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RESEARCH ARTICLE

DETECTION OF RHODAMINE VIA SURFACE ENHANCED RAMAN SPECTROSCOPY UTILISING AG NANOWIRES

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ABSTRACT

Rhodamine, which is extensively used as a synthetic dye in food industry, is regarded as an illegal additive by European Food Safety Authority because of its carcinogenic and toxicological properties. Since it's a colourless material at low concentrations such as 10^{-7} M, its detection via spectroscopical methods is very challenging and crucial in terms of food safety issues. In this study Ag nanowires were synthesized, their crystal structure is characterized via XRD analysis, their surface morphology and radius are determined via SEM images and their chemical composition was determined by EDS analysis. Afterwards, rhodamine solutions which were prepared at 10^{-4} – 10^{-7} M were dropcasted onto Ag nanowire solutions. After selecting three characteristic Raman peaks belonging to the Rhodamine molecule, which are located at 612, 1189 and 1362 cm^{-1}, detection of Rhodamine was performed at these ultra low concentrations. As a result, one can conclude that Ag nanowires can be utilized as possible SERS substrates for detection of Rhodamine at low concentrations by exhibiting significant reproducibility, stability and recyclability features.

Keywords: Rhodamine, SERS, Detection, Ag nanowires

1. INTRODUCTION

Raman spectroscopy has gained a significant importance by the discovery of utilising surface enhancement to boost Raman signals in order to detect molecules even at ultra low concentrations [1]. This phenomenon, which is referred as Surface Enhanced Raman Spectroscopy (SERS), provides a fast and cheap method for detection of such molecules at ultra-low concentrations. It is based on the principle of boosting Raman signals, which have low intensity by nature, by utilising metal nanoparticles in order to enhance Raman signals by creating hot-spots. At this point, the signal enhancement primarily depends on the distribution, shape, size, morphology and density of the nanoparticles implented on the substrate [2, 3]. To obtain uniform and reproducible SERS substrates, various nanomaterials utilised, while most preferred ones are Ag, Au, ZnO and ZnO/Ag [4].

The signal enhancement in Raman spectrum depends on two components, namely, electromagnetical enhancement and chemical enhancement. The electromagnetical component, being the main contribution to the signal enhancement, arises due to the surface plasmons on the metallic nanomaterials. Basically, free electrons of the nanomaterial substrate (generally Ag or Au) oscillate at the same frequency, when these plasmons resonate at the identical frequency with the laser light. Thus, analyte molecules adsorbed on the metal surface are stimulated in a more effective way, with the existence of the enhanced electromagnetic field [5]. The second contribution is of chemical nature and has a shorter range compared to the electromagnetic contribution. It simply depends on molecular polarization, where charge-transfer complexes occur between the metal nanomaterial substrate and the analyte molecule. This type of contribution needs so-called active-sites where analyte molecules should adsorb directly on the nanomaterial substrate [6].

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Rhodamine B, with the chemical formula $C_{28}H_{31}ClN_2O_3$, extensively used as a synthetic dye in food industry, is accepted as an illegal additive by European Food Safety Authority because of its carcinogenic and toxicological properties for human beings and as well as animals [7]. In this sense, detection of Rhodamine at even low concentrations is an important issue, in terms of human and animal health. Up to present, various methods have been utilised in order to determine Rhodamine presence in condiments and peanuts[8]. Among them, one can mention High Performance Liquid Chromatography (HPLC) [7], Spectrofluorimetry [8], Liquid Chromatography - Mass Spectrometry (LC-MS) [9]. However, these methods have several situation-specific disadvantages, such as long preparation times, expensive equipment necessities and complex and long pre-treatments. Moreover, these methods are not suitable for on-site analysis [10]. At this point, SERS emerges as a useful alternative, for rapid and precise detection of Rhodamine at even low concentrations.

In this study, SERS method was utilised in order to detect Rhodamine molecule at varying concentrations from 10^{-4} M to 10^{-7} M, where Ag Nanowires were employed as the substrate in order to form hotspots on rhodamine solution.

2. EXPERIMENTAL SECTION

2.1. Reagents

Ethylene glycol (EG) (99.8 %), ethanol (99.9 %), polyvinylpyrrolidone (PVP, Mw 55.000) and silver nitrate ($AgNO_3$) (99 %) were purchased from Sigma Aldrich. During all experimental procedures, ultrapure water was utilized.

2.2. Synthesis of Ag Nanowires

5 ml EG was poured into a three necked balloon and was stirred for 20 minutes at 600 rpm, 160^0C. Afterwards, it was flashed via Ar gas for one minute. A solution of 0.25 mg $AgNO_3$, which was prepared in 5 ml EG was slowly added into the first EG solution at 160^0C. Afterwards, 1.56 g PVP was prepared in 10 ml EG and was added into the first EG solution after the $AgNO_3$ solution. This mixture was left to stir for 2 hours at 160^0C. At the end of this process, the mixture was put into ice bath in order to terminate the reaction and was left to cool down at ambient temperature for 20 minutes. Then, the centrifugation procedure was performed in order to purify ag-nanowires. The first centrifugation was performed via acetone at 2000 rpm for 30 minutes, while the next two cycles were performed via distilled water with the same rpm and time settings [11].

2.3. Characterization

For the visual verification of the $AgNO_3$ nanowire synthesis, Hitachi Regulus 8230 FEG-SEM instrument was utilized and the elemental analysis was carried out via the Oxford EDS detector attached to this electron microscope. For determining the $AgNO_3$ nanowires' crystal structure, X-Ray Diffraction (XRD) method was utilized. The instrument was a Panalytical Empyrian diffractometer which operates with 45-kV anode voltage and 40-mA filament current settings. Nickel-filtered Cu Kα radiation had a wavelength of 0.1542 nm, the goniometer of the diffractometer had a scanning speed of 0.133°/s with a step size of 0.01° and it diffracted X-rays in the 2θ of 30°–90°. For obtaining the Raman spectra, Renishaw Invia Raman instrument was utilized. For all Raman spectrums, the excitation energy was 532 nm and the objective lens was 20x. For measurements, a silicon wafer was preferred as the standart material, while the raman peak for this material is observed at 520 cm^{-1} and all spectra were acquired between 400 cm^{-1} – 2000 cm^{-1}.

2.4. Preparation of SERS Substrate

As a first step, glass was disinfected via ethanol and acetone. Then, 10 µl $AgNO_3$ nanowire solution was dropped onto glass. Rhodamine solutions in distilled water were prepared with concentrations varying from 10^{-3} M to 10^{-7} M. Afterwards, Rhodamine solutions were dropped onto $AgNO_3$ nanowire deposited glasses. Later, glasses were left to dry at room temperature for 20 minutes, following this procedure the SERS measurements were performed.

3. RESULTS AND DISCUSSION

3.1. Raman spectrum of Rhodamine Molecule

As the first step of the Raman experiments, Raman spectrum of Rhodamine was obtained from the powder form of the sample directly and the resulting spectrum is presented in Figure 1 As seen from the figure, the peak observed at 612 cm^{-1} belongs to the C-C-C in-plane vibration mode, while the peak observed at 774 cm^{-1} belongs to the C-H out-of plane bending vibration mode. The peaks observed at 1189 cm^{-1} and 1509 cm^{-1} belong to C-H in-plane bending, C-H bending and C-C stretching vibration modes, respectively, while the peaks at 1362 cm^{-1} and 1650 cm^{-1} are attributed to aromatic C-C stretching modes. The obtained Raman spectra of powder sample are in agreement with the literature findings [12-16].

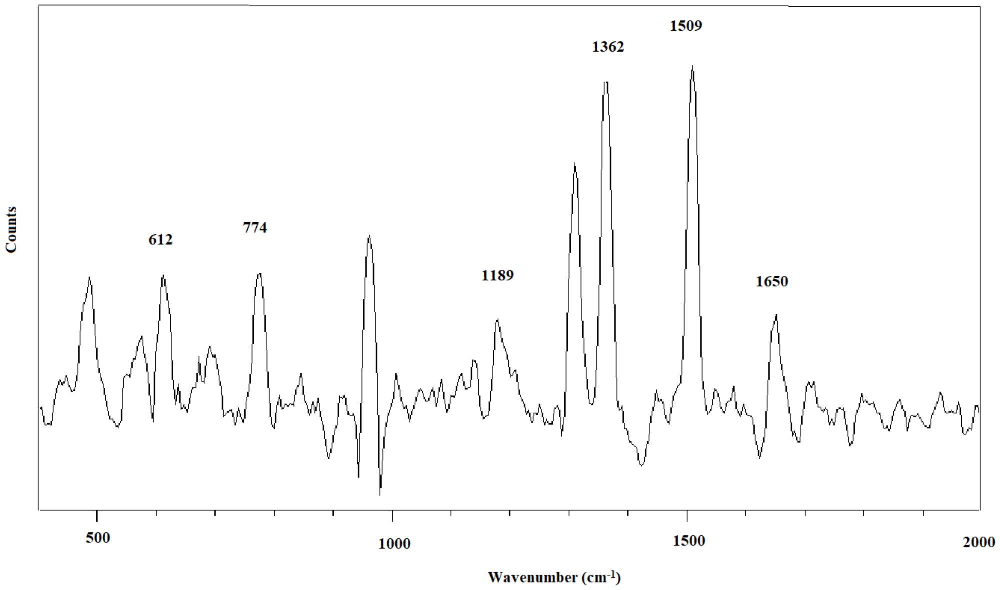

Figure 1. Raman spectrum of powder Rhodamine.

3.2. Characterization of Ag Nanowires as SERS Substrate

Ag nanowires' XRD pattern is presented in Figure 2. The crystalline peaks belonging to Ag nanoparticles are located at 38.192, 44.391, 64.585, 77.578 and 81.735, which is in accordance with the library values. The Miller index values belonging to these peaks are [111], [200], [220], [311] and [222], respectively. When Figure 2 is examined, one can clearly see that the intensity of the peak located at 38.192 with [hkl] value [111] is greater than the other Ag peaks, revealing that the development of the structure is towards silver nanoparticles [17,18]. Thus, one can conclude that XRD pattern of the structure reveals that the Ag nanoparticle synthesis is successful. The rest of the peaks located at 27.732, 32.130, 46.077, 54.632, 57.280 and 62.206, which are labeled with Asterix (*) are residues of the NaCl solution which was utilized during the synthesis.

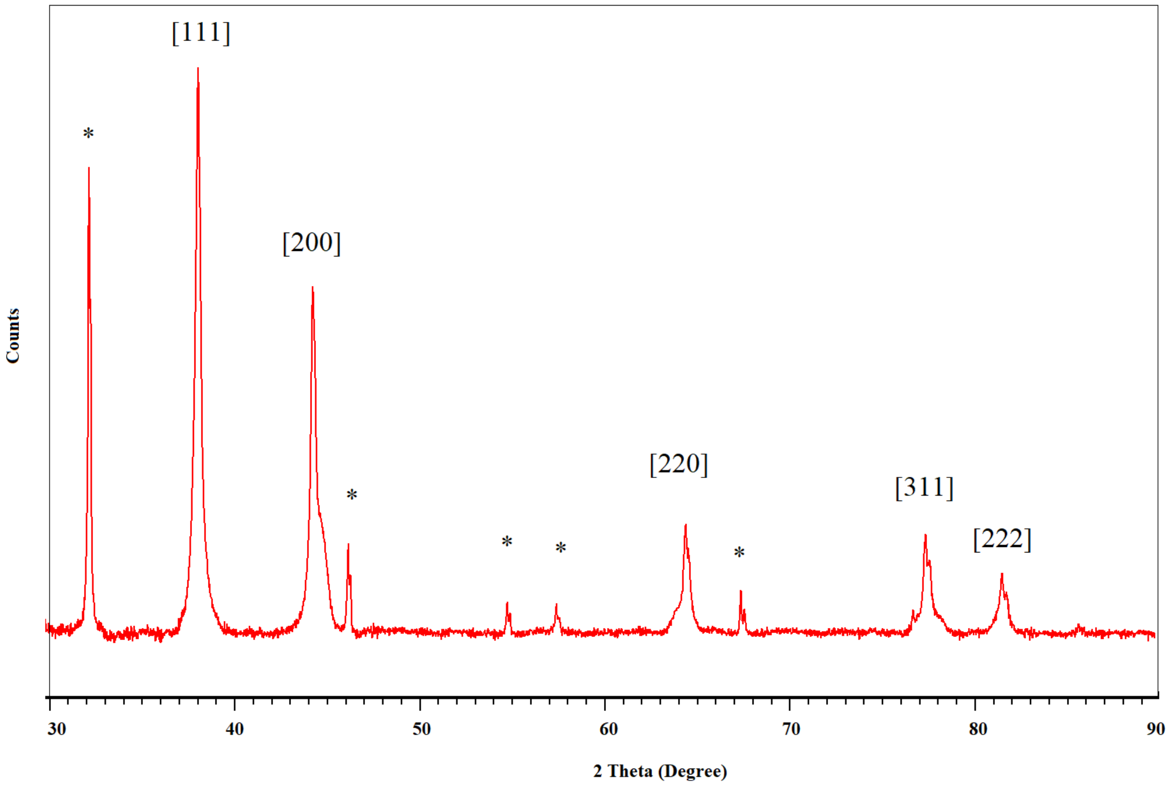

Figure 2. XRD Pattern of Ag Nanowires

SEM was utilized in order to examine the morphology of the Ag nanoparticles. Figure 3 exhibits the SEM image belonging to the nanoparticles. As the image reveals, nanoparticles have an average diameter of 90-100 nanometers. Furthermore, EDS analysis was performed via an Oxford Ultima EDS detector, which shows that the selected area in the SEM image consists of 96.50 % Ag, 2.28 % C and 1.22 % O in percentage.

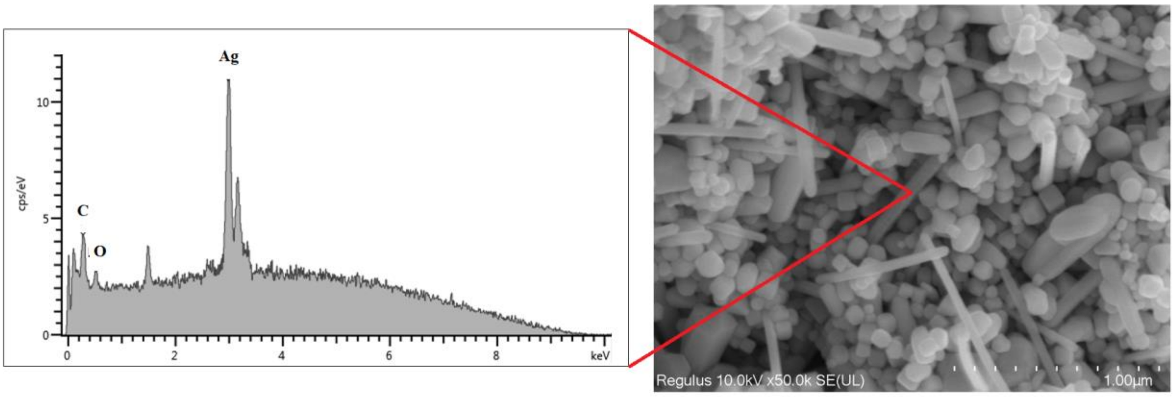

Figure 3. EDS Spectrum and SEM image of Ag Nanowires

3.3. SERS Studies of Rhodamine

In order to verify the SERS performance of Ag nanowires, various experiments were conducted for several concentrations of Rhodamine solutions. In these experiments, Rhodamine and Ag nanowires were utilized as the analyte molecule and the SERS substrate, respectively.

In Figure 4, SERS spectrum of Rhodamine molecule is presented at concentrations from 1x10^{-4} M to 1x10^{-7} M. As seen from Figure 4, Rhodamine's characteristic peak intensities decrease as the Rhodamine concentration decreases. In this study, three distinct peaks were selected for SERS studies. These peaks, which were selected because of their discrete locations, were observed at 612, 1189 and 1362 cm^{-1}. Another reason for their selection is their visibility at even ultra-low concentrations. For the selected 3 peaks, concentration versus intensity graphics were obtained in order to demonstrate the relation between Rhodamine concentration and peak intensity. These graphs are presented in Figure 4. The correlation coefficients (R^2), which were calculated for the peaks located at 612, 1189 and 1362 cm^{-1} were calculated as 0.9691, 0.9572 and 0.9628, respectively. These (R^2) values clearly demonstrate that Ag nanowires can be used for detection of Rhodamine at even low concentrations.

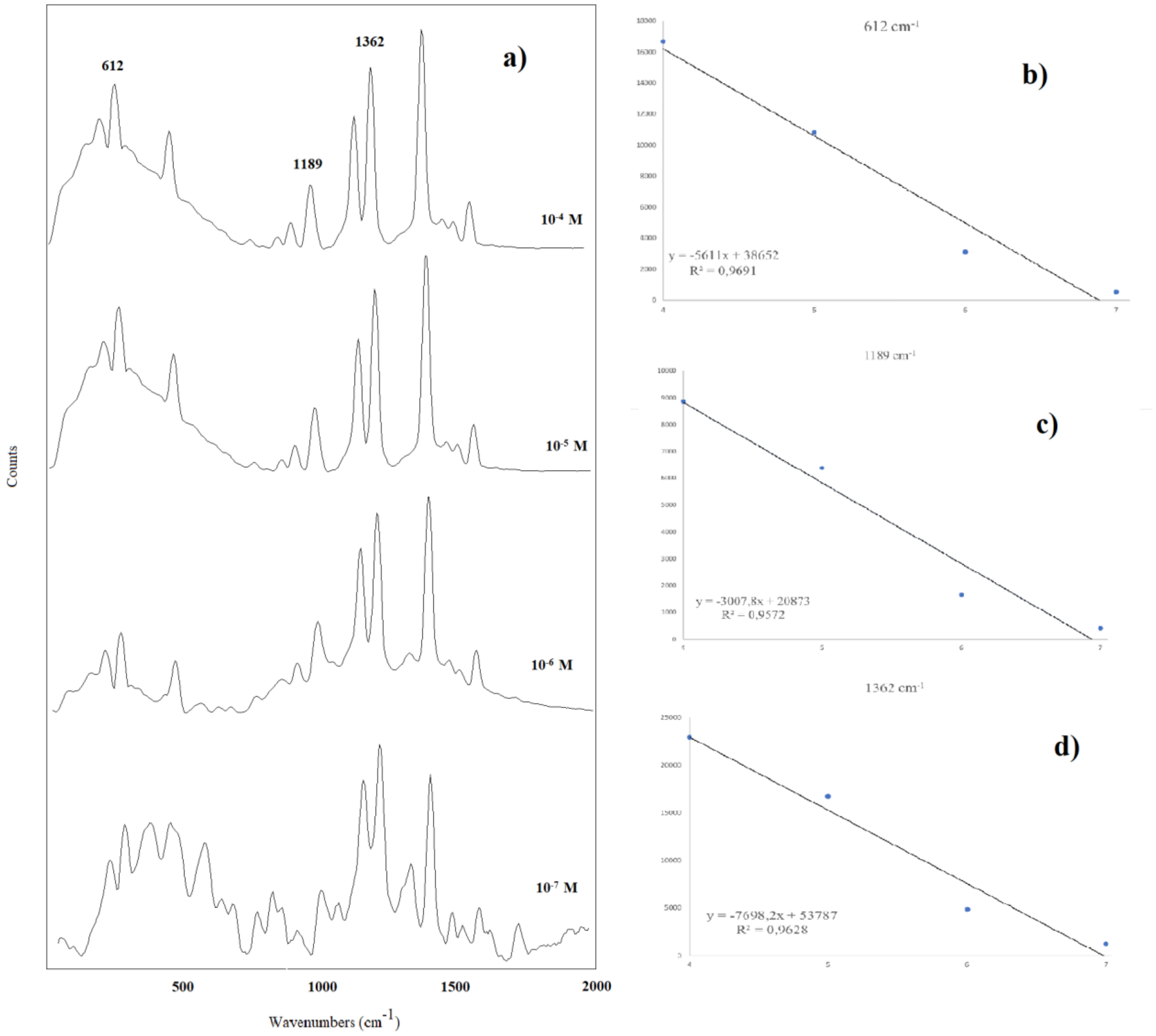

Figure 4. a) Raman spectrum of Rhodamine at concentrations from 10^{-4} to 10^{-7} and b,c,d) R^2 values for the selected peaks.

The next step is to calculate the enhancement factor (EF) which is another feature demonstrating the Ag nanowires' SERS capability. In order to calculate EF, the following formula was utilized [19]:

$$EF = \frac{I_{SERS} x C_0}{I_0 x C_{SERS}}$$

Here, I_{SERS} is Rhodamine's SERS intensity, I_0 is Rhodamine's normal Raman intensity, C_{SERS} (10^{-4} M) is Rhodamine concentration adsorbed on Ag Nanowires and C_0 is only Rhodamine concentration. Using the peak located at 612 cm^{-1}, Rhodamine's EF was computed as 1.39×10^6.

In the literature, various studies discussed EF of several nano-scaled systems with different sizes and geometries. For example, Dikmen calculated the enhancement factor of the silver nanocubes as $3.13\text{x}10^8$ for the characteristic peak observed at 1456 cm^{-1} of the amoxicillin molecule [20]. In another study, Francis et.al. calculated the enhancement factor for the characteristic peak of Rhodamine located at 1648 cm^{-1} as $6.93\text{x}10^{13}$ [21].

Additionally, the limit of detection (LOD) was determined using the following formula [22]:

$$LOD = \frac{3.3\delta}{k}$$

where, δ is the peak intensities' standard deviation and k is the calibration curve's slope.

In this study, LOD was calculated as $1.8\text{x}10^{-8}$ M, concluding that, depending on the SERS experiments conducted, Ag Nanowires can be utilized for detection of Rhodamine as SERS substrates at low concentrations.

3.2 Reproducibility and Stability of Ag Nanowires

Reproducibility is another important feature when utilizing specific detection applications. Two samples which were prepared under equal conditions were used in order to test the reproducibility of Ag nanowires. Four random points were selected on each sample and Raman spectrums were obtained from a total of eight points. The spectra belonging to these eight points are demonstrated in Figure 5 As seen from this figure, there are no discrepancies between the shapes of the peaks. For the selected peaks located at 612, 1189 and 1362 cm^{-1}, relative standard deviation (RSD) values were calculated as well. These values were 10.285 %, 9.887 % and 4.815 % for 612, 1189 and 1362 cm^{-1} respectively, which clearly exhibits that Ag nanowires can be utilized for detection of Rhodamine in terms of their reproducibility features.

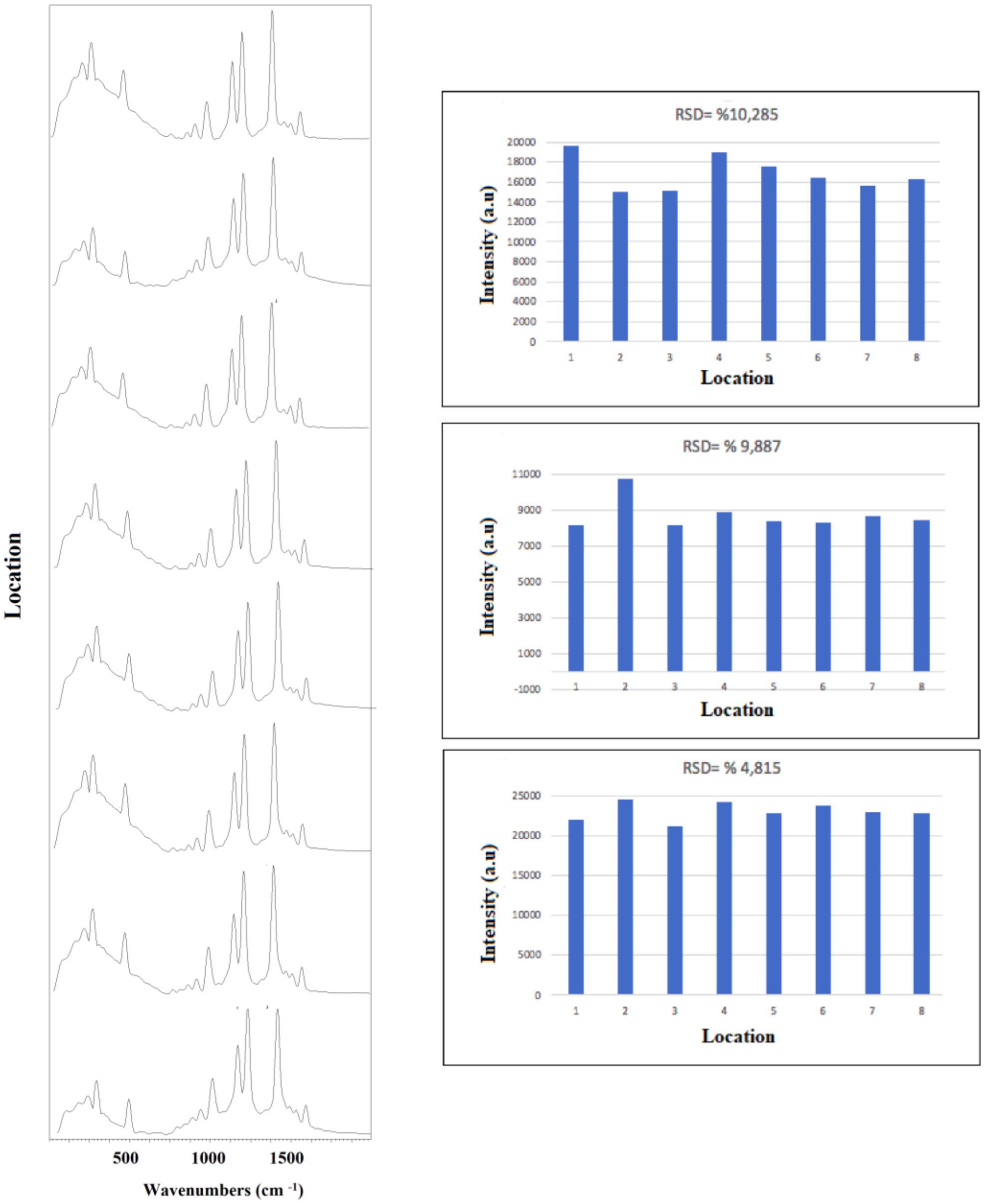

Figure 5. 8 random points' Raman intensities for Rhodamine adsorbed Ag NWs (left), RSD % values at 612, 1189 and 1362 cm^{-1}(right).

Reproducibility is a characteristic property which is easily affected by the stability of the substrate. For this reason, the substrate's stability should be checked when concluding about their convenience to be used as SERS substrates. In this study, Ag nanowires were left to ambient environment for 40 days and at the end of 40 days, their Raman spectra were collected again and the spectra were examined in order to check characteristic peak intensities at 612, 1189 and 1362 cm^{-1}. Figure 6 exhibits the spectrum obtained within 40 days of storage at ambient environment. The spectra demonstrate that there is no significant change in the intensities of the characteristic peaks, indicating that Ag nanowires are chemically stable substrates for Rhodamine detection via SERS method.

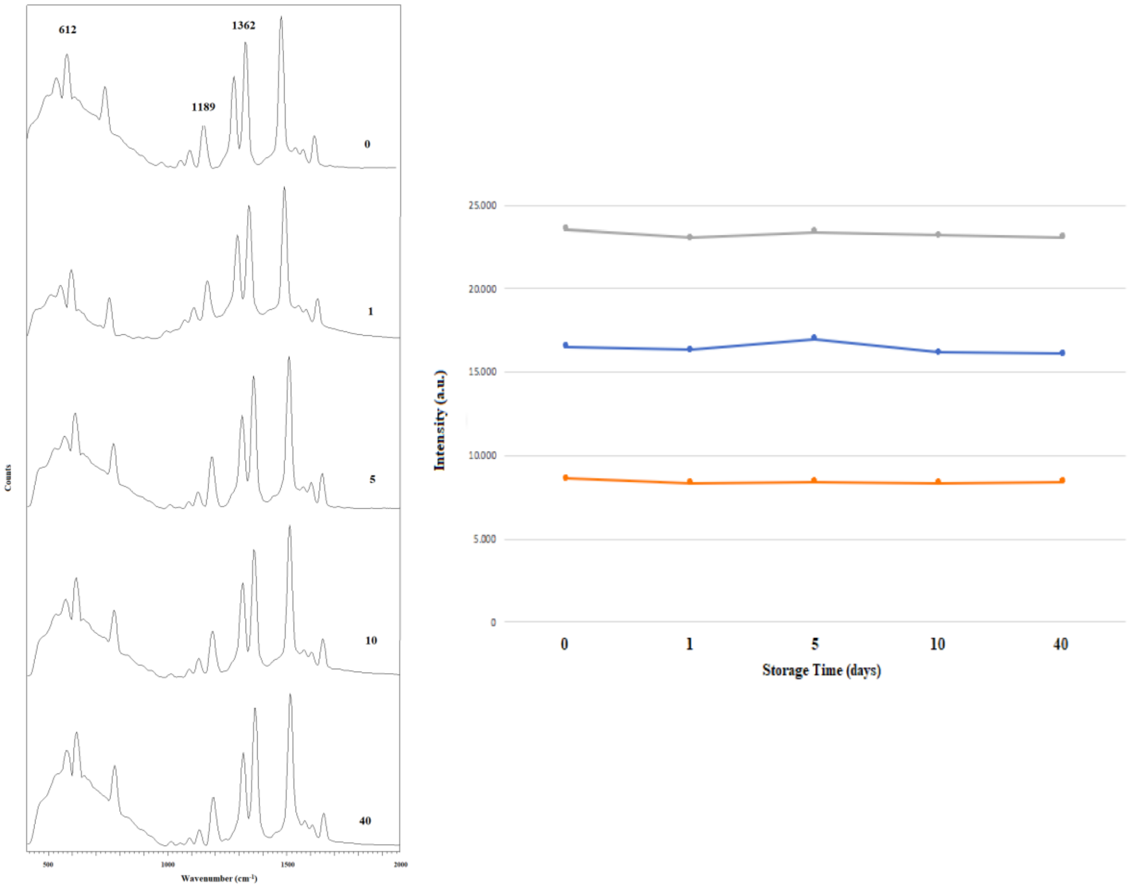

Figure 6. SERS spectra of Rhodamine on Ag NWs substrate stored within 40 days (a) and peak intensities of characteristic peaks of Rhodamine and storage times.

4. CONCLUSION

In this study, Ag nanowires were synthesized and this nanostructure was utilized as a SERS substrate in order to detect Rhodamine molecule. Rhodamine concentrations varying from 10^{-4} to 10^{-7} were prepared for SERS experiments. Afterwards, SERS experiments were conducted and three of Rhodamine's characteristic peaks, which are 612, 1189 and 1362 cm^{-1} were selected for SERS studies. LOD and EF values for Ag nanowires were calculated as $1.8x10^{-8}$ M and 1.39 x 10^{6} respectively. In the study, Ag nanowire substrates made the Rhodamine detection possible at even low concentrations such as 10^{-7} M, by boosting up the Raman signals via electromagnetic and chemical enhancements. Moreover, this nanostructure exhibited high sensitivity, chemical stability and reproducibility for detection of Rhodamine molecule. Finally, it can be concluded that, Ag nanowires can be used for detection of Rhodamine at ultra low concentrations via SERS method as a reliable and sensitive substrate.

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CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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