Author's Accepted Manuscript

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PII: S0167-577X(14)00949-5
DOI: http://dx.doi.org/10.1016/j.matlet.2014.05.137
Reference: MLBLUE17069

To appear in: *Materials Letters*

Received date: 23 April 2014
Accepted date: 21 May 2014

Cite this article as: N Ravi Chandra Raju, Hemoglobin detection on AgO surface enhanced Raman scattering (SERS)-substrates, *Materials Letters,* http://dx.doi.org/10.1016/j.matlet.2014.05.137

Hemoglobin detection on AgO surface enhanced Raman scattering (SERS)-substrates

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Abstract

Human hemoglobin (2.2×10^{-4} M) detection has been demonstrated on AgO surface enhanced Raman scattering (SERS)-substrates. Hot spots that enable detection of hemoglobin using SERS are formed at the silver nanoclusters induced by the photo-activation of AgO under Raman excitation wavelength (633 nm). Higher enhancement is observed at integration time (20 s) and threshold energy density (12 MJ/cm^2). At higher excitation energy densities photo-chemical (or photo-thermal) activity of the hemoglobin molecules are dominated. These results are critical to the future use of AgO films as SERS substrates for the detection of biological molecules.

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Keywords: silver oxide, surface enhanced Raman scattering, hemoglobin, silver nanoclusters

1. Introduction

Surface-enhanced Raman scattering (SERS) is an extraordinary technique for detecting and characterizing biological and biomedical molecules [1, 2]. Hemoglobin is an oxygen transport protein in the human blood and has a major clinical significance. The early detection and structural information with respect to any changes in the blood caused by the diseases, infections are of very importance. Nie and Emory [3] and Kneipp and co-workers [4, 5] were demonstrated that using colloidal silver nanoparticles the effective Raman cross section of small aromatic molecules could be increased by 14–15 orders of magnitude. Even though the chemical molecule detection has been reported on silver oxide SERS substrates [6, 7, 8], our focus of the present letter is the successful demonstration of biological molecule detection on AgO based SERS substrates. Early studies on hemoglobin detection by Xu et al [9] demonstrated up to single molecule level (1×10^{-11} M) by dispersing with the colloidal silver nanoparticles. Kang Y et al [10] have studied SERS studies of oxy and de-oxy hemoglobin on silver films prepared by electrolysis. Enhancement in the Raman signal can be achieved with silver based nanostructures because silver has a lower dielectric loss due to inter-band damping of plasmon resonance when compared to other plasmonic metal elements [11, 12]. However due to silver being highly reactive to the ambient species, it is often a challenge to prepare stable and reproducible silver based SERS substrates.

In this letter we present the detection of human hemoglobin (hHb) on AgO based SERS substrates. It is easy to prepare AgO SERS substrates using any thin film deposition method. AgO photo-activation process is the underlying mechanism to enable hot spots

of silver nanoclusters under Raman excitation. We identified the critical excitation energy density for the detection of hHb beyond which the structural changes of hHb molecule occur due to photo-chemical and /or photo-thermal reactions.

2. Experimental section

AgO SERS substrates were prepared using the pulsed laser deposition (PLD) method [13, 14]. Hemoglobin molecules of three different concentrations (2.2×10^{-4} M, 5.8×10^{-4} M and 1.3×10^{-3} M) were prepared; micro drops of these dilutions are dried on AgO substrates a few minutes before conducting the SERS measurements. Complete details on the substrate deposition and hemoglobin separation process are provided in the supplementary material [15, 16]. SERS studies are conducted at room temperature using a Jobin Yvon HR 800UV Raman spectrometer with 633 nm as an excitation wavelength. Spectral resolution of 0.6 cm^{-1} is achieved with a holographic grating of 1800 lines/mm and slit opening of 100 μm. Scanning electron microscope (SEM) images were collected on a high resolution Raith 150 Two instrument.

3. Results and discussion

Fig 1 shows the normal Raman spectra of hHb molecules of various concentrations dried on plain glass cover slips. All spectra are at an equal acquisition time of 20 s. The detection limit of hHb molecules with normal Raman spectroscopy is observed for the concentration of 2.2×10^{-4} M by noticing the absence of spectral features. However for the higher concentrations we can observe the spectral modes of hHb (nearly at 755, 1252 and 1576 cm^{-1}) agreeing well with the reported literature [9, 10].

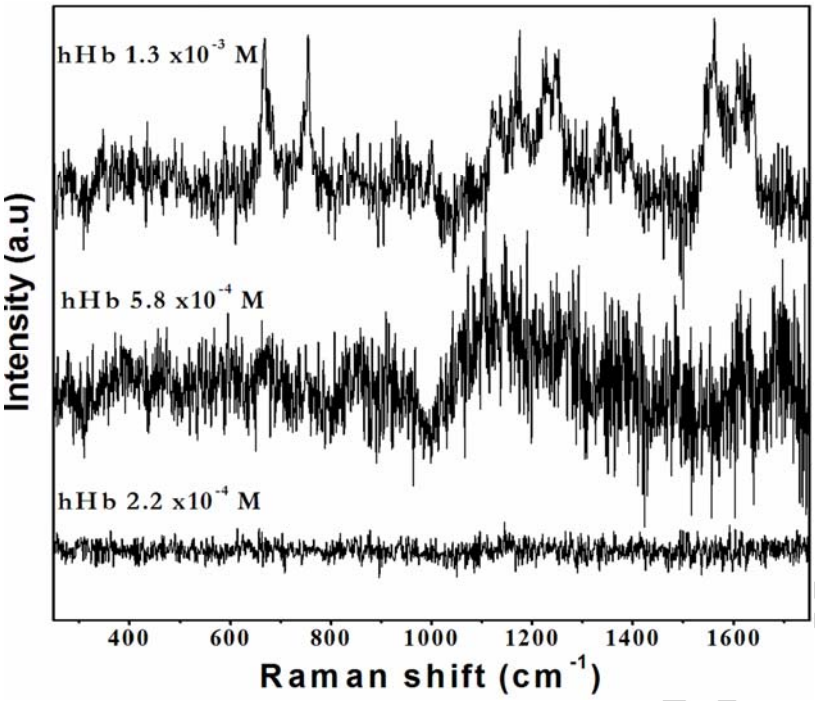

FIG. 1. Raman spectra of human hemoglobin of various dilutions dried on glass cover slips.

In order to detect hemoglobin on AgO SERS substrates we used the lowest concentration 2.2×10^{-4} M hHb which is undetectable by the normal Raman method. The detection process using AgO SERS substrates is quite different from the normal SERS methods. Under a normal SERS experiment, the detection molecules are directly sitting on the surface of metal plasmonic nanostructures, because of which the enhancement in the molecule signal increases. In the case of AgO based SERS studies, though the geometry of the substrate and detecting molecule is the same, the substrate is purely AgO and it is highly insulating in nature. Preparation of AgO SERS substrates is simple and reproducible using any thin film deposition method. In contrast the usual commercial

SERS substrates of nanostructure and pattern silver are involved rich process technologies.

Under Raman excitation, the AgO SERS substrate undergoes photo-activation to produce silver nanoclusters. These silver nanoclusters then act as hot spots in enhancing the Raman signal of any nearby chemical or biological detecting molecule. Unlike in normal SERS studies the excitation laser here is also involved in creating hot spots. Detection is possible with AgO due to unique nature of photosensitivity and it can easily decompose into silver nano clusters by the visible light interaction.

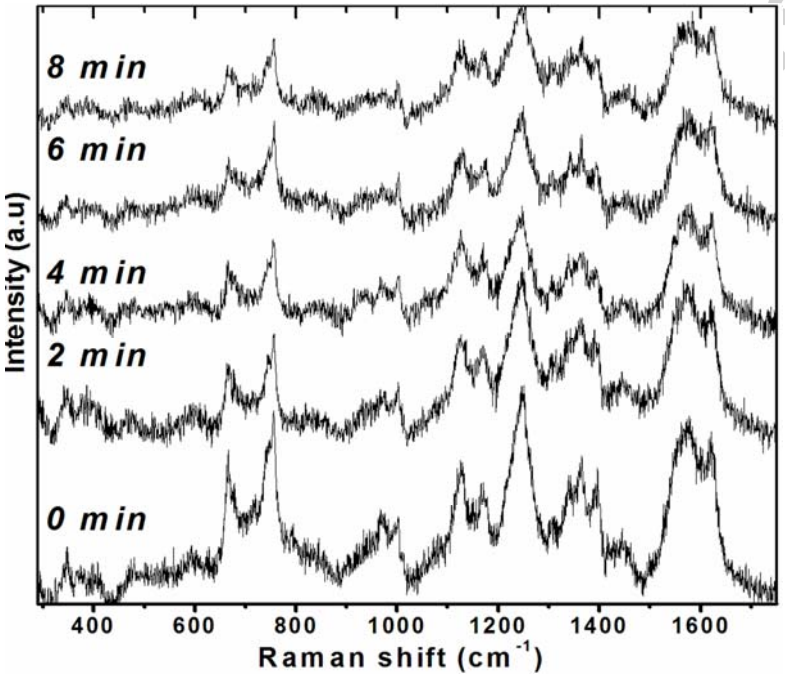

FIG. 2. SERS spectra of 2.2×10^{-4} M human hemoglobin dried on AgO substrate, recorded from 0 min (immediately after laser incident) to 8 min irradiation time intervals using 633 nm laser light at 0.6 MW/cm^{-2} with 20 sec data acquisition time for each spectra collected in the range 300-1750 cm^{-1}.

Fig 2 shows the SERS spectra of the lowest concentration hHb molecules on the AgO SERS substrate. The laser energy density is the critical parameter for the photo-activation

process to occur in AgO. We chose a lower energy wavelength of 633 nm as an excitation laser light and performed the photo-activation process of the AgO substrate without any hemoglobin on the surface at various laser energy densities. It was determined that 0.6 MW/cm^{-2} with the total spectra acquisition time of 20 s is necessary to photo-activate the AgO substrate. Complete details of the AgO photo-activation experiment is discussed in the supplementary material [15]. Using these critical laser parameters we have collected the SERS spectra of hHb molecules dried on the AgO substrate.

In order to understand the effect of silver nano cluster (photo-activated AgO) on the detection molecule we collected spectra at five different time intervals from 0 min (immediately after laser incidence) to 8 min. As seen in Fig 2, spectra collected immediately after laser incidence hHb Raman modes are sighted; whereas the same concentration of hHb on a bare glass cover slip (without AgO SERS substrate) is undetectable (as observed in Fig. 1). This enhancement in the Raman signal of hHb is possible only when the hot spots are developed near the hHb molecules on the AgO surface under the 633 nm excitation laser spot. Here the hot spots are the silver nanoclusters created by the photo-activation of AgO. In the next series of spectra we can notice a slight variation in the relative spectral intensities of the hHb modes without changing the Raman shift.

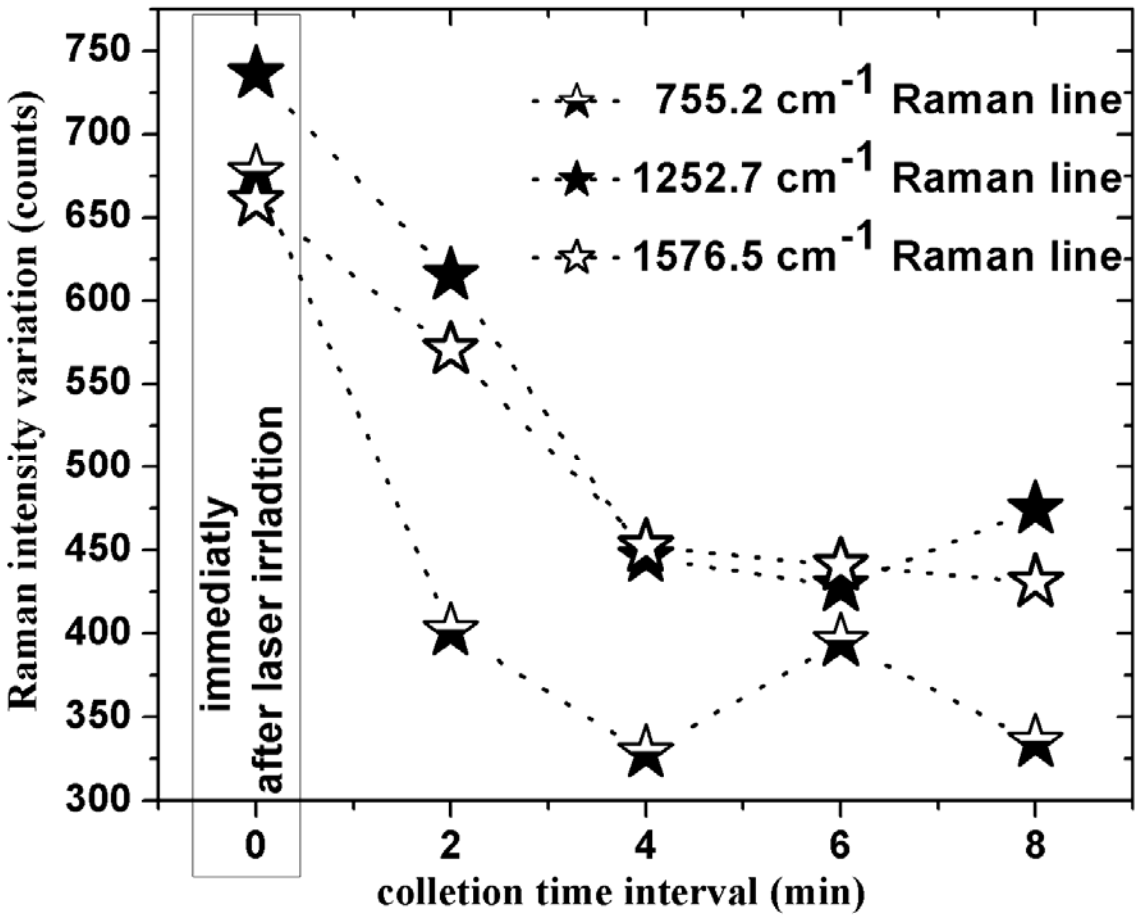

FIG. 3. SERS intensity variation of 2.2×10^{-4} M human hemoglobin on AgO substrate irradiated using 633 nm laser light at 0.6 MW/cm^{-2} at different collection time intervals.

Variation in intensity of hHb modes at different collection intervals are plotted in Fig 3. The non linear variation in the intensity of the modes corresponds to the dynamic variation of the hot spot (size of silver nanoclusters) near the hHb molecule under the excitation laser interaction spot. Under the interaction of light, properties of the dielectric media present in the vicinity of the silver nanocluster are highly sensitive due to the plasmonic fields of the cluster [17].

In order to further understand the nature of the hotspot, we have recorded the SEM image of the AgO SERS substrate immediately after 8 min of irradiation.

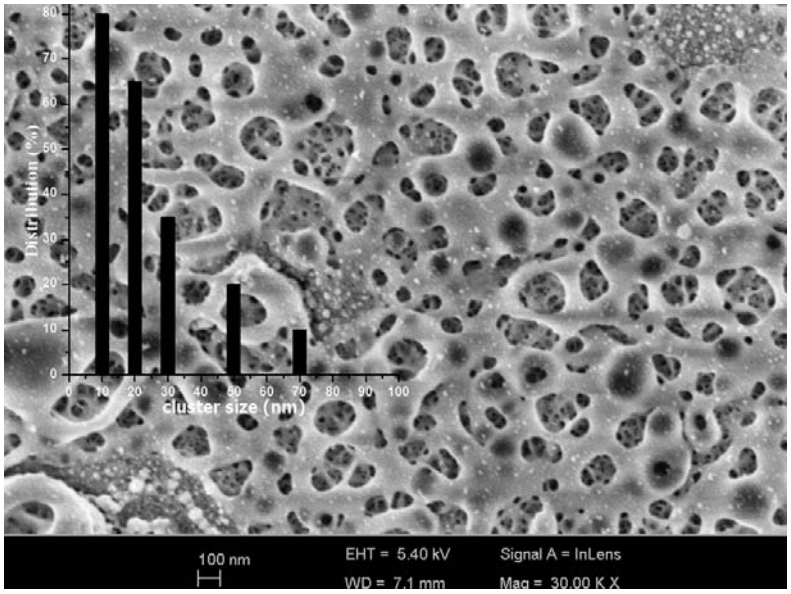

FIG. 4. SEM image of 8 min irradiated (using 633 nm laser light at 0.6 MW/cm^{-2}) AgO SERS substrate having hHb (2.2×10^{-4} M) dried on the surface. Inset of the figure shows the histogram of silver nanoclusters distributed on the same AgO SERS substrate.

Fig 4 shows the SEM image of an 8 minute irradiated AgO SERS substrate. From the SEM image we can observe that the size distribution of silver clusters ranged from10 nm to 70 nm. These silver nanoclusters yield the electromagnetic enhancement under laser interaction and are responsible for the amplification of hHb Raman signal. To amplify the hHb Raman signal further we increased laser energy density to 1.2 MW/cm^2. The position and relative intensities of the hHb Raman modes drastically changed and some of the modes completely disappear. The change in Raman modes implies the structural change of the hHb molecule. This may be due to the photo-chemical or photo thermal processes occurring due to high laser energy density. High intensity SERS experiments are discussed in the supplementary material [15]. These findings indicate that the threshold energy density should be maintained while detecting biological molecules on AgO SERS substrates. As the AgO photo-activation is a dynamic process under the laser

interaction spot, in-situ real time experiments must be conducted in order to rationalize the Raman modes by correlating with respect to the dynamic size distribution. Further experiments are in progress to understand the complete process of AgO photo-activation.

4. Conclusions

In conclusion, we demonstrated the application of AgO SERS substrates for the detection of biological samples. Raman modes of the 2.2×10^{-4} M human hemoglobin on the AgO SERS substrates were identified. The results indicate that a photo-activation process of AgO is responsible in creating hot spots (nano silver clusters) which further contribute to the enhancement of hHb Raman signal. Optimum laser (633 nm) threshold intensity (0.6 MW/cm^2) and acquisition times (20 s) are identified for the AgO photo-activation by conducting photo-activation studies on Raman spectroscopy. These are the important parameters for the realization of SERS detection of biological samples using AgO substrates.

Acknowledgements

The technical support and discussions from A. Subrahmanyam, IIT Madras, Chennai, India and Paul Ramesh Thangaraj, Apollo Hospitals, Chennai, India is gratefully acknowledged.

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Figure Captions

Fig.1. Raman spectra of human hemoglobin of various dilutions dried on glass cover slips.

Fig.2. SERS spectra of 2.2×10^{-4} M human hemoglobin dried on AgO substrate, recorded from 0 min (immediately after laser incident) to 8 min irradiation time intervals using 633 nm laser light at 0.6 MW/cm^{-2} with 20 sec data acquisition time for each spectra collected in the range 300-1750 cm^{-1}.

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The present manuscript has the following highlight points for the consideration of review:

- Novel silver oxide surface enhanced Raman scattering substrates
- Hemoglobin detection
- Threshold energy density of silver oxide photo-dissociation
- Optimized conditions for the detection of hemoglobin on silver oxide SERS substrates