Research Article

Electrochemical Sensing System Utilizing Simazine-Imprinted Polymer Receptor for the Detection of Simazine in Tap Water

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Received 30 December 2008; Accepted 21 May 2009

Recommended by Yongxiang Li

A simazine sensing system, composed of column packed with a molecularly imprinted polymer (Sim-MIP) and an electrochemical analyzer, was scaled down in order to easily determine the concentration of simazine, an environmentally restricted chemical, in tap water. In order to enhance the detection limit, the ratio of the eluent (dilution rate) in the electrolyte was optimized to 10%. A new in-house built column size with $\varnothing = 1.5$ mm was prepared, and 3 mg of Sim-MIP particles was packed in the column. During the sensing process, 90% of the simazine loaded to the column was collected by elution. The reductive current of simazine was determined up to $1–10 \mu M$. Solid phase extraction through the Sim-MIP column enabled simazine to be selectively detected from a mixed aqueous solution containing structural analogues in the range of 10–40 nM. Whether the concentration of simazine in tap water had reached environmentally restricted levels (10–40 nM) was determined within 1 hour using this system.

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1. Introduction

Recently, triazine herbicides have been widely used to effectively increase the quantity of agricultural produce. Triazine herbicides are therefore distributed in the soil or irrigation water and may be spread to adjacent areas by wind, stream runoff, or evaporation. These herbicides are persistent in the natural environment and are therefore restricted to certain concentration levels for safety reasons [1].

Among the triazine herbicides, simazine has been commonly used around suburban areas, such as golf courses and dry riverbeds. However, simazine is suspected of being an endocrine disrupting chemical. According to the water-purity standards of the World Health Organization (WHO), tap water that is contaminated with 10 nM (2 ppb) of simazine is considered to be harmful. However, it is difficult to remove such trace amounts of simazine from tap water using common water purification systems.

In addition, there are few reports of simazine monitoring systems, despite numerous reports of sensors for atrazine, which belongs to the group of s-triazine herbicides that includes simazine [2–7]. Therefore, a monitoring method that can be used to trace concentrations of simazine in untreated water is required.

In order to detect simazine, focus was made on molecularly imprinted polymers (MIPs) as a recognition element and a selective binder to simazine. We investigated the preparation of an imprinted polymer for simazine (Sim-MIP) from methacrylic acid (MAA) andethylene glycol dimethacrylate (EDMA) as a sensor receptor for the construction of a simazine sensing system [8]. The electrochemical reduction of simazine by voltammetry was reported using a dropping mercury electrode [9]. We proposed a novel sensing system for simazine based on determination of its electrochemical coupling with Sim-MIP, which functions as a recognition element. The prepared Sim-MIP is more selectively bound to simazine under aqueous and acidic conditions through a hydrogen bond with the carboxyl group of MAA, compared with other triazines, such as atrazine and propazine. The sensing system was constructed from Sim-MIP particles packed in a column for the selective collection (solid phase extraction) of simazine and an
electrochemical analyzer, in which an amalgamated gold electrode was used as the working electrode in place of a dropping mercury electrode [10]. Use of the amalgamated electrode with electrochemical analyzing system provided a linear relationship between the concentration of simazine and the reductive current of simazine in the range of 10–30 μM. For the detection of 10 μM of simazine, 10 mL of a 100 nmol simazine solution was applied to the Sim-MIP packed column and eluted to a volume of 10 mL. The simazine in the eluent was then determined in a commonly used electrochemical vial, in which the electrodes were placed. Simazine at a level of 10 nM (WHO water-purity standard) could be detected with this system. The distinctive feature of the Sim-MIP solid-phase column is that a simazine concentration as low as 10 nM could be extracted. Therefore, for the detection of 10 nM simazine, a large volume (10 L) of 100 nmol simazine sample solution was applied to the column and eluted to a volume of 10 mL and then electrochemically analyzed. The results indicated that the column concentrated the simazine significantly; however, it took more than 10 hours to apply the entire sample solution to the column at a flow rate of 5 mL/min. Moreover, the obtained response showed low stability, because the large volume of sample solution was continued to be loaded onto the column. Although the performance showed that the system was applicable to a low concentration of simazine, it took an excessive amount of time, and also stable extraction was not easily achieved. Therefore, improvement of the sensing system is necessary to analyze the legally restricted concentration of tap water samples.

To establish a more efficient system for the evaluation of practical concentrations of simazine in the environment, the sensing system was improved to detect environmentally restricted concentrations of simazine in tap water within a short time frame. In order to reduce the time, the electrochemical cell setup and the Sim-MIP packed column were optimized. Basically, reduction of the volume of sample solution loaded to the column is required, which takes most of the time for the sensing process. Considering this, the system was scaled down by constructing an in-house built disposable column to realize a practical monitoring system. The optimum sensing system enabled simazine at environmentally restricted concentrations in tap water to be easily monitored within a time frame of 1 hour.

2. Experimental

2.1. Reagents. 6-Chloro-N,N-diethyl-1,3,5-triazine-2,4-diamine (simazine) and propazine were purchased from Wako Pure Chemicals (Osaka, Japan) as standard chemicals, and atrazine was purchased from Kanto Chemicals (Tokyo Japan). The structures of these herbicides are shown in Figure 1(a). Dimethylformamide (DMF), MAA, and EDMA were distilled prior to use in order to remove stabilizers (Figure 1(b)). Aqueous solutions of the triazine herbicides were prepared using water purified to 18.3 MΩ with a Milli-Q SP system (Millipore). All other chemicals were of laboratory grade and used without further purification. Stock solutions of the herbicides were prepared at 2.0 mM by dissolving an appropriate amount of the triazine herbicide in methanol. Sample solutions were prepared by dilution of the stock solutions with 0.1 M KCl.

2.2. Preparation of MIP. The MIP for simazine was prepared using a previously reported procedure [8]. Simazine (1.34 mmol), MAA (9.99 mmol), EDMA (26.88 mmol), and aminobutyronitrile (0.61 mmol) were mixed and dissolved in DMF (30 mL). After the solution was purged with dry nitrogen gas for 10 minutes, it was polymerized using UV light irradiation at 4°C for 5 hours. The obtained polymer was crushed in a mortar and sieved to between 30 and 60 μm diameter size. To remove the template (simazine), the polymer was washed three times in methanol/acetone mixture (7:3 v/v).

2.3. Electrochemical Determination. In order to scale down the system, the electrochemical measurements were performed using a computer-controlled electrochemical analyzer (ALS 1200, Tokyo, Japan) and a three-electrode electrochemical cell setup in a glass vial that required only 1 mL of analyte solution. By reducing the volume of the electrochemical measurement solution from 10 to 1 mL, the initial sample solution used to load the column can be reduced. An amalgamated electrode as a working electrode was prepared on a gold electrode (Φ = 1.6 mm, BAS-Au) according to the recommended procedure given in the BAS Co. technique guide [11]. An Ag/AgCl (3 M NaCl) reference electrode and a auxiliary platinum wire electrode (BAS, Tokyo) were used.

All cyclic voltammetry electrochemical measurements were carried out in a glass vial containing a deoxygenated electrolyte (0.1 M KCl, pH 3.0) at room temperature. Simazine exhibited a reductive current at −1.08 V during the measurement at the scan rate of 0.1 V s⁻¹. The cathodic current difference at −1.08 V, with and without simazine, was recognized as the reductive current of simazine.

2.4. Sim-MIP Packed Column and Solid Phase Extraction (SPE). In our previous study, a commercially available column (I.D. = 3 mm, Eyela) was used, in which 20 mg of dried Sim-MIP particles were packed (Figure 1(c)) [10]. In this study, a small in-house built column (I.D. = 1.5 mm; Figure 1(d)) was prepared as an inexpensive setup to reduce the volume of the sample solution and to allow more stable and simpler extraction of simazine than the previous sensor setup. 3 mg of Sim-MIP particles were packed in the column. The procedure for sample application to the column was performed according to our previous report, as shown in Figure 1(e) [10]. In the first step, the sample solution was loaded and adsorbed onto the column packed with Sim-MIP. Dichloromethane was used to wash impurities from the Sim-MIP column resulting from sample solutions containing impurities, such as atrazine and/or propazine, as well as simazine. After removal of the impurities, methanol was loaded onto the column as the eluent to break the hydrogen bond between simazine and MAA. The eluent
Figure 1: Schematic diagram of electrochemical sensing system for simazine. (a) Triazine compounds, (b) structural formula of MAA as functional monomer and EDMA cross-linking monomer, (c) the column packed with 20 mg of Sim-MIP, (d) the column packed with 3 mg of Sim-MIP, and (e) electrochemical sensing system combining the Sim-MIP packed column with electrochemical analyzing setup, a: an amalgamated electrode, (b) a vial containing 1 mL of solution, and (c) Ag/AgCl reference electrode.
KCl solution as the electrolyte [8]. Simazine is electroreductive; the current of simazine was obtained for a pH 3.0 cathodic peak was plotted against the concentration.

In our previous study, the highest electrochemically measured by cyclic voltammetry. The flow rate of the loading solutions was 2.0 mL/min.

3. Results and Discussion

3.1. Electrochemical Measurement of Simazine on Amalgamated Gold Electrode. In our previous study, the highest reductive current of simazine was obtained for a pH 3.0 KCl solution as the electrolyte [8]. Simazine is electrochemically reduced through protonation, which precedes the electron-transfer reaction [9]. The electrochemical reaction of simazine has to be performed in acidic media. However, the reductive current of simazine is hardly detectable at a pH lower than 2.0; therefore, 0.1 M KCl (pH 3.0) was used as the electrolyte in the experiment.

The electrochemical detection of simazine was then performed after elution, in which simazine was eluted by methanol and mixed with the electrolyte. To reduce the detection time, reduction of the volume of simazine solution loaded onto the column is required; however, this would also result in reduction of the amount of simazine available for the electrochemical measurement. Therefore, optimum conditions for the sensitive electrochemical determination of simazine must be determined for this sensing process. For this purpose, the smaller volume of electrolyte is better, because simazine in the eluent would not be diluted. In other words, the dilution rate should be as low as possible.

Therefore, dilution of the eluent (methanol) by the electrolyte was examined at 1%, 10%, 20%, and 30%. The reductive current and peak potential of simazine (10 μM) at various dilutions were compared. The reductive current difference with and without simazine was also compared. The peak current of simazine was the highest at 10% methanol in the electrolyte, while there was no significant difference between the peak current at 1% and 10% dilution.

Each peak potential was −1.02 V at 1% methanol and −1.08 V at 10%. Higuera et al. reported that the peak current of simazine increased with the decrease in the peak potential in the range from −1.0 to −1.1 V due to changing proton concentration [9]. The results obtained are consistent with their study; therefore, the electrochemical measurement of simazine was performed at 10% methanol. For ratios higher than 10%, the reductive current of simazine decreased and was not observed at 30%. The peak potential shifted in the negative direction and overlapped the reductive potential of the proton itself, at around −1.2 V, and the electrochemical reduction of simazine and the proton compete at a higher concentration of methanol.

Based on this result, for the electrochemical determination, one hundred μL of the eluent was mixed with 900 μL of electrolyte solution to determine the concentration of simazine in 1 mL of the measurement solution. In addition, at volumes less than 1 mL of solution, the simazine did not reduce for stable electrochemical determination because of the change in dilution. Basically, it is difficult to completely maintain the diluted concentration of a mixed solution composed of methanol with the electrolyte at every measurement, because methanol is a volatile solvent. Moreover, simazine is a poorly water-soluble compound, so that precipitation would occur, even for a slight decrease in the methanol ratio of the mixed solution for a highly concentrated simazine solution. Thus, it was determined that for the electrochemical determination of simazine in a mixed measurement solution, 100 μL of methanol with 900 μL of electrolyte would be the optimum condition. As a result, the reductive peak current was dependent on the concentration of simazine up to 10 μM, and the electrochemical detection limit of simazine was 1 μM, as shown in Figure 2.

3.2. Improvement of the Sim-MIP Packed Column. The environmental standard for the simazine concentration in water is legally restricted to 19.8 nM (4 ppb) by the U.S. Environmental Protection Agency, 9.9 nM (2 ppb) by the World Health Organization, and 14.9 nM (3 ppb) in Japan. In our previous study, a sensing system was constructed by packing 20 mg of Sim-MIP particles in a commercially available column, which was coupled to an electrochemical analyzing system including an amalgamated gold electrode, and this system could detect the environmental restricted concentration of simazine [10]. In this system, a 100 nmol simazine sample solution (10 nM × 10 L) was loaded on the Sim-MIP packed column and eluted using 1 mL methanol; however, a large volume of the simazine solution (10 L) must be loaded to collect 100 nmol of the simazine, requiring more than 10 h. This is not a suitable amount of time to detect a trace concentration of simazine. 1 μM of simazine in 1 mL (containing 1 nmol of simazine) was shown to be the limit of detection for the electrochemical system. To reduce the column loading time, we have proposed scaling down of the system, and the volume was reduced to 10% of that used in the previous study. However, the detection limit of the amalgamated gold electrode is 1 μM, which is higher than the environmental limit for simazine (10 nM).
In tap water. Therefore, from such a trace concentration of simazine, 100 nmol has to be collected in the fabricated sensing system.

The examined column conditions are listed in Table 1. The recovered simazine in the eluent was determined by measurement of the UV light absorbance of simazine (λ_{max} = 220 nm). When 1000 mL of the sample solution (10 nM) was loaded on a column packed with 20 mg of Sim-MIP, the recovery was 96.5%; however, in the case of 100 mL of sample loaded on the column, simazine was not detected in the eluent (0.10 mL). We consider that the volume of eluent may be insufficient for the size of the column. In order to take enough time to elute simazine with a small volume of eluent, the inner diameter of the column was reduced to half of the previous column, and was packed with 3 mg of Sim-MIP. When 100 mL of the sample was loaded on this column, the recovery in the eluent was 90%, so that it can be used as a small volume (1 mL) for electrochemical detection (Figure 1(e)b). When 100 mL of the sample was loaded on the column and eluted by 100 μL of methanol, elution was completed within 1 h at a flow rate of 2 mL/min, as shown for system 3 in Table 1. Based on the calibration curve obtained, using this system, a linear relationship was observed in the concentration range of 10–40 nM. The sensitivity and reduction of the measurement time were improved by utilizing a small 3 mg Sim-MIP packed tube column, which was cheaper than that previously used (Figure 1(c)).

3.3. Selective Detection Of Simazine. With the use of an amalgamated gold electrode, triazine compounds such as atrazine and propazine exhibit similar CV profiles and cathodic peaks similar to simazine. In our previous study, we examined the interference of atrazine and propazine in the determination of simazine, and the washing process using dichloromethane was so effective in removing the nonspecific adsorption of these compounds, and simazine was selectively detected, even from a sample solution also containing atrazine and propazine [10]. If compounds other than triazine herbicides are present in the tap water, these compounds would not be electrochemically detected in the negative potential area of the simazine cathodic peak, because water soluble and hydrophilic compounds are not adsorbed on the hydrophobic polymer of the Sim-MIP column.

In order to examine the effect of other triazine compounds, calibration plots of simazine with and without impurities such as atrazine (10 μM) and propazine (10 μM) were compared.

The sample solutions were eluted through the Sim-MIP packed column using the extraction procedure shown in Figure 1(e). The reductive current of the sample solution, both with and without impurities, showed a linear concentration dependence, and the slope was −0.0204 μA/μM and −0.0200 μA/μM, respectively, and the calibration plots were almost the same in the examined range of simazine concentration (Figure 3). Therefore, when using this procedure, the presence of other triazine compounds has no effect on the measurement sensitivity.

3.4. Simazine Detection in Tap Water. Sample solutions of tap water spiked with 10, 20, or 40 nM of simazine were examined, and the concentrations were determined as 8.75, 22.5, and 43.5 nM, respectively. The simazine concentration found in tap water was almost the same as that expected, suggesting that there was no significant influence from unknown impurities in the tap water. Thus, it was shown that the electrochemical sensing system proposed in this study is applicable for the simple detection of environmentally restricted concentrations of simazine (10–40 nM) in tap water within a period of 1 hour.

4. Conclusion

A new electrochemical sensing system was fabricated using an in-house built column packed with 3 mg of Sim-MIP, coupled with an electrochemical analyzing system with an amalgamated gold electrode and a cell setup requiring 1 mL of analyte solution. 100 mL of a trace concentration of simazine solution was applied to the column, and simazine
was eluted by 100 μL of methanol. One volume of eluent was mixed with nine volumes of electrolyte (0.1 M KCl, pH 3.0) for the electrochemical determination. The fabricated sensing system could specifically detect simazine in the range of 10–40 nM, even though there were other triazine compounds present. The environmental restricted concentration of simazine (10–40 nM) in tap water was determined within 1 hour. This sensing system provides a portable and low-cost method for the practical application of simazine sensing.

Acknowledgments

This work was supported by the Grant-in-aid from the Ministry of Education, Culture, Sports and Technology and the Open research project from Soka University and from Health Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST).

References


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