

# Surface Enhanced Raman Spectroscopy on Silver Nanoparticles

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**Abstract** The main purpose of this study is to detect different ecologically harmful substances in aqueous solutions using surface enhanced Raman spectroscopy (SERS) as a tool. Therefore, assigning the vibrational modes is essential to identify the substances. SERS using silver nanoparticles was used to obtain reproducible intense signals for detection of molecules in aqueous solutions. The study extends the use of thiol layers for attracting molecules onto the silver surface. Optimization for SERS measurements during scanning was made by scanning different points per line and different lines per image. The dependence of SERS intensity on scan speed had been demonstrated using descriptive figures. The spectra in pure solid forms and aqueous solutions of the carbamazepine (CBZ) sample helped for assigning which band belongs to which bond in the compound.

**Keywords** Lithography, Monolayer, Nanostructures, Organic Compounds, Raman Spectroscopy and Scattering

## 1 Introduction

Surface Enhanced Raman scattering (SERS) is the enhancement of Raman signal by rough metal surfaces. Fleischman et. al used silver electrode and silver nanoparticles adsorbed with pyridine, and discovered high Raman signals in 1974 [1]. His group concluded that the observed large signal is due to the number of molecules scattered on the surface; however, they were not aware of that it was actually a major enhancement effect [2]. Raman spectroscopy can be applied to any Raman active samples without pretreatment of the sample. Since only a small number of the incident photons are inelastically scattered, limited number of molecules are detected for dilute solutions. To circumvent this problem, extraordinary Raman signal-enhancing techniques can be applied by using SERS for instance. This method can enhance the intensity of inherently weak Raman signal by several orders of magnitudes, so as to boost the possibility to investigate low concentration samples [3]. The SERS effect corresponds to the enhancement of the Raman scattering of a molecule situated in the vicinity of nano-sized metallic structures. SERS activity has been found so far for silver, copper, and gold [3]. Since silver can fulfill the resonance condition in the visible and near infrared (NIR), a frequency

range found in many common lasers, it is widely used and best substrate for SERS as compared to the other metals.

There is energy transfer between the incident radiation and the quantized and non-degenerate states of the scattered system. The incident photons either lose (Stokes) or gain (anti-Stokes) on the vibrational rotational electronic level [7].

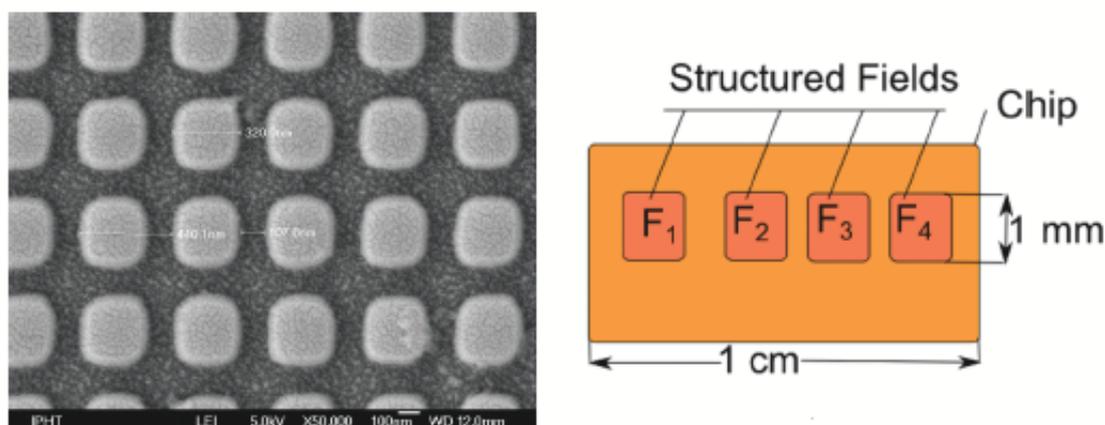
The shift in photon energy is the difference between the initial and final states of rotational and vibrational energies [7, 8].

Although, SERS gives the same information as normal Raman spectroscopy with very much enhanced signals, still there is some differences due to selection rules and electric field gradient effect [11]. It is very easy to assemble plasmonic nanoparticles at low cost so as to produce intense electric field in localized volumes between the nanoscale metallic gaps. This localized intense fields are called hot spots [14]. SERS is characterized by this hot spots where the Raman signal can be amplified to enable single molecule detection [15].

Since SERS enhancement consists of electromagnetic and chemical effects, hot spots are required. The hot spots describe the field enhancement that exist only for selection of some plasmonic materials in the visible range [16]. It is the local extreme enhancement of electromagnetic field between neighboring nanoparticles [17]. The enhancement of the Raman signal therefore depends on the hot spots besides to the size of the nanoparticle clusters [18].

Carbamazepine (CBZ) is a compound usually exists in different pharmaceuticals and it is mainly used to treat epilepsy [19]. It is unstable in its unhydrate forms under humid conditions and aqueous dispersions and converts immediately in to its dihydrate (DH) when immersed in water [20].

One of the techniques for managing the surface properties of metallic substrates is self-assembled monolayers (SAMs) [21]. In this regard, the adsorption of n-alkanethiols on metal substrates has great considerations as models for ordered organic surfaces [22]. Organic monolayers prepared by self-assembly of thiols on metal substrates have attracted a steadily increasing interest throughout the past few years [23]. For Ag nanoparticles, by far the most popular method is to use self-assembled monolayers (SAMs) of substituted thiols that spontaneously bond to the surface via strong covalent metal-thiol bonds [24].



**Figure 1.** Video image of a single field for silver substrate detected by CCD camera under white light illumination. It shows the area to be scanned in a single field from the substrate

## 2 Experimental Methods

### 2.1 Substrate preparation

A quartz layer with a resist serves as a starting material for substrate preparation. Electron beam lithography (EBL) technique have been employed to form nanostructures on the quartz layer. A 10 nm silver was deposited on these nanostructured quartz layers to form rough surface for excitation of plasmons since silver is reported to be the best material for SERS [30]. For optimization while conducting the experiment, a solution of crystal violet with concentration  $10^{-5}$  M and three times rinsed with water has been placed over the silver sample to coat the nanostructured surface thereby acts as a model analyte molecule. The solution was abandoned for 20 minutes on the silver thin film substrate until it forms a monolayer.

To measure the solid phase spectra and the spectra of aqueous solutions, CBZ grains and  $10^{-4}$  M concentration aqueous solution of CBZ had been prepared. While preparing the solution 127 ml of CBZ had been used. The grains of CBZ were placed on a tape, which is stick to a glass surface; while the solutions were poured into a cuvette. In order to measure the SERS spectra, the best scanning conditions for SERS had been chosen, and then  $1 \mu\text{l}$  saturated aqueous solution of CBZ sample adsorbed onto the silver surface and incubated for 20 minutes until it forms a monolayer.

### 2.2 Experimental procedures

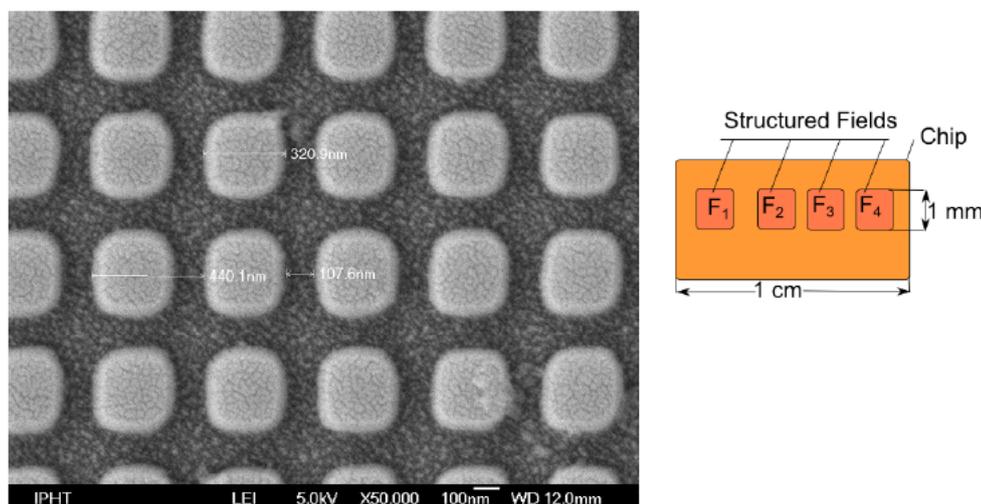
An argon-ion laser of excitation wavelength 488 nm had been connected to the coupler unit of a microscope by a fiber cable. The laser power incident on the sample was measured with laser power meter (from COHERENT company) and adjusted at  $20 \mu\text{W}$ . When the light was then incident on the sample some part of the light had been reflected, while some part was scattered. An in built notch filter had been used to suppress the intense elastically scattered light. However, the inelastically scattered light were collected by a converging lens and detected by a CCD camera. Once the assembled setup had been adjusted, an incident power of  $575 \mu\text{W}$  had been measured and the prepared CBZ biomedical sample (in solid form) were placed on a tape which was glue onto glass. Single spectrum measurements with an integration time of 1 s for 100 accumulations points had been taken to investigate

the sample. After the solid phase measurements have been carried out, the two week age prepared aqueous solutions of CBZ sample was poured into a cuvette. But first the spectrum of the pure cuvette had been measured to observe the background effects. The same measuring conditions as the solid samples were then employed to study the spectra of the solutions. However, the single spectrum measurement in this case was carried out with integration time of 5 s for 20 accumulations.

To optimize the best scanning condition for 10 nm silver sample for further study, crystal violet was used as a model analyte molecule to form a monolayer on the silver sample. Different image scans were carried out on the fields. This means that spectral arrays were taken while the laser beam is moving in lines and rows on the surface. The scanning condition was conducted over  $60 \mu\text{m} \times 60 \mu\text{m}$  area.

Different spectral measuring conditions have been carried out for 10 scans per field in each spectra for single point integration time of 0.3 s on the fields  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  as shown in fig 4. To determine the SERS intensity from the spectrum of crystal violet, integration of the Raman mode in the wavenumber range between  $1151 \text{ cm}^{-1}$  and  $1205 \text{ cm}^{-1}$  using the LabSpec software had been carried out. Once the optimal condition had been chosen  $1 \mu\text{l}$ , saturated aqueous solutions of CBZ was adsorbed in field  $F_2$  of the silver surface and incubated for 20 minutes until it develops a monolayer. The silver sample was put on glass, and then placed at the base of the microscope. The sample was irradiated through 100X magnification objective of the microscope. An image scan in rows and lines (10 scans per field) was conducted and the corresponding SERS spectra were detected for CBZ sample.

The spectra information observed using the above techniques were helped us to arrive at the conclusion that still analyte molecules of CBZ that was attached to the silver surface has to be increased. Therefore, to attract more analyte molecules onto the substrate, the silver sample was coated with octadecanethiol. The CBZ sample was dissolved with ethanol and washed with ethanol again so as to attract more analyte onto the surface.

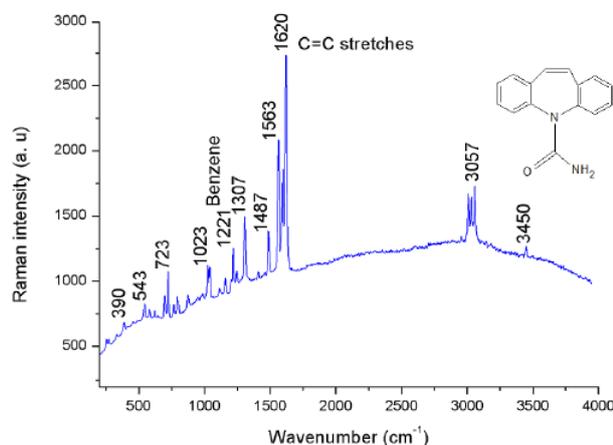


**Figure 2.** Scanning electron microscope of silver nanoparticle on quartz layer with etching depth into the fused silica wafer 50 nm-60 nm. There are fields  $F_1$ ,  $F_2$ ,  $F_3$ , and  $F_4$  in the arrays of silver nanoparticles on the quartz substrate where the light is being focused.

### 3 Results and Discussions

#### 3.1 Raman spectra of solid CBZ sample

One of the widely used biomedical substances which can be used for treatment of various infectious diseases is CBZ. In this work the Raman spectrum measurement for solid CBZ has been carried out. Fig 3 illustrates the spectrum of CBZ.



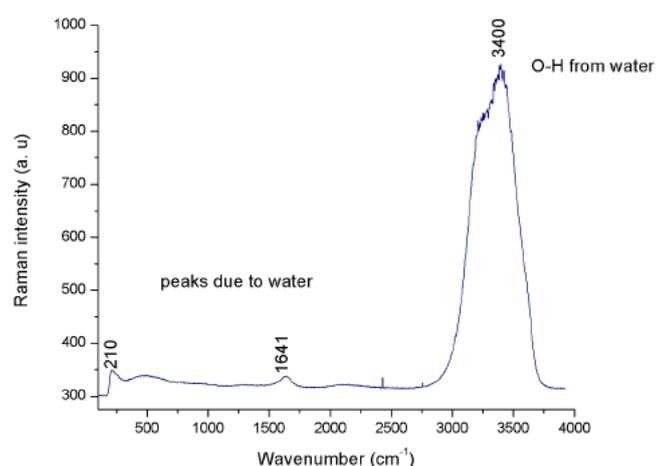
**Figure 3.** Raman spectrum of solid carbamazepine. There are well defined peaks in the spectrum which can be assigned to different bonds.

In this spectrum we see that the highest peak appears at frequency of  $1620\text{ cm}^{-1}$ , which is assigned to the coupled modes of C=C stretches. The peak at  $1221\text{ cm}^{-1}$  can be assigned to benzene ring vibrations.

#### 3.2 Raman spectra of aqueous solutions of CBZ sample

The aqueous solution of CBZ lacks clear spectrum peaks and has weak Raman intensity as compared to the pure CBZ.

Fig 4 depicts the Raman spectrum of this solution with broad band peak at  $3400\text{ cm}^{-1}$ , which can be assigned to the O-H vibration from water. The broad bands at  $1641\text{ cm}^{-1}$  and  $210\text{ cm}^{-1}$  also belongs to  $\text{H}_2\text{O}$ .



**Figure 4.** Raman spectrum of aqueous solution of carbamazepine. The intensities get lost as the concentration decreases due to less scattering

#### 3.3 Optimization of measuring conditions for scanning

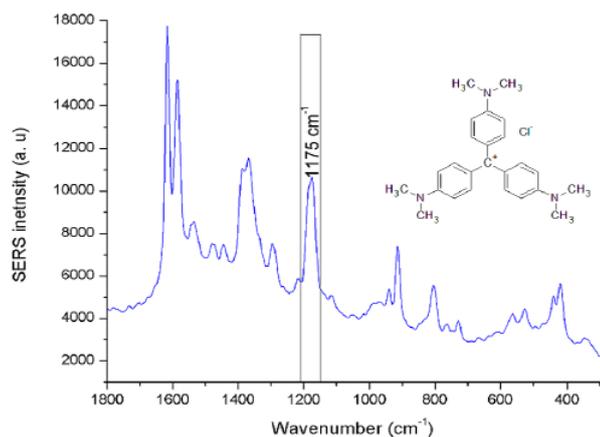
In order to optimize the SERS scanning condition, crystal violet was used as a model analyte molecule and its spectrum is measured. Fig 5 illustrates the spectrum of crystal violet. The marked mode is the integrated Raman mode for evaluation of SERS intensity. This Raman mode lies in the Raman shift range between  $1151\text{ cm}^{-1}$  and  $1205\text{ cm}^{-1}$ .

The best scanning condition was taken to probe the biomedical substances in all SERS measurements as well as hydrophilic and hydrophobic samples.

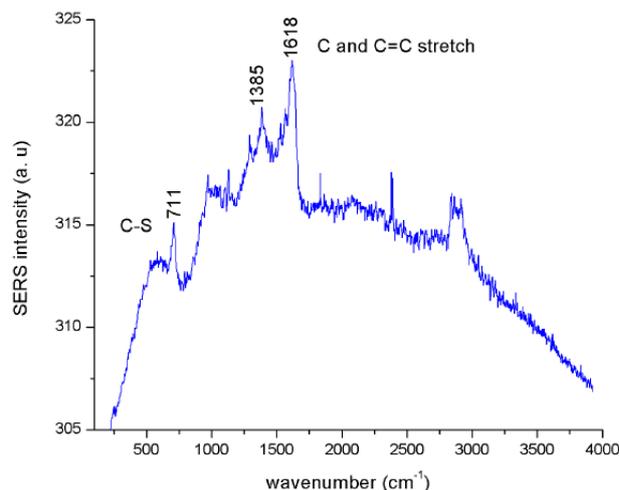
#### 3.4 SERS spectra of the aqueous solutions CBZ sample

The Raman spectrum of pure CBZ shows well defined peaks for identification of different band assignments. However, the Raman spectrum of aqueous solution of CBZ do not show distinguishable peaks for identification of the existing bonds in the compound. Therefore, SERS is employed to enhance the signal that can be used for detection of molecules.

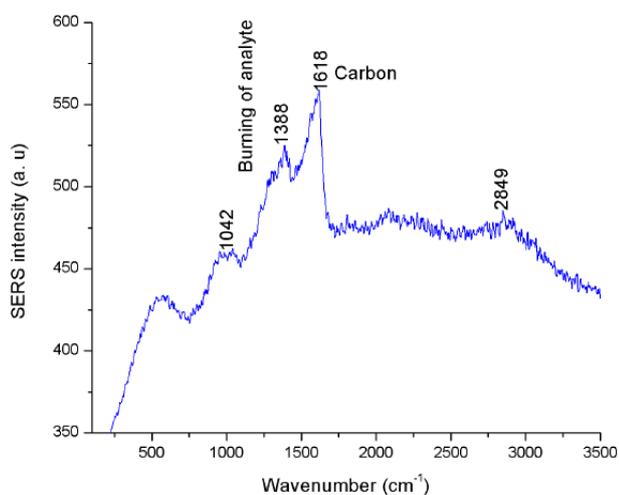
Fig.6 shows the SERS spectrum of  $1\ \mu\text{l}$  saturated solution of CBZ dropped on to silver and dried. The intense peak at



**Figure 5.** The marked Raman mode shows the integrated peak for SERS optimization through scanning



**Figure 7.** SERS carbamazepine with octadecanethiol



**Figure 6.** SERS spectrum of carbamazepine solution

$1618\text{ cm}^{-1}$  belongs to the carbon signal. The weak broad band peak at  $1388\text{ cm}^{-1}$  can be assigned to the burning of the analyte.

### Carbamazepine onto octadecanethiol

In order to observe better SERS intensity for further investigation of CBZ, measurement had been carried out using thiol layer (octadecanethiol).

Fig 7 depicts the SERS spectrum of  $1\ \mu\text{l}$  CBZ-ethanol solution adsorbed on octadecanethiol to trap analyte molecules onto silver substrate and dried. It can be observed that this spectrum is similar to the aqueous solution spectrum of CBZ. However, there is additional peak at  $711\text{ cm}^{-1}$  due to the presence of octadecanethiol and it belongs to C-S stretch. Moreover, the band that corresponds to the wavenumber  $1618\text{ cm}^{-1}$  belongs to carbon due to burning effects and C=C stretch. The Raman shift at  $1385\text{ cm}^{-1}$  also represents the carbon background.

## 4 Conclusion and future outlook

In order to detect molecules reproducible SERS signals are required. Such signals can be obtained using reproducible SERS substrates which can be produced by electron

beam lithography. Since silver supports surface plasmon resonance, silver nanostructures can be used as SERS substrates for producing intense reproducible signal that can be used to detect molecules. Nanostructures of silver metal adsorbed with crystal violet as a model analyte molecule for optimization can enhance the weak Raman signal. This enhanced signal is used for detection of biomedical compounds even in dilute solution. The widely used biomedical substance; CBZ is studied using Raman spectroscopy. This compound display different spectral information which is suitable for band assignments in the compound. The stretching modes in this biomedical sample corresponds to different peaks in the spectra. Aqueous solution of this biomedical sample do not show well defined spectra for assigning which peak belongs to which bond. Therefore, to detect small amounts of molecules in aqueous solutions, enhancement of the Raman signal is needed, which had been achieved using 10 nm silver nanoparticles.

For further study of the CBZ sample, thiol layers was used taking in to account that the thiol layer will attract more analyte molecules to the silver surface. In general, the band assignments made for different bonds using SERS in this paper is in agreement with the results done by other researchers [31–34].

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