

Attomole Detection of Glyphosate by Surface-Enhanced Raman Spectroscopy Using Gold Nanorods

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Summary

Surface-enhanced Raman spectroscopy (SERS) was used for the detection of glyphosate using gold nanorods derivatized with 4-mercapto phenylboronic acid. Since the introduction of the SERS, nanomaterials have been of significant interest as SERS substrates due to their tunable optical properties. The design and fabrication of a variety of nanomaterials with large SERS enhancement factor have been reported. Gold nanorod particle with controllable size and numerous surface functionalities can be easily synthesized and used as SERS substrates. SERS analysis of the glyphosate at different concentrations in the presence of gold nanorods was carried out and a calibration graph was drawn. According to this procedure, determination of glyphosate in tomato juice was carried out by using developed SERS substrate.

Key Words: SERS, glyphosate, gold nanorods.

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Altın Nano Çubuklar Kullanılarak Yüzeyde Güçlendirilmiş Raman Spektroskopisi ile Attomole Seviyesinde Glifosat Tayini

Özet

Yüzeyde güçlendirilmiş Raman spektroskopisi (Surface Enhanced Raman Spectroscopy, SERS) yöntemi ile 4-merkaptofenil boronik asitle türevlendirilmiş altın nano-çubuklar kullanılarak glifosat tayini yapılmıştır. SERS yönteminin uygulanmaya başlamasından itibaren nano malzemeler, optik özelliklere sahip olmaları nedeniyle SERS yüzeyleri olarak kullanımda büyük öneme sahiptirler. Büyük oranda SERS güçlendirme faktörüne sahip çeşitli nano materyallerin üretimi ve dizaynı mevcuttur. Çeşitli sayıda yüzey fonksiyonuna ve istenilen büyüklüğe sahip altın nano-çubuk partikül kolaylıkla sentezlenebilir ve SERS yüzeyi olarak kullanılabilir. Bu çalışmada altın nano çubukların varlığında farklı derişimde hazırlanan glifosatın SERS analizi gerçekleştirilmiş ve kalibrasyon grafiği oluşturulmuştur. Buna bağlı olarak, hazırlanan SERS yüzeyinde domates suyundaki glifosat tayini gerçekleştirilmiştir.

Anahtar Kelimeler: SERS, glifosat, altın nanoçubuklar.

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INTRODUCTION

Glyphosate (*N*-phosphonomethylglycine) is a post-emergence nonselective herbicide (1) and has long been considered as environmentally safe due to its rapid inactivation in soil, both by degradation and by adsorption (2). Due to its relatively low toxicity to mammals; it has become the most widely used herbicide. The widespread application generates problems regarding environment contamination and therefore, determination of glyphosates in crops, vegetables, and fruits has gained increasing importance (3). For decades, the long-term environmental and ecological effects of glyphosates have been the targets of researches and discussions (4-8).

Glyphosate contains a C-P bond, which is highly resistant to chemical breakdown, and degradation of glyphosate is therefore a biological process involving the enzyme C-P lyase(9). Many microorganisms degrade glyphosate via the degradation products AMPA or sarcosine. In soil, the adsorption of glyphosate is further influenced by phosphate, because glyphosate and phosphate compete for adsorption sites, so application of phosphate may enhance bioavailability and mineralization of glyphosate. On the other hand, in pure-culture studies, phosphate has been found to inhibit the degradation of glyphosate (10).

Most of the methods reported in the literature are based on chromatography separation and determination. Gas chromatographic determination is performed after derivatization of glyphosate to convert it to volatile and thermally stable derivative (11-15). In liquid chromatographic method, UV-visible and fluorescence detections are used for glyphosate derivatives (3,16-18). The glyphosate derivatives show high sensitivity and selectivity in GC and LC determination, but the derivatization procedures are quite complicated and also generate unstable products (19). Ion chromatography (20-23) and capillary electrophoresis (24-26) were also used for determination of glyphosate in different samples. The lack of adequate chemical groups in glyphosate molecule (e.g. chromophores or fluorophores) hampers its measurement by conventional detectors (27).

Since the introduction of SERS, nanomaterials have been of significant interest as SERS substrates, due to their tunable optical properties. The design and fabrication of a variety of nanomaterials with large SERS enhancement factor have been reported (28). Gold nanorod particle with controllable size and numerous surface functionalities can be easily synthesized and used as SERS substrate (29). It is also demonstrated that SERS enhancements were always greater for aggregated gold nanorods than for aggregated spherical gold nanoparticles (30). In this work, we report for the first time, the gold nanorods derivatized with 4- mercaptophenylboronic acid to detect glyphosate by SERS.

EXPERIMENTAL

Instrumentation

Absorption spectra for rod shaped gold nanoparticle solutions were recorded with a UV-Vis spectrophotometer (SpectronicGenesys™ 2PC). A DeltaNu Examiner Raman microscope (Deltanu Inc., Laramie, WY) with a 785-nm laser source and a CCD detector was used. Transmission electron microscopy (TEM) measurements were performed on a JEOL 2100 HRTEM instrument (JEOL Ltd., Tokyo, Japan). TEM samples were prepared by pipetting 10 μ L of nanoparticle solution onto TEM grids and allowed to stand for 10 min.

Chemicals

Hydrogen tetrachloroaurate (HAuCl_4), hexadecyltrimethylammonium bromide (CTAB), 4-mercaptophenyl boronic acid (MPB) and L-ascorbic acid (AA) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Silver nitrate (AgNO_3) and sodiumborohydride (NaBH_4) were obtained from Merck (Darmstadt, Germany). Absolute ethanol was obtained from Sigma-Aldrich (Taufkirchen, Germany). All solutions were prepared using deionized water, 18.2 M Ω .cm free of any organic matter, obtained from a Millipore purification system.

Synthesis and characterization of rod shaped gold nanoparticles

Gold nanorods were prepared using a seed-mediated growth technique with slight modification (30). Seed solution was prepared mixing 7.5 mL of 0.1 M CTAB

and 250 μL of 0.01 M HAuCl_4 solution. Once mixed, 600 μL of 0.01 M ice-cold NaBH_4 was added rapidly to the resulting solution, and allowed to stand for 5 min to form the seed solution.

To prepare rod-shaped gold nanoparticles, 4.75 mL of 0.1 M CTAB, 250 μL of 0.01 M HAuCl_4 , and 60 μL of 4×10^{-3} M AgNO_3 were mixed, respectively. Then 250 μL of 0.1 M AA was added drop wise to the resulting solution, to afford the stock solution. The solution turned colorless after adding 250 μL of AA. Then, 5 μL of seed solution was added to the stock solution. The final mixture was stirred for a few seconds and allowed to stand for 3 h at room temperature to afford nanorods. Gold nanorods solution was centrifuged to remove CTAB from the surface of gold nanorods and washed with absolute ethanol three times.

Absorption spectra for gold nanorods were recorded using a UV-VIS spectrophotometer (Spectronic Genesys™ 2PC). Transmission electron microscopy (TEM) measurements were carried out by a JEOL 2100 HRTEM instrument. TEM samples were prepared by pipetting 10 μL of nanoparticle solution onto TEM grids and the measurements were performed.

Surface modification of gold nanorods and glyphosate sensing

The gold nanorods were interacted with 2 mL ethanol solution containing 20 mM MPB overnight stirring. The gold nanoparticle solution was centrifuged and washed with absolute ethanol three times to remove excess MPB. Then, glyphosate at different concentrations and gold nanorods derivatized with MPB were shaken in ethanol overnight. Remaining glyphosate molecules were removed by washing with ethanol. The prepared solutions were dropped on silicon wafer and ethanol was evaporated for 5 min. This procedure was repeated three times. Then, glyphosate concentrations were detected monitoring the MPB signals by the SERS platform.

RESULTS and DISCUSSION

Gold nanorods were prepared using a seed-mediated growth technique. Thus the seed was synthesized using sodium borohydride as reducing agent. Then,

aqueous colloids of gold nanorods were synthesized using ascorbic acid as reducing agent in the presence of the surfactant CTAB. Figure 1 shows TEM image of rod shaped gold nanoparticles. Based on these images, the gold nanorods have diameters of 40 and lengths of 10 nm. Figure 2 shows the UV-Vis absorption spectra for gold nanorods, which was used to decide the shape of the gold nanoparticles. The first Plasmon band of the gold nanorods indicates the transverse Plasmon band at 524 nm, and the second Plasmon band of gold nanorods indicates the longitudinal Plasmon band at 675 nm.

The gold nanorods were interacted with MPB. The best glyphosate response was obtained when the gold nanorods were interacted with 20 mM MPB. The SERS spectra of gold nanorods are shown in Figure 3, after they were interacted with MPB and different concentrations of glyphosate. In the relevant figure, the Raman shift at 613 nm was

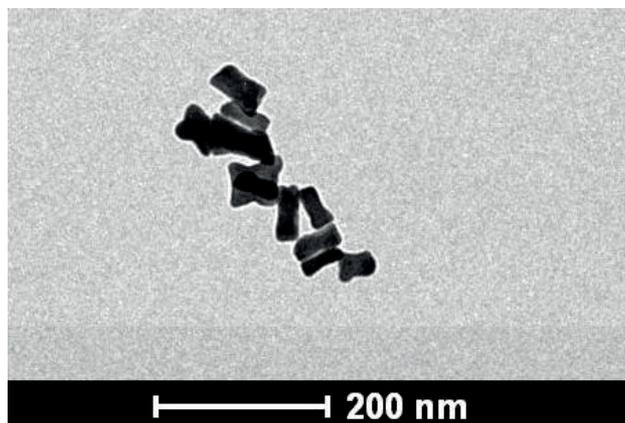


Figure 1. TEM image of gold nanorods.

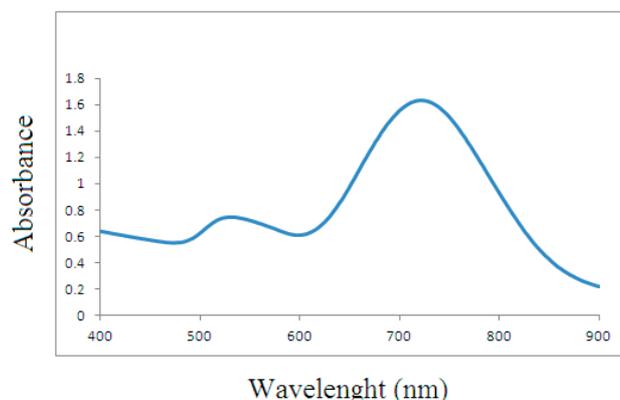


Figure 2. UV-Vis absorption spectra for gold nanorods.

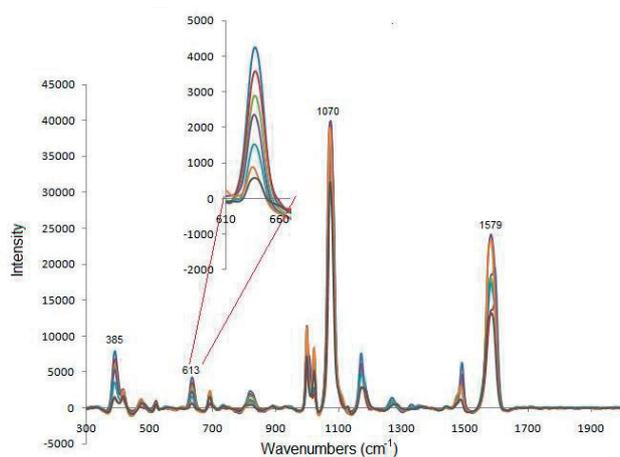


Figure 3. The SERS signals of MPB at different glyphosate concentrations obtained with gold nanorods

increased by increasing concentration of glyphosate, regularly. In Figure 4, a calibration curve plotted for the concentration of glyphosate, is shown. The SERS signal exhibits a linear dependence within the glyphosate concentration range of $1 \cdot 10^{-16}$ mM. The concentration–intensity curve was observed for high correlation coefficients (R^2) as 0.9736.

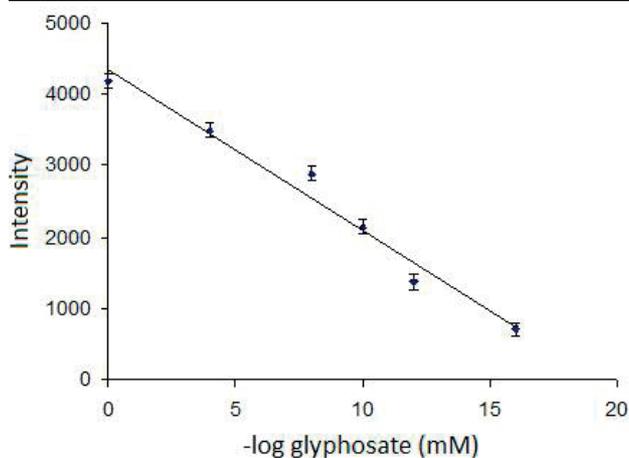


Figure 4. The calibration curve plotted, for the concentration of glyphosate.

Figure 5 shows the structure of the glyphosate molecule. The interaction of glyphosate molecules and MPB is based on diol groups in the structure of glyphosate. When glyphosate molecules were attached to MPB molecules, no new Raman signal was observed. On the other hand, the Raman shift at 613 nm was increased by increasing the

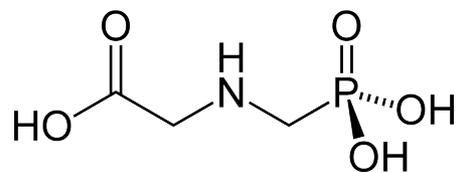


Figure 5. The structure of the glyphosate molecule.

concentration of the glyphosate, regularly. The effect of the MPB concentrations on the AuNPs surface was investigated.

For gold nanorods derivatized with 4-mercaptophenylboronic acid as SERS substrates, raman spectrum were investigated. The peaks at 613, 698, 1000, 1024, 1070, 1184, 1290, 1490, 1583 cm^{-1} were obtained on gold nanorods derivatized with MPB (Figure 5). The SER peaks appeared at 698 cm^{-1} and small peaks at 1024 cm^{-1} due to C-S stretching mode, and 1000 cm^{-1} due to phenyl groups (31,32). The peaks at 1290 and 1490 cm^{-1} were attributed to C-C and C=C stretching modes of the phenyl groups, respectively (33). Raman bands at 1070 cm^{-1} and 1184 cm^{-1} illustrate the B-OH and B-C stretching mode, respectively.

In order to demonstrate the application of the glyphosate sensor in the real sample, we tested the concentration of glyphosate of tomato juice. For this reason, we santrifuged tomato juice at 13000 rpm for 10 min. The residue was discarded and the supernatant was collected. Then, the supernatant was interacted with the gold nanorods derivatized with 4- mercaptophenylboronic acid overnight stirring. Figure 6 shows the SERS signals of MPB in glyphosate in tomato juice obtained with gold nanorods. The recovery was found as 90% (RSD=2.48, n=5) for 10^{-6} mM glyphosate in tomato juice.

Recently, the use of the surface Plasmon properties of Au and Ag nanoparticles have been reported.

We produced gold nanorods derivatized with 4-mercapto phenylboronic acid to develop an easier method to detect glyphosate in juice using the surface Plasmon property of Au.

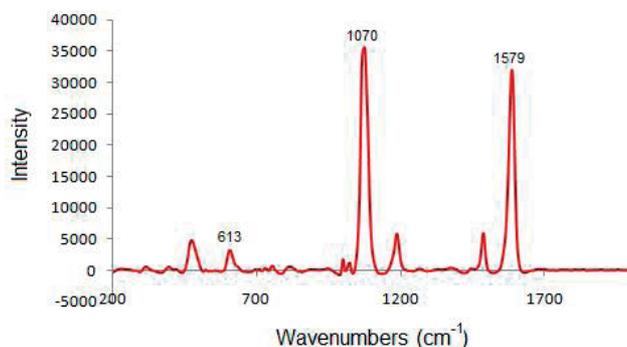


Figure 6. The SERS signals of MPB in glyphosate in tomato juice obtained with gold nanorods.

CONCLUSIONS

The SERS spectra of glyphosate were obtained by 785 nm laser excitation and a calibration diagram was plotted. In conclusion, a method for detecting a pesticide is presented using SERS on gold nanorod. We demonstrated the ability to accurately detect glyphosate at different concentration ranges based on the SERS spectrum of glyphosate with gold nanorod derivatized with 4-mercaptophenylboronic acid.

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