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Microchip Capillary Electrophoresis Based Electroanalysis of Triazine Herbicides

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Abstract The number of pesticides used in agriculture is increasing steadily, leading to contamination of soil and drinking water. Herein, we present a microfluidic platform to detect the extent of contamination in soil samples. A microchip capillary electrophoresis system with in-channel electrodes was fabricated for label-free electroanalytical detection of triazine herbicides. The sample mixture contained three representative triazines: simazine, atrazine and ametryn. The electropherogram for each individual injection of simazine, atrazine and ametryn showed peaks at 58, 66 and 72 s whereas a mixture of them showed distinct peaks at 59, 67 and 71 s respectively. The technique as such may prove to be a useful qualitative and quantitative tool for the similar environmental pollutants.

Keywords Triazine herbicide · Amperometric detection · Capillary electrophoresis · Microchip

The cumulative number of studies on the effect of pesticides over the last two decades has increased substantially, especially for organophosphates, organochlorines, carbamates and triazines (Kohler and Triebskorn 2013). Due to potential over exposure of pesticides on non-target species, it became a prime concern to find a cost-effective, real-time, highly sensitive and specific in-field easy to operate sustainable microdevices to monitor the level of the chemical agents. Among the triazines, atrazine (2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), simazine (2,4-Bis-ethylamino-6-chloro-1,3,5-triazine) and ametryn (2-Ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine) are herbicides used to control broadleaf weeds and annual grasses. Owing to their close chemical structure and properties, specific sensing from a mixture of the frequently used herbicides is of great importance. Although many advances have been made in this area in recent years, much is yet to be accomplished.

According to the United States Environmental Protection Agency, people who are exposed to atrazine through drinking water could experience problems with their cardiovascular system or have reproductive difficulties (Strandberg and Scott-Fordsmand 2002). Particularly, exposure to simazine could cause problems with blood, while ametryn could cause liver toxicity. Studies have also shown that these herbicides are potent carcinogens and can induce congestion of the heart, lungs and kidneys; hypotension; antidiuresis; muscle spasms; weight loss, adrenal, retinal and cardiovascular damage. Long term exposure to triazine may even lead to Parkinson's disease (Tchounwou et al. 2000).

So far the analysis of pesticides are carried out by gas chromatography equipped with flame thermionic detection, electron capture detection, nitrogen phosphorus detection or mass spectrometric detection (Albanis and Hela 1995; Jiménez et al. 1997) and liquid chromatography with diode array detection, fluorescence detection or mass spectrometric detection (Gong and Ye 1998; Tanabe and Kawata 2004). However, these methods require sample pretreatment, enrichment or extraction steps. These additional steps make the detection laborious and time-consuming, and require sophisticated technical equipment.

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Electroanalytical techniques have gained importance for the analysis of environmental samples. Their advantages include simple and inexpensive operation, sensitivity, and selectivity. The advent of microfabrication technology has also made techniques highly portable. Methods based on biological or electrochemical principles are available to a certain extent for the analysis of these pollutants, e.g., biosensor (Anh et al. 2004), immunochemical methods (Bhand et al. 2005), square wave voltammetry with the hanging mercury drop electrode (Dossantos et al. 2004), etc. The method applied should ideally be able to determine simultaneously multi-component pesticide mixtures with good reproducibility, high recovery and low limit of detection (LOD).

In this regard, capillary electrophoresis (CE) coupled with optical and electrochemical detection methods is becoming an advantageous tool for determining pesticide residues in environmental matrices (Felhofer et al. 2010). Apart from its ability to analyze even mixtures of compounds, analysis in a miniaturized format is interesting for the routine analysis of samples containing hazardous pesticides (Lin et al. 2000; Tsai et al. 1998). However, most of these reported CE devices suffer the drawbacks of low separation efficiency for closely related analytes and often have low detection sensitivity and non-reproducibility in small microchannel configuration. Alternate methods were devised by various groups with some degree of success by fabricating spiral microchannel configuration in order to pack longer separation length on microdevices (Joung et al. 2007).

While addressing some of the drawbacks related to CE devices, herein, we report a microfluidic chip for the separation and detection of three most common triazines namely simazine, atrazine and ametryn. The sensing principle of this microfluidic sensor is based on the amperometric detection (AD). A sieving medium (agarose gel) was maintained for their effective separation prior to detection. The proposed method was also used for analyzing the herbicides in soil extract samples.

Experimental

Atrazine, simazine and ametryn were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and chemicals were of analytical grade. All solutions were prepared and diluted using deionized (DI) water. The positive photoresist (PR) AZ-1512, AZ developer AZ-300 and negative PR used in microfabrication were from Micro-Chem (Newton, MA). The negative PR SU-8 2075 and SU-8 developer were procured from the same company. For fabrication of microchannel, the PDMS with brand name Sylgard 184 was procured from Dow Corning (Midland, MI). Electrochemical analyses were performed using CHI 800B (CH Instrument, Texas).

Surface soil grab samples were taken from corn fields representative of agricultural land. 50 mL of DI water and methanol (1:1) was added to 10 g of soil sample. The soil samples were thoroughly mixed at room temperature to ensure homogeneity. Sample extracts were clarified by filtration through a Whatman no. 40 filter paper. Thereafter, appropriate volumes of methanolic solutions of individual triazine herbicides were spiked to a portion of soil extract and were used directly.

The devices were fabricated using standard photolithographic techniques (Islam et al. 2011). For making the electrode pattern, the PR (AZ-1512) was spin-coated on the HMDS-coated glass followed by UV exposure through the photo mask and Au-vapor deposition. The chip consisted of two reservoirs acting as inlet and outlet along with a microchannel made from PDMS. The dimension of microchannel was 200 μm (width) × 120 μm (height) × 5 cm (length). The configuration of the microfluidic chip is shown in Fig. 1. For fabrication of microchannel, 120 μm-thick PR (SU-8 50) was spin-coated and patterned on the silicon wafer. PDMS was chosen as the channel material because of its inertness with the reagents and transparency for easy observation of various changes inside the channel such as bubble formation, filling of liquids, corrosion due to high voltage, etc. The PDMS layer was fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto the molding master, followed by curing for at least 1 h at 72°C. The cured PDMS was separated from the mold, and reservoirs were made at the end of each channel using a 3 mm circular punch. Finally, bonding of PDMS layer on glass substrate containing the electrodes was performed using UV-Ozone cleaner.

A three-electrode system comprising platinum wire as auxiliary, Ag/AgCl as reference and gold electrode as working was used for all the electrochemical experiments in bulk system. Through the cyclic voltammetry experiments, we could find the detection voltage(s) to be applied in CE–AD device and the peak current range that these

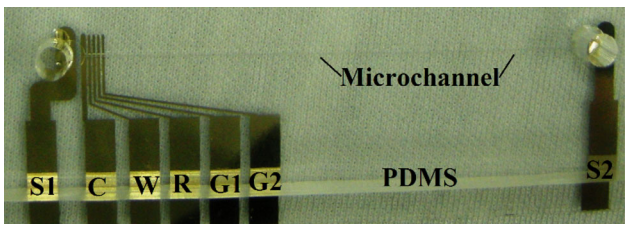

Fig. 1 CE–AD microchip showing *microchannel* engraved in *PDMS* mold, sample reservoirs, gold electrodes (*S* separation, *C* counter, *W* working, *R* reference, *G* decoupling)

chemicals would generate. Electrochemical measurements were carried out in a solution of 200 mM aqueous KCl and methanol (1:1 v/v). For the electrophoresis, at first, the microchannel was cleaned with the supporting electrolyte, pumped using silicone tubes and a precision syringe pump (KD Scientific, USA). Then the channel was filled with 1.5 % agarose gel (prepared in supporting electrolyte) while avoiding air bubble formation inside the channel and left for 30 min to let it semi-solidified. The two reservoirs were completely filled with the electrolyte to avoid negative hydrodynamic pressure on electrophoretic migration of analytes.

Subsequently, 1 μL of the triazine sample (diluted to appropriate concentration) was injected into sample reservoir close to the microchannel opening using a micropipette and an electric field of 100 V was applied immediately between the inlet and the waste reservoir. The separation potential was switched on immediately after sample addition to limit the mixing of sample in reservoir. The AD was performed with three-electrode configuration (Fig. 1) placed in the path of analyte flow. The three gold electrodes represent the working, counter and pseudo reference electrodes for electrochemical detection. Two decoupling electrodes were also used to reduce noise during the detection. Redox reaction of atrazine, simazine and ametryn from testing analytes on the working electrode generated current peaks in the amperometric i–t curve, which was recorded and stored on a computer using the electrochemical analyzer.

Results and Discussion

The CE–AD microchip was fabricated on a glass substrate with PDMS mold containing separation microchannel pattern. The PDMS mold was formed on a micro-patterned SU-8 molding master. The choice for all-gold electrode material was due to their inertness to redox reactions and ease in fabrication over having conventional Au, Pt, and Ag/AgCl electrode configuration. The design of our CE–AD separation channel was considerably different from previous reports incorporating double-T structure, where samples are added in a reservoir perpendicular to the main separation channel (Jang et al. 2011). In such configuration, the sample is first pre-concentrated in the main channel before being subjected to electrophoretic separation. While a double-T injector configuration is more commonly reported throughout the literature, this configuration requires prior knowledge of migration time for analytes to some extent. In an event where more than a few unknown species are present in sample, for instance, soil samples containing pesticides of different kinds, double-T configuration is not easy to implement. Therefore, in this study, we adopted a simple straight channel configuration for ease in operation and effective separation of complex samples.

In addition, we maintained low sample size (1 μL) to avoid the problem of continuous sample injection, as usually present with straight channel CE configuration.

Simazine, atrazine and ametryn are triazines with different substituents that gives each triazine its specific selectivity. The cyclic voltammetric (CV) analysis of atrazine, simazine and ametryn was the pre-requisite for the knowledge of detection voltage to be applied in CE–AD procedure and peak current range that these chemicals would generate. Conventional CV analysis of these three triazines was performed using three electrodes in our previous work (Islam et al. 2012). In brief, a mix solution of 200 mM KCl in DI water and methanol (1:1 v/v) was used as the solvent as well as supporting electrolyte. For CV, the potential was swept between 0.4 and −1.0 V with a rate of 100 mV/s. Both atrazine and simazine produced defined reduction peaks in the cathodic scan at −0.70 V, whereas ametryn reduced at −0.80 V. These reduction peak voltages were subsequently applied for CE–AD analysis with a correction for the possible coupling effect taking place between the applied separation voltage and the detection voltage, which was −0.75 V for atrazine and simazine and −0.90 V for ametryn.

Atrazine, simazine and ametryn are closely related compounds with only a small difference in molecular weight. Therefore, as expected, attempts to resolve these compounds using conventional CE in 5 cm long straight microchannel failed. To achieve a distinct separation, the microchannels were thus filled with 1.5 % agarose gel which has a molecular sieving action. The agarose was dissolved in 200 mM KCl in methanol: H_2O (1:1) and injected into the prewashed (with methanol-KCl) microchannel through reservoir using a syringe pump. The agarose was allowed to get semi-solidified by keeping for 30 min.

Atrazine, simazine and ametryn were subsequently analyzed on the microfluidic chip at their peak detection voltages by injecting 1 μL of 1 mM concentration of each into the reservoir. The separation voltage was kept at 100 V, thus, effectively creating field strength of 20 V/cm, which is one of the mildest conditions used in any CE–AD analysis. A low separation voltage ensures low corrosion in electrodes. Figure 2 shows the resulting electropherograms of individual migration times for simazine, atrazine and ametryn are 58, 66 and 72 s respectively. The corresponding reproducibility of these herbicides were found to be 94.7 % for simazine (SD 3.08 s, n = 6), 95.2 % for atrazine (SD 3.13 s, n = 6) and 96.2 % for ametryn (SD 2.79 s, n = 6) in our experiment.

After this initial success, the separation and detection was attempted for a mixture of triazines on the same

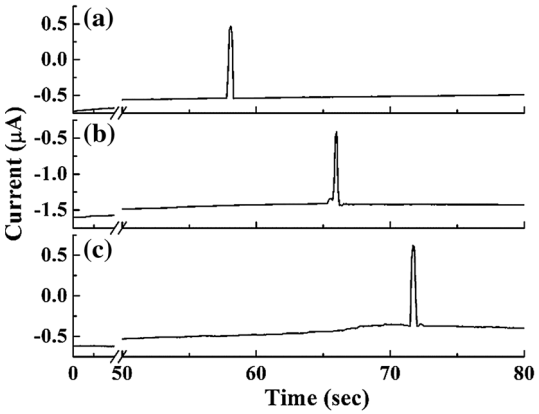

Fig. 2 Electropherograms for CE–AD of *a* simazine, *b* atrazine and *c* ametryn showing detection peaks at 58, 66 and 72 s respectively. Concentration of each: 1 mM, Separation voltage: 100 V

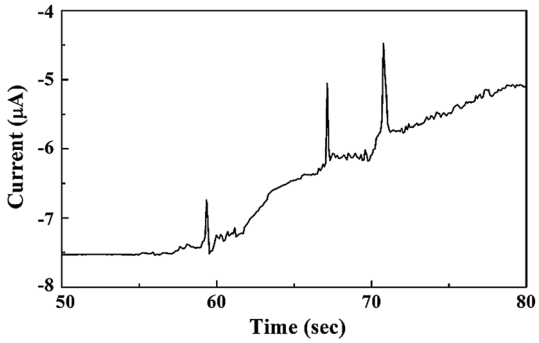

Fig. 3 Electropherograms for separation of a mixture containing three triazines with peaks at 59, 67 and 71 s respectively. Separation voltage: 100 V

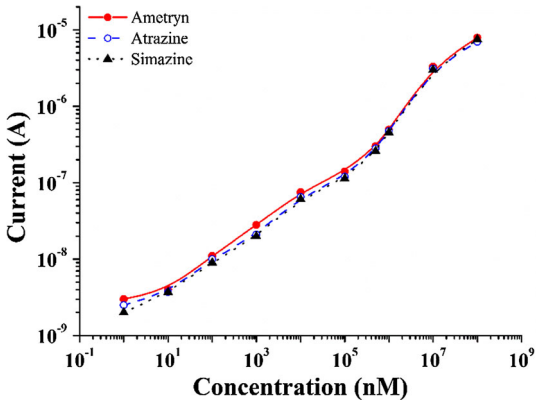

Fig. 4 Calibration curves for simazine (*filled circle*), atrazine (*filled triangle*) and ametryn (*open circle*). The *X* and *Y* axis are shown in logarithmic scale

microchip. In order to perform such separation, we made four combinations with three triazines as: simazine and atrazine, atrazine and ametryn, simazine and ametryn and finally the mixture of all three triazines. After loading an initial sample mixture (1 μL) into the agarose gel by applying the electric field of 20 V/cm, the sample mixture resolved in the channel. The results of the separated band were recorded by the electrochemical detector. After separation the mixture of analytes (data not shown) simazine and atrazine showed separated peaks at 59 and 67 s respectively. The mixture of simazine and ametryn showed separated peaks at 56 and 73 s respectively. Similarly, the mixture of atrazine and ametryn showed separated peaks at 65 and 73 s respectively. A mixture of all three triazines showed separated peaks at 59, 67 and 71 s for simazine, atrazine and ametryn respectively (Fig. 3). In our study we found that the mixtures showed much higher current than the individual herbicides along with unstable baseline. The reason is unclear but may be attributed to the combined electronegativity of the compounds in the mixture.

In order to accurately calibrate the system for quantitative analysis, a correlation between electropherogram peak heights (in terms of cathodic current) and migration time for analytes was desirable. For this reason such correlation was established by measuring peak heights obtained by electrophoretic separation of different concentrations of triazines. The relation between the peak current and the concentration of simazine, atrazine and ametryn is shown in Fig. 4. Each concentration was measured four times or more. The calibration plots obtained for each analyte over a concentration range of 1 nM–100 mM represented low standard deviation between measurements (only around ±0.6 μA SD for the highest concentration of each analytes). The response time for detection of these herbicides was <90 s. Moreover, the LOD for the sensor was calculated to be 0.36, 0.45 and 0.55 nM for simazine, atrazine and ametryn respectively considering a constant noise of 0.37 nA as obtained during CE–AD studies in plain separation medium. This suggested that the chip can successfully detect the concentration of simazine, atrazine or ametryn in soil or water.

Although simazine, atrazine and ametryn have been studied extensively, the details of their pathways to complete degradation and mineralization are not well understood. However, there is a general agreement that the first step in major routes of degradation is biotic N-dealkylation and abiotic hydrolytic dechlorination (Tanabe and Kawata 2004). These processes may occur simultaneously and perhaps competitively, depending upon the local soil environment. Hence, major site for accumulation of these pesticides in the environment lies in the soil itself. Therefore, we analyzed soil samples collected from local sources using proposed CE–AD method. The soil extract was analyzed as it is and also after deliberately spiking it with a mixture of three triazine herbicides. The electropherogram in Fig. 5a represents analysis of spiked mixture consisting

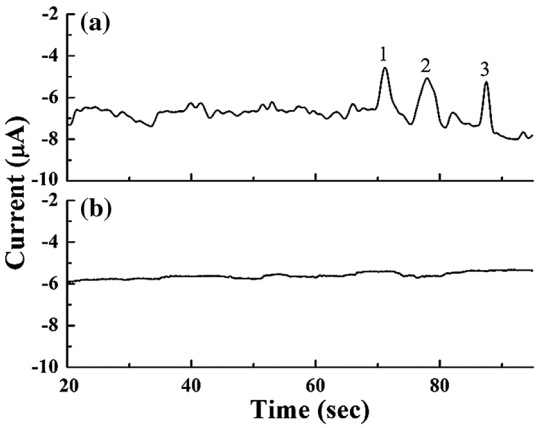

Fig. 5 Electropherograms for CE–AD analysis of *a* soil extract spiked with 5 mM each of simazine, atrazine and ametryn and *b* unspiked soil extract

of 5 mM of each compound while Fig. 5b represents the response of analyzing the soil extract. The results show satisfactory resolution of herbicides in spiked sample.

In conclusion, we have shown the use of available methodology such as CE–AD for separation and detection of a mixture of pesticides from the environment. Such application of existing methodology for simplified detection of herbicides has not been demonstrated in past. The triazines were successfully separated and detected using the microfabricated chip. The results showed that simazine, atrazine and ametryn were separated and analyzed within 1.25 min without any pretreatment of the electrode surface. This CE–AD technique may prove to be a useful qualitative and quantitative tool for similar environmental pollutants.

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