

# Development of Silver Nanoparticles-Based Sensor for Detection of Quinolones by Surface-Enhanced Raman Spectroscopy: a Case-Study Using Levofloxacin

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Numerous studies have shown that a variety of pharmaceuticals such as quinolones have been detected in the vicinity of municipal wastewater discharges and livestock agricultural facilities. Many of those works have been employed conventional analytical techniques and traditional methods of detection. However, in many cases these detection methodologies are time consuming and generate waste, which can be overcome using Surface-enhanced Raman spectroscopy (SERS) as analytical technique. In the present work, silver nanoparticles (AgNP) films were prepared on amine-modified glass slides with different number of depositions. The AgNP aggregates films were tested as SERS substrates using 4-aminobenzenethiol (4-ABT) as model Raman molecule. A trend between the 4-ABT SERS response and the number of AgNP deposition could be identified, which can be associated with the formation of AgNP aggregates. The aggregation effect could be quantified by calculating the enhancement factor (*EF*), which was on order of  $10^6$  for the film with five AgNP depositions. This optimized AgNP film was employed as SERS sensor of Levofloxacin in different concentrations, which was easily detected from  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  mol L<sup>-1</sup> solution. Also, the SERS spectral profile are rich in information, which allowed to describe the chemical interaction between the Levofloxacin molecules and the AgNP sensor surface. This study provides an efficient method for detecting of Levofloxacin using AgNP SERS sensor, which can be extended for others quinolones.

## 1. Introduction

Levofloxacin, a third-generation quinolone antimicrobial drug is a human and veterinary antibiotic, which is widely used in the treatment of respiratory and urinary tract infections, and soft tissue infections (Willmott et al., 1994). Although Levofloxacin is generally tolerated, in some cases it may cause serious adverse reactions. Some reported adverse effects occurred after Levofloxacin treatment such as tendon damage, heart problems, and muscle wasting. Therefore, it is important to determine this compound in biological fluids, pharmaceutical formulations, urine, and wastewater. Many analytical techniques have been employed for determining of Levofloxacin such as high performance liquid chromatography, capillary electrophoresis, and ELISA test (Santoro et al., 2006; Huet et al., 2006). Although these techniques have been employed for determining this kind of analyte, the preparation steps, use of chemicals, generation of wastes, and long time of analysis have been assigned as unfavorable. On the other hand, Surface-enhanced Raman spectroscopy (SERS) has emerged in recent years owing to its potential to be employed as an extremely sensitive technique for analytical applications (Le Ru and Etchegoin, 2009). SERS technique is a highly sensitive vibrational spectroscopy with the ability for ultra-sensitive detection and it has been widespread applied in chemistry, environmental analyses, and biologic sciences (Fan et al., 2011). For example, Alula (2014) has reported the potential application of the SERS technique to detect creatinine in urine using silver nanostructures as SERS sensor. Using gold nanoparticles with an ultra-thin silica-shell as sensor, Hughes

(2014) has reported the ultra-trace detection of bioactive molecules by SERS. These works and others are excellent examples that show the efficient application of the SERS technique for molecules detection in different matrices.

The most used SERS substrates are based in silver and gold colloidal nanoparticles. Formed primarily by the reduction of gold or silver salts solutions, nanoparticles can be easily synthesized in the laboratory. Furthermore, a number of strategies exist for controlling the nanoparticles aggregation, allowing researchers to tailor the nanoparticles to their applications (Fraire et al., 2013). One of the most popular and simple methods for controlling nanoparticles aggregation consists in adding diluted salts solutions in the colloidal nanoparticles solution (Pamies et al., 2014). Controlling the nanoparticles aggregation is an efficient strategy to control the generation of hot spots, where the maximum SERS enhancement factor (*EF*) typically occurs. In this work, it was synthesized silver nanoparticles (AgNP), which were deposited on modified glass slides. This AgNP material was employed as SERS sensor of Levofloxacin in low concentrations.

## 2. Materials and Methods

### 2.1 Silver nanoparticles synthesis and preparation of the SERS sensor

Ag nanoparticles were prepared according to the Lee and Meisel method (Lee and Meisel, 1982), with some modifications. The functionalization of glass slides for preparation of nanoparticles-based SERS sensor was described in previous work by our group (Santos et al., 2013).

### 2.2 Materials characterization

UV-Vis spectra of the AgNP colloidal solution and AgNP films were obtained using a spectrometer Agilent Cary probe 50. The AgNP films scanning electron microscopy (SEM) images were obtained using Field Emission Gun microscope model Inspect, with acceleration voltage of 15 kV. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100 microscope (200 kV, 0.25 nm point resolution). The sample was prepared by drop-drying the colloidal AgNP on a holey-carbon coated Cu grid.

### 2.3 SERS measurements and Levofloxacin detection

All Raman measurements were accomplished using a confocal Jobin-Yvon T64000 Raman spectrometer system, equipped with a liquid N<sub>2</sub> cooled CCD detector. The excitation source was a laser at 633 nm. The laser power at the sample surface was about 2.5 mW. The laser was focused with a 100x focal-lens objective to a spot of about 1 μm. For all measurements, the laser exposure time was 10 s. For SERS measurements, 50 μL of 10<sup>-6</sup> mol L<sup>-1</sup> 4-aminobenzenethiol (4-ABT) solution was dropped onto AgNP films. After the solvent evaporation the substrates were ready to be analyzed. For all samples, Raman mappings were carried out in a selected area on the AgNP films surface, resulting in a total of 49 spectra for each sample. Also, the drop-drying procedure was used when 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> mol L<sup>-1</sup> Levofloxacin aqueous solution were analyzed.

## 3. Results and discussion

The optical absorption spectra of AgNP colloidal solution and of the AgNP films on glass slides with different depositions are not shown. The AgNP formation is confirmed by the presence of the surface plasmon resonance (SPR) absorption band at 433 nm, which is a typical signature of silver nanoparticles (Stamplecoskie and Scaiano, 2011). The SPR absorption of AgNP is also observed in the absorption spectra of the AgNP films. TEM images (data not shown) show that the AgNP have a sphere-like shape morphology. Although the majority of AgNP size is centered at around 40-70 nm, counting the diameter of AgNP in several TEM images was obtained a size distribution between 5 and 90 nm. The AgNP deposition methodology employed resulted in the formation of nanoparticles aggregates, which grows increasing the number of depositions (Figure 1). These aggregates exhibit a closed-packed formation, and they are distributed on the glass slide surface. The first AgNP deposition on the amine-modified glass slides generates aggregates without addition of any salt to induce aggregation as commonly reported. After successive depositions (3D and 5D), the generation of AgNP aggregates involves the APTMS sol gel used as linker, where the nanoparticles can be chemisorbed. The interparticle gap should be very small, once the AgNP are separated by the sol gel linker, which is an important condition to generate hot spots, where the maximum SERS enhancement is observed.

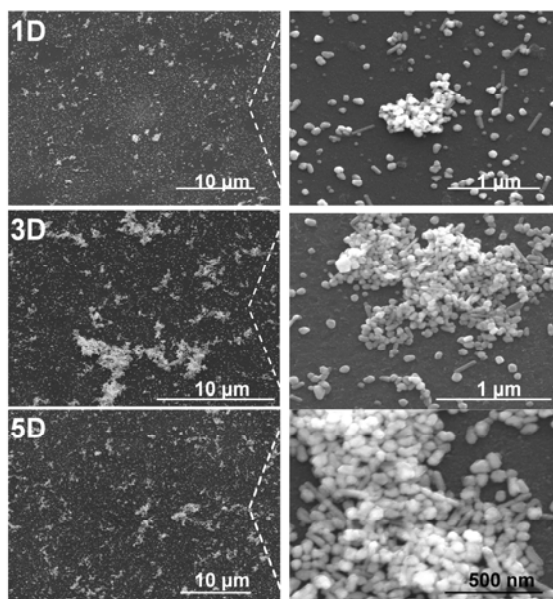


Figure 1. SEM images of the AgNP films (1D, 3D and 5D). Right side: SEM images in high magnification.

Before investigating the SERS activity of the AgNP aggregates films, it is important a description of the Raman spectrum of 4-ABT (data not shown). This molecule is commonly used as Raman probe to investigate the SERS effect (Kim et al, 2013). As can be observed in Figure 2, the intensities of the 4-ABT bands and their spectral pattern are dependent on the number of AgNP depositions. This result suggests that the AgNP aggregation should play an important role in the SERS response of 4-ABT on AgNP aggregates films. To evaluate the influence of the AgNP aggregates on 4-ABT SERS response, it was mapped one defined area for each AgNP aggregates film. The spatial distribution of the band at  $1070\text{ cm}^{-1}$  is different for each substrate as can be observed analyzing its intensity in Figure 3. For the substrate 1D the distribution of intensities is more uniform than in the substrate 3D. This result indicates that increasing the number of AgNP deposition generates aggregates with different sizes and, consequently, a high oscillation in the SERS signal is observed. For the substrate 5D is observed a more uniform distribution of the intensities than in the substrate 3D (Figure 3 (c)), suggesting that five depositions of the AgNP aggregates should be more appropriate than 3D. This result indicates that the generation of AgNP aggregates is directly related with the number of AgNP deposition. Consequently, it plays a strong influence on the SERS signal obtained spot-to-spot as well as sample-to-sample.

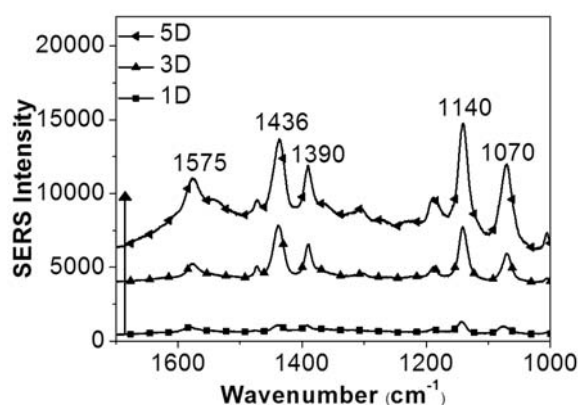


Figure 2. Raman spectra of  $10^{-6}\text{ mol L}^{-1}$  4-ABT on AgNP films (AgNP depositions: 1D, 3D and 5D).

The formation of AgNP aggregates is an important issue in SERS. In this kind of structure are generated hot spots due to plasmon coupling in the small interparticle gaps. This explain the dependence of the SERS signal on AgNP aggregates for the different substrates showed in Figure 3.

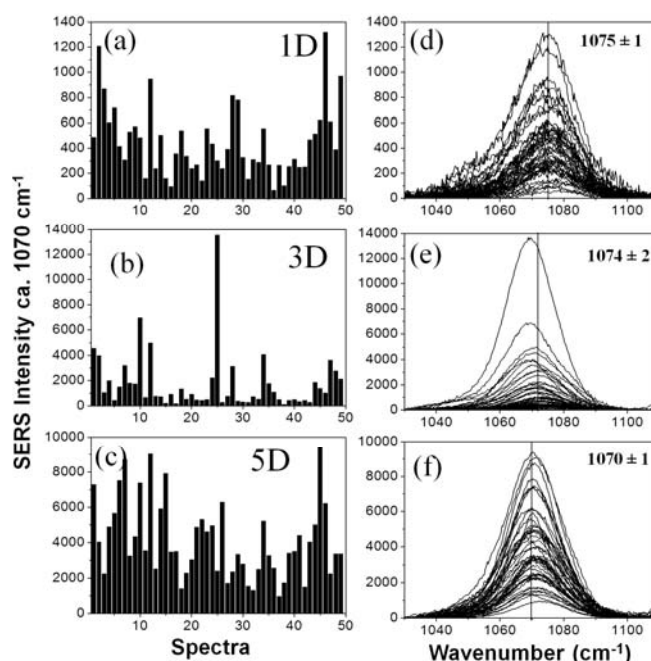


Figure 3. Left side: (a, b, c) SERS intensity distribution at ca.  $1070\text{ cm}^{-1}$  for AgNP films. Right side: (d, e, f) SERS spectra of  $10^{-6}\text{ mol L}^{-1}$  4-ABT on AgNP films, highlighting the band at ca.  $1070\text{ cm}^{-1}$ .

Other effect is observed analyzing the intensity and the position of the band at  $1070\text{ cm}^{-1}$ , attributed to  $\nu(\text{C-S} + \text{C}=\text{C})$ , for the three AgNP aggregates films (Figure 3 (d), (e), (f)). There is a variation in the maximum position of this band as well as in the SERS intensity, which can be related to different orientation of the 4-ABT molecules adsorbed on AgNP aggregates. This is a binding effect, where fluctuations in the SERS intensity and/or position of the Raman bands are commonly observed due different possibilities of molecules to interact with the metallic substrate surface (Sivapalan et al., 2013). In Figure 3 the average position shifts from  $1075\text{ cm}^{-1}$  (for substrate 1D) to  $1070\text{ cm}^{-1}$  (for substrate 5D). This shift is related with the formation of chemical bond between AgNP and 4-ABT by the thiol group, indicating that there is a dependence on the formation of AgNP aggregates. This result suggests that increasing the number of AgNP aggregates more particles are available to interact with the 4-ABT molecules. Consequently, the formation of stronger links could be benefited from it.

The AgNP aggregation effect on 4-ABT SERS response can be quantified calculating the enhancement factor ( $EF$ ) by  $EF = (I_{SERS} / I_{Raman}) \times (C_{Raman} / C_{SERS})$ , where  $I_{SERS}$  corresponds to the highest SERS intensity ca.  $1070\text{ cm}^{-1}$ ,  $I_{Raman}$  is the Raman intensity at  $1089\text{ cm}^{-1}$ ,  $C_{Raman}$  corresponds to the concentration of 4-ABT deposited on glass slide without AgNP, and  $C_{SERS}$  is the concentration of 4-ABT deposited on AgNP SERS substrates. It was determined the  $EF$  for all SERS substrates. As showed in Figure 4, the  $EF$ s increase with the number of AgNP deposition, which can be associated with the larger number of AgNP aggregates on the substrates. Although the high standard deviation, it can be identified a trend in the magnitude of the  $EF$  as a function of the AgNP aggregates formation (Figure 4). This result is a clear demonstration of the dependence of the SERS signal on the AgNP aggregates. The AgNP aggregates films exhibit enhanced factors with magnitudes around  $10^6$ , which is comparable to others commonly used SERS substrates (Qu et al., 2012). They can be optimized controlling the formation of aggregates, which is a function of the number of AgNP deposition as showed above. From the substrate 1D to the substrate 5D the  $EF$ s is improved in one order in magnitude, indicating that the enhancement is an important parameter to be used probing the substrate SERS response. The AgNP 5D film was chosen to be employed in the Levofloxacin detection because it exhibits an high  $EF$  and it presented less variations in the 4-ABT SERS response.

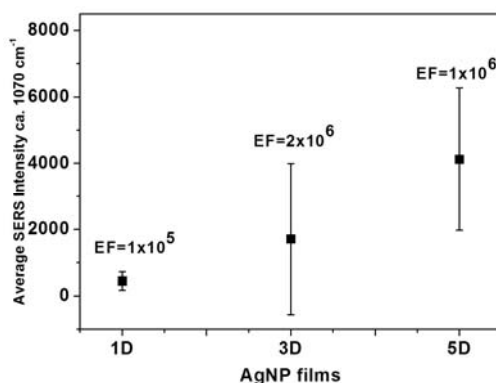


Figure 4. Average SERS intensity at ca. 1070 cm<sup>-1</sup> for the AgNP films, showing the highest calculated EF.

The Raman spectrum of the compound Levofloxacin and the SERS spectra of Levofloxacin in different concentrations at pH 6.5 are presented in Figure 5. The most significant bands are indicated with a dashed line to compare with the bands observed in the Raman spectrum of the compound used as reference. The bands at 1620 cm<sup>-1</sup> assigned to the stretching  $\nu(\text{C}=\text{C})$ , at 1399 cm<sup>-1</sup> assigned to the stretching vibration of the quinolone ring system  $\nu(\text{C}=\text{O})$ , at 1552 cm<sup>-1</sup> assigned to  $\nu(\text{C}=\text{O})$ , and at 1442 cm<sup>-1</sup> assigned to  $\delta(\text{CH}_2)$  are commonly observed in the Raman spectra of quinolones (Neugebauer et al, 2005). As can be seen in Fig. 5, these and others bands are shifted in SERS condition due to the binding effect, and also, due to different orientations of Levofloxacin molecules adsorbed on AgNP substrate. These effects lead to differences in intensities and positions of some bands when comparing with the Raman spectrum of Levofloxacin.

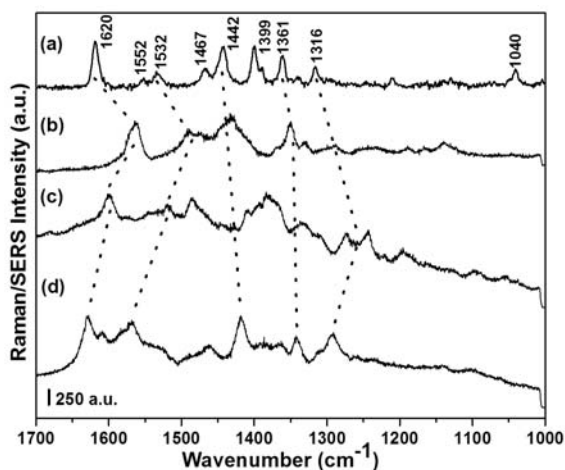


Figure 5. (a) Raman spectrum of solid Levofloxacin, and (b, c, d) SERS spectra of 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> mol L<sup>-1</sup> Levofloxacin, respectively.

The observed variations in the SERS spectra of the Levofloxacin can be explained based on its chemical structures. Mitscher (2005) has reported that when the pH is below 7 the equilibrium between the cationic and the zwitterion structure is predominant for Levofloxacin. Since the AgNP are negatively charged, due to citrate used as capping agent, the interaction between Levofloxacin molecules and the AgNP should be preferentially by the groups of the molecule positively charged. Consequently, the structures can interact with the AgNP substrate in different ways due their different charges. Also, as can be observed in Figure 5, the binding effect on SERS spectra of Levofloxacin appears to be minimized for more diluted solutions. In the SERS spectrum for Levofloxacin at 10<sup>-6</sup> mol L<sup>-1</sup> the Raman bands are less shifted when comparing with the SERS spectra obtained for other two concentrations.

#### 4. Conclusions

In this report, we have investigated the effect of aggregates formation on the 4-ABT SERS response for different AgNP films. The variation of the AgNP deposition number leads to formation of more aggregates on the glass slides as well as the formation of larger aggregates. It was observed an increasing trend between the number of AgNP deposition and the 4-ABT SERS response. This effect could be quantified by calculating the enhancement factor, and values on the order of  $10^6$  could be achieved. Taking advantage of the high SERS enhancement from AgNP film with five depositions, it was employed as SERS sensor. The fast detection of Levofloxacin and a high signal/noise ration are two important advantages obtained using the AgNP films prepared. Furthermore, the SERS results provided valuable information about the chemical interaction between the Levofloxacin molecules and the AgNP sensor surface.

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#### References

- Alula, M.T., Yang J., 2014, Photochemical decoration of magnetic composites with silver nanostructures for determination of creatinine in urine by surface-enhanced Raman spectroscopy, *Talanta* 130, 55-62.
- Fan M., Andrade G. F.S., Brolo A. G., 2011, "A review on the fabrication of substrates for surface enhanced Raman spectroscopy and their applications in analytical chemistry", *Anal. Chim. Acta* 693, 7-25.
- Hughes J., Izake E. L., Lott W. B., Ayoko G. A., Sillence M., 2014, Ultra sensitive label free surface enhanced Raman spectroscopy method for the detection of biomolecules, *Talanta* 130, 20-25.
- Kim K., Lee S. H., Kim K. L., Shin K. S., 2013, Visible light response of silver 4-aminobenzenethiolate and silver 4-dimethylaminobenzenethiolate probed by Raman scattering spectroscopy, *J. Raman Spectrosc.* 44, 518-524.
- Kim K., Yoon J. K., Lee H. B., Shin D., Shin K. S., 2011, Surface-Enhanced Raman Scattering of 4-Aminobenzenethiol in Ag Sol: Relative Intensity of  $a_1$ - and  $b_2$ -Type Bands Invariant against Aggregation of Ag Nanoparticles, *Langmuir* 27, 4526-4531.
- Le Ru E. C., Etchegoin P. G., 2009, *Principles of Surface-Enhanced Raman Spectroscopy*. Elsevier, Amsterdam, Netherland.
- Lee P. C., Meisel D., 1982, Adsorption and surface-enhanced Raman of dyes on silver and gold sols, *J. Phys. Chem.* 86, 3391-3395.
- Mitscher L. A., 2005, Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents, *Chem. Rev.* 105, 559-592.
- Neugebauer U., Szeghalmi A., Schmitt M., Kiefer W., Popp J., Holzgrabe U., 2005, Vibrational spectroscopic characterization of fluorquinolones, *Spectrochim. Acta A* 61, 1505-1517.
- Pamies R., Cifre J. G. H., Espín V. F., Collado-González M., Baños F. G. D., Torre J. G., 2014, Aggregation behavior of gold nanoparticles in saline aqueous media, *J. Nanopart. Res.* 16, 2376-2386.
- Qu L. L., Li D. W., Xue J. Q., Zhai W. L., Fossey J. S., Long Y. T., 2012, Batch fabrication of disposable screen printed SERS arrays, *Lab Chip* 12, 876-881.
- Santoro M. I. R. M., Kassab N. M., Singh A.K., 2006, Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography, *J. Pharmaceut. Biomed. Anal.* 40, 179-184.
- Santos E. B., Sigoli F. A., Mazali I. O., 2013, Surface-enhanced Raman scattering of 4-aminobenzenethiol on silver nanoparticles substrate, *Vib. Spectrosc.* 68, 246-250.
- Sivapalan S. T., DeVetter B. M., Yang T. K., Schulmerich M. V., Bhargava R., Murphy C. J., 2013, Surface-enhanced Raman Spectroscopy of Polyelectrolyte-Wrapped Gold Nanoparticles in Colloidal Suspension, *J. Phys. Chem. C* 117, 10677-10682.
- Stamplecoskie K. G., Scaiano J. C., 2011, Optimal Size of Silver Nanoparticles for Surface-Enhanced Raman Spectroscopy, *J. Phys. Chem. C* 115, 1403-1409.
- Willmott C.J. R., Critchlow S. E., Eperon I. C., Maxwell A., 1994, The complex of DNA gyrase and quinolone drugs with DNA forms a barrier to transcription by RNA polymerase, *J. Mol. Biol.* 242, 351-363.