

SINGLE BACTERIUM DETECTION USING SERS

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Abstract

This work is devoted to the study of a single *Staphylococcus aureus* bacterium detection using surface-enhanced Raman spectroscopy (SERS) and resonant Raman spectroscopy (RS). It was shown that SERS allows increasing sensitivity of predominantly low frequency lines connected with the vibrations of Amide, Proteins and DNA. At the same time the lines of carotenoids inherent to this kind of bacterium are well-detected due to the resonance Raman scattering mechanism. The reproducibility and stability of Raman spectra strongly depend on the characteristics of nanostructured substrate, and molecular structure and size of the tested biological object.

1. Introduction

Pathological bacteria are the cause of many serious diseases. Their early diagnostics is the indispensable condition of the successful fight against these diseases. Many methods have been developed for detection of bacteria which are the causative agents of human illnesses. Bacteria invasion into the cerebral spinal fluids (CSF) is a serious problem leading to the development of meningitis – a dangerous bacterial form of the central nervous system illness with a high level of mortality. First bacteria identification is very important for the early diagnosis and treatment of this illness. The current tools available for detecting the bacteria penetration into CSF are cytological, bacteriological, and serological analysis techniques. They are time-consuming and not always reliable, simple, and inexpensive. At present the optical methods of CFS analysis unfortunately are poorly developed.

In view of its potential for the fast and noninvasive analysis at a molecular level the Raman spectroscopy has been the method of precise research of biological objects for the last years. Raman spectroscopy undoubtedly holds promise for CSF analysis, because it is a prompt and noninvasive technique capable of providing reliable information about the kind of microorganisms in a few seconds [1-5]. An increase in measurement sensitivity becomes actual if the detection of a single bacterium is the goal.



This work is devoted to the study of bacterium detection using surface-enhanced Raman spectroscopy (SERS), which combines the molecular fingerprint specificity with an increased sensitivity due to an enhancement of the Raman signal intensity for molecules of interests in close proximity to a plasmonic nanostructure. The theory of this phenomenon has been still poorly developed. In spite of this, experimental study showed a very high gain of spectral lines using the plasmonic effect. This fact was experimentally demonstrated for the cases of simple molecular substances detection. Concerning the bacteria as the objects of the study the spectroscopic results are more modest [6-9].

Pure bacterial cultures of *Staphylococcus aureus* were chosen for the study in vitro. This kind of bacteria is a causative agent of meningitis. As known, this bacterium can be effectively detected using resonant version of Raman spectroscopy (RS) due to carotinoids content [10,11]. As a result, the detection efficiency of resonance version of Raman spectroscopy and surface-enhanced Raman spectroscopy could be compared in the frames of this work.

2. Materials and Methods

2.1. Nanoparticles

Gold sphere nanoparticles (Au-NPs) of ≈ 40 nm in diameter were used for SERS. Au-NPs were fabricated by pulsed laser radiation (1030 nm, 300 fs) focused on a gold plate. In so doing, the gold plate was placed under a water or isopropyl alcohol layer two millimeters thick. Au-NPs were put on the substrate of silver coated glass plate. The silver film was deposited on the glass plate by magnetron unit SC7620 Mini Sputter Coater. Colloidal Au-NPs were dropped onto the substrate surface (1-22 drops) that was rotated in a centrifuge at a speed up to 400 rpm. As a result, the substrates with different Au-NPs concentration and the substrate without NPs were prepared for the bacteria deposition.

2.2. Bacterium samples

Cultivation and preparation of in vitro cultured bacterium of *Staphylococcus aureus* were performed by the bacteriological laboratory of the Moscow Infectious Clinical Hospital No 2. The bacterial species were cultured in nutrition medium and their colonies suspended afterwards in phosphate buffered saline solution. About 1 ml of the suspension were spotted onto the clean surface of a substrate and dried at room temperature. 70% ethanol solution was used for bacteria inactivation. As was shown earlier [2], a bacterium sample in the form of a droplet dried at room temperature keeps the main characteristic features of its Raman spectrum.

2.3. Spectroscopic instrumentation

The spectral measurements were fulfilled with the help of Nicolet Almega XR spectrometer supplied by a 532-nm laser excitation source (Nd:YAG CW laser producing 20-mW second-harmonic radiation). The spectrometer was equipped with a microscope capable of X10, X50 and X100 magnification. The diameter of the probe laser beam spot was 1 μm in the microscopic research. The Stokes shift components in the range of 400–3100 cm^{-1} at the spectral resolution of 2 cm^{-1} were used in our analysis. Wave number calibration was performed by using the characteristic silicon reference peak at 521 cm^{-1} . The phenomenon of photobleaching for 30 seconds prior to the recording of the Raman spectra was used to reduce the fluorescence influence. The experimental spectra were computer processed using the OMNIK software.

3. Experimental Results

The measurements showed that maximum absorption of Au-NPs in water solution just after their preparation takes place at the plasmonic resonance wavelength of 525 nm (Fig. 1). Because of NPs aggregation, the absorption is decreasing and plasmonic resonance is broadening in time. So, the absorption coefficient is degraded as much as twice in two days.

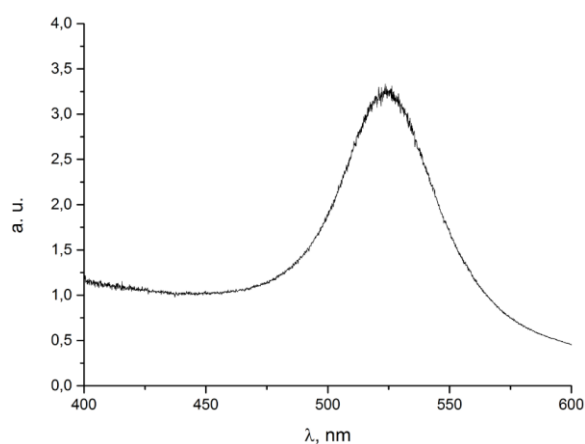


Figure 1. Absorption spectrum for gold sphere nanoparticles (≈ 40 nm in diameter) in water

Raman spectra of *Staphylococcus aureus* dried samples registered using resonant RS (without NPs) and SERS (with Au-NPs) are demonstrated in Fig. 2. It can be seen that the advantage of plasmonic effect using is equal to one order and more for the lines, connected with the vibrations of Amide, Proteins and DNA (1673.39 , 1547.97 , 1498.89 , 1449.82 , 1321.67 и 730 cm^{-1}) [12]. At the same time the lines of carotenoids (in the range of 1152 cm^{-1} and 1528 cm^{-1}) typical of *Staphylococcus aureus* are amplified insignificantly because of the plasmonic effect. However, they are well-detected due to the resonance Raman scattering mechanism because the excitation wavelength (532 nm) is within the carotenoids absorption band [13,14].

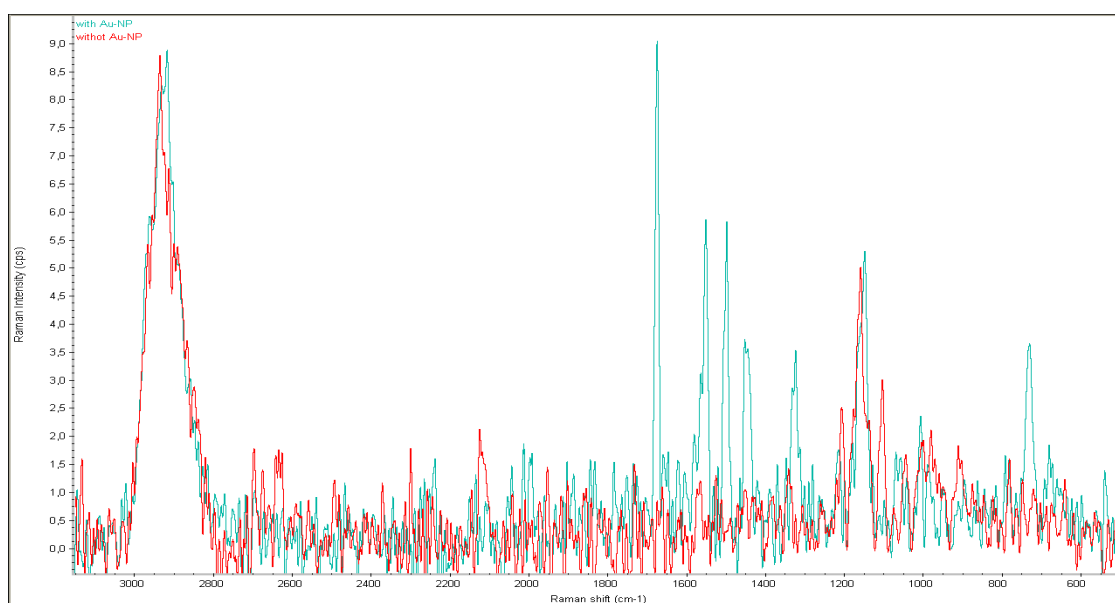


Figure 2. Raman spectra of *Staphylococcus aureus* under excitation of radiation with the wavelength of 532 nm for resonant RS (red) and SERS (green)

It was also observed that some spectral lines intensities are changing from one measurement to another. This fact of the spectra registration non-reproducibility and specific difference between the resonant RS and SERS spectra mentioned above is obviously the result of joint action of substrate parameters, bacteria orientation on substrate surface and the distance between the surface and bacteria. The influence of these factors was discussed during spectral analysis of single molecules [8,15]. In the case of bacteria as the test object these factors can be manifested more specific and complicated ones because of the large difference between characteristics of nanostructured substrate surface and molecular structure, and the size of bacterium.

4. Conclusion

The potentiality of surface-enhanced Raman spectroscopy (SERS) for a single bacterium detection as compared to the resonant Raman spectroscopy (RS) is demonstrated in this work. Specific difference between the resonant RS and SERS spectra and their non-reproducibility can be explained by large difference between the parameters of nanostructured substrate, and the size and molecular structure of bacterium and their spatial orientation in the vicinity of substrate surface.

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