

## CHARACTERIZATION OF SILVER NANOPARTICLES SUBSTRATES USING LASER INDUCED REDUCTION TOWARD SURFACE-ENHANCED RAMAN SPECTROSCOPY

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ARTICLE INFO	ABSTRACT
<b>Received:</b> 11/3/2024	Surface-enhanced Raman spectroscopy (SERS) has garnered significant attention due to its efficacy in elucidating chemical and structural information from pharmaceutically active compounds (PhACs). The advancement and practical utilization of SERS technology rely heavily on the fabrication of suitable SERS substrates. Integration of SERS substrates within microfluidic systems introduces additional complexity to this task. This investigation employs a laser-induced reduction technique to fabricate silver nanoparticle substrates directly within microfluidic environments. The study aims to evaluate the influence of irradiation time and laser power on substrate quality. Subsequently, these substrates are employed for the characterization of Rhodamine B. The findings reveal that an irradiation time of 30 seconds and a laser power of 0.11 mW yield optimal results, manifesting nanoparticle sizes within the range of 20-40 nm with a well-organized arrangement. Notably, the system exhibits a sevenfold increase in Raman signal compared to non-SERS conditions, thereby validating its efficacy.
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## KHẢO SÁT ĐỂ NANO BẠC SỬ DỤNG PHƯƠNG PHÁP KHỬ BẰNG LASER ĐỊNH HƯỚNG CHO TĂNG CƯỜNG TÍN HIỆU PHỔ RAMAN

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THÔNG TIN BÀI BÁO	TÓM TẮT
<b>Ngày nhận bài:</b> 11/3/2024	Hiệu ứng tăng cường tín hiệu Raman bề mặt đã thể hiện sự ưu việt của mình trong việc phát hiện và phân tích các hợp chất có hoạt tính dược (PhAC). Sự tiện lợi và ứng dụng thực tế của SERS phụ thuộc nhiều vào việc chế tạo để kim loại. Điều này còn phức tạp hơn khi được triển khai bên trong lòng các kênh dẫn vi lưu. Trong nghiên cứu này của nhóm, kỹ thuật khử bằng laser đã được sử dụng để tạo ra các hạt Bạc nano trên đế kính trong kênh. Các thông số như thời gian chiếu và công suất laser đã được khảo sát để thu được để SERS tốt nhất. Các để này sau đó sẽ được sử dụng để phân tích hoạt chất Rhodamine B. Các kết quả cho thấy, với thời gian chiếu là 30 giây tại công suất 0,11 mW thì để SERS thu được cấu hình tốt nhất với các hạt nano có kích thước nằm trong dải từ 20-40 nm và có độ phân bố đồng đều. Đáng chú ý, tín hiệu Raman thu được có mức tăng cường lớn hơn 7 lần khi không có để, qua đó xác nhận được hiệu quả của nghiên cứu.
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<b>TỪ KHÓA</b>	
SERS	
Hạt nano bạc	
Phương pháp khử bằng laser	
Rhodamine B	
Vi lưu	

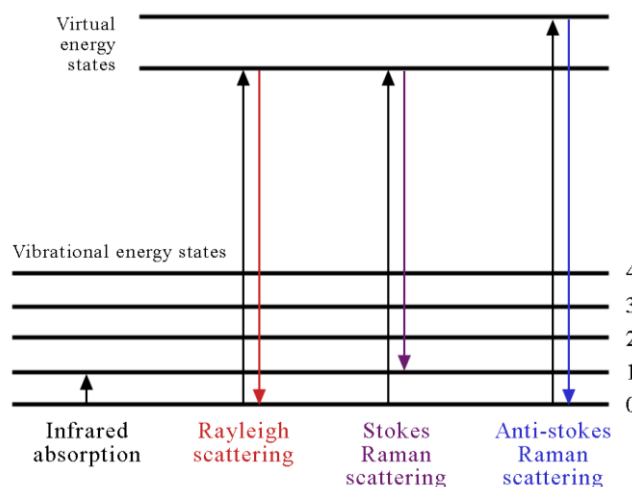
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## 1. Introduction

In the dynamic landscape of contemporary society, an extensive array of pharmaceutically active compounds, notably antibiotics, is manufactured and extensively employed on a daily basis. The pharmaceutical industries in numerous low and middle-income nations generate wastewater, often subjected to inadequate treatment, subsequently finding its way into the environment or municipal sewage systems [1], [2]. This pervasive presence and dissemination of antibiotics in the environment contribute to the emergence of pathogenic resistance. Consequently, antibiotics and other medications become progressively less effective in treating microorganisms that have developed resistance [3]-[5]. Given the urgency of addressing this issue, there is a contemporary imperative to explore techniques for accurately determining the concentrations of pharmaceutically active substances and antibiotics in wastewater.

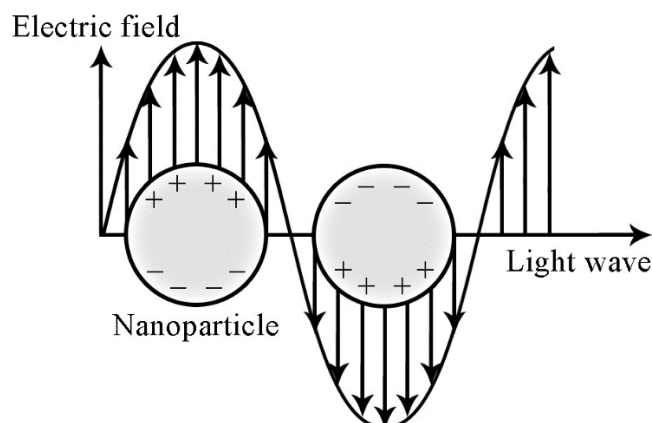
While numerous detection techniques, such as electrochemical and fluorometric methods, have been developed and utilized for many years [6]-[8], each approach possesses inherent limitations in the context of detecting these substances. Consequently, in response to the escalating demand, Surface-enhanced Raman spectroscopy (SERS), an adaptation of the analytical technique named after the Indian scientist C. V. Raman, has garnered significant interest. SERS is valued for its efficacy in extracting chemical and structural information from samples. Relying on the inelastic scattering of photons, Raman spectroscopy involves the interaction of monochromatic light with molecular vibrations. The resultant shift in energy, as illustrated in Figure 1, provides valuable information about the vibrational modes within the system. Moreover, Raman spectroscopy proves instrumental in substance identification through the discernment of characteristic Raman 'fingerprints'.



**Figure 1.** Energy-level diagram showing the states involved in Raman spectra [9]

Surface-enhanced Raman Spectroscopy has been developed as a remedy for the inherent issue of weak signals associated with Raman scattering. This is achieved by introducing a plasmonic metal particle surface, facilitating the absorption of analyte molecules [10]. The outcome is a pronounced enhancement of the Raman scattering signal, commonly denoted as SERS. This enhancement is attributed to the amplification of light resulting from the stimulation of localized surface plasmon resonances (LSPRs). The underlying mechanism is grounded in the electromagnetic effect.

In the conventional scenario, incident light striking the surface of a bulk ferromagnetic sample prompts the excitation of electrons in the metal surface layer by photons. Subsequently, these electrons propagate parallel to the metal surface, a phenomenon recognized as surface plasmon resonances (SPRs) [11].



**Figure 2.** Schematic diagram illustrating the localized surface plasmon on a nanoparticle surface [11]

When the material is reduced to the nanoscale, significantly smaller than the specific wavelength of the incident photon (approximately a couple of hundred nanometers), a distinct phenomenon occurs. In this scenario, where the material exists at the nanoscale, all existing electrons are excited, initiating oscillations and resonance throughout the material, as depicted in Figure 2. The frequency of these electron oscillations is heavily reliant on factors such as the density of electrons, effective electron mass, and the shape and size of the charge distribution. This leads to a concentration of amplified light in the interparticle gaps when nanoparticles are positioned closely together, particularly evident in plasmonic materials like silver, gold, and copper with nanoscale features. Consequently, there is a substantial increase in amplitude, with the technique capable of achieving enhancing factors in the range of  $10^{12}$ – $10^{14}$  orders of magnitude [12].

Meanwhile, microfluidics have a significant development in the field of biochemical analysis and detection. These systems are called Lab-on-a Chip (LOC) [13], and has several benefits as low sample consumption, fast reaction, high sensitivity and portability. SERS-microfluidics was combined by a SERS substrate inside micro channel which enable to work with low-volume sample as drugs, oligonucleotides, biomolecules and proteins [14]. Over the years, various methods have been employed for the preparation of SERS substrates [15]-[17]. However, each of these methods has its inherent limitations, such as challenges in achieving ordered and high enhancement factors in microfluidic chips or issues related to poor controllability [18].

Recently, the in situ synthesis of SERS substrate on microfluidic chip technology has been developed as a better integration method between SERS detection and microfluidic technique. Thank to precise control of liquid inside microchannel, the more controllable and flexible substrate was easier to achieve. Using the femtosecond laser induced photo-reduction was proposed first time by Xu et al [18]. The work shown the whole catalytic reduction can be monitored by SERS detection on the microfluidic chips since the silver substrates exhibit high SERS enhancement. Moreover, the laser induced reduction process was also applied to prepare other SERS substrate as Ag-Graphene Oxide for DNA detection [19], nanofilm on PDMS [20], 2D periodic metal structure [21]. In general, SERS integrated microfluidics is a potential analytical tool however, challenges still exist, especially on the preparation SERS substrate inside microfluidics.

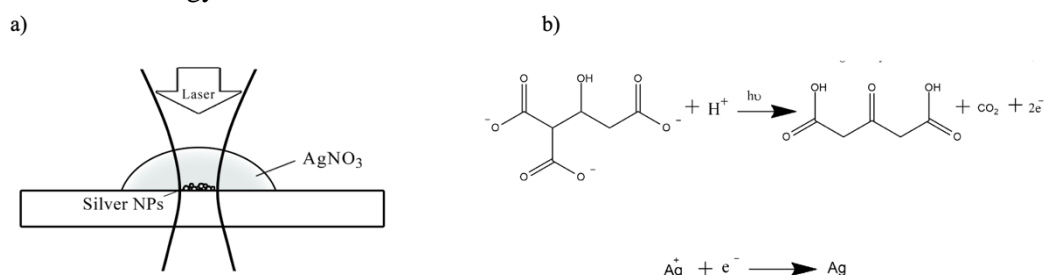
In this paper, the synthesis of silver nanoparticles substrate using laser induced reduction technique is considered to achieve the better SERS signal. By changing the power of laser, time of exposure, the size distribution of silver NPs are discussed. The enhanced Raman signal of Rhodamine B was provided to confirm the effect of obtained substrates.

## 2. Materials and Methods

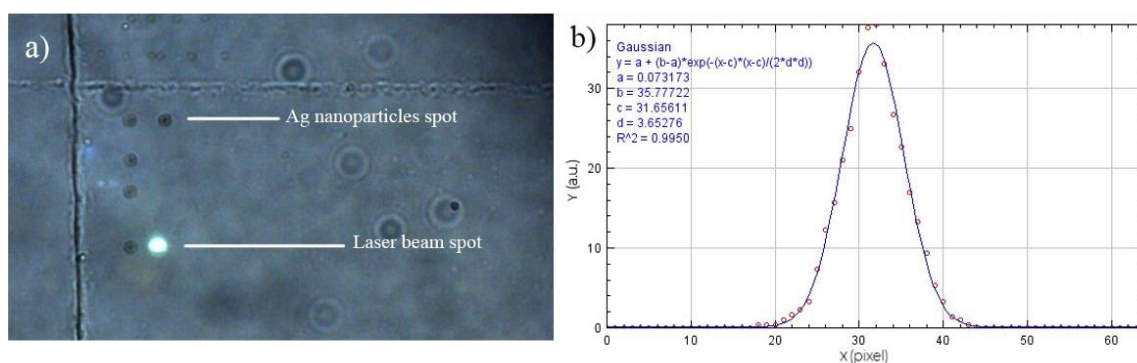
### 2.1. Synthesis of Ag nanoparticle substrates

Isotropic and anisotropic metallic nanoparticles, such as gold and silver, have gained significant attention for the creation of SERS substrates. This is attributed to their tunable optical properties, controllable particle size distribution, straightforward synthesis procedure, long-term stability, and high biocompatibility. The size and shape of these nanoparticles can be precisely controlled, offering diverse optical properties and distinct SERS signals [22].

The synthesis of Ag NPs with a homogeneous size distribution was pursued using a laser-induced reduction technique. The reduction of Ag<sup>+</sup> cations from silver nitrate (AgNO<sub>3</sub>) occurred on a glass substrate, resulting in the formation of Ag NPs precisely within the region where the laser spot was focused on the substrate, as depicted in Figure 3a) [23]. The silver reduction mechanism also is shown in the Figure 3b). Firstly, the sodium citrate was decomposed to form acetone-1,3-dicarboxylate and free electrons by the laser. Consequently, the silver ions will combine with electron to reduce to silver [24]. The size and SERS effectiveness of the synthesized silver nanoparticles were systematically investigated under various experimental conditions, including laser power, laser irradiation time. The experimentation was conducted using an ECLIPSE Ti2 (Nikon Instrument) inverted microscope provided by the Institute of Physics, Vietnam Academy of Science and Technology (VAST). Subsequent analysis of the results was performed using a scanning electron microscope (FE-SEM) at the University of Science and Technology of Hanoi, VAST.



**Figure 3.** a) Schematic view of the laser induced reduction process for Ag NPs and b) the mechanism of silver reduction using tri-sodium citrate



**Figure 4.** a) Focused beam spot in the synthesizing process under a 10x optical microscope and b) the Gaussian profile of the laser (position versus intensity, with 1 pixel = 0.46 μm)

The fabrication process initiates with the mixture of 0.1 mM AgNO<sub>3</sub> (Sigma Aldrich, Singapore) and 0.08 mM C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub> (Sigma Aldrich, Singapore) in a 1:1 volume ratio. Subsequently, a 20 μL droplet of the mixture is dropped onto a common microscope glass slide. A laser source (Roithner Lasertechnik GmbH) with a wavelength of 473 nm, operating at two distinct powers 0.07 mW and 0.11 mW, is then focused on the surface of the slide to generate Ag

NPs at predefined positions. Various irradiation times: 5 s, 10 s, 30 s, and 60 s are employed in this process. The focused laser beam spot and the positions of Ag NPs on the interface are illustrated in Figure 4, with a Gaussian profile employed for calculating the diameter of the laser spot. Following this, the substrate undergoes rinsing with diluted water and is allowed to dry at room temperature before using for further characterizations and experiments.

## 2.2. SEM characterization

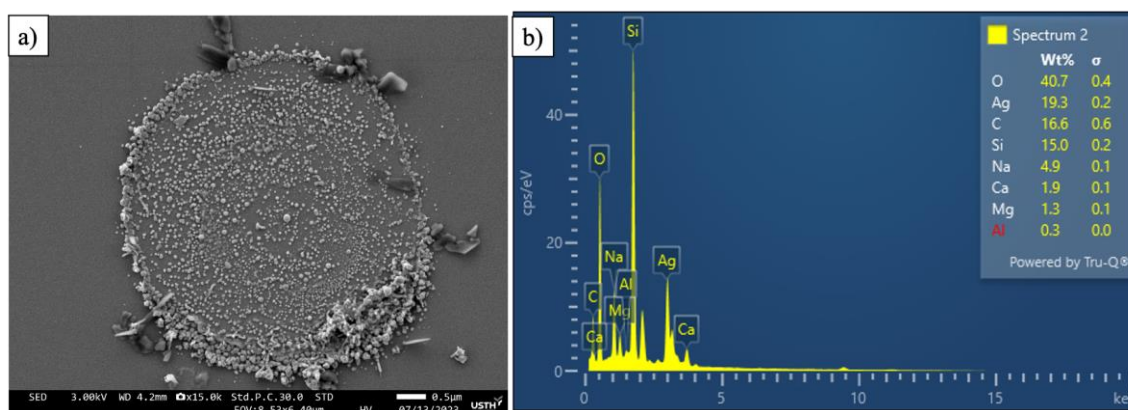
After exposure, the Ag NPs substrate was rinsed by distilled water 3 times and let dry at room temperature before characterizing by Scanning Electron Microscope as well as EDX (FE-SEM, Jeol).

## 2.3. SERS experiments

A 10 $\mu$ L drop of solution of Rhodamine B (Sigma Aldrich, Singapore) in deionized water were deposited onto the prepared SERS substrate and let it dry at the room temperature. The recording of Raman spectra was conducted utilizing a 532 nm laser at a power of 0.1 mW (LabRAM HR Evolution, Horiba).

## 3. Results and Discussion

### 3.1. Size and morphology of Silver nanoparticles



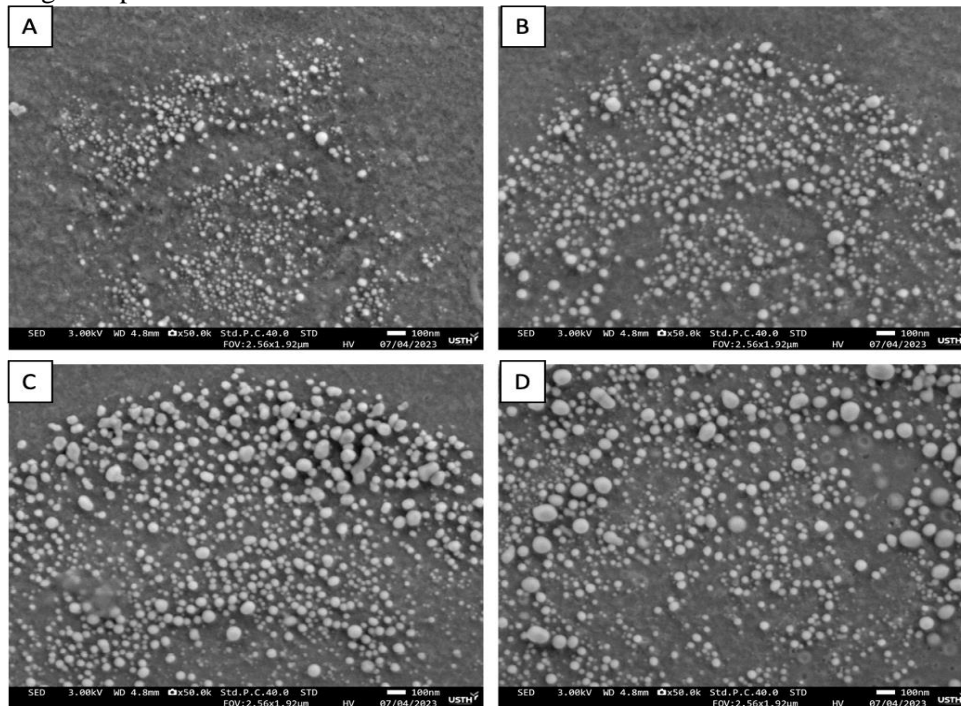
**Figure 5.** a) The SEM image of whole Silver NPs substrate and b) the EDX (Energy-dispersive X-ray) signal of synthesized silver nanoparticles

The SEM image of entire silver nanoparticles substrates was illustrated in the Figure 5a. It is clear to see that the shape of silver substrate was a 7 $\mu$ m diameter which was fit to the diameter of laser beam. It was worth to notice that in the center of substrate the size of silver NPs was quite homogeneous than the border. The convection of solution during the laser exposure involved to aggregation of NPs was mentioned on other publications [25]. The Figure 5b shown the EDX signal of the nanoparticle on the substrate. The weight percentage of silver was by 19.3% this means that the silver nanoparticles was successful synthesized. For other elements, they were belonged to the glass substrate, and other contaminations which not be rinsed enough but they are not effect to achieved results.

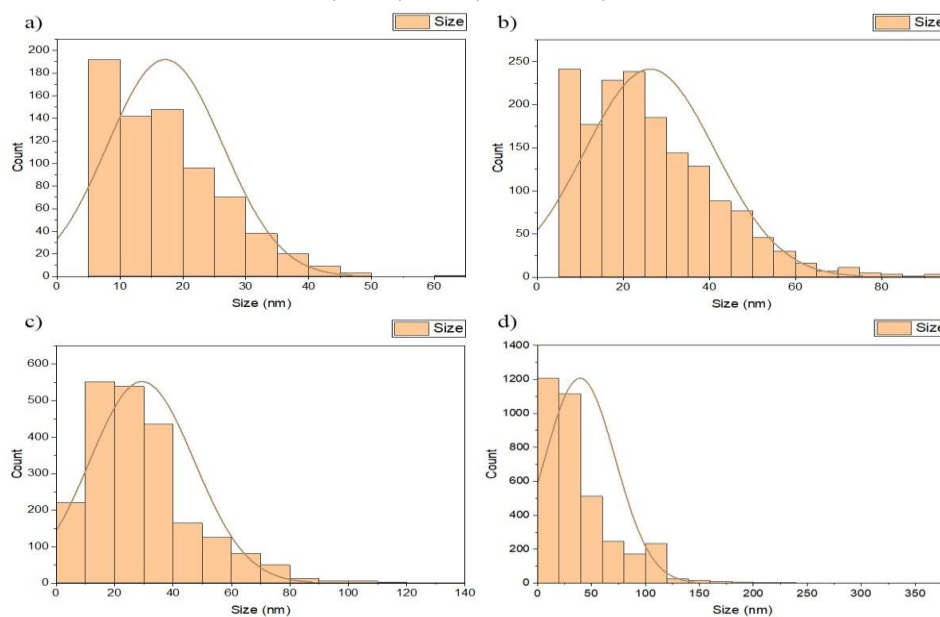
#### 3.1.1. Dependence on time of irradiation

The SEM images of synthesized silver nanoparticles by laser-induced reduction at power of laser by P=0.11mW at different durations as t=5s; 10s; 30s and 60s were demonstrated in the Figure 6 and the size distribution of size of each case was shown in the Figure 7, respectively (a), (b), (c) and (d). It was clear to see that the number of nanoparticles in the same scale of image will increase when the duration increased. It can be explain that more time, more energy will conduct more and more nucleation of silver nanoparticles as well as their aggregations. For

instance, the countable number of NPs increased from 200 to 1200 while increasing the exposure time from 5s to 60s. From these figures, it was noted that the size of NPs was gradually increased. The size and distribution of Ag NPs were combination of both nucleation and growth/aggregation, hence, the irradiation time at 30s was shown a better size distribution of than other. And the time of irradiation will be used to characterize the change of NP's morphology when change the power of laser.

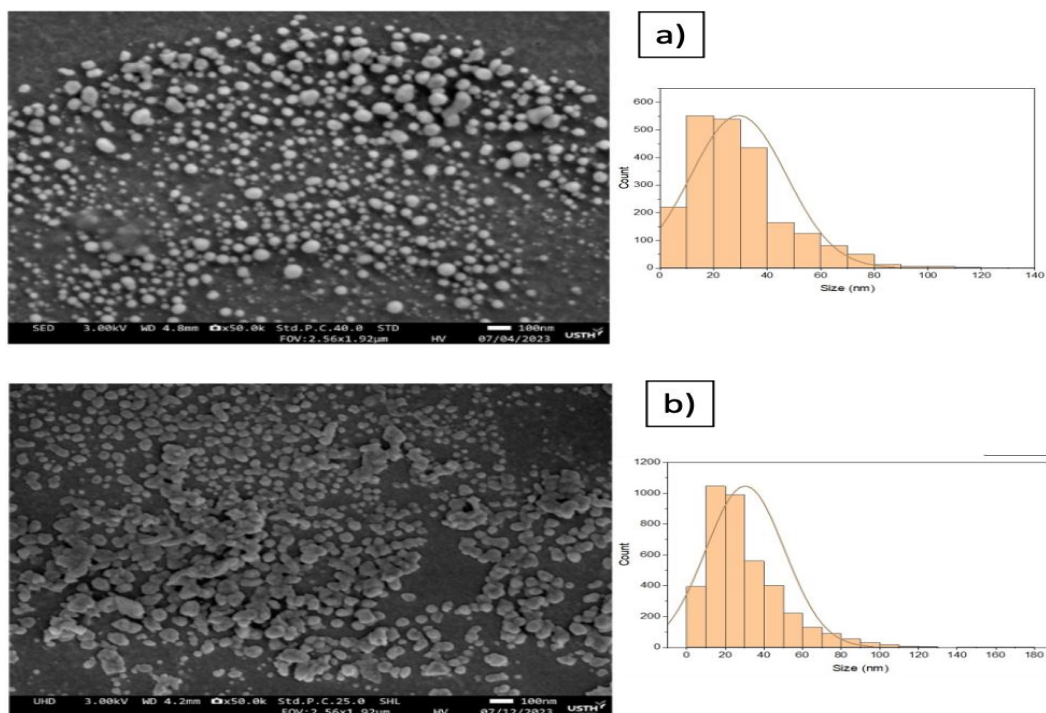


**Figure 6.** SEM images for Ag NPs with  $P = 0.07\text{mW}$  at different times of irradiation: a) 5s, b) 10s, c) 30s, and d) 60s



**Figure 7.** Particles count and size distribution of silver nanoparticles at time of radiation a) 5s, b) 10s, c) 30s, and d) 60s

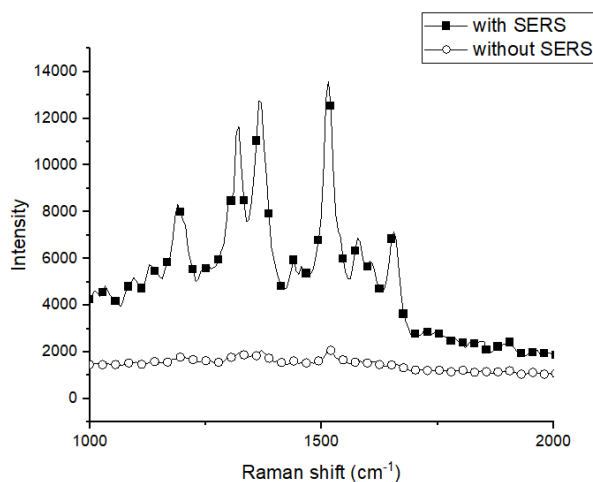
### 3.1.2. Dependence on power of laser



**Figure 8.** SEM images and size distribution of synthesized Ag NPs with a)  $P = 0.07$  mW and b)  $P = 0.11$  mW at irradiation time = 30s

By changing the power of laser between 0.07 mW and 0.11 mW, the difference in size and size distribution for each condition can be observed in the SEM images of Ag NPs synthesized at irradiation time by 30 s and at two different power by 0.07 mW and 0.11 mW, as illustrated in Figure 8. It was clear to see that the distribution of particles for the case with laser power by 0.11 mW yields more tight gaps between particles and more even in size distribution mostly in a range from 20-40 nm. In results, the laser power by 0.11 mW and exposed in 30 s was chosen to fabricate the Ag NPs substrate for SERS experiments.

### 3.2. SERS measurement



**Figure 9.** Raman spectra of Rhodamine B with SERS and without SERS

As a result acquired from previous parts, an ideal condition for this project is to use silver particles synthesized with the laser of power 0.11 mW with 30 seconds of irradiation time. The obtained Raman signals have SERS and no-SERS of Rhodamine B were shown in the Figure 9. The SERS substrate enhanced the Raman signal for Rhodamine B with distinctive peaks at  $1200\text{ cm}^{-1}$ ,  $1350\text{ cm}^{-1}$ , and  $1500\text{ cm}^{-1}$  with about 7 times of intensity comparing to the one without the effect, rising from 2100 to 13600. There also appears to have a large peak rising to the left, indicating the fluorescence in Rhodamine B.

#### 4. Conclusion

The SERS substrate fabricated by this method served its purpose as an improvement for Raman spectroscopy analysis with enhanced intensities in signals and the ability to acquire Raman “footprints” of substances that are commonly unachievable with the basic method, such as tetracycline at low concentration. SERS substrates with silver nanoparticles was successfully created, and the condition of having irradiation time of 30 seconds using a 473 nm laser with power of 0.11 mW with time of nucleation of a day has been chosen for the most homogenous in size distribution. The intensity for rhodamine B increased from 2100 to 13600 under the effects of SERS, without losing any “signature” signal that is distinctive for the substance, and this also helped solving the problem with measuring signals in fluorescence. This method opens the door for implementing in microfluidic chips for on-field detection of antibiotics in wastewater in this time.

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