Portable Agrichemical Detection System for Enhancing the Safety of Agricultural Products Using Aggregation of Gold Nanoparticles

Seung Hoon Baek,†§ Seung Woo Lee,†§ Eun Ju Kim,† Dong-Hyuk Shin,‡ Seog-Won Lee,‡ and Tae Jung Park*†§

†Department of Chemistry, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Republic of Korea
‡Well Korea Corporation, 44 Techno 11-ro, Yuseong-gu, Daejeon 34036, Republic of Korea

Supporting Information

ABSTRACT: Organophosphorus (OP) and triazole chemicals have been commonly used as insecticides and fungicides to protect agricultural foods from harmful insects and fungi. However, these agrichemicals sometimes remain after the foods are distributed, and this can result in serious health and environmental issues. Therefore, it is essential to detect OPs and triazole chemicals in agricultural products. Nowadays, many detection techniques for OPs and triazole chemicals are expensive and time-consuming and require highly trained technicians. Thus, particularly rapid, simple, and sensitive detection methods are in demand for on-site screening of agrichemicals. Gold nanoparticles (AuNPs) have been utilized for applications in analytical assays and real-time monitoring in the biosensor field because of their biocompatibility and outstanding size-dependent optical properties. In this study, we used AuNPs as a detection probe, which have a size of 17 nm in diameter, a red color, and the absorbance peak at 520 nm. When imidazole was added to AuNPs mixed with the agrichemicals, the AuNPs aggregated and their colors changed to purple, causing the appearance of a new peak at 660−670 nm, which could be measured within approximately 20 s. Moreover, we developed a novel device for multiple agrichemical detections using an AuNP-aggregation-based spectrometric detection system. This portable device is light, simple, fast, and highly sensitive as well as selective. With this system, agrichemical residues can be easily detected on the spot at a low cost and in a short reaction time.

INTRODUCTION

To protect agricultural foods from harmful insects and fungi, insecticides and fungicides composed of organophosphorus (OP) and triazole chemicals have commonly been used.1−3 However, these agrichemicals sometimes remain after the foods are distributed, and this can result in serious health and environmental issues.4−7 When people ingest agrichemical residues from agricultural products, for example, the residues work as inhibitors of acetylcholinesterase, which is a crucial enzyme that catalyzes the hydrolysis of acetylcholine.8−10 This could eventually lead to serious health problems such as cancer, neurological disorders, Parkinson’s disease, asthma, and lymphoma.11−13 For this reason, there has been much interest in the detection of agrichemical residues, and many detection methods have been researched and developed.

The widely used methods for detecting agrichemicals are high-performance liquid chromatography (HPLC),14,15 gas chromatography-mass spectrometry,16,17 enzyme inhibition, enzyme-linked immunosorbent assay,18,19 and surface-enhanced Raman scattering.20−22 These methods show high sensitivity and selectivity, which enable the detection of agrichemicals at low concentrations in food, water, and soil. However, most of them are difficult to handle and time-consuming and require complicated equipment and expensive reagents. Therefore, a portable, simple, and rapid detection system for the determination of agrichemical residues is urgently needed.

Recently, many detection techniques using the aggregation of nanoparticles such as iron-oxide, silver, and gold nanoparticles (AuNPs) have been developed to overcome the disadvantages of the previous methods.23−25 These detection approaches are suitable for on-site detection systems because nanoparticle
aggregation is fast, simple, and easy to confirm through the color and state of the resultant solution. There are notable examples to show this simple and fast detection approach trend. Gao et al. reported a magnetic lateral flow immunochromatographic assay for detecting residues of paraoxon-methyl by using iron-oxide particle aggregates. Xiong and Li have presented a colorimetric pesticide detection method. Calixarene-modified silver nanoparticles underwent aggregation after being combined with specific pesticides and changed color. An AuNP-aggregation-based spectrometric detection system was developed in our group. When agrichemicals were added to the AuNP solution with imidazole, strong aggregation occurred, and the agrichemical concentrations could be calculated using the absorbance response at 670 nm. However, they reported about the detection of agrichemical residues on only grains but not fruits and vegetables.

Herein, we confirmed whether an AuNP-aggregation-based detection system that was previously developed by our group is also suitable for fruits, particularly strawberries. Furthermore, we designed a small and lightweight device with five channels for on-site and multiplex detection (Scheme 1). Finally, we analyzed the sensitivity and selectivity of the developed device. Moreover, the reliability of this device was investigated by comparing its results with HPLC results. The proposed portable device is a promising candidate for practical application in on-site trace analysis in food safety, homeland security, and environmental monitoring.

## RESULTS AND DISCUSSION

### AuNP-Based Agrichemical Detection System

The key to the detection system using AuNPs is their aggregation. The fact that the aggregation of AuNPs is strongly induced by imidazole and agrichemical complexes rather than only imidazole was shown in previous research.

As shown in Figure 1A, the absorbance peak of AuNPs was observed at 520 nm, whereas the peaks of the other chemicals, imidazole and tebuconazole, were not. In addition, when AuNPs were added to the germicide solution, only the absorbance peak of AuNPs appeared. When an AuNP solution was added to the imidazole solution, weak aggregation of the AuNPs was observed. As a result, a broad absorbance peak appeared between 600 and 700 nm. When the 1 ppm tebuconazole solution was added to the above-mentioned solution, a strong aggregation of AuNPs occurred and a sharp absorbance peak appeared at 670 nm. Figure 1C–E depicts the treatment of AuNP solutions with different substances. Transmission electron microscopy (TEM) was employed to verify the aggregation of the AuNPs. Weak aggregation was observed after the addition of the imidazole solution (Figure 1D); however, with 1 ppm tebuconazole, the AuNPs showed more aggregation (Figure 1E).

The AuNP-based optical sensor was used to detect tebuconazole using the developed portable device, and the ultraviolet–visible (UV–vis spectra) at 670 nm of the AuNPs were measured at different tebuconazole concentrations ranging from 0.0 to 1.0 ppm (0, 0.05, 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0 ppm) (Figure 1B). As the AuNPs form longer chains with increasing tebuconazole concentration, the absorption peak at 520 nm remained and the absorbance intensity at 670 nm increased.²⁶

![Scheme 1. Illustration of the Optical Detection of Agricultural Chemicals through the Aggregation Effect of AuNPs](image-url)
We also investigated the sensitivity of the optical sensing platform using three different harmful agrichemicals, edifenphos, iprobenfos, and diazinon, using the same experimental procedures as those used for tebuconazole. Figure S1A,B shows the changes in the absorbance peak at 600 to 700 nm with various diazinon and edifenphos concentrations (0, 0.05, 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0). Figure S1C shows that the absorbance response at 680 nm increased when the iprobenfos concentration increased from 0 to 0.7 ppm. The absorbance responses of iprobenfos, edifenphos, and diazinon were saturated when their concentrations were higher than 1 ppm. An iprobenfos concentration higher than 0.7 ppm also led to saturation in the absorbance graph. This result suggests that this system allows both the quantitative and qualitative analyses of agrichemicals.

**Design of Portable Device for Fast and Simple Detection.** So far, we have demonstrated a simple and fast system for agrichemical detection. On the basis of this system, we designed a portable device with a five-channel system for spontaneous analysis and with enhanced portability for easy real-field analysis by decreasing the size of the device. Because a clear spectrum of the aggregated AuNPs can be obtained with a darker detecting chamber, the device was developed to be light-tight. The display was a 7 in. touch screen, and the sample holder can be manipulated with a button for automatic loading−unloading. Moreover, to improve the performance of the device and for smooth sample movement, the sample holder system employed a linear motion guide rail. An ESD200 Bluetooth module from Chipsen was introduced; this could enable wireless telecommunication with devices containing a universal asynchronous receiver/transmitter interface within a range of 30 m. For the liquid sample measurement, we designed a spectroscopic optical system using a white LED (450−750 nm) as a light source and a charge-coupled device detector (0.5−2.0 Abs). Using the existing spectroscopic equipment (Synergy H1), the linearized standard curve at 670 nm wavelength was obtained for Tebuconazole. After that, linear equation was incorporated into the developed device for further experimental analysis. Therefore, when the tebuconazole sample was put into the device, the absorbance intensity at 670 nm was measured. Finally, this intensity value was converted to agrichemical concentration from the standard curve equation, and then the value (in ppm) was displayed on the screen. Figure 2 shows the recently developed spectrophotometer system. The development of optical instrument was used for all experiments.

**Detection of Agrichemicals.** The sensitivity of the convenient developed instrument was examined to determine the limit of detection (LOD) for tebuconazole. Figure 3A shows the absorbance spectrum plot at 670 nm of the imidazole−AuNP system treated with tebuconazole at various concentrations. The absorbance response at 670 nm increased as the tebuconazole concentration increased because of the aggregation of AuNPs. Dynamic light scattering (DLS) measurements were performed to verify the aggregation of AuNPs (Figure 3B). The graph shows that the diameter of the AuNP aggregation increased from 415.9 ± 7.7 to 548.8 ± 8.4 nm as the concentration of tebuconazole increased from 0.0 to 1.0 ppm.

A linear relationship between the tebuconazole concentration and the absorbance at 670 nm was observed as the tebuconazole concentration (0.0−1.0 ppm) increased, with a correlation coefficient (R²) of 0.9910 (inset table in Figure 3A).

The LOD for tebuconazole using this assay was 52.0 ppb according to a 3σ calculation. Additionally, other agrichemicals, namely edifenphos, iprobenfos, and diazinon, were also examined using the procedure described above (Figure S3). The results indicated that these samples showed similar limits of detection (~50 ppb) and high linearity and linear regression correlation values. These results satisfy the acceptable limits for...
Agricultural residues presented in the regulations of local governments and the Ministry of Food and Drug Safety in the Republic of Korea for agrichemical concentration. On the basis of these experimental results, the device has the potential to be adopted in real-field analysis owing to its high sensitivity, rapidity, and portability.

Comparison of the Proposed Assay with HPLC. The reliability of the developed device was determined by comparing its results with the calculated values obtained using HPLC, which is one of the sensitive agrichemical detection methods. The present method was used to quantify the tebuconazole concentrations, as shown in Table 1. To demonstrate the accuracy of the developed portable device, HPLC was used to assay several tebuconazole samples.

The results from the developed instrument were generally more accurate than those obtained using HPLC at various tebuconazole concentrations. Also, it was confirmed that the developed instrument was accurate for the detection of diazinon, iprobenfos, and edifenphos at various concentrations (Table S1−S3). Table 1 shows that the developed instrument is more rapid, portable, accurate, and convenient. The results are in good agreement with those from the established instrument. The recoveries of tebuconazole in the samples were between 98.7 and 100.5%. The results indicated that the developed instrument is accurate.

Selectivity in Detection. To evaluate the selectivity of the optical sensor in detecting agrichemicals, various chemicals with structures similar to those of the agrichemical samples, namely, 10% methanol, phosphoric acid, phenol, 1,2-dichlorobenzene, toluene, and xylene, were analyzed. After 1 ppm of the above-mentioned substances was added to the AuNP−imidazole solution, the interferences were tested under the same conditions as those used for the agrichemicals. As shown in Figure 4, no distinct changes in the absorbance peak at 670 nm were observed as each of these interferences was added when compared with the peaks from the agrichemical results. These results clearly indicate that this strategy using the developed device can be used to detect agrichemicals with high selectivity.

CONCLUSIONS

In this study, we developed a simple sensor for the detection of OP and triazole agrichemicals based on the aggregation of AuNPs. The agrichemicals were successfully detected in strawberries using the developed device. The limits of detection calculated for this device were 52, 54, 48, and 45 ppb for tebuconazole, iprobenfos, diazinon, and edifenphos, respectively. These results show that this portable, simple, rapid, and cost-effective device has good sensitivity and selectivity. Moreover, it has a wireless data-transfer function and a central monitoring program. We are convinced that this system based on bioinformation fusion technology can be used in farms, markets, and homes and will contribute to the enhancement of human health.
added to 50 mL of phosphate-buffered saline (50 mM, pH 7.4) solution.

**Determination of the Chemicals.** In a cuvette, 350 μL of a 9 nM AuNP solution was added to 700 μL of agrichemical residue solution and 350 μL of 0.3 mM imidazole solution (AuNP/agrichemical residue/imidazole solution ratio = 1:2:1). UV–vis spectroscopy was conducted at 670 nm using the developed instrument after the above solution had reacted for 20 s. The standard agrichemical residues were dissolved in methanol after dilution, and the solutions were filtered using a 0.2 μm syringe filter. The OP chemicals were quantified using HPLC (Agilent 1260, Santa Clara, CA) using a C 18 column (10 cm length and an inner diameter of 4.6 mm, Eclipse Plus, Agilent). The agrichemical residues were analyzed at 35 °C and monitored using the UV absorbance at 250 nm. The agrichemical residue solution was chromatographed with a premixed mobile phase composed of 65:35 (v/v) methanol/water at room temperature. The samples of 100 μL were injected at a flow rate of 1 mL/min.

**Characterization.** TEM images were obtained using a Tecnai G2 F30 S-Twin (FEI, Hillsboro, OR) microscope at an operating acceleration voltage of 300 kV. UV–vis absorption spectra were recorded using a Synergy H1 (BioTek, Winooski, VT) instrument. DLS measurements were completed on a particle size analyzer ELSZ-1000 system (Photol OTSUKA Electronics, Tokyo, Japan). Agrichemical detections were performed with the developed device (WellKeeper) (Well Korea, Daejeon, Korea) within 90 s.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00477.

Additional figures, including figures showing the absorbance changes according to the concentration of agrichemicals, interior and exterior design maps and pictures of the developed device, and standard curve calculated using the absorbance intensity at 670 nm, and comparison of detection results from the developed device with those from HPLC (PDF)

**AUTHOR INFORMATION**

Corresponding Author

*E-mail: tjpark@cau.ac.kr (T.J.P.).

**ORCID**

Tae Jung Park: 0000-0001-8918-0957

Author Contributions

S.H.B. and Seung Woo Lee contributed equally to this work. This study was designed, directed, and coordinated by S.H.B., Seung Woo Lee, E.J.K., and T.J.P. T.J.P. provided conceptual and technical guidance for all aspects of the project. S.H.B performed optical spectrometry and analyzed the data with E.J.K. Seung Woo Lee contributed to the particle size and TEM analyses. Seog-Won Lee and D.-H.S designed and manufactured the portable device. B.S.H performed and analyzed the data from the HPLC and developed the device with Seog-Won Lee. Sensitivity and selectivity experiments were performed by Seung Woo Lee and S.H.B. The manuscript was written by S.H.B., Seung Woo Lee, and T.J.P. and commented on by all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

AChE, acetylcholinesterase; GC/MS, gas chromatography-mass spectrometry; ELISA, enzyme-linked immunosorbent assay; SERS, surface-enhanced Raman scattering; LFIAs, lateral flow immunochromatographic assay; TEM, transmission electron microscopy; UV–vis, ultraviolet–visible; AuNPs, gold nanoparticles; LOD, limit of detection

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