Microelectronic Engineering 97 (2012) 391–395

Contents lists available at SciVerse ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

Fast detection of triazine herbicides on a microfluidic chip using capillary electrophoresis pulse amperometric detection

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ARTICLE INFO

Article history:
Available online 9 April 2012

Keywords:
Pulsed amperometric detection
Capillary electrophoresis
CE–PAD microchip
Triazine herbicide
Lab-on-a-chip

ABSTRACT

We report simple and rapid capillary electrophoresis (CE) separation followed by in-channel pulsed amperometric detection (PAD) of three common triazine herbicides: simazine, atrazine and ametryn that are used to control broad leaf weeds and annual grasses. For their detection in soil and groundwater samples, a CE–PAD microfluidic chip was fabricated using standard photolithography methods. Cyclic voltammetry was conducted on these herbicides that exhibited a characteristic cathodic peak at −0.70 V for simazine or atrazine and −0.80 V for ametryn, without any anodic peak at reverse scan, indicating that the cathodic peaks were irreversible electron transfer processes. For effective CE–PAD separation of triazine complex, the capillary was filled with 1.5% agarose. The pulsed amperometric detection of these chemicals ensured better sensor response and low electrode fouling. The average electropherogram of simazine, atrazine and ametryn showed single peaks at 58, 66 and 74 s, respectively at 20 V/cm separation potential. A mixture of all three herbicides showed similar separated peaks. HPLC was also conducted in a soil spiked with these pollutants to compare the method. The results hold the promise of detecting triazines within a very short time.

1. Introduction

Simazine (2,4-bis-ethylamino-6-chloro-1,3,5-triazine), atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and ametryn (2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine) are atrazine herbicides used to control broadleaf weeds and annual grasses. The widespread use of these herbicides can cause groundwater contamination [1] leading to acute health effects including congestion of the heart, lungs and kidneys; hypotension; antidiuresis; muscle spasms; weight loss, adrenal, retinal and cardiovascular damage; carcinogenicity and long term exposure may even lead to Parkinson's disease [2,3]. Because of the increase in incidence of mammary gland tumors in female laboratory animals exposed to triazine herbicides, these compounds are classified in Group C, and are therefore considered as possible human carcinogens [4].

The analysis of these herbicides are usually carried out by gas chromatography (GC); mass spectrometric detection; high performance liquid chromatography (HPLC) [5]. However, these methods require sample pretreatment, enrichment or extraction steps. Therefore, they are mostly laborious and time-consuming, and require complicated cleanup procedures and sophisticated technical equipment. Beside these analytical technologies, methods based on biological or electrochemical principles are available to certain extent for sample analysis of these pollutants, e.g., biosensor [6,7], square wave voltammetry with the hanging mercury drop electrode (HMDE) [8], etc. Compared to these methods, capillary electrophoresis (CE) coupled with optical and electrochemical detection methods is becoming an advantageous tool for determining pesticide residues in environmental matrices because of its advantages, such as shorter analysis times, higher separation efficiency and very small consumption of expensive reagents and toxic solvents [9,10]. Initially introduced as a technique for separation of biological macromolecules, CE has since attracted much interest in other application areas, including pesticide-residue determination [11]. The capability to conduct analysis in a miniaturized format (microchip technology) is interesting for the routine analysis of samples containing hazardous pesticides. However, most of these reported CE–AD 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. While addressing some of the drawbacks related to CE–AD devices, we fabricated a microfluidic chip for the detection and separation of three most common triazines. Cyclic voltammetry was conducted due to accurately resolve different detection voltage of structurally similar compounds. The sensing principle of this microfluidic sensor is based on the

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http://dx.doi.org/10.1016/j.mee.2012.03.034

capillary electrophoresis pulsed amperometric detection (CE–PAD), while maintaining a sieving medium (agarose) for their effective separation prior to detection which is an improved and modified version of our previous CE–AD studies [12–14]. The pulsed amperometric technique of detection, rather than the common amperometric method was to ensure better sensor response and prevent electrode fouling during continuous CE operation. The proposed method was also compared with existing HPLC method while analyzing the herbicides in soil samples.

2. Materials and methods

2.1. Device design and fabrication

The devices were fabricated using standard photolithographic techniques as per the schematics shown in Fig. 1. The chip consisted of two reservoirs acting as inlet and outlet along with a microchannel made from PDMS. The dimensions of each microchannel were 200 μm (width) × 200 μm (height) × 5 cm (length). The configuration of the microfluidic chip is shown in Fig. 2. For fabrication of microchannels, 200 μm-thick photoresist (SU-8 2075) was spin-coated and patterned on the silicon wafer. The PDMS layer was fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto a 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. At the same time, gold electrodes were fabricated on a glass substrate using standard photolithographic methods. The three electrodes namely working, reference and counter electrodes were fabricated by thermal evaporation. Finally, bonding of PDMS layer on glass substrate containing the electrodes was performed with UV-Ozone cleaner to get improved bond strength.

2.2. Device operation and electrochemical measurements

Cyclic voltammetric (CV) and Amperometric measurement were performed using CHI 800B electrochemical workstation. A three-electrode system comprising a platinum wire as auxiliary,

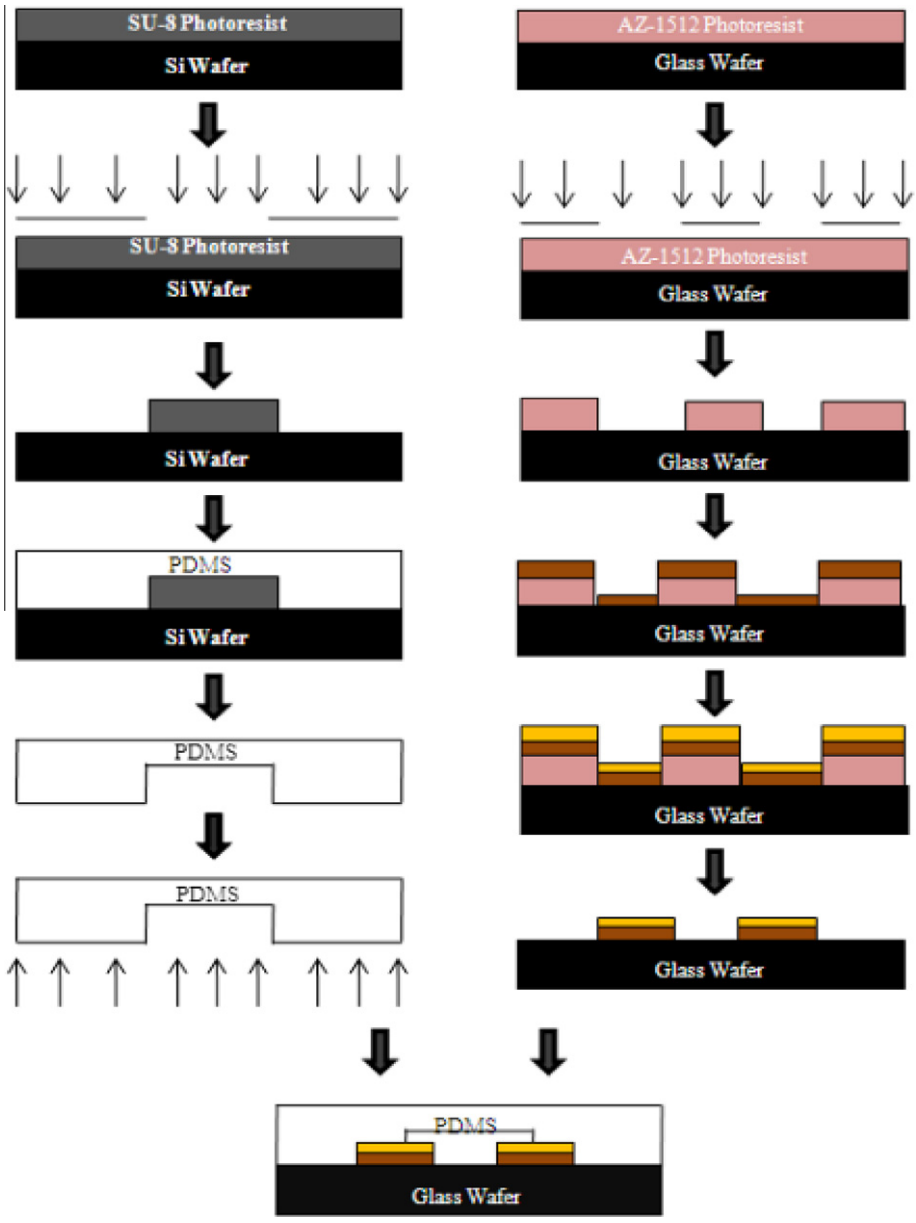


Fig. 1. Schematics for the fabrication process of the microchip.

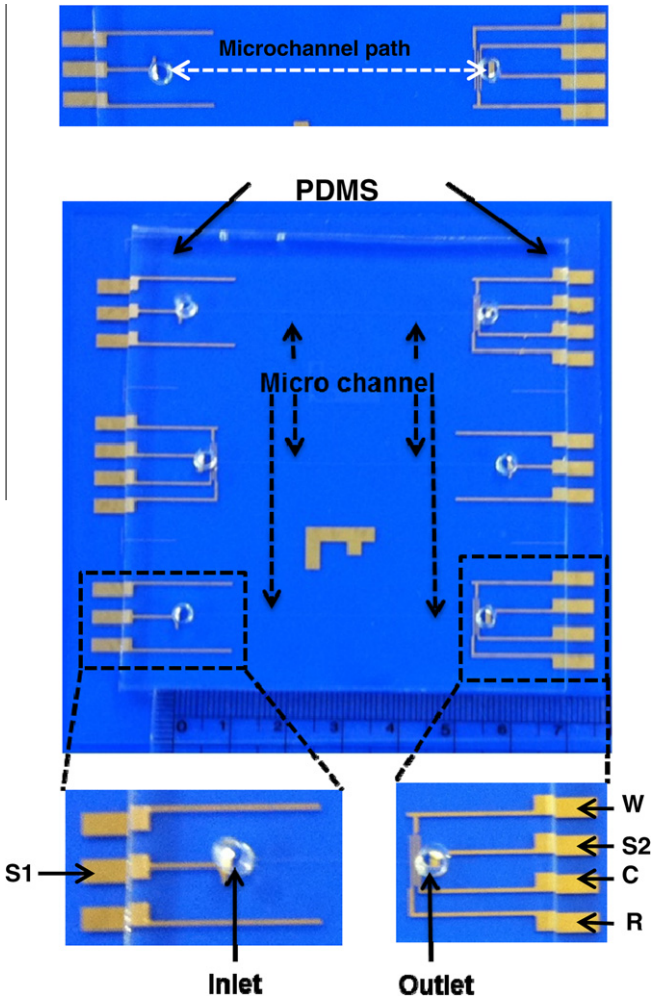


Fig. 2. Image of CE–AD microchip showing microchannel (5 cm in length) engraved in PDMS mold, sample reservoirs, silicon tubes, gold electrodes (S1 and S2: separation electrodes, C: counter, W: working, R: reference). The sample is injected into the inlet and gets resolved within the microchannel while migrating towards outlet reservoir.

an Ag/AgCl electrode as reference and a gold electrode as working electrodes were used for all electrochemical experiments in bulk system. Through these CV experiments, we could find the detection voltage(s) to be applied in CE–AD device and the peak current range that these chemicals would generate. On the other hand, pulsed amperometric detection was performed using in-house built potentiostat.

The potentiostat array device having two potentiostat channels was assembled in our laboratory using simple opamp circuits. The electronic circuit within the potentiostat housed two general purpose opamps for each measurement channels. One of them (LM 348N form Texas Instruments, USA) was used as voltage followers and comparator. The precision opamp OP 177AZ (from Analog Devices, USA) was used as current to voltage converter. This analog circuit was interfaced with analog to digital converter (ADC) card (NI USB 6212) form National Instruments, USA. The device was controlled using a program developed with LabVIEW (National Instruments, USA) code. It was possible with this device-software interface to apply fixed or variable bias on counter/reference electrode combinations and read the output current as a consequence of redox activity on working electrodes of each channel simultaneously. The measurement data could be plotted online as well as stored for offline use. The same principle was applicable while maintaining pulsed amperometric bias on the in-channel microelectrodes on the device, while measuring the detection current using potentiostat in a sensitive manner.

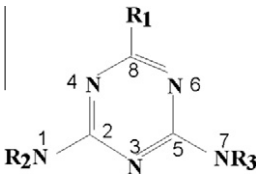
Electrochemical measurements were carried out in aqueous solution in the presence of 200 mM of KCl in methanol (1:1) as supporting electrolyte for detection voltage determination. At first, this separation medium (as well as supporting electrolyte) was filled in the microchannel using silicone tubes and precision syringe pump (KD Scientific, USA) while avoiding air bubble formation inside the channel. The volume of each reservoir was about 35 μL. These were completely filled with supporting electrolyte to avoid negative hydrodynamic pressure on electrophoretic migration of analytes due to volume difference between reservoirs. Then the channel was filled with 1.5% agarose (prepared in separation medium by warming over a magnetic stirrer for 20 min) and left for 30 min to let it semi-solidified within the channel. Care was taken to keep the temperature of agarose solution to about 40 °C while injecting it into the microchannel.

Subsequently, 1 μL of the triazine sample (also diluted to appropriate concentration with separation medium) 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 reservoir and the waste reservoir. The separation potential was switched on immediately after sample addition, thereby limiting the rate of sample mixing into reservoir; therefore, the concentration of the analyte that we reported throughout this study represented 1 μL volume that was injected into the reservoir. The pulsed amperometric detection was performed with three-electrode configuration (Fig. 2) placed in the path of analyte flow, while maintaining a 2 s interval for a pulse of 0 and −0.8 V (peak to peak). 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 lab-built potentiostat.

3. Results and discussion

Simazine, atrazine and ametryn with their corresponding structures, molecular weights, and selected ion monitoring (SIM) are summarized in Table 1. Conventional CV analysis of these three triazines was performed using three electrodes (gold working, Pt counter and Ag/AgCl reference) in a beaker, using 1 mM of each triazine samples. A solution of 200 mM KCl in (1:1) methanol:H_2O was used as the solvent as well as supporting electrolyte because atrazine, simazine and ametryn molecules have poor solubility in water. The KCl solution also proved useful, as triazine molecules were redox inactive at neutral or alkaline pH, under present experimental conditions. For CV, the potential was cycled between 0.4 and −1.0 V with a scan rate 100 mV/s. Both atrazine and simazine produced defined reduction peaks in the cathodic scan at −0.70 V, whereas ametryn showed at −0.80 V, without any anodic peak at

Table 1
Three *s*-triazine herbicides with corresponding structure and molecular weights.



Triazines	R_1	R_2	R_3	Molar mass (g/mole)
Simazine	Cl	Ethyl	Ethyl	201.66
Atrazine	Cl	Ethyl	Isopropyl	215.68
Ametryn	SCH_3	Isopropyl	Isopropyl	227.33

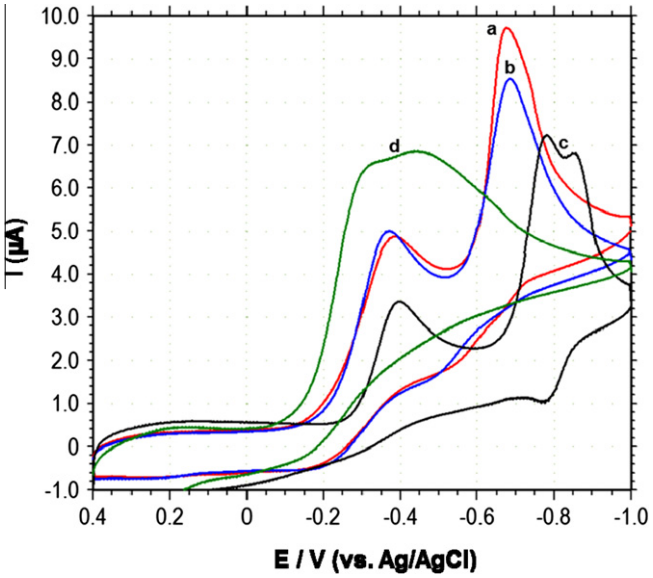


Fig. 3. Cyclic voltammograms of atrazine (a), simazine (b), ametryn (c) in 200 mM KCl in (1:1) methanol:H_2O using conventional electrodes. They showed a characteristic cathodic peak at −0.70 V for both simazine and atrazine, and −0.80 V for ametryn, without any anodic peak at reverse scan between a potential of −1.0 to 0.40 V at scan rate of 100 mV s^{-1}. Green line (d) represents control CV of 200 mM KCl in (1:1) methanol:H_2O. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reverse scan, indicating that the cathodic peaks were due to irreversible electron transfer process (Fig. 3). These reduction peak voltages were subsequently applied for pulsed amperometric analysis.

To achieve a distinct separation, the microchannels were filled with 1.5% of agarose gel. The agarose was dissolved in 200 mM KCl in (1:1) methanol: H_2O for 30 min followed by cooling down to around 40 °C before injecting it into the prewashed (with methanol-KCl) microchannel through reservoir using a syringe. 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 a small aliquot of 1 μl 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. The resulting electropherograms are shown in Fig. 4. In these experiments, it is found that the migration time of simazine (Fig. 4 curve-a, 58 s, SD 3.08 s, n6, reproducibility = 94.7%), atrazine (Fig. 4 curve-b, 66 s, SD 3.13 s, n8, reproducibility = 95.2%) and ametryn (Fig. 4 curve-c, 74 s, SD 2.79 s, n4, reproducibility = 96.2%) could be resolved satisfactorily using agarose. These results indicated the feasibility to use this system in quantitative analysis of such pesticides by separating them from a mixture, which otherwise should have been a cumbersome and expensive task. On the negative side, the device could not be used for multiple analysis due to electrode poisoning after one measurement. Nevertheless, as it was designed as a disposable chip, we could still use it for single use for sensing herbicides in a fast and sensitive manner.

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 established by measuring peak heights obtained by electrophoretic separation of different concentrations of triazines ranging from 1 nM to 1 mM (Fig. 4, curves-d). The calibration plots obtained for each analyte represented straight line correlation between peak current and concentration. The LabVIEW software used along with our potentiostat permitted a peak-by-peak statistical evaluation for

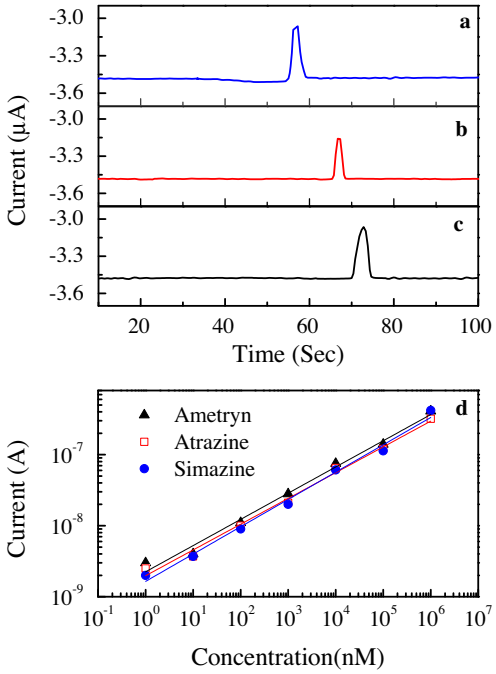


Fig. 4. Electropherograms for CE–AD of (a) simazine, (b) atrazine and (c) ametryn showing detection peaks at 58 s, 66 s and 74 s respectively; (d) Calibration curves for simazine (●), atrazine (▲) and ametryn (○). Error bars represent standard deviation ($n = 4$) of data. The *X* and *Y* axis are shown in logarithmic scale.

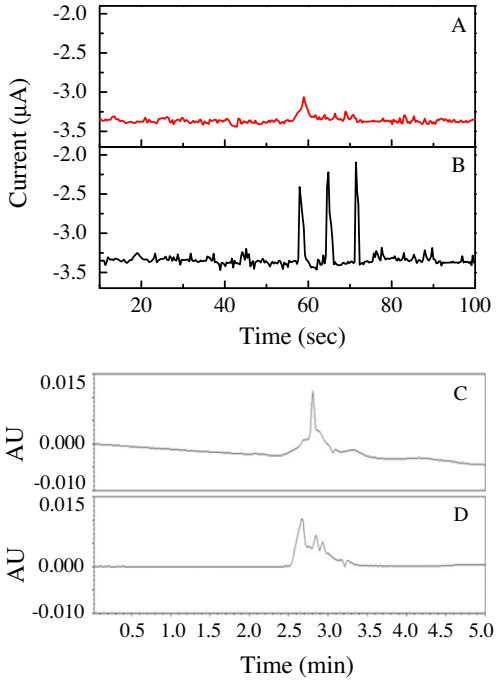


Fig. 5. Electropherograms for CE–AD analysis of (A) soil extract and (B) soil extract spiked with 5 mM each of simazine, atrazine and ametryn; analysis of same samples using HPLC: (C) soil extract and (D) soil extract spiked with 5 mM each of simazine, atrazine and ametryn.

each peak of the chromatogram to establish the repeatability of instrument performance for assessing compliance within specified regulatory guidelines. The sensor thus had a working range between 1 nM and 1 mM with low standard deviation between measurements (only around 0.6–0.7 μA SD for the highest concentration of each analytes). Due to difficulties in solubilizing

these herbicides beyond 1 mM concentration, no measurements were obtained beyond this level. The response time for detection of these herbicides was less than 90 s, which was impressive when compared to HPLC analysis (around 3 min), which is one of the most established protocols for detecting these chemicals (Fig. 5). Moreover, the limit of detection (LOD) for the sensor was calculated as 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 (data not shown). This suggested that the chip can successfully detect the concentration of simazine, atrazine or ametryn in soil or water.

We analyzed soil samples collected from local sources using proposed CE–AD method vis-à-vis well established existing method of HPLC. The chromatogram in Fig. 5A represents the response of analyzing the soil extract while Fig. 5B represents analysis of spiked mixture consisting of 5 mM of each compound. The results show a small amount of simazine was natively present in the soil extract. The samples were reanalyzed using HPLC method with CAP column and UV–VIS detector (220 nm). The results thus obtained (Fig. 5C and D) proved the validity of CE–AD method, which also showed the presence of simazine in native soil extract and to some extent could resolve the mixture of herbicides in spiked sample (5 mM mixture each of three triazines). The prominence of the analyte peaks obtained using CE–AD method in spiked soil extract, while in comparison of HPLC, is apparent and illustrates the effectiveness of the microfluidic chip over the HPLC in regard to separation or herbicide mixture in soil.

4. Conclusion

We have successfully tested a microfluidic chip for separation and detection of a mixture of pesticides from the environment. Information on the structure of CE-separate triazine peaks could be determined from pulsed amperometric data, even when the triazines were of the same charge and similar structure. 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.

Acknowledgments

This work was supported by Chinese Academy of Sciences visiting professorship for senior international scientists. We are also deeply thankful to Regional Research Center at Myongji University for the support of HPLC experiments.

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