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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.

2.5. Sample preparation and solid-phase extraction procedure

Shrimp samples were obtained from local market in Thailand. A solid-phase extraction (SPE) using Microcolumn *VertiPak*™ HCP was employed for preconcentration and extraction of SAs in shrimp samples [33]. 2 g of homogeneous shrimp sample and 10 mL of Na_2EDTA– McIlvaine buffer were added into a tube and mixed in a vortex mixer (Mixer Uzusio LMS. Co. Ltd., Japan) with a high speed for 5 min. To achieve distribution of SAs from shrimp to supernatant, a mixture was placed in an ultrasonic bath and centrifuged (MSE limited, UK) at 3500 rpm for 10 min. For an SPE procedure, the SPE microcolumn was connected to a 12-position vacumn manifold system, followed by a conditioning step with 5 mL of methanol, Milli-Q water, and Na_2EDTA–McIlvaine buffer, respectively. Then, 10 mL of supernatant was loaded into the microcolumn, and then 7 mL of methanol was used to elute SAs. Finally, the extracted solution was filterted through a 0.20 μm nylon membrane to a vial before transferring to an autosampler of UPLC system.

3. Results and discussion

3.1. Surface morphology of G/PANI nanocomposite modified electrode

The solution of G/PANI (1:1) was electrosprayed on a screen-printed carbon electrode to generate the G/PANI nanocomposite on the surface. The well dispersed G/PANI nanocomposites without severe agglomeration were observed. The surface morphology of modified electrode and G distribution within the nanocomposites were characterized by SEM and TEM images as shown in Fig. 1a and b, respectively. The homogeneous distribution of G/PANI nanocomposites on the electrode surface contains unprecedented number of G, leading to increased surface area and thus electrochemical sensitivity of the modified electrode.

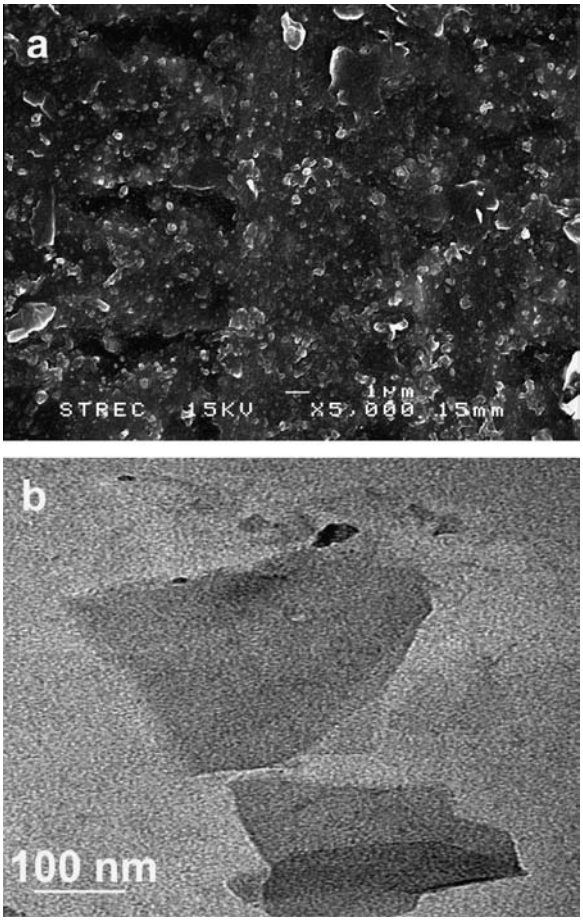

Fig. 1. SEM (a) and TEM (b) images of G/PANI nanocomposite modified screen-printed carbon electrode prepared by electrospraying using a needle diameter of 0.45 mm, an applied voltage of 10 kV, 5 min collecting time and 10 cm collecting distance.

3.2. Electrochemical characterization of G/PANI nanocomposite modified electrode

To characterize the G/PANI nanocomposite modified electrode, the electrochemical behavior of the modified electrode was investigated using standard 1 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe by cyclic voltammetry (CV) and then compared with PANI modified electrode and unmodified screen-printed carbon electrode as shown in Fig. 2.

As seen in Fig. 2, the G/PANI-modified screen-printed carbon electrode exhibited a well-defined oxidation and reduction peak, which indicates reversible electron transfer kinetics on G/PANI-modified electrode surface. Compared to PANI-modified electrode and unmodified electrode, the highest anodic (i_{pa}) and cathodic (i_{pc}) current responses were obtained from G/PANI-modified electrode indicating the highest electrochemical sensitivity. Moreover, the G/PANI-modified electrode demonstrated a lower peak-to-peak separation (ΔE_p) value compared to PANI-modified

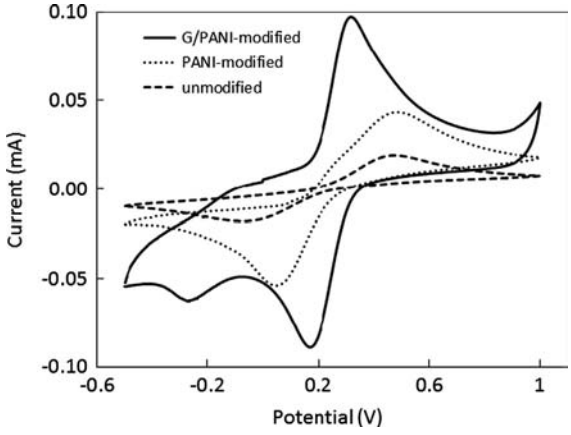

Fig. 2. Cyclic voltammograms of 1 mM $[Fe(CN)_6]^{3-/4-}$ measured on G/PANI modified screen-printed carbon electrode, PANI modified screen-print carbon electrode and unmodified screen-printed carbon electrode vs Ag/AgCl with scan rate 100 mV s^{-1}.

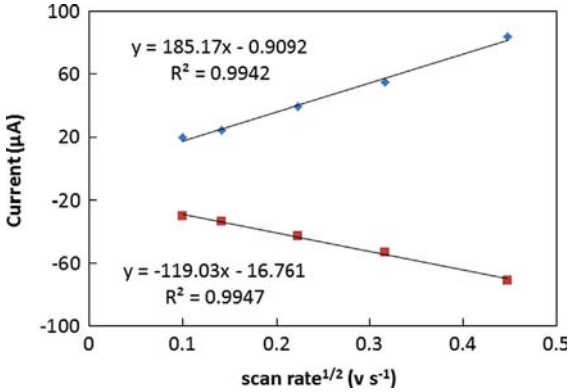

Fig. 3. The linear relationship between square root of scan rate and current response of 1 mM $[Fe(CN)_6]^{3-/4-}$ solution in 0.1 M KCl measured on G/PANI modified screen-print carbon electrode.

electrode and unmodified electrode; a decrease of ΔE_p value on the G/PANI-modified electrode was attributed to a fast electron transfer rate as a result of conductive layer on the modified electrode surface as well as the electrocatalysis. This result verifies that G/PANI-modified screen-printed carbon electrode proposed in this study substantially improves the electrochemical performance of screen-printed carbon electrode. These promising results indicated that G/PANI-modified screen-printed carbon electrode might be an effective tool for sensitive determination of trace levels of SAs.

To study the mass transfer process on G/PANI modified screen-printed carbon electrode, the square root of scan rate was plotted as a function of current. The rate of mass transfer was studied by cyclic voltammetry of standard 1 mM $[Fe(CN)_6]^{3-/4-}$ solution in 0.1 M KCl. As shown in Fig. 3, a good linearity for both anodic (i_{pa}) and cathodic (i_{pc}) peak currents with a correlation coefficient (R^2) between 0.9942 and 0.9947 was achieved indicating that the diffusion controlled mass process occurred on the G/PANI modified screen-printed carbon electrode.

3.3. Cyclic voltammetry of eight SAs

Cyclic voltammetry was used to investigate the electrochemical behavior of each SA on G/PANI-modified screen-printed carbon electrode compared to unmodified screen-printed carbon electrode and boron-doped diamond electrode, respectively. The chemical structures of eight SAs are shown in Fig. S1 in the Supporting information. Eight SAs can be electrochemically oxidized at $–NH_2$ group; however, the reduction of $–SO_2–$ group can occur only at the higher negative potential value. Therefore, only

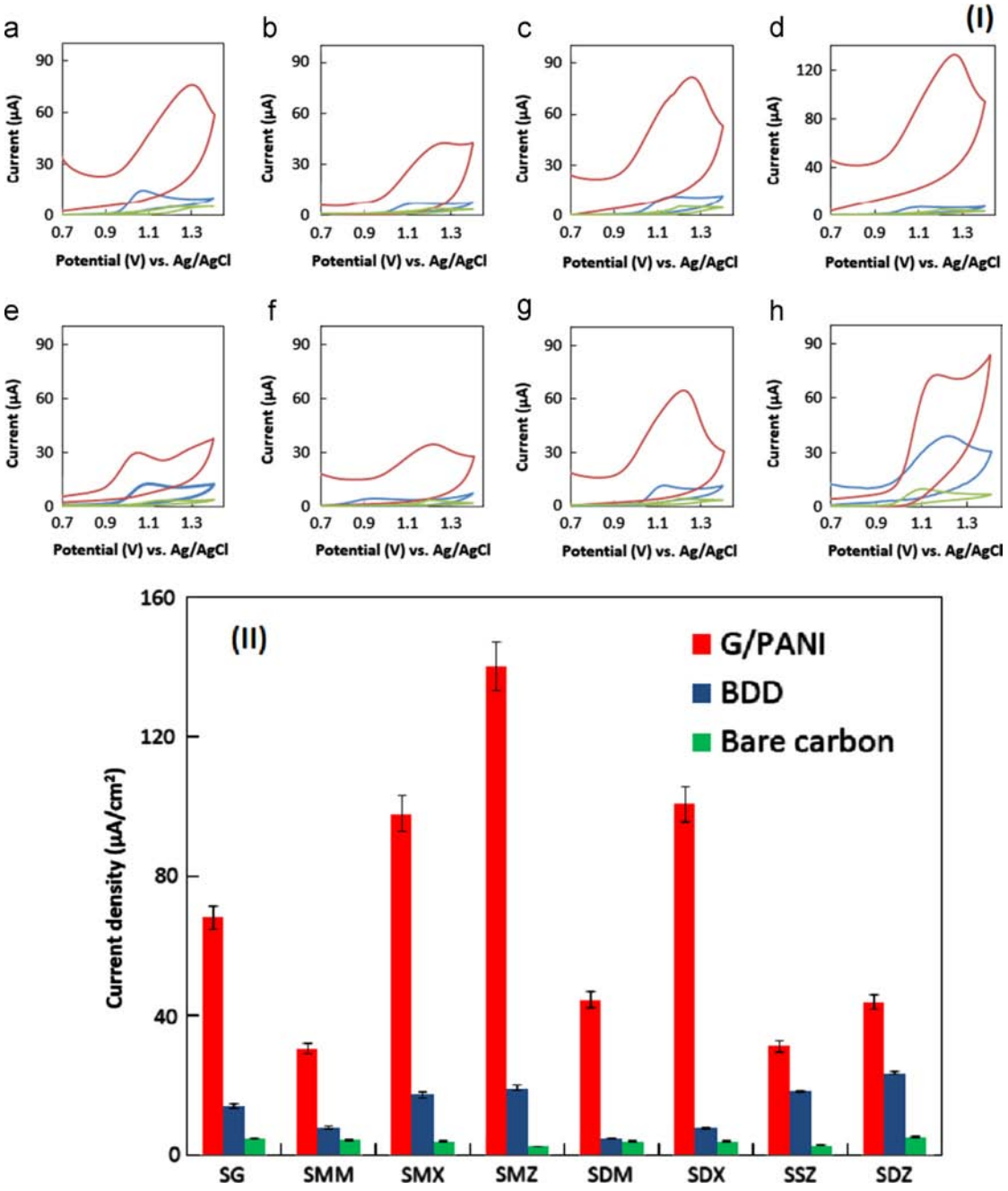

Fig. 4. (I) Cyclic voltammograms of (a) SG, (b) SDZ, (c) SMZ, (d) SMM, (e) SDX, (f) SMX, (g) SSZ, and (h) SDM, and (II) comparison of oxidation current density between (—) G/PANI-modified screen-printed carbon electrode, (—) boron-doped diamound electrode, and (—) unmodified electrode, vs Ag/AgCl at the concentration of 50 μg mL^{-1} in 0.1 M phosphate solution pH 3, scan rate 100 mV s^{-1}.

anodic peak of eight SAs was investigated as shown in Fig. 4. The CV results show that the larger capacitance current by using the G/PANI-modified electode compared to other electrodes was obtained. We believe that it was because of the accumulation of charge obatined from the PANI conducting polymer deposited on the electrode surface. However, the use of G/PANI-modified electrode for SAs detection exhibited the highest current response signal to background ratio and well-defined irreversible cyclic voltammogram when compared to other electrodes including boron-doped diamound electrode and unmodified carbon electrode.

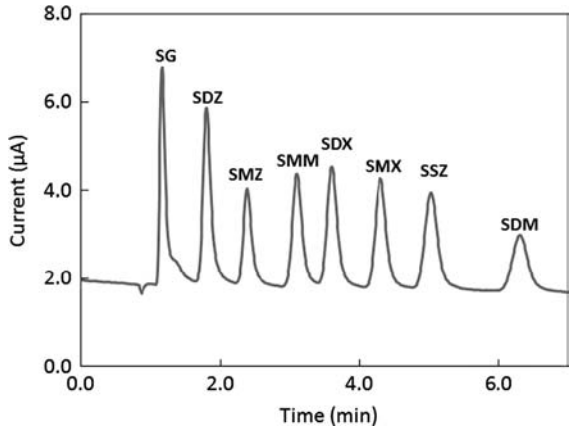

Fig. 5. UPLC–ECD chromatogram of a standard mixture of eight SAs (10 μg mL^{-1}) measured on G/PANI-modified screen-printed carbon electrode. The mobile phase contains 0.05 M potassium hydrogen phosphate solution (pH 3.0):acetonitrile: ethanol (70:25:5 v/v/v) with the injection volume of 25 μL, and the flow rate of 1.5 mL min^{-1}.

Table 1
Linear range, limit of detection (LOD), and limit of quantitative (LOQ) of eight SAs.

SAs	LR (μg mL^{-1})	Equation $y=bx+a$	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
SG	0.01–10	$y=71.991x+71.738$	0.9934	1.162	3.336
SDZ	0.01–10	$y=38.970x+34.603$	0.9913	1.601	5.337
SMZ	0.01–10	$y=29.614x+15.320$	0.9922	2.900	9.667
SMM	0.01–10	$y=29.236x+28.891$	0.9954	2.467	8.224
SDX	0.01–10	$y=33.708x+74.571$	0.9912	2.995	9.983
SMX	0.01–10	$y=42.024x+13.735$	0.9920	2.513	8.376
SSZ	0.01–10	$y=32.494x+22.453$	0.9951	3.287	10.957
SDM	0.01–10	$y=20.657x+15.135$	0.9949	6.127	20.425

These results indicated that G/PANI-modified carbon screen-printed electrode might be a promising electrode for SAs detection in real sample. Due to low-cost material and simple fabrication process of G/PANI-modified electrode, it can be an alternative choice of working electrode that can replace the high cost electrode (i.e. boron-doped diamond) in the future. Subsequently, G/PANI-modified screen-printed carbon electrode will be coupled with UPLC separation system for simultaneous determination of eight SAs.

3.4. Optimization of the detection potential for eight SAs

The effect of detection potential for amperometric measurement was investigated in a potential range of +1.1 V to +1.5 V for eight SAs. Oxidation of $-NH_2$ accounts for the anodic property, while the alkyl group shows negligible influence on the oxidation potentials of SAs. As described in Fig. S2, the anodic current (i_{pa}) of all eight SAs increased when the detection potential was increased until the detection potential of +1.4 V. Above +1.4 V, the anodic current significantly decreased. Therefore, +1.4 V was selected as an optimal potential for amperometric detection of eight SAs.

3.5. UPLC separation coupled with amperometric detection of eight SAs on G/PANI-modified electrode

In this study, the reversed phase C4 column was used for UPLC separation along with amperometric detection (UPLC–ECD) on G/PANI-modified screen-printed carbon electrode. Under optimal conditions, the mixture of 0.05 M potassium hydrogen phosphate solution (pH 3):acetonitrile:ethanol (70:25:5 v/v/v) was used as a mobile phase with a flow rate of 1.5 mL min^{-1}, and injection volume of 25 μL. Eight SAs were completely separated within 7 min and the high current responses were observed for all SAs as shown in a UPLC–ECD chromatogram in Fig. 5. It can be seen that this proposed system provides both rapid analysis and high sensitivity for simultaneous determination of eight SAs.

3.6. Analytical performance of the system

The analytical performance of this proposed system was studied. A calibration curve was established by measuring between the concentration and the current response signal of SAs. Under the optimal conditions, all SAs measured on G/PANI-modified screen-printed carbon electrode showed a good linearity in a concentration range of 0.01–10 μg mL^{-1}. All the correlation coefficients exceed 0.9912 for eight SAs. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from $LOD=3S_B/b$ and

Table 2
Recent report using carbon nanotube composite modified electrode and boron-doped diamond electrode for electrochemical determination of sulfonamide derivatives.

Modified electrode	Detection method	Linerity range	LOD (ng mL^{-1})	Analyte	Real sample	Reference
MWCNT-modified CPE	DPV	0.35–30 μg mL^{-1}	100	SMX	Drug tablet	Arvand et al. [59]
MIPs-modifed CPE	DPV	3.98–398 ng mL^{-1}	1.83	Sulfasalazine	Human serum	Sadeghi et al. [60]
Paraffin/MWCNT-SbNPs composite electrode	DPV	0.04–0.30 μg mL^{-1}	6.10	SMX	Natural water	Cesarino et al. [61]
SWCNT/pDAN –modifed EPPG	SWV	3.1–919 μg mL^{-1}	67.4	Sulfacetamide	Eye drops	Yadav et al. [62]
BDD	Amperometry	0.01–120 μg mL^{-1}	1.2–3.4	SG, SDZ, SMZ, SMM, SMX, SDM, and SQ	Shrimp	Sangjarusvichai [37]
G/PANI-modified SPCE	Amperometry	0.01–10 μg mL^{-1}	1.2–6.1	SG, SDZ, SMZ, SMM, SDX, SMX, SSZ, and SDM	Shrimp	This work

Abbreviations: LOD, limit of detection; DPV, differential pulse voltammetry; SWV, square wave voltammetry; MWCNTs, multi-walled carbon nanotubes; CPE, carbon paste electrode; MIPs, molecularly-imprinted polymer; SbNPs, antimony nanoparticles; SWCNTs, single-walled carbon nanotubes; pDAN, poly1,5-diaminonapthalene; EPPG, edge plane surface of pyrolytic graphite; BDD, boron-doped diamond; G, graphene; PANI, polyaniline; SPCE, screen-printed carbon electrode.

LOQ = $10S_B/b$, respectively (S_B as a standard deviation of the mean value for 10 signals of the blank and b as a slope of the straight line in the linearity curve). LOD was found between 1.162 and 6.127 ng mL^{-1}, and LOQ was found in a range of 3.336–20.425 ng mL^{-1}. All data including linear range, equation, R^2, LOD, and LOQ of eight SAs were summarized in Table 1. Moreover, the electrochemical performance of the G/PANI-modified electrode was compared to the other previous electrodes used for the SAs detection as shown in Table 2. It was observed that our proposed electrode shows a relatively high electrochemical sensitivity for SAs detection.

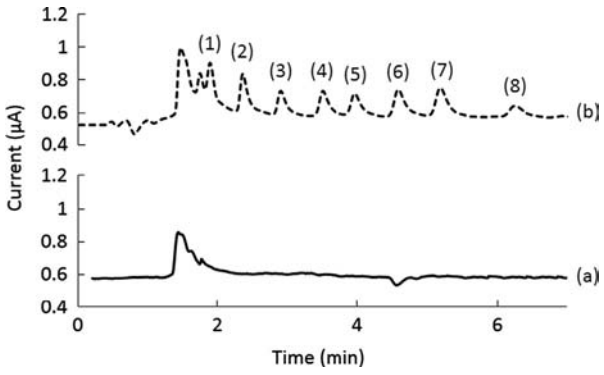

Fig. 6. UPLC–ECD chromatogram of (a) blank shrimp sample and (b) shrimp sample with spiked (1) SG, (2) SDZ, (3) SMZ, (4) SMM, (5) SDX, (6) SMX, (7) SSZ and (8) SDM at a level of 5 μg mL^{-1} measured on G/PANI-modified screen-printed carbon electrode. The mobile phase contains 0.05 M potassium hydrogen phosphate solution (pH 3.0):acetonitrile:ethanol (70:25:5 v/v/v) with an injection volume of 25 μL and a flow rate of 1.5 mL min^{-1}.

3.7. Application in real samples

Eventually, the proposed system was applied for the determination of SAs in real samples. The standard addition method was chosen to investigate a reliability of this proposed system. UPLC–ECD chromatogram (Fig. 6) shows the peak current of eight SAs in blank (Fig. 6a) and in shrimp samples with spiked SAs at a level of 5 μg mL^{-1} (Fig. 6b). In order to assess the reproducibility, four different concentrations of standard SAs (1, 3, 5 and 9 μg mL^{-1}) were spiked in shrimp samples. The precision of analytical procedure was determined by calculating the relative standard deviation (RSD, $n=3$), and the accuracy was determined by calculating percent recovery. The inter- and intra-day precision and recovery of this novel system UPLC–ECD for the determination of eight SAs in shrimp samples were summarized in Table 3. These results illustrated percent recovery in a range of 81.10–108.91% with RSD < 5. Therefore, this novel method might be an alternative approach for rapid separation and simultaneous determination of SAs.

To validate the developed method, a novel UPLC–ECD system was compared to the standard method of UPLC coupled with ultraviolet detection (UPLC–UV). A paired t-test at 95% confidential interval was performed on the results obtained by spiked three concentrations of standard SAs including 1, 3 and 5 μg mL^{-1} in sample. The experimental t-values ($t_{calculated}$) obtained by this novel method are −1.27 at the concentrations of 1 μg mL^{-1}, −2.02 at the concentrations of 3 μg mL^{-1}, and −0.16 at the concentrations of 5 μg mL^{-1}. These $t_{calculated}$ values were significantly lower than the critical t-values 1.89, 2.36 and 0.87, at concentrations of 1, 3 and 5 μg mL^{-1} respectively. It can be concluded that there is no significant difference for two sets of results between UPLC–ECD system and conventional UPLC–UV method. Thus, UPLC coupled with G/PANI-modified screen-printed

Table 3
Inter-day and intra-day of eight SAs.

SAs	Spiked level (μg mL^{-1})	Intraday Mean of recovery (X ± SD)	%RSD	Interday Mean of recovery (X ± SD)	%RSD
SG	1	93.80 ± 3.02	3.22	92.48 ± 1.98	2.14
	3	101.84 ± 2.70	2.65	92.05 ± 2.85	3.10
	5	96.94 ± 1.46	1.50	101.65 ± 1.83	1.79
	9	99.56 ± 0.80	0.80	99.34 ± 1.01	1.02
SDZ	1	88.35 ± 0.46	0.52	88.72 ± 0.22	0.25
	3	89.86 ± 3.77	4.20	87.95 ± 0.24	0.27
	5	98.01 ± 0.34	0.35	102.74 ± 0.18	0.17
	9	100.73 ± 1.11	1.10	100.22 ± 0.56	0.56
SMZ	1	81.80 ± 0.83	1.02	81.10 ± 0.16	0.19
	3	84.99 ± 1.68	1.97	83.87 ± 0.81	0.96
	5	99.58 ± 0.34	0.34	102.13 ± 0.68	0.67
	9	100.15 ± 0.75	0.75	100.74 ± 0.12	0.12
SMM	1	96.48 ± 0.34	0.35	91.02 ± 1.42	1.56
	3	96.52 ± 0.50	0.52	92.52 ± 0.64	0.96
	5	101.58 ± 0.64	0.63	104.22 ± 1.85	0.67
	9	99.43 ± 0.57	0.50	100.57 ± 0.36	0.12
SDX	1	84.11 ± 0.63	0.75	84.59 ± 0.52	0.61
	3	86.82 ± 1.86	2.15	86.69 ± 0.99	1.15
	5	102.32 ± 3.27	3.19	102.94 ± 0.93	0.90
	9	99.15 ± 1.23	1.24	101.01 ± 0.21	0.20
SMX	1	85.41 ± 0.99	1.16	86.59 ± 0.12	0.13
	3	86.46 ± 1.80	2.08	87.57 ± 0.39	0.45
	5	100.48 ± 0.25	0.25	96.79 ± 0.32	0.33
	9	101.37 ± 0.73	0.72	102.21 ± 0.06	0.06
SSZ	1	85.22 ± 0.58	0.68	84.63 ± 0.14	0.16
	3	88.60 ± 1.27	1.43	85.09 ± 0.24	0.28
	5	101.68 ± 0.81	0.79	99.67 ± 0.32	0.32
	9	101.42 ± 0.95	0.94	100.42 ± 0.26	0.25
SDM	1	92.33 ± 0.60	0.65	90.84 ± 1.45	1.60
	3	108.91 ± 2.10	1.93	91.52 ± 0.12	0.13
	5	98.96 ± 0.28	0.28	94.98 ± 0.46	0.48
	9	101.02 ± 0.35	0.34	103.06 ± 0.77	0.74

carbon electrode might be a novel system that is acceptable and reliable.

4. Conclusion

G/PANI modified screen-printed carbon electrode was first coupled with UPLC system for rapid separation and sensitive determination of eight SAs. By using this system, eight SAs were completely separated within 7 min with the high current response signal. Compared to boron-doped diamond electrode, the proposed electrode offers wider linear range and lower LOD for SAs determination. Moreover, this electrode is much cheaper than boron-doped diamond electrode. Eventually, this system was successfully applied for simultaneous determination of eight SAs in shrimp samples. The results exhibit a good recovery, high precision and low LOD suggesting that this system might be an alternative tool for routine analysis of SA residues in shrimp.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.004.

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