Highly Sensitive Pesticide Detection using
Electrochemically Prepared Silver-Gum Arabic
Nanocluster-based SERS Substrates

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Chip fabrication process

- SiO₂/Si
- Spin coating of photoresist
- Photolithography
- Physical Vapour deposition of the metal electrode layer
- Lift off the extra part
- Deposition of Si₃N₄ protecting layer

Figure S1 Chip fabrication process flow.

Electrochemical deposition setup

- Portable electrochemical device
- SERS substrate fabrication setup
- Chip holder for SERS test
- Chip layout

Figure S2 (A) portable electrochemical device; (B) SERS substrate fabrication setup. (C)(D) chip holder for SERS test. (E) chip layout.

Data processing: (A) Raw data for 0.001 nM 2,4-D; (B) Spectra after baseline subtraction. (C) Sum average of the ten measurements in (B).

Optimization of the Ag-GA nano structure
Figure S4 (A) Current (nA)-time (s) graph for four Ag-GA nanostructures. On the same chip, for structure 1-4, deposition current is around 70 nA, 60 nA, 90 nA, 120 nA respectively; (B) Corresponding SEM images for the four structures; (C) Corresponding microscope (x50) images for the four structures.
Figure S5 (A)-(D) SEM image of the structures one to four.

Figure S6 Particle size marked for structure four.
Figure S7 SEM image tilted at 35° that showed the isolated growth of the nanoclusters on SiO₂.

![SEM Image](image1)

Figure S8 Current (µA)-time (s) graph for Ag deposition without Gum Arabic. (change the order)

![Current-time Graph](image2)

To optimize the Ag-GA nano structure, different concentrations of AgNO₃, voltage settings, concentrations of Gum Arabic, deposition times were studied. With the sample voltage and deposition time (step 1: -0.2 V, 2 s; step 2: -0.8 V, 12 s; step 3: -1.2 V, 2 s), and the same concentration of AgNO₃ (5 mM), by tuning the concentration of Gum Arabic solvent of 1 g/L, 2 g/L, 3 g/L, 10 g/L, 100 g/L, the volume and morphologies of the Ag NPs are different. As shown in figure S3 (A), (B), (C), (D), (E). When the concentration of Gum Arabic larger than 3 g/L, the silver nano particles tend to aggregate, while when the concentration of Gum Arabic
was below 3 g/L, the silver nano clusters distributed separately on the SiO2/Si substrate with nano gap forming in between the silver nano clusters. For 1 g/L Gum Arabic solvent, the silver nanoparticles were decorated by small size nano dendrite on the top; For 2 g/L Gum Arabic solvent, the silver nanoparticles had thicker and larger size nano dendrite decoration on the top compared to 1 g/L Gum Arabic solvent. To study the dependence between deposition time and morphology, 2 g/L Gum Arabic solvent and 5 mM AgNO3 solvent were used, voltage setting: setting 1: step 1: -0.2 V, 2 s; setting 2: step 1: -0.2 V, 2 s; step 2: -0.8 V, 8 s; setting 3: step 1: -0.2 V, 2 s; step 2: -0.8 V, 12 s; setting 4: step 1: -0.2 V, 2 s; step 2: -0.8 V, 16 s; As shown in figure S3 (F)(G)(H)(I), the deposition time increased, the nano dendrites grow bigger. With the same voltage setting (step1: -0.2 V, 2 s; step2: -0.5 V, 8 s; step3: -0.6 V, 2 s.) and the same Gum Arabic concentration (1g/L), different AgNO3 concentration (1 mM, 5 mM, 0.5 M) was used to show the effect of the concentration of AgNO3. As shown in figure S3 (J)(K)(L), at low AgNO3 concentration (1 mM), there was a thin layer of Ag-GA nano particles grow on the SiO2/Si substrate; At AgNO3 concentration of 5 mM, a thicker layer of Ag-GA nano particles grew on the SiO2/Si substrate, as shown in figure S3 (J); at high AgNO3 concentration (0.05 M), the Ag-GA dendrite tended to grow in 3D as shown in figure S3 (L). As a result, an AgNO3 concentration of 5 mM was the optimized parameter for this electrochemical platform. As shown in figure S3 (K), there was a dense coverage of Ag-GA nano particles providing numerous hotspots and the size of the nano particles have good resonance with the laser wavelength (514 nm). To determine the optimized deposition voltage, we used different voltage combinations at the same concentration of AgNO3 (5 mM) and Gum Arabic (2 g/L). Combination 1: step1: -0.2 V, 2 s; step2: -0.5 V, 8 s; step3: -0.6 V, 2 s; combination 2: step1: -0.2 V, 2 s, E2: -0.8 V, 8 s, E3: -1.2 V, 2 s; combination 3: step1: -0.2 V, 2 s, step2: -1.2 V, 8 s; step3: -1.5 V, 2 s; as shown in figure S3 (M)(N)(O). At lower voltage combination (combination 1: step1: -0.2 V, 2 s, step2: -0.5 V, 8 s, step3: -0.6 V, 2 s), the Ag-Ga nano dendrites grow near the Au electrode; at voltage combination 2 (step1: -0.2 V, 2 s, E2: -0.8 V, 8 s, E3: -1.2 V, 2 s), the Ag-Ga nano particles grew on the SiO2/Si substrate with nano dendrites that were decorated on the top of the nano particles; At higher voltage combination (combination 3: step1: -0.2 V, 2 s, step2: -1.2 V, 8 s, step3: -1.5 V, 2 s), irregular growth of the Ag-GA particles occurs, this inhomogeneous growth of Ag-GA particles resulted in dramatically different Raman signal at different points during the 2,4-D detection process. In this electrochemical platform, a voltage below 1.2 V was used.
Figure S9 SEM images of different deposition parameters. (A)(B)(C)(D)(E): different Gum Arabic concentration: 1 g/L, 2 g/L, 3 g/L, 10 g/L, 100 g/L; (F) deposition voltage at -0.2 V, 2
s; (G)(H)(I) E: -0.8 V, different deposition time: 8 s, 12 s, 16 s; (J)(K)(L) different AgNO₃ concentrations: 1 mM, 5 mM, 0.05 M. (M)(N)(O) different voltage settings which effected the growth pattern of the nano gaps. AgNO₃: Gum Arabic=1:1 in all the experiments. All the nanostructures had been electrochemical deposited at least 3 times.

![Chemical structures of Gum Arabic unit. Gum Arabic contains around 39-42% of galactose units, 24-27% of arabinose units, 12-16% of rhamnose units, 15-16% of glucuronic acid units, 1.5-2.4% of protein moieties and 12-16% of moisture. These percentages are different with different gums depending on the acacia trees ages or locations.](image)

Figure S10 Chemical structures of Gum Arabic unit. Gum Arabic contains around 39-42% of galactose units, 24-27% of arabinose units, 12-16% of rhamnose units, 15-16% of glucuronic acid units, 1.5-2.4% of protein moieties and 12-16% of moisture. These percentages are different with different gums depending on the acacia trees ages or locations.¹

![Spectra for the bare substrate in air and after applying DI water.](image)

Figure S11 Spectra for the bare substrate in air and after applying DI water.
SERS substrate fabricated by commercial Gum Arabic

The same experiment was also carried out by using the commercial Gum Arabic, similar peaks for detecting 2,4-D: 290 cm\(^{-1}\), 926 cm\(^{-1}\), 1128 cm\(^{-1}\), 1397 cm\(^{-1}\) show up. The deposition parameters (-0.2 V, 2 s; -0.8 V, 8 s; -1.2 V, 2 s; 5 mM AgNO\(_3\); 20 g/L Gum Arabic) were different from the lab-used one because the type or quality of the gum Arabic were different. Different concentration from 1 nM to 0.06 mM were used for linear calibration. The logarithm trend for \(I_{1130}\ cm^{-1} / I_{1400}\ cm^{-1}, I_{293}\ cm^{-1} / I_{1400}\ cm^{-1}\) VS concentration (in nM) was shown in figure 12 (C). The linear relation between \(I_{1130}\ cm^{-1} / I_{1400}\ cm^{-1}\) (y) and \(\log_{10}\) (concentration (in nM)) (x) was \(y = 0.51 + 0.04\log_{10}(x)\) with \(R^2 = 0.99\). The linear relation between \(I_{290}\ cm^{-1} / I_{1400}\ cm^{-1}\) (y) and \(\log_{10}\) (concentration (in nM)) (x) and was \(y = 0.29 + 0.027\log_{10}(x)\) with \(R^2 = 0.939\). (See figure 12 (D)). The lowest concentration that had been detected by this SERS substrate was 1 nM. The reproducibility of this SERS substrate for \(I_{1130}\ cm^{-1} / I_{1400}\ cm^{-1}, I_{293}\ cm^{-1} / I_{1400}\ cm^{-1}\) is shown in figure 12 (E), the RSD was 5% and 7%.

Figure S12 SERS substrate that was deposited from Gum Arabic for 2,4-D detection. (A) the SEM image for the Ag - GA nano structure; (B) Spectra of 2,4-D at different concentration on SERS substrate. (C) logarithm trend for \(I_{1130}\ cm^{-1} / I_{1400}\ cm^{-1}, I_{293}\ cm^{-1} / I_{1400}\ cm^{-1}\) VS concentration (in nM); (D) Linear fitting line for \(I_{1130}\ cm^{-1} / I_{1400}\ cm^{-1}, I_{293}\ cm^{-1} / I_{1400}\ cm^{-1}\) VS \(\log_{10}\) (concentration) (in nM); (E) the reproducibility of the SERS substrate for 2,4-D at 1 \(\mu\)M. The error bar was from the standard deviation (SD) of ten measurements at random points.
Table 1 Mineral composition of commercial mineral water.

| Mineral composition | water composition mg/L | Mineral composition | water composition mg/L |
|---------------------|-------------------------|---------------------|-------------------------|
| Calcium (Ca$^{2+}$) | 12                      | Potassium (Pb$^{2+}$) | 6                       |
| Sulphates (SO$_4^{2-}$) | 9                      | Silica (SiO$_2$) | 32                       |
| Magnesium (Mg$^{2+}$) | 8                      | Chlorides (Cl$^-$) | 15                       |
| Sodium (Na$^{+}$) | 12                      | Nitrates (N$^3$) | 7.3                      |
| Bicarbonates (HCO$_3^-$) | 74                      |                      |                          |

Table 2 Comparison with literature for 2,4-D study by SERS.

| SERS substrate                                      | Pre-treatment of 2,4-D | LOD       |
|------------------------------------------------------|------------------------|----------|
| functionalized Au@Ag bimetallic; 1h incubating and Shaking. |                         | 0.05 ppb$^2$ |
| functionalized silver nanoparticles (AgNPs)          | Drop the targeted solvent. Add extract chemicals and dry. | 0.1 ppb$^3$ |
| Ag NPs on SiO$_2$                                    | 5.0% ethanol as the solvent | 10 ppb$^4$ |
| AuNPs templated from mesoporous silica film          | No pre-treatment        | 0.01 ppb$^5$ |
| Molecular imprint                                    | Pre-treatment and dry the solvent. | 0.01ppm$^6$ |
| citrate functionalized AgNPs                         | No pre-treatment        | 0.1 ppb$^7$ |
| magnetic-based molecular imprinted polymer nanoparticles (Mag@MIP NPs) | No pre-treatment | 1.47 ppt $^8$ |
| This work                                            | No pre-treatment        | 0.221 ppt  |
Table 3 Comparison with literature for 2,4-D study by other methods.

| Substrate | Detection method | Real sample | LOD       |
|-----------|------------------|-------------|-----------|
| 2,4-D-bovine serum albumin (2,4-D-BSA) conjugate to an integrated micro ring resonator | optofluidic chip | different types of water samples | 4.5 ppt<sup>9</sup> |
| nitrobenzoxadiazole (NBD) and CdTe quantum dots (QDs) | microfluidic paper-based analytical devices | real food and environmental samples | 29.88 ppb<sup>10</sup> |
| pyrrole on a glassy carbon electrode | electrochemical sensor | environmental water samples | 0.183 ppm<sup>11</sup> |
| molecularly imprinted gold nanoparticles-polypyrrole polymer (MIP) modified BiOI nanoflake arrays (BiOINFs)MIP@BiOINFs | visible-light photoelectrochemical (PEC) sensor | soil samples | 0.04 ppb<sup>12</sup> |
| MIP PPy@TiO2 NTs | hotoelectrochemical sensor | / | 3.32 ppb<sup>13</sup> |
| porous graphene (PGR) | electrochemical impedance spectroscopy | toothpaste | 0.332 ppb <sup>14</sup> |
| / | high-performance liquid chromatography | water samples | 0.004 ppb<sup>15</sup> |
| / | liquid chromatographic | soil | 0.1 ppm<sup>16</sup> |
| This work | SERS sensor | River water sample and mineral water | 0.221 ppt |
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