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Capabilities of Surface Enhanced Raman Spectroscopy for Identifying Multiple Pigments in a Complex Organic Mixture

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Abstract—The authors propose a way of identifying the composition of paints by means of surface enhanced Raman spectroscopy using signal-amplifying substrates with arrays of vertically standing silver nanowires. A model tempera paint based on egg white with inorganic pigments (red lead, massicot, and emerald green) is used to show that with a reduced concentration of pigment, substrates can greatly improve sensitivity when detecting pigments in lower concentrations up to 0.01 g, compared to signals from the Raman scattering of light on foil. Reinforcing substrates allows the sensitivity of the technique to be improved in order to accurately identify components used individually and in mixtures.

Keywords: Surface enhanced Raman spectroscopy, nanowires, protein, pigment
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INTRODUCTION

The development of ways of synthesizing reinforcing surfaces is allowing low-intensity Raman spectroscopy to expand its functionality in a wide range of applied tasks, thanks to surface-enhanced Raman scattering (SERS) [1–9]. A striking application of SERS is analyzing the composition of paints in studying works of art [10–18]. The most important problem in such an analysis is creating an effective nondestructive way of determining the composition of paints in complex mixtures that can replace the set of means currently used in laboratories. The use of reinforcing surfaces (so-called SERS substrates) opens up prospects for adapting enhanced Raman spectroscopy to analyzing trace concentrations of components in mixtures.

Nowadays, paintings are dated by analyzing the binders in paint, since the evolution of the latter is the reason for the former. The evolution of a binder is influenced by several factors, e.g., the availability and cost of ingredients, the development of technology and scientific research, changes in fashion, and customer tastes [19]. The simplest and most accessible binders have always been such natural ones as egg yolk, milk, vegetable oils, animal fat, and resin [20]. Such components have always been available, but were not particularly resistant to the effects of time and the environment. In the Middle Ages and Renaissance, artists began to experiment with more resistant binders like animal glue or oil base, which allowed them to create more resistant and durable paints that could retain their brightness and beauty for many years. New technologies and chemical binders like synthetic resins, acrylic, and latex appeared in the 19th and 20th centuries, making it possible to create higher-quality and more durable paints that are resistant to weather conditions, mechanical influences, and wear [21].

The study of pigments and paint components allows us to determine which materials an artist used [22]. This can also help determine the age or origin of a painting [23]. Several ways of analyzing paints are used in practice, most often in combination with one another. The most common way is radiocarbon dating, based on carbon analysis and allowing us to determine the time a picture was created by measuring the content of the radioactive isotope of carbon C-14 (however, its accuracy ranges from hundreds to thousands of years). Another way of analyzing the composition of paints is chromatography, in which the components that make up a paint are separated and their chemical composition is determined. It is therefore possible to determine whether modern chemical compounds were used that did not exist at the time a painting was created. This can help identify a forgery. Chromatography can also help determine the origin of the paints to establish where and by whom a painting was created. This in turn can also help in the restoration of paintings. Such means of nondestructive analysis as IR and Raman spectroscopy have developed rapidly in

recent years [24–26], since they allow us to quickly determine the composition of pigments and binders used in painting a picture.

Raman spectroscopy is not effective when studying small amounts of a test substance, or when there is strong fluorescence of a pigment. SERS on plasmon nanostructures is used to enhance the low-intensity spectra obtained with Raman spectroscopy. Surfaces with plasmonic nanostructures allow the sensitivity and accuracy of an analysis to be improved, allowing us to register the spectra of molecules that cannot be isolated under normal conditions [27]. There are two factors that explain the amplification of a Raman signal near plasmonic nanostructures: chemical and electrodynamic. The latter is based on localizing the electromagnetic field near the tips and defects of plasmonic nanostructures, and in the nanometer gaps between them [28]. On the other hand, the chemical effect is associated with a special mechanism of a substance's adhesion to a SERS substrate, and an increase in the concentration of molecules of the studied substance in a region characteristic of hydrophobic SERS substrates [29]. It is worth noting separately the quenching of the studied substance's fluorescence near plasmon nanostructures, which also expandings the boundaries of SERS application [30].

The simplest and most accessible way of obtaining SERSS spectra using plasmon structures is to use colloidal solutions of noble metal nanoparticles as reinforcing surfaces [31]. A solution with colloidal particles is applied to a test surface or mixed with small fragments of scrapings [32]. A disadvantage of this approach is the strong agglomeration of nanoparticles and thus the instability of the received signal.

One way of solving the agglomeration problem is to introduce nanoparticles inside the polymer matrix. The authors of [33] described the development and synthesis of a specially manufactured active film of methylcellulose with inclusions of silver nanoparticles. This approach allowed them to form a solid substrate on the studied surface and minimize the agglomeration of nanoparticles. Since the methylcellulose film is transparent, it does not interfere with analysis. After it dries completely, the film is easily removed from the test surface without damaging it.

The above problem of agglomeration is also avoided by using a substrate with an array of ordered nanostructures. Fragments of a sample are applied to the substrate's surface in the form of a crushed powder or a solution.

The potential of a substrate based on silver nanoparticles formed inside a matrix of hydroxypropyl cellulose (HPC) was demonstrated in [34], where the SERS procedure was tested using a variety of approaches: direct application, soaking (incubation) of a sample in a substrate, and hydrolysis with vapors of a hydrofluoric acid (HF). The SERS substrate (AgHPC1) was prepared by adding an aqueous solution of silver nitrate (15 g 2% $AgNO_3$) to an HPC solution (15 g 1.2% HPC) in a weight ratio of 1.0 : 0.6. The solution was stirred at room temperature for at least 15 min prior to irradiation. The samples were irradiated for 24 h using two ultraviolet table lamps with a dominant spectral peak at 365 nm (UVP LLS, United States). Substrates in closed quartz glasses were placed on stands at a sufficient distance from the lamps. The intensity of radiation was then set and averaged to 15 W/m^2.

The authors of [35] reported a 100× increase in the Raman scattering of light by ultramarine microcrystals when interacting with silver nanoparticles in films and powders. Theoretical modeling predicts a maximum gain of 10^{10} times in the immediate vicinity of a spherical silver nanoparticle (0.24 nm) with a rapid drop in the gain to 1 in the range of approximately 50 nm. The results are considered an important extension of traditional SERS spectroscopy in the direction of larger inorganic probes.

In this work, we propose using new substrates with arrays of silver nanowires to obtain the SERS spectra of a model tempera paint based on egg white and yolk with inorganic pigments. The task was to analyze a complex organic mixture of various pigments and a protein base in order to develop a way of applying paint to a solid substrate and demonstrate the capabilities of reinforcing nanostructured substrates with ensembles of nanowires, and to study the spectra of proteins in low concentrations on SERS substrates.

EXPERIMENTAL

We used template synthesis to manufacture SERS substrates [36–38]. The first of several steps was to apply a silver layer to one side of a track membrane (TM) made of polyethylene terephthalate with a pore diameter of 100 nm in order to create a conductive layer via resistive spraying, with subsequent reinforcement using a layer of copper 10–15 μm thick. In the next step, the silver was electroplated into the pores of the track membrane. Before using the substrate, the polymer TM was dissolved for 2 h in a 6 M solution of sodium hydroxide at a temperature of 85°C. SERS substrates with ensembles of vertically standing silver nanowires on their surfaces were obtained as a result.

Raman and SERS spectra were recorded on a portable TruScan Raman spectrometer (Thermo Fisher) equipped with a laser having a wavelength of 785 nm and a maximum power of 265 MW. The spot of laser illumination was 110 μm in diameter. The obtained spectra were analyzed using the Spectrogryph [39] and Origin software packages.

The tempera (a mixture of egg white and three pigments: red lead, emerald green, and massicot) was used to create the model paint. Three samples, two of which had their own pigment with egg white, were prepared to obtain reference spectra. The reference sam-

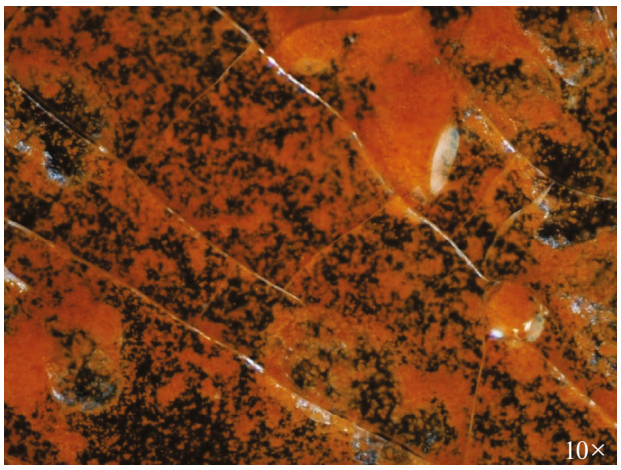

Fig. 1. Photo of a mixture under a microscope.

ples were examined on foil, along with samples having pigment concentrations >0.1 g. The SERS substrates were used to analyze samples with lower pigment contents. The concentrations of protein were 1.5–3 g on the foil for recording Raman spectra and from 0.337 to 3 g on a substrate for recording SERS spectra. The time needed to record the Raman spectra was ≤10 min, and on the order of several seconds for the SERS substrate.

The samples were prepared by mixing pigments and protein with five milliliters of water, after which the samples were dried at 100°C. A Nikon Eclipse LV 100 optical microscope with NIS-Elements D software was used to check the uniformity of each mixture (Fig. 1).

RESULTS AND DISCUSSION

The first stage of this work was to obtain Raman spectra of the protein and three pigments separately to identify characteristic peaks that were then used to analyze the mixtures' spectra (Fig. 2). The most intense characteristic peaks were at 1006 cm^{-1} (for protein), 548 cm^{-1} (for red lead), 389 cm^{-1} (for massicot), and 644, 748, 1143, 1338, and 1527 cm^{-1} (for emerald green).

After measuring the individual spectra of the pigments and protein, we moved on to analyzing the mixture. However, the determination of protein in the

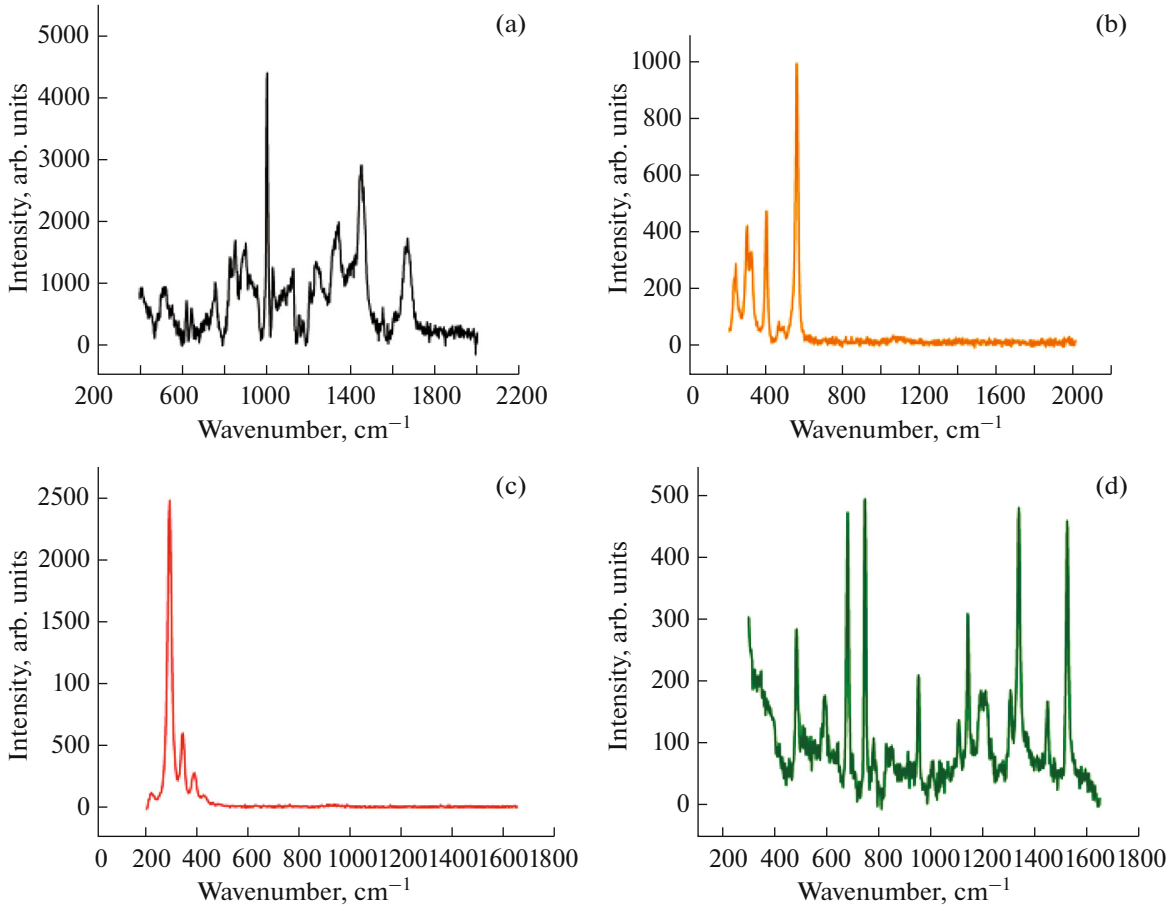

Fig. 2. Spectra of components: (a) egg white, (b) red lead, (c) massicot, and (d) emerald green.

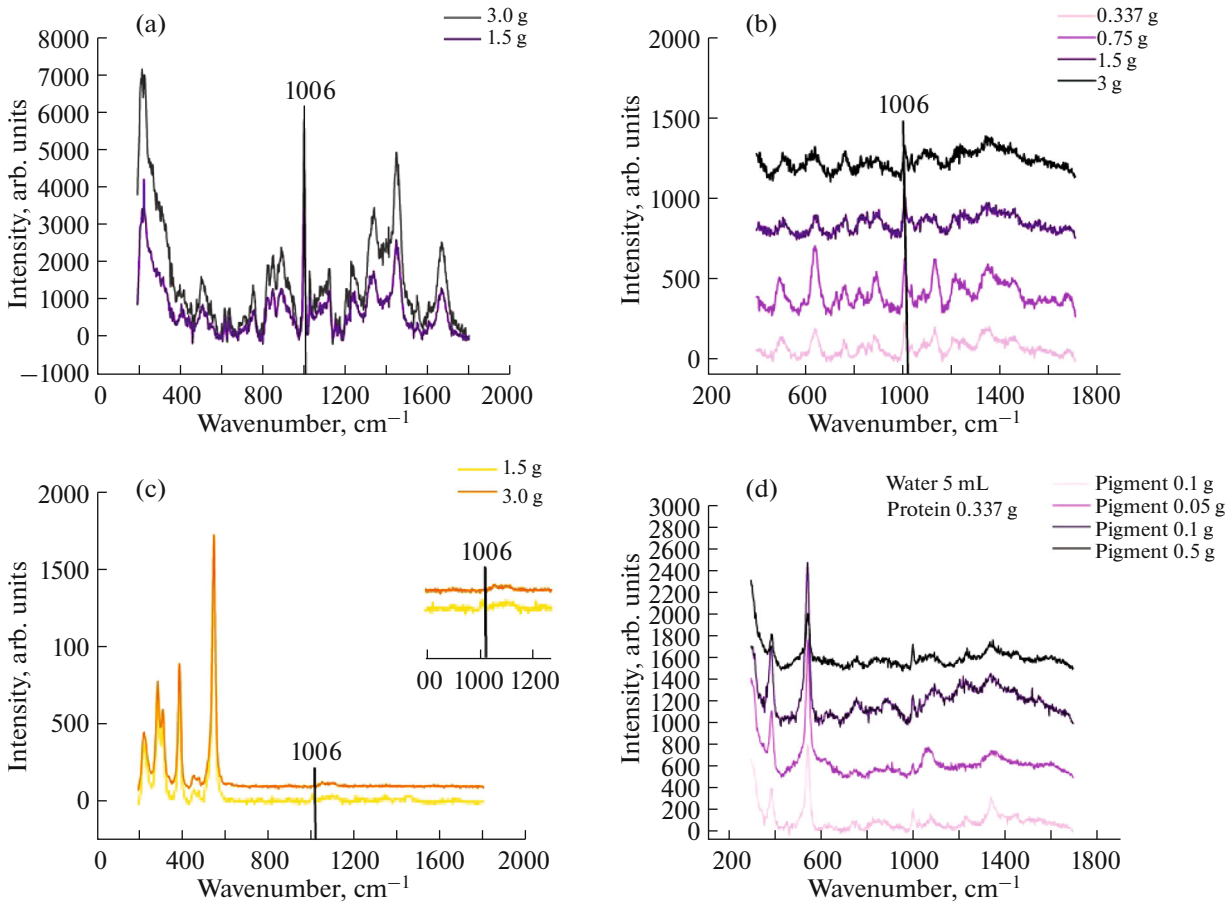

Fig. 3. Spectra of (a) protein on foil, (b) protein on a substrate, (c) protein with pigment on foil, and (d) protein with pigment on a substrate.

spectrum becomes almost impossible when mixing protein with a high concentration of pigment, since the pigment's spectrum is much more intense than that of the protein spectrum. To solve this problem, we switched from basic Raman spectroscopy to SERS using nanostructured reinforcing substrates. It should be noted that in order to obtain amplification, the sample must be applied to the substrate in a thin layer. Otherwise there will be no amplification of the spectra, since effective signal amplification is achieved at distances ≤10 nanometers from the nanostructures.

We prepared paint samples with red lead to determine the concentration dependence. The minimum recorded concentration of pigment when using Raman spectroscopy was 0.1 g. It was an order of magnitude lower on SERS substrates with nanowires: 0.01 g.

Figure 3 shows spectra of different concentrations of protein on foil and a substrate. Their characteristic peaks are clearly visible [40, 41]. To determine the visibility of a protein and compare the intensity of the signal in pure form and on the SERS substrates, we compared the peak intensity in the region of 1006 cm^{-1} (the characteristic peak of the phenylalanine contained in egg white) [42] for each spectrum.

It should be noted that the time needed to accumulate spectra from the surfaces of samples with different concentrations of protein differed (it was 5 min for 3 g and 10 min for 1.5 g) because it was impossible to set one period of detection for all samples on the portable spectrometer used in this work. After obtaining protein spectra with the SERS substrates (Fig. 3b), there was a notable doubling of signal intensity when comparing spectra of the same concentration (1.5 g) on the foil and the substrate

We next mixed the protein with one of the pigments (red lead). At this stage, we tried to find the minimum concentrations of paint components that could be detected with SERS, in order to determine the technique's capabilities. Figure 3 shows the spectra of mixtures with different concentrations of pigment obtained on (c) foil and (d) substrate. At these concentrations of pigment (0.01, 0.05, 0.1, and 0.5 g), peaks belonging to both pigment and protein are distinguishable everywhere, showing that SERS allows us

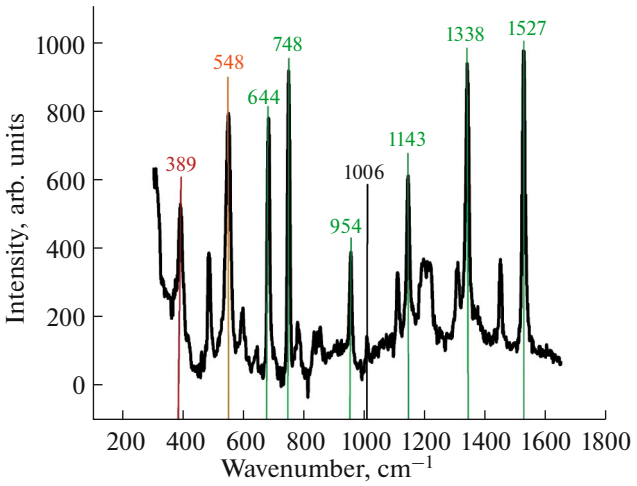

Fig. 4. Spectrum of a mixture: (1) massicot (red), (2) red lead (orange), (3) emerald green (green), and (4) egg white (black).

to detect concentrations 150 times lower than when using basic Raman scattering.

A mixture consisting of three pigments was investigated last. Since they had different dispersities, the pigments were used in different quantities: 0.5 g of red lead, 2 g of massicot, and 0.01 g of emerald green (Fig. 4). The emerald green pigment had more peaks than the other paint components. The most intense of these were located at 644, 748, 1143, 1338, and 1527 cm^{-1}.

Only the most intense peaks at 389 and 548 cm^{-1} are observed in the mixture for the massicot and red lead, respectively. The protein is barely distinguishable in the mixture. These results open up prospects for studying the composition of the complex paints with many pigments using of surface enhanced Raman spectroscopy.

CONCLUSIONS

We investigated the possibility of using SERSS with specially manufactured metal nanowire surfaces (substrates) for the multicomponent identification of pigments in mixtures of paints of organic origin for art history applications.

Results from spectrum analysis showed that emerald green pigment was the most intense of our components, but the spectra of all three pigments were distinguishable in mixtures along with protein, due to using SERS substrates. The use of the SERS substrates while measuring increased the intensity of the obtained spectrum at lower concentrations of ≤ 0.01 g. The time needed to accumulate a useful signal was also reduced, greatly simplifying the analysis.

It was shown that a signal can be amplified by several orders of magnitude using a SERS substrate with nanowires, compared to the Raman scattering of light recorded on foil, and we can detect the pigments in a protein with a concentration ratio of 30 to 1. This opens up prospects for restoring paintings through detailed replication of the original composition of paints.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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