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RESEARCH ARTICLE

Enhancing the Raman Scattering for Diagnosis and Treatment of Human Cancer Cells, Tissues and Tumors Using Cadmium Oxide (CdO) Nanoparticles

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Abstract

In the current paper, the Localized Surface Plasmon Resonance (LSPR) effect induced by Cadmium Oxide (CdO) nanoparticles is used to observe Raman spectrum of human cancer cells, tissues and tumors. The diagnosis and treatment of human cancer cells, tissues and tumors in sample is investigated through Nanomaterial Surface Energy Transfer (NSET) process from human cancer cells, tissues and tumors to the surface of nanoparticles, and Surface Enhanced Raman Scattering (SERS) process, as effective factors for Raman spectrum detection. For interaction of human cancer cells, tissues and tumors with Cadmium Oxide (CdO) nanoparticles, colloidal state and Self-Assembled Monolayer (SAM) methods were used. Both methods have shown good agreement with each other in detecting the Raman spectrum. It should be noted that these methods and techniques can be applied on different types of human's cancer cells, tissues and tumors, respectively.

Keywords

Surface Enhanced Raman Scattering (SERS), Localized Surface Plasmon Resonance (LSPR), Nanomaterial Surface Energy Transfer (NSET), Self-Assembled Monolayer (SAM), Diagnosis and Treatment of Human Cancer Cells, Tissues and Tumors, Cadmium Oxide (CdO) Nanoparticles

Introduction

Since the discovering time of Raman scattering, a great effort has been begin to enhance Raman signal for increasing the detection limit and sensitivity of this method due to inherent low scattering cross section of Raman. Today, Plasmon structures are widely used to enhance Raman signal. This method is known as Surface Enhanced Raman Scattering (SERS) [1-27]. The Enhancement Factor (EF) of Raman signal can reaches up to 1015 times. The main mechanism that affects EF of signal is electromagnetic mechanism and is induced by enhancing the scattered light by the Localized Surface Plasmon Resonance (LSPR) of metallic nanoparticles or in sharp points and other curvatures of Plasmon structures. In this method, molecule should be placed at distance lower than 10 (nm) from the surface of nanoparticle [28-43].

In recent years, a considerable attention has been paid to pair and enhance the surface Plasmon fields in the connection point of metallic nanoparticles through creating various arrays and geometries [44-63]. In order to produce an ideal SERS substrate, various methods such as Electron-Beam Lithography, Nanoimprint Lithography, Self-Assembling of nanoparticles and etc. are used. The first two methods can create very regular and uniform structures with high repeatability. However, such methods need high cost and special laboratory conditions. In contrast, Self-Assembling of nanoparticles is used as a low cost and high productivity method [64-75].

In addition, utilizing Plasmon structures leads to increase the maximum effective distance of Forster (fluorescence) Resonance Energy Transfer (FRET) from 10 (A°) to 220 (A°). This process of energy transfer between fluorophore and surface of nanoparticle named as Nanomaterial Surface Energy Transfer (NSET). In this condition, the quantum gain of energy transfer is defined as:

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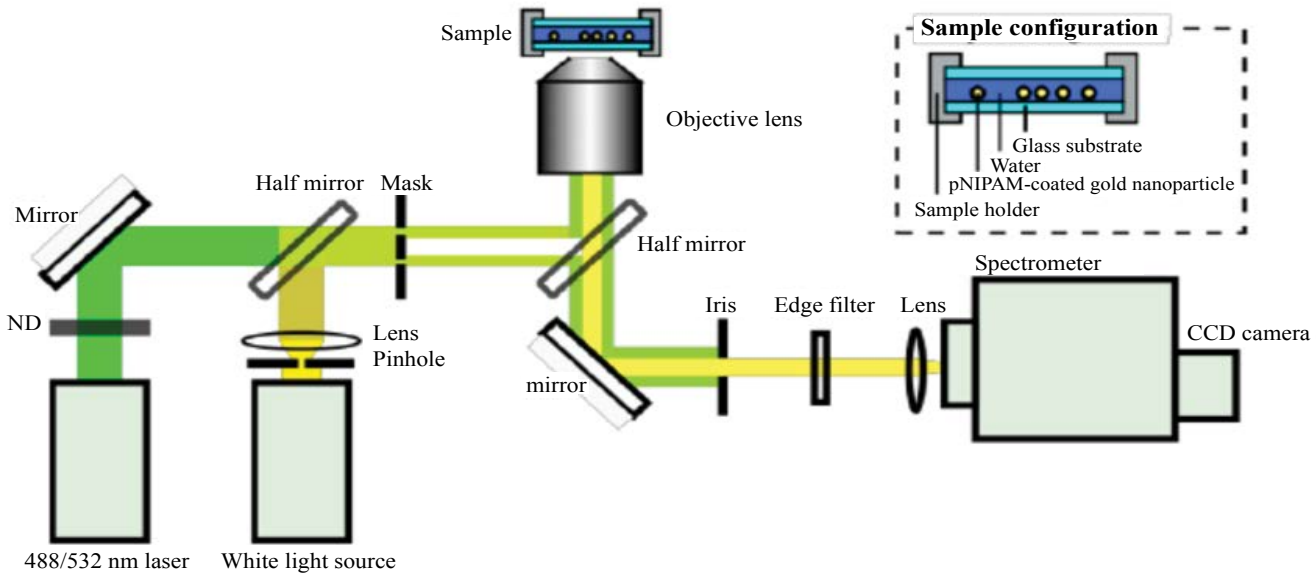


Figure 1: Schematic view of experimental arrangement for Raman Spectroscopy.

$$\phi_{Tr} = \frac{1}{1+\left(\frac{d}{d_0}\right)^4} \qquad (1)$$

where d_0 is the distance in which fluorophore has a same probability for emission and energy transfer to nanoparticle [76-106]. The emitted fluorescence signal by molecule can be enhance by Plasmon field at the vicinity of nanoparticle either at emissive wavelength or at excitation wavelength. At the other hand, fluorescence signal of molecule can be transferred to the neighboring nanoparticle through non-radiant process of NSET. As Plasmon modes are mainly damped in non-radiant form, this process can be led to considerable reduction of fluorescence in the sample. Domination of each of these two processes and its intensity are depend on factors such as overlapping of nanoparticle absorption spectrum with fluorescence spectrum of sample, distance and relative direction between them, size and form of nanoparticles and etc. [107-161].

In the current paper, SERS spectrum of a very light emissive sample of human cancer cells, tissues and tumors is obtained at colloidal condition and over the substrate monolayer produced using Self-Assembled Monolayer (SAM) method and the effects of enhanced Raman scattering and NSET are studied for observing the spectrum of these human cancer cells, tissues and tumors. It should be noted that these methods and techniques can be applied on different types of human's cancer cells, tissues and tumors, respectively [162-191].

Martials, Research Method and Experimental Techniques

Materials and tools

Cadmium Oxide (CdO) nanoparticles, Sodium Citrate ($Na_3C_6H_5O_7$), Sulfuric Acid, Hydrogen Peroxide, HPLC, Water and APTES (3-Aminopropyl-Triethoxysilane) were supplied from Sigma-Aldrich Corporation.

SERS spectra for samples were obtained using the designed arrangement for Raman spectroscopy. A schematic view of this arrangement is shown in (Figure 1). A laser with 532 (nm) wavelength was used as excitation source. Laser light was focused on the sample by a 4X thing and the scattered Raman signal was collected by that thing and was sent to spectrometer for analysis.

Sample preparation method

Nanoparticles were synthesized based on Alireza Heidari and Christopher Brown method [1]. Briefly, 18 (mg) Cadmium Oxide (CdO) nanoparticles solution was heated up to boiling point at 90 (ml) water. Then, 2 (ml) of 1% citrate solution was added to it and was maintained at boiling point for 90 minutes [1].

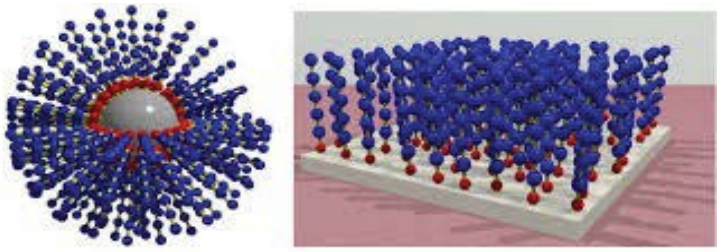
The glassy substrates activated with hydroxyl group were placed in 1% solution of APTES for 4 hours. After washing with toluene, substrates were submerged into colloidal Cadmium Oxide (CdO) nanoparticles solution for 24 hours and then, were maintained into the water up to the time for using. To measure the Raman spectrum of human cancer cells, tissues and tumors, substrates were placed in 1 (µM) solution of sample and then, Raman spectrum was measured.

To observe SERS signal at colloidal state, at least 3 hours before the test, 0.01 (M) solution of NaCl and 1 (µM) solution of human cancer cells, tissues and tumors were added to Cadmium Oxide (CdO) nanoparticles.

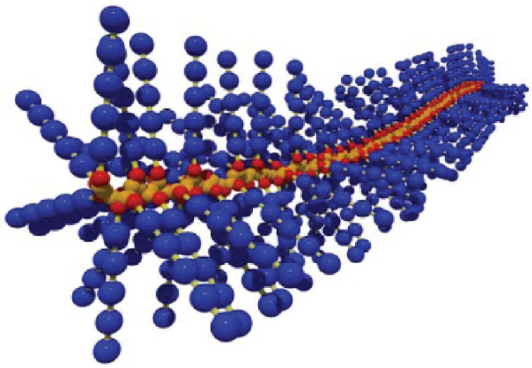
Results and Discussion

The general scheme of formation mechanism of substrate is shown in (Figure 2).

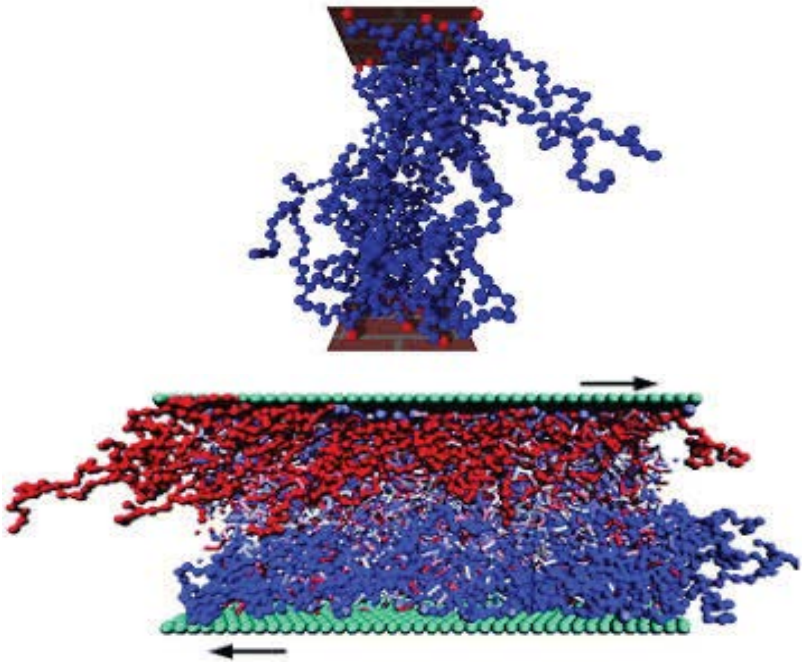
Figure 3 shows absorption spectrum of Cadmium Oxide (CdO) nanoparticles at colloidal and self-assembled



(a)



(b)



(c)

Figure 2: Various stages of formation of substrate; a) Cleaning and activating the glassy medium; b) Surface functionalizing using silane group and; c) Connection of nanoparticles to the surface using silane group.

states over a glassy medium. Maximum Plasmon resonance at colloidal state is about 453 (nm). However, this maximum for the substrates produced using self-assembled method shows a shift towards shorter wavelengths (418 nm). This shift can be attributed to dipole-dipole interaction between arrayed nanoparticles in a two-dimensional structure. Further, a new peak is emerged around 710 (nm) due to pairing of nanoparticles.

Figure 4a and Figure 4b show the enhanced Raman spectrum of human cancer cells, tissues and tumors over substrate and at colloidal state, respectively. Detecting such spectrum from such human cancer cells, tissues and tumors which has very strong absorption and fluorescence in utilized wavelength of laser (Figure 3) can be attributed to Surface Enhanced Raman Scattering phenomenon at the presence of nanoparticles and to reduction in fluorescence due to energy transfer to nanoparticles through NSET process. Figure 3 shows considerable overlapping of fluorescence human cancer cells, tissues and tumors spectrum with absorption spectrum of nanoparticles, especially when

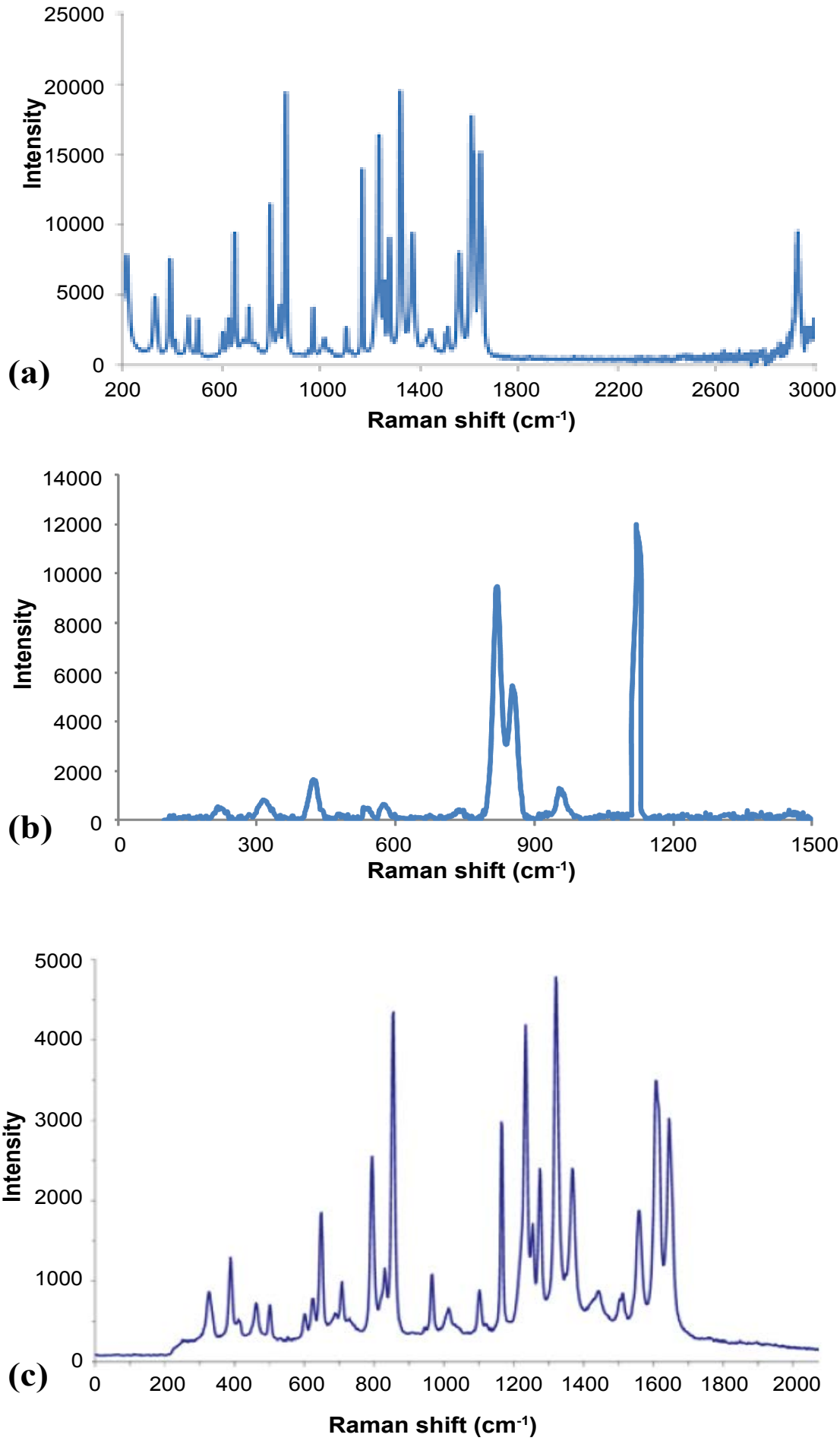


Figure 3: Absorption spectrum of cadmium oxide (CdO) nanoparticles; a) At colloidal state; b) After self-assembling over substrate and; c) Fluorescence spectrum of human cancer cells, tissues and tumors.

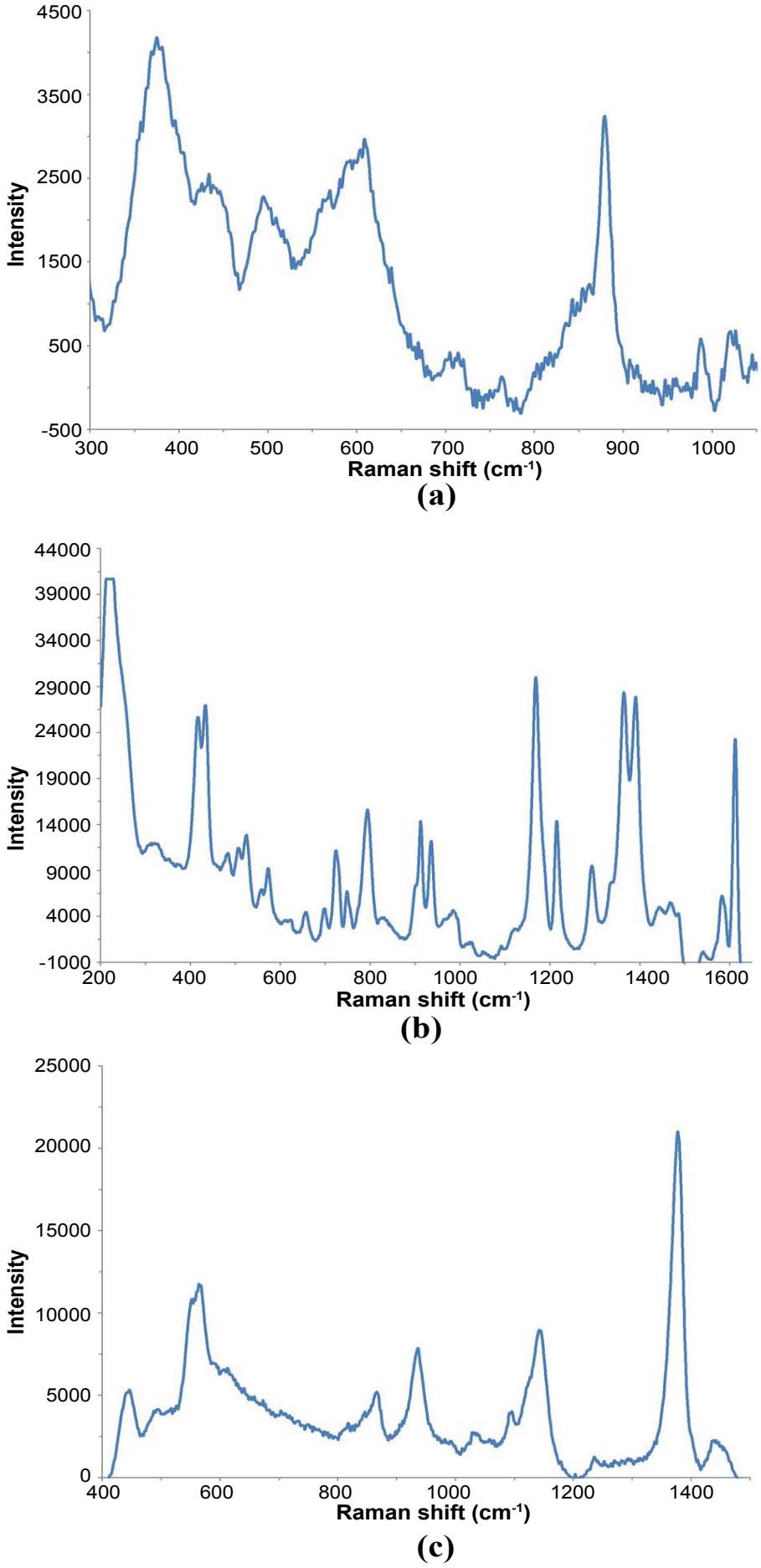


Figure 4: a) Raman spectrum of SERS from human cancer cells, tissues and tumors over self-assembled substrates of cadmium oxide (CdO) nanoparticles; b) Raman spectrum of SERS from human cancer cells, tissues and tumors at colloidal state and; c) Raman spectrum of human cancer cells, tissues and tumors at normal state.

Table 1: Raman vibrational modes of human cancer cells, tissues and tumors molecules.

Raman vibrational mode	Experimental frequency shift (cm^{-1})	Reference frequency shift (cm^{-1})
Bending C-H	1192	1199
Ring stretching C-C	1333	1323
Ring stretching C-C	1384	1385
Ring stretching C-C	1527	1543
Ring stretching C-C	1573	1589
Ring stretching C-C	1691	1697

placed as two-dimensional arrays over glassy medium. This overlapping is necessary for energy transferring. As the synthesized nanoparticles are mainly spherical, energy transferring process is independent of emission direction of incident light. Spectroscopy was performed during 5 seconds for all samples. In this comparison, fluorescence human cancer cells, tissues and tumors spectrum is attenuated up to 25 times due to high concentration of fluorescence in 1 (μM) solution of human cancer cells, tissues and tumors molecules and saturation of spectrometer (Figure 4c).

Table 1 shows vibration modes related to human cancer cells, tissues and tumors molecules. Frequency shift of this test is compared with a reference which its results show good agreement with reference results [1].

Conclusions, Perspectives, Useful Suggestions and Future Studies

The results obtained in the current study show the effect of Cadmium Oxide (CdO) nanoparticles on Raman spectrum detection through two mechanisms of SERS and NSET. In order to confirm the effect of nanoparticles on various conditions, colloidal and self-assembled monolayer mediums were used. The results obtained from both states are in good agreement with each other.

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