer than 20 min [18–20].

To decrease the separation time for simultaneous determination of SAs, ultra-performance liquid chromatography (UPLC) [21–24] has become a promising method. UPLC offers several advantages

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including fast analysis, high resolution, high sensitivity, and high throughput [25,26]. In general, UPLC detection techniques used for SAs determination are ultraviolet–visible (UV–vis) [27,28], fluorescence [29,30], and mass spectrometry [31,32]. Although these techniques are very sensitive and selective, they require expensive equipment and specialist for operation [20,23,33,34]. Alternatively, an electrochemical detection coupled with UPLC is an attractive system for SA determination since this detection technique is simple, rapid, sensitive, and inexpensive [35–40]. Various types of working electrode have been applied for SA detection, such as boron-doped diamond electrode [35–37], carbon paste electrode [39], aluminum oxide–gold nanoparticle-modified carbon paste electrode [38], and polycrystalline gold electrode [40]. To improve the electrochemical sensitivity of working electrode, design and modification of working electrode became an interesting issue for ongoing research. Recently, it has been reported that modification of working electrode with nanomaterials, such as metallic nanoparticles [41,42] and carbon nanotubes (e.g. SWCNT [43–45] and MWCNT [46–50]) can increase both surface area and electrochemical sensitivity of modified electrodes.

Graphene (G), a two dimensional sheet of sp^2 carbon atom, has become a promising nanomaterial in electrochemistry [51–53] due to its high surface area, high electrical conductivity, high mechanical strength and potentially low manufacturing cost. Recently, G has been applied for electrode surface modification to improve electrochemical property of the electrodes [54,55]; however, G can easily agglomerate together and form graphite. To improve the distribution of graphene on the electrode surface, a conducting polymer is selected to create G/conducting polymer nanocomposite. Among conducting polymers, polyaniline (PANI) is an attractive material for electrode surface modification because of its excellent electrochemical properties, biocompatibility and environmental stability [56–58].

In this work, G/PANI nanocomposite is developed and used for electrode surface modification along with UPLC separation for simultaneous determination of eight SAs. Under optimal conditions, this novel system is applied for sensitive determination of eight SAs in real shrimp samples.

2. Material and methods

2.1. Reagents and solutions

Eight standard SAs including sulfadiazine (SDZ), sulfamerazine (SMZ), sulfaguanidine (SG), sulfisoxazole (SSZ), sulfadimethoxine (SDM), sulfamonomethoxine (SMM), sulfadoxine (SDX) and sulfamethoxazole (SMX) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile (HPLC-grade), ethanol, methanol, ortho-phosphoric acid 85%, dimethylformamide (DMF), di-sodium hydrogen phosphate dehydrate (Na_2HPO_4), polyaniline and camphor-10-sulfonic acid ($C_{10}H_{16}O_4S$) were obtained from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate (KH_2PO_4) was acquired from BDH laboratory supplies (VWR International Ltd., England). Graphene (G) was obtained from A.C.S (Medford, USA). Milli-Q water from Milipore ($R \geq 18.2\ M\ \Omega\ cm^{-1}$) was used throughout this experiment. All stock standard solutions of SAs ($1000\ \mu g\ mL^{-1}$) were prepared by dissolving 10 mg of each SA in acetonitrile: Milli-Q water (ratio of 1:1) to final volume of 10 mL in volumetric flask and then stored at 4 °C. To prepare working standard solution, the stock solution was diluted to suitable proportions in acetonitrile: Milli-Q (50:50; v/v). All solutions and solvents were filtered by 0.22 μm nylon membranes prior to use in UPLC separation.

2.2. Fabrication and modification of electrode

The novel electrodes were fabricated and modified using screen-printing and electrospraying technique. An in-house carbon electrode was prepared by sequentially printing conductive inks onto the polyvinyl chloride (PVC) substrate. Whilst carbon ink was printed for using as a working electrode area, silver/silver chloride (Ag/AgCl) was printed for using as a conductive pad. The printed electrode was allowed to dry in an oven at 55 °C for 1 h. For the electrode modification, electrospraying was used to produce the G/PANI nanocomposite on the screen-printed carbon electrode surface. Graphene solution was simply prepared by dispersing 20 mg graphene nanopowder into 10 mL dimethylformamide (DMF). The graphene solution was sonicated by ultrasonic bath and left overnight. For preparation of polyaniline solution, camphor-10-sulfonic acid ($C_{10}H_{16}O_4S$) was used as a doping agent to generate the conducting form of PANI (emeraldine salt). The solution of PANI was prepared by mixing 100 mg of polyaniline (emeraldine base) and 129 mg of camphor-10-sulfonic acid ($C_{10}H_{16}O_4S$) by using a mortar and a pestle, and after that the mixture was transferred into 15 mL of chloroform. Then, the homogeneous solution was stirred for 2 h. Finally, to achieve G/PANI nanocomposite solution, the solution with ratio of 1:1 between graphene and polyaniline was mixed together. After that G/PANI nanocomposite solution was electrosprayed on a screen-printed carbon electrode attached on a ground collector for 5 min with a constantly applied high voltage of 10 kV.

2.3. Cyclic voltammetry

The electrochemical measurements were performed on a CH instrument potentiostat 1232A (CH Instrument, Inc., USA) with a standard three-electrode system. The reference and auxiliary electrodes were Ag/AgCl and platinum wire, respectively. The working electrodes used in this study are boron-doped diamond (BDD), bare screen-printed carbon electrode, PANI-modified screen-printed carbon electrode and G/PANI-modified screen-printed carbon electrode. For the cyclic voltammetric measurement, the standard solution of 1 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ in 0.1 M KCl and $50\ \mu g\ mL^{-1}$ individual SA in 0.1 M phosphate buffer (pH 3) were examined. All experiments were performed at room temperature and covered in a Faraday cage.

2.4. UPLC separation and electrochemical detector

The UPLC system consisted of a 20 ADXR solvent deliver unit (Shimadzu Corporation, Japan), an auto sampler (SIL-20A) with 0.1–100 μL loop, an Inertsil C4 packed column (150 mm × 4.6 mm i.d.; particle size, 5 μm, GL science), a thin-layer flow cell (GL Science Inc.), and an amperometric detector. The thin-layer flow cell consisted of a reference Ag/AgCl electrode (Bioanalytical system Inc., USA), a working G/PANI electrode, and a stainless steel tube counter electrode. A 1 mm thick silicon rubber gasket was used as a spacer in flow cell for limiting the geometric area of working electrode. The separation of SAs by the UPLC–ECD system was carried out using a mobile phase of 0.1 M phosphate solution (pH 3): ACN: EtOH (70:25:5 v/v/v ratios) with an injection volume of 25 μL, flow rate of $1.5\ mL\ min^{-1}$ and an applied potential at +1.4 V vs Ag/AgCl. For hydrodynamic voltammetry, the applied potential in a range of 1.1–1.5 V vs Ag/AgCl was examined in amperometric detection. The hydrodynamic voltammogram was plotted between peak current and applied potential. For the precision of intra-day and inter-day, four concentrations of SAs (1, 3, 5 and $9\ \mu g\ mL^{-1}$) were investigated for 3 times within a day and three different days. To validate this proposed method, UPLC coupled with ultraviolet (UV) detection was carried out using the same stationary phase and mobile phase. In addition, the accuracy of recovery for spiked SAs determination was compared between the standard UPLC–UV method and the proposed UPLC–ECD method.