# DETECTION OF GLYPHOSATE BY SURFACE-ENHANCED RAMAN SPECTROSCOPY


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## Introduction

Glyphosate (N-(phosphonomethyl) glycine) (Fig.1) is the most commonly used active ingredient of all time for plant protection in the world. It is used as a non-selective herbicide for weed control before germination and for drying weeds before harvest. If glyphosate is used just before harvest, it can appear in food. Investigations about the harmfulness of glyphosate are still ongoing [1]. Detection of glyphosate is mainly based on chromatographic methods. In gas chromatography, glyphosate is derivatized into a volatile and thermally stable derivative, while in liquid chromatography, glyphosate derivatives are detected by UV-Visible detector and fluorescence detector.

Raman spectroscopy method is fast and simple, and its advantage is the possibility of analysis in aqueous solutions. But problems, such as insufficient sensitivity and the frequent occurrence of fluorescence, limit the use of Raman spectroscopy. Since its discovery in 1974, Surface-Enhanced Raman Scattering (SERS) has gained great popularity as a sensitive method suitable for examining the vibrational properties of molecules adsorbed on rough metal surfaces of nanometer dimensions even at very low concentrations [2]. SERS is a very sensitive method, and the possibility of combining it with other techniques allows it to be widely used. One of the significant advantages of the SERS method is the ability to analyze photoluminescent compounds. The time of sample preparation for analysis is extremely short, as well as the recording of the spectrum itself, which allows a significant number of processed samples in a short time. The analysis of many compounds are performed in situ using small portable Raman spectrometers. The sensitivity of the SERS method depends on the type of substrate which can be either a colloidal solution of metal nanoparticles or a metal surface with a suitable nanostructured topology. Today, suspensions of silver and gold nanoparticles (colloids) are most often used as SERS substrates. The sensitivity and repeatability of the Raman signal can be significantly increased by using metal particles of precisely defined size and shape. The most common method of preparing metal nanoparticles for SERS is chemical reduction of metal salts. The particle size suitable for SERS ranges between 10 and 80 nm. The size and shape of the particles can be controlled in part by the choice of colloid preparation method.

In this work glyphosate is detected by surface-enhanced Raman scattering (SERS) using three laser excitations. The SERS substrates used for glyphosate detection were silver and gold nanospheres.

### Synthesis of Silver Nanoparticles (Ag NPs)

The colloid suspension of Ag NPs was synthesized by a modified Leopold, Lendl method. 10 mL of $AgNO_3$ (10 mM) was added to 90 mL of a hydroxylamine hydrochloride solution (1.67 mM) containing 3.33 mM NaOH at room temperature under stirring. The mixture was kept stirring for 15 min.

### Synthesis of Gold Nanoparticles (Au NPs)

The colloid suspension of Au NPs was synthesized by a modified Turkevich method using sodium citrate as a reducing. 200 mL of $HAuCl_4$ (1 mM) was vigorously stirred and heated in a flask fitted with a reflux condenser. Then, 10 mL of 38.8 mM sodium citrate was rapidly added to the boiling solution and kept boiling for 15 min, when its color changes from pale yellow to wine-red. The resulting 0.95 mM colloid solution was stable for several months at 4 °C.

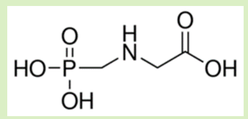


**Figure 1**. Structural formula of glyphosate (N- (phosphonomethyl) glycine).

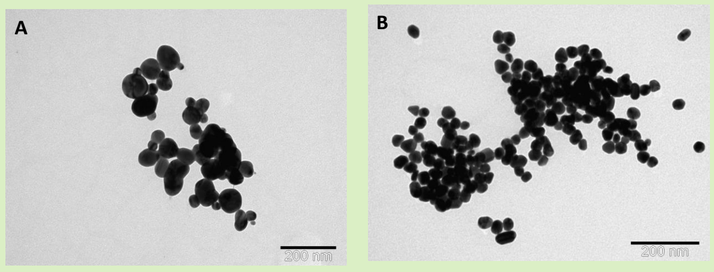


**Figure 4.** TEM images of a) Ag NPs and b) Au NPs.

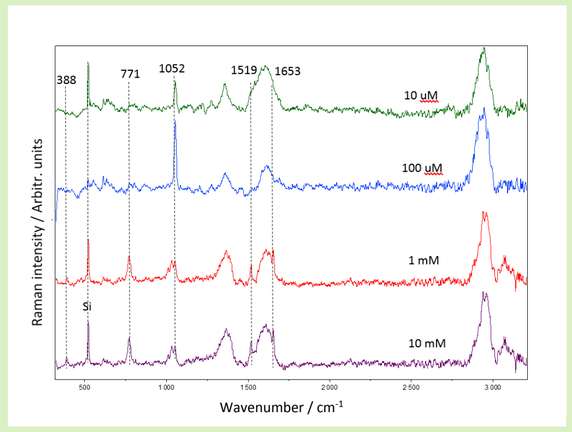


**Figure 6**. SERS spectra (baseline corrected) of different glyphosate concentrations with silver NPs (AGH) at 532 nm laser excitation.

## Nanoparticles characterization

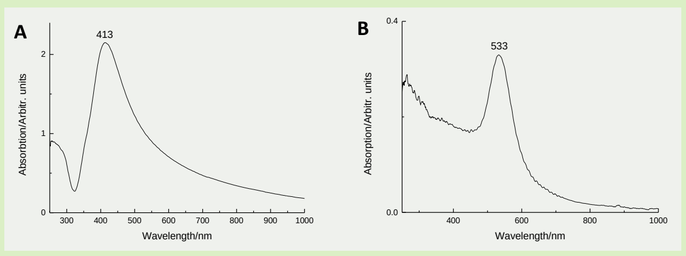


**Figure 2.** The UV–Vis spectra of the synthesized a) silver and b) gold nanoparticles.

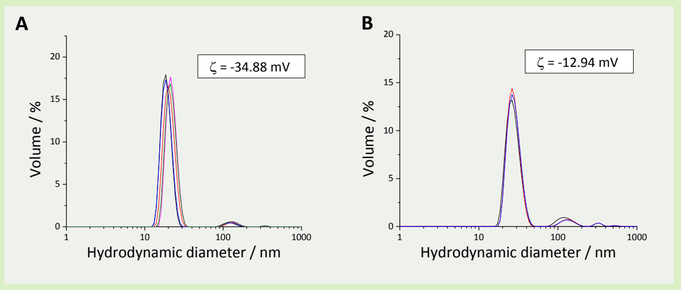


**Figure 3.** The hydrodynamic size distributions by volume for a) Ag and b) Au NPs, measured using Multiple Angle Dynamic Light Scattering (MADLS) technology. The results of zeta potential (ξ) measurements are given in the insets.

**Table 1.** The pH values of glyphosate and NPs solutions.

| | Ag NPs | Au NPs | Glyphosate (10 mM) | Gly (10 mM):Ag NPs = 1:1 | Gly (10 mM):Au NPs = 1:1 |
|---|---|---|---|---|---|
| **pH** | 6.5 | 4.7 | 2.7 | 3.0 | 2.8 |

## Raman and SERS measurements

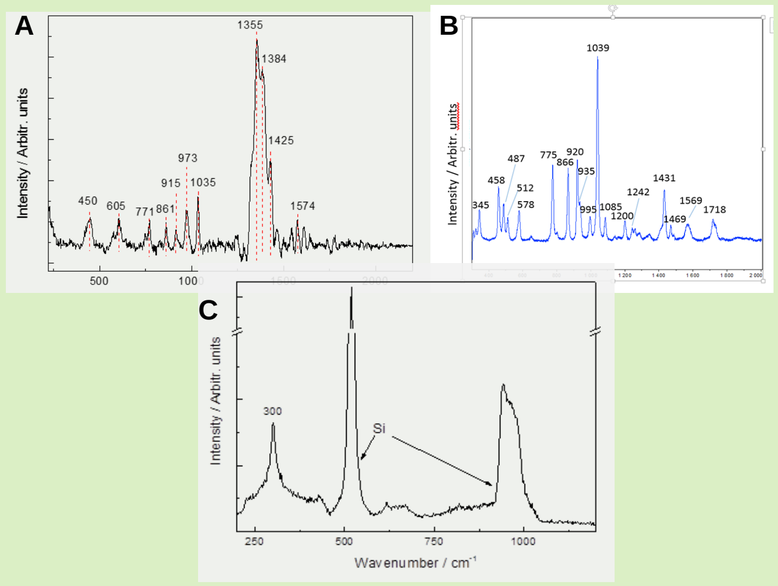


**Figure 5**. Raman spectra of glyphosate powder a) at 785 nm laser excitation (Cora 5000), b) at 532 nm excitation and c) 10 mM solution of glyphosate at 633 nm on Si wafer.

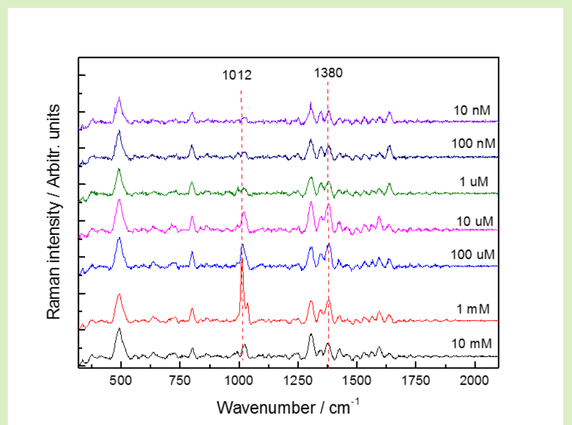


**Figure 7**. SERS spectra of different glyphosate concentrations with gold NPs at 785 nm laser excitation (Cora 5000).

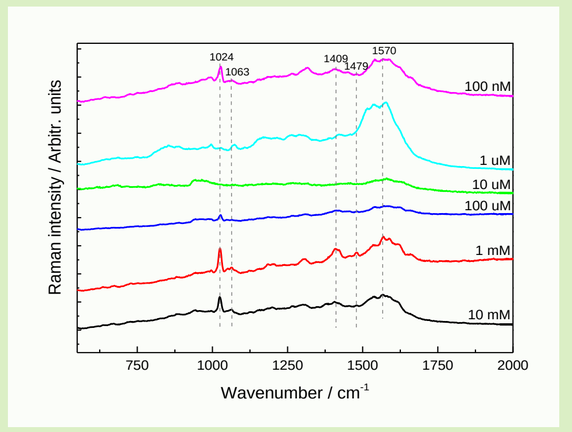


**Figure 8**. SERS spectra of different glyphosate concentrations with gold NPs (AuC) on Si wafer at 633 nm laser excitation.

## Conclusion

Nowadays due to the large amounts of food needed, the use of pesticides is widespread. Therefore, there is a need to detect low concentrations of pesticides in soil, water and food, since the pesticides are potentially harmful to human health. Detection of low concentrations of compounds in different matrices is not trivial. It is necessary to find a quick, simple and sufficiently sensitive method of detection. The SERS method has the advantages of easy sample preparation and fast analysis, but still has reproducibility issues. Also, the search for a cheap, reliable, reproducible and general SERS substrate is still ongoing.

Here, the silver and gold colloids were prepared and used as SERS substrates. The colloid suspension of Ag NPs was synthesized by a modified Leopold-Lendl method, while the colloid suspension of Au NPs was synthesized by a modified Turkevich method using sodium citrate as a reducing agent. The prepared suspensions of NPs were investigated with respect to their morphology and SERS activity. The calculated mean particle size from TEM analysis for Ag NPs was about 32 and 72 nm, while Au NPs had an approximate diameter of 27 nm (Fig. 4). The synthesized NPs were quite stable in aqueous media and had zeta potential -34.9 mV (Ag NPs) and -12.9 mV (Au NPs) (Fig. 3). The samples were analyzed using 532, 633 and 785 nm laser excitations. As already reported in the literature, Raman and SERS spectra strongly differed. SERS spectra quite varied when Ag or Au NPs were used. For example, at 532 nm excitation with Ag NPs three prominent SERS glyphosate peaks are at 771, 1519 and 1653 $cm^{-1}$ (Fig. 6). Using Au NPs and 633 nm excitation, the most prominent peaks are at 1024 $cm^{-1}$ and at 1409 $cm^{-1}$ (Fig. 8). Au colloidal solution and 785 nm excitation resulted in spectra where the most pronounced peak is at 1012 $cm^{-1}$ (Fig. 7). The results suggest different ways of glyphosate absorption to the Au and Ag NPs which is also dependent on the pH of the solution. The lowest limits of detection obtained for glyphosate water solution were 100 μM.


**References:**

[1] A.H.C. Van Bruggen, M.M. He, K. Shin, V. Mai, K.C. Jeong, M.R. Finckh, J.G. Morris, Jr., Sci. Total Environ. 2018, 616-617, 255-268.
[2] M. Moskovits, B.D. Piorek, J Raman Spectrosc. 2021, 52(2), 279-284.



**Acknowledgment**

This work was supported by the project “Hybrid Silicon Nanostructures for Sensing” nr. IP-2014-09-7046, funded by the Croatian Science Foundation and by the 2018-2.1.12-TET-HR-2018-00003 Croatian-Hungarian bilateral project.