Formation of SERS-substrate based on Ag-Cu bimetallic nanoparticles for detection of ultra-small amounts of substance

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Abstract. The aim of this work was to determine the sensitivity threshold and enhancement factor of a planar SERS substrate based on an array of composite Ag - Cu nanoparticles. The nanoparticles were deposited using the method of vacuum-thermal evaporation followed by annealing at 300 ° C. Methylene blue was chosen as the analyte. The possibility of detecting the micro- and nanomolar concentration of methylene blue using a SERS-substrate with an active Ag-Cu layer at a laser wavelength of 632.8 nm is shown. The use of such arrays of nanoparticles as an active layer makes it possible to achieve an analytical enhancement factor of the SERS substrate of the order of $6 \times 10^5$.

1. Introduction
Raman spectroscopy is a common chemical analysis technique that provides information on the composition and structure of a test substance. The presence of plasmonic nanostructures near the analyte surface can lead to significant enhancements (up to $\sim 10^{12}$) of the Raman signal of the substances under study. The effect of surface-enhanced Raman scattering (SERS) of light arises due to the excitation of plasmon resonance in metallic nanostructures. SERS can be used to detect substances of low concentration, which makes it a promising method for such areas as materials science (determination of the composition and structure of nanoscale objects), pharmacology (control of the composition of dosage forms), forensics (determination of explosives or drugs), early diagnosis of many diseases, including cardiovascular and viral, as well as malignant tumors [1-8]. Due to their unique optical properties, Ag, Au, and Cu [9] and their alloys [10] are traditional materials used in the formation of SERS substrates.

In our previous work [11], using thin layers of amorphous carbon as an analyte as an example, we showed that the use of composite Ag-Cu arrays for SERS allows a high amplification of the Raman signal in the red spectral region, in which copper arrays are usually effective. However, the enhancement for Ag-Cu turns out to be higher than that for arrays of pure copper particles: it turns out to be comparable with the enhancement of an array of pure Ag nanoparticles in the blue spectral region.
region. In this work, using planar SERS substrates based on arrays of composite Ag-Cu nanoparticles, a solution of methylene blue (MB) dye at various concentrations (normal, mil-, micro-, nanomolar), which is a standard marker for assessing the gain, was studied.

2. Experimental
SERS substrates were planar structures on silicon containing a reflective metal layer (Al), a thin dielectric layer (SiO$_2$), and an SERS-active layer (Ag-Cu NPs). The manufacturing process of SERS substrates and their cross section are schematically shown in Figure 1.

![Figure 1. Fabrication steps of SERS-substrate based on Ag-Cu alloy nanoparticles.](image)

Arrays of Ag-Cu nanoparticles were formed by vacuum-thermal evaporation and condensation of a substance on substrates, followed by thermal annealing in vacuum at 300 °C. In this case, the ratio of the weight portions of the evaporated metals was chosen so that the formed condensate corresponded to the eutectic composition (Ag - 5.0 mg; Cu - 2.0 mg). Studies in [11] show that the particles obtained in this way are precisely composite and consist of parts of Cu and Ag.

In this series of experiments, we used a LabRAM HR Evolution Raman spectrometer (Horiba). The measurements were carried out at room temperature at a wavelength of 632.8 nm. The nominal laser power was 10 mW. Methylene blue (Sigma-Aldrich, CAS Number 122965-43-9) converted into solutions of nano-, micro-, milli- and normal concentrations was used as an analyte. 10 μL of the analyte was applied to a substrate by pipette, followed by drying the samples in an oven at room temperature.

3. Results and discussion
At the first stage of the study of methylene blue using a SERS-substrate with Ag-Cu nanoparticles, an experiment was carried out to identify the optimal laser power for obtaining the Raman spectra of the analyte. Figure 2 shows the spectra of MB obtained at a laser power of 0.1 to 10% of its nominal power. At 10% power, the average intensity is about 11300 arbitrary units. For a power of 5%, the average intensity is 8100 a.u. For a power of 2.5%, the average intensity is approximately 4650 a.u. For a power of 1%, the average spectrum intensity is 2200 a.u. For a power of 0.1%, the average spectrum intensity is about 160 a.u.
Figure 2. Raman spectra for SERS substrate based on Ag-Cu MB millimolar concentration at laser power 0.1%, 1%, 2.5%, 5%, 10% of its nominal power.

As can be seen from Figure 2, with decreasing laser power, the intensity of the spectra decreases. It is important to note that when the laser power is increased, the formation of new peaks does not occur, which may appear due to the thermal effect of laser radiation on the analyte. The revealed spectral modes of MB millimolar concentration (Figure 2) using the developed SERS substrate are in full agreement with the measurement data of other authors [12, 13].

Figure 3. Raman spectra for a SERS substrate based on Ag-Cu methylene of blue milli-, micro- and nanomolar concentrations at a laser wavelength of 632.8 nm.

Figure 3 shows the spectra of MB solutions of 1 milli-, micro-, and nanomolar concentrations obtained at a laser wavelength of 632.8 nm and 1 mW power. It can be noted that lowering the concentration to micromolar does not affect the clarity of the Raman spectra peaks. At the same time, for the nanomolar concentration of MB, the peaks of most of the spectral lines are not clearly detected, while some of them are shifted. However, it should be noted that a part of the spectrum for nanomolar concentration is similar to the spectra of micro- and millimolar concentrations. However, the clear
peaks for nanomolar concentration are reduced to 10, while for millimolar and micromolar concentrations are around 17. Figure 4 shows the Raman spectra of MB with micromolar concentration on a glass substrate and on a SERS substrate with an active layer of composite Ag - Cu nanoparticles. It should be noted that in the case of a glass substrate, none of the characteristic MB spectral modes is observed, even for the strong vibrations of MB attributed to the skeletal deformation of C-N-C (at 448 and 500 cm⁻¹) and the ring stretching of C-C (at 1623 cm⁻¹).

![Raman spectra of MB](image)

**Figure 4.** Raman spectra of the micromolar MB concentration on a glass substrate and on a SERS substrate with an active layer of composite Ag-Cu nanoparticles.

The analytical enhancement factors (AEF) calculated for spectral modes at 1623 and 440 cm⁻¹ for planar SERS substrates with an active layer based on an array of Ag-Cu nanoparticles for a laser with a wavelength of 632.8 nm was ~ 5.78×10⁵ (1623 cm⁻¹) and 5.71×10⁵ (440 cm⁻¹). The calculation of the AEF was carried out according to the well-known method [14]. The obtained AEF values turned out to be quite close to the enhancements reported in [12 - 14], in which the authors studied MB using Au nanoparticles (EF 2.6 × 10⁵) [12] and NPs Ag of various shapes (AEF 3.7 × 10⁵) [14]. The relative standard deviation (RSD) of the Raman intensity at 1623 cm – 1 was about 23%, which is significantly higher than our previous estimates when detecting a thin a – C film (~ 11%) [11]. This difference is probably related to the nonuniform distribution of the surface concentration obtained upon drying of a droplet of micromolar MB concentration, and not to the bulk of Ag - Cu nanoparticles itself. Previous measurements on a-C film are more accurate in terms of evaluating the uniformity of Raman signal amplification by the SERS-active layer, since the analyte distribution over the surface was more uniform.

The enhancement for MB obtained in this work is not one of the best, for example, there are reports on the detection of femtomolar concentrations of MB using structures of complex shape with a developed surface based on Ag – Au nanoparticles [15]. Nevertheless, our result indicates that the use of the Ag - Cu composite in SERS detection is promising. Undoubtedly, the use of nanoobjects of this material of a more complex shape can increase the sensitivity of SERS substrates and limiting quantitative determination. This should become one of the tasks of further research.

4. Conclusions
The work shows the possibility of detecting the micro- and nanomolar concentration of MB using a SERS-substrate based on composite Ag-Cu nanoparticles at a laser wavelength of 633 nm. The use of
such arrays of nanoparticles as an active layer makes it possible to achieve an analytical enhancement factor of the SERS substrate of the order of $5.7 \times 10^5$. It has been demonstrated that when using different laser powers, no new peaks appear due to the interaction of laser radiation with MB.

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