Application of silver films with different roughness parameter for septic human serum albumin detection by Surface Enhanced Raman Spectroscopy

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Abstract. In this paper, the rough silver films parameters investigation, used as media for surface enhancement Raman spectroscopy for health and septic human serum albumin (HSA) study results have been presented. The detection of small concentrations of HSA isolated from blood serum and its main vibrational groups identification has been done.

1. Introduction
Raman spectroscopy is widely used in biomedical research [1], including non-invasive [2] and label-free [3] biomolecules diagnostics. Surface Enhanced Raman Spectroscopy (SERS) is used to identify both single biomolecules and the processes occurring in them [4].

SERS is explained by the effectiveness of the analysis of trace amounts of substances, which is claimed in various fields, such as biology, medicine, criminalistics, ecology, etc. The main advantages of the method include the simplicity of sample preparation, high sensitivity, ability to detect ultra-low concentrations of substances under study up to single molecules, molecules qualitative determination and detailed information on their structure and orientation by characteristic spectra obtaining [5-7].

This paper performs the investigation of silver films roughness parameters for both septic and health HSA SERS study. The main structural groups of the septic human albumin in the middle-band region with a frequency shift of 500-1700 cm⁻¹ have been analyzed and compared with its healthy condition. It has been shown that surface plasmons (SPs) appear on a rough silver film when interacting with the optical radiation and multiply (~10¹ – 10²) Raman scattering signal for an albumin biomolecule.

2. Materials and Methods
The human plasma was thawed for 30 minutes to room temperature before experiment, then, a weak solution of 5-10% aqueous sulfasalicylic acid solution (C₇H₆O₆S) was prepared. Extraction of HSA was prepared by placing 2 ml of unfrozen plasma in an Eppendorf tube, then the solution of
sulfosalicylic acid was added to plasma dropwise, as a result, the hydrate coat of the protein was destroyed and its solubility in water decreased, and HSA precipitated in the bottom of the tube. Special attention was paid to a soft change process of the pH solution, both for the gradual withdrawal of albumin into the sediment, and for the subsequent renaturation of a part of the protein. The supernatant was neatly removed with a pipette, the pH of the protein was adjusted to 5.5 (near the isoelectric point of the protein) by successive washing with distilled water and, as a result, adding up to 5 ml in the tube. Thus, the denaturing agent was partially removed and part of the HSA was transferred to its original state.

SERS substrates were prepared as follows: copper substrates were stripped and polished from one side, to gain a metallic luster. After polishing, the surface was washed with 96% ethanol, after it was degreased in a 5% solution of sodium hydroxide, washed with distilled water, and dried at 60°C. After drying and washing procedures, silver electrodeposition was deposited at 20-22°C temperature with current density of 5 mA/cm² for 15 minutes. Thickness of the silver film was measured to be 5 μm. Then, the roughness and porosity of one part of the sample surface was changed by anodic dissolution of the silver film onto 0.25; 0.5; 0.75 μm-thick layers at current density of 5 mA/cm². After electrodeposition and anodic dissolution, the samples were washed for 10 minutes with running water to remove the electrolyte salts and dried. The surface roughness was controlled by method described in [8] by SPM Certus Standart module with optical registration system (LTD «NanoScanTechnology», Russia) and tapping mode atomic force microscopy (AFM) use.

Raman spectra were obtained by CENTAUR U unit with diode-pumped solid-state laser (DPSS) excitation (λ = 633 nm, radiation power of 37 mW). Raman spectra from SERS surface with a drop of protein solution on the substrate at 11 mg/ml concentration were recorded using a CCD detector matrix with 120 s signal accumulation.

3. Results and Discussion
Figure 1 shows AFM image of SERS substrate with different anodic dissolution of the silver film layer (Figure 1A-1C) and SERS substrate with protein solution of high concentration (Figure 1D).
**Figure 1.** The morphology of the porous silver film surface, controlled by AFM for 0.75 μm (a), 0.5 μm (b), 0.25 μm (c) anodic dissolution of the silver film layer and for 0.5 μm and anodic dissolution of the silver film layer with 45 mg/ml protein solution on the surface (d)

The obtained results showed that increase in the anodic dissolution value from 0.25 μm to 0.5 μm leads to increase in silver clusters size from 800 nm to 2 μm, which consequently causes increase in the overall amplitude of the Raman signal and a decrease in efficiency of SERS spectral resolution. In a sample with an anodic dissolution layer thickness of 0.75 μm, decrease in the size of silver clusters to 800-600 nm was observed. Regardless up to 84% of clusters have a size of 800-600 nm, 2 μm clusters were observed also. We suppose that the cluster size change leads to a decrease or the repeatability of the recorded SERS spectra (for the AP sample with anodic dissolution layer of 0.75 μm value). This may be due to the substrate pores size decrease and their deepening, along with changes in the geometry of the excitation radiation optical rays path on the sample. The experiment demonstrated that the samples had both inhomogeneous structure and significant difference in the degree of the detected signal amplification (up to 41 times), depending on the laser beam position on the surface. Figure 2 illustrates the SERS signal with best resolution, registered with 0.5 μm anodic dissolution value sliver substrate film.

**Figure 2.** SERS spectra of septic (red line) and health (blue line) HSA on the SERS substrate surface, prepared with anodic dissolution of the silver film with 0.5 μm thick.

Based on the aforementioned study results, the protein structure of healthy patients and the protein of patients with sepsis were identified. The main characteristic frequencies in the frequency range of Δν = 500-1000 cm⁻¹ are the skeletal vibrations of the protein molecule [9], Δν = 1200-1400 cm⁻¹
denote the regions of the Amide I band [10], $\Delta \nu = 1600$-1700 cm$^{-1}$ denote the regions of the Amide III [10]. Identified vibrational groups are performed in Table 1.

| Protein group | Raman band position of health HSA, cm$^{-1}$ | Raman band position of septic HSA, cm$^{-1}$ | SERS Enhancement factor |
|---------------|---------------------------------------------|-----------------------------------------------|-------------------------|
| Amide III     | 1288                                        | 937, 1259, 1311, 1359                           |                         |
| Amide I       | 1610                                        | 1616                                          | 41                      |
| Skeletal      | 807                                         | 776, 893                                      |                         |

According to the SERS spectroscopy method, the main frequency bands of the spectrum of the protein molecule were identified and deciphered. According to sepsis pathology, deformation was found to be affected by Amide III group. Its structure become more chaotic, the amino acid residue of tryptophan 214 appears on the surface of the protein globule [11], as well as the amino acid residue of tyrosine is packaged inside the molecular structure.

4. Conclusion
The methodology for creating silver films has been developed and optimal experimental parameters for recording the Raman spectra of human serum albumin in sepsis has been performed. It has been shown that the use of the developed substrates with deposited silver films makes it possible to obtain amplified Raman signal. The surface structure with 0.25, 0.5 and 0.75 dissolution AFM results have been presented. Protein molecules structure for normal and septic conditions for the main protein vibration groups in the 500-1700 cm$^{-1}$ band has been analyzed.

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