Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Surface-enhanced Raman scattering (SERS) of riboflavin on nanostructured Ag surfaces: The role of excitation wavelength, plasmon resonance and molecular resonance

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ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form 16 January 2018
Accepted 19 January 2018
Available online xxxx

Keywords:
SERS
Riboflavin
Ag nanoislands
Excitation profile
LSPR

ABSTRACT

Optimization of surface-enhanced Raman scattering (SERS)-based sensors for (bio)analytical applications has received much attention in recent years. For optimum sensitivity, both the nanostructure fabrication process and the choice of the excitation wavelength used with respect to the specific analyte studied are of crucial importance. In this contribution, detailed SERS intensity profiles were measured using gradient nanostructures with the localized surface-plasmon resonance (LSPR) condition varying across the sample length and using riboflavin as the model biomolecule. Three different excitation wavelengths (633 nm, 515 nm and 488 nm) corresponding to non-resonance, pre-resonance and resonance excitation with respect to the studied molecule, respectively, were tested. Results were interpreted in terms of a superposition of the enhancement provided by the electromagnetic mechanism and intrinsic properties of the SERS probe molecule. The first effect was dictated mainly by the degree of spectral overlap between the LSPR band, the excitation wavelength along with the scattering cross-section of the nanostructures, while the latter was influenced by the position of the molecular resonance with respect to the excitation wavelength. Our experimental findings contribute to a better understanding of the SERS enhancement mechanism.

1. Introduction

Fabrication of plasmonic platforms for surface-enhanced spectroscopic methods, such as surface-enhanced Raman scattering (SERS), surface-enhanced fluorescence (SEF) or surface-enhanced infrared absorption (SEIRA), has been under thorough investigation for the last ~20 years [1]. To date, close attention has been devoted to optimization of the SERS efficiency by tuning the nanoparticle dimensions, geometry, composition, degree of aggregation as well as their dielectric environment [2–5]. Correlation between the maximum SERS enhancement and details of the nanoobject fabrication procedure mentioned above was in place already in the 1990s [6]. Supposing the size of the metallic nanoobjects is much smaller than the laser wavelength (electrostatic approximation), the oscillating charge density at the metal-dielectric interface may be viewed as an oscillating electric dipole and the term dipolar plasmon resonance is often used. However, when the dimensions of the nanoobjects become comparable to the excitation wavelength, other terms of the multipole expansion have to be taken into account. This gives rise to higher order plasmon resonances (usually quadrupolar) that do not couple to light very effectively [2]. For this reason, it is generally recognized that there exist optimum dimensions of the metallic objects that provide the biggest enhancement, usually in the range ~5–200 nm. This is evidenced by a vast body of experimental data using both metallic colloids [7,8] as well as regular SERS-active platforms [9,10]. Generally, with the increasing size of the metallic nanoobjects and/or reducing the mutual distance, the LSPR is shifted to longer wavelengths and progressively broadens, accompanied by reduced local field enhancements due to retardation effects. Moreover, special care should be devoted to the proper choice of the excitation wavelength used. Information on the plasmonic properties of the substrate is very often derived from extinction [6,11] or reflection [9,12] spectra, although this approach has led to several misconceptions found in literature [13,14]. This confusion stems from overseeing the difference between the "near-field" (Raman-enhancing) and the "far-field" properties of a given substrate. In the average SERS regime, spectral position of the highest SERS enhancement was found near the spectral position of the localized surface-plasmon resonance (LSPR) maximum. In this case, the optimum excitation wavelength tends to be slightly blue-shifted with respect to the LSPR with the LSPR peaking approximately halfway between the incident and the Raman frequency so that both fields can be optimally enhanced [15]. However, this relationship may no longer be true for hot-spot dominated SERS-active

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https://doi.org/10.1016/j.saa.2018.01.055
1386-1425/© 2018 Published by Elsevier B.V.

Please cite this article as: M. Šubr, et al., Surface-enhanced Raman scattering (SERS) of riboflavin on nanostructured Ag surfaces: The role of excitation..., Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (2018), https://doi.org/10.1016/j.saa.2018.01.055

systems (such as colloids) where the maximum enhancement is often red-shifted with respect to the LSPR maximum [2,13]. In the case of more complicated SERS-active platforms, no correlation between these two phenomena was found [16–18]. Moreover, the SERS enhancement may be coupled with molecular resonance enhancement or charge transfer effects, which in turn influences the SERS excitation profile (dependence of the SERS intensity on the excitation wavelength) [19–21].

In this contribution, we use gradient nanostructured silver surfaces, i.e. surfaces with gradually changing optical properties in one direction, to study the dependence of the SERS enhancement factor on the excitation wavelength and the LSPR position in the range from 450 nm to 700 nm. We show that not only the scattering cross-section of the nanostructures, but also the spectral position of the molecular resonance of the analyte used (riboflavin in this study) and its relation to the excitation wavelength used possess a key role in the observable characteristics. Our approach facilitates the way for modern analytical techniques, biosensing and medical applications [22,23].

2. Experimental Section

2.1. Fabrication of the SERS Nanostructures

The SERS nanostructures were prepared on polished Si wafers (~3 cm × 1 cm). The fabrication procedure was carried out based on a step-by-step optimization process described in our previous work [11]. The produced coatings had a three-layer structure schematically depicted in Fig. 1a.

The topmost layer consisted of Ag nanoislands (responsible for the SERS effect) deposited by means of DC magnetron sputtering of a silver target in argon atmosphere (pressure 0.1 Pa, magnetron current 200 mA). The gradient character of properties of silver nanoislands was achieved using a movable mask introduced in between the substrate and the magnetron during the process of deposition of nanoislands (Fig. 1b) [24]. The mask initially shielded the entire substrate area from the flux of incoming silver atoms emitted from the magnetron target. The position of the edge of the mask in this situation is denoted as 0 mm. The mask was subsequently moved with the constant speed along the sample length which in turn gradually diminished the shielded fraction of the substrate. As a result of this, different locations on the samples experienced different fluence of silver atoms that led to Ag structures with different morphologies at different spots on the substrate.

The intermediate, 80 nm thick layer in the SERS active samples was composed of magnetron-sputtered polytetrafluoroethylene (PTFE) film. This film, that was produced using applied RF power of 40 W and argon as the working gas (pressure 1 Pa), served as a dielectric separation layer between the Ag nanoislands and the continuous smooth silver that represented the bottom layer of the produced structure. As we demonstrated in our previous study, the introduction of such a base layer, which acts as a mirror for the incoming laser beam during SERS measurements, contributes to the total enhancement factor by approximately an order of magnitude [11].

The morphology of prepared surfaces along the sample length was investigated by scanning electron microscopy (SEM, TESCAN Mira 3, 15 kV accelerating voltage). Optical properties of silver nanoislands deposited onto glass slides were investigated by UV–Vis spectrophotometry (Hitachi U-2910). No metallic back-reflector was used in this case.

2.2. Raman Measurements

SERS spectra were recorded at room temperature using an integrated confocal Raman microscopic system LabRam HR800 (Horiba Jobin-Yvon), equipped with a diffraction grating with 300 grooves per mm and a liquid nitrogen cooled CCD detector. Two excitation sources were used: He–Ne laser, operating at the wavelength of 633 nm, and Ar^+ laser with the lines at 488 and 515 nm. The laser beam was focused onto the sample at a spot of approximately a 1 μm diameter using a 100× objective, NA = 0.9. Laser powers were adjusted using grey filters to approximately the same laser power around 0.05 mW for all three wavelengths used. Scattered radiation was collected in a back-scattering geometry and filtered by an edge filter for Rayleigh rejection before focusing it onto the 100 μm entrance slit of the spectrometer. SERS intensities were determined as height of the Raman band centered around 1077 cm^{-1} above spectral background and normalized using a Si wafer as an external intensity standard.

2.3. Studied Biomolecule

Riboflavin (vitamin B2) was used as the model biomolecule. Riboflavin serves as a cofactor of many key enzymes and absorbs light in the spectral region between ~400–500 nm. Thus, it is a suitable molecule for the study of both SERS and resonance SERS in the spectral region determined by the LSPR of our structures. Riboflavin powder was dissolved in deionized water. Final riboflavin concentration 3×10^{-5} M was obtained by subsequent dilution of the stock solution and used for SERS testing.

3. Results and Discussion

The first step of this study involved characterization of produced SERS-active surfaces from the point of view of their morphology and

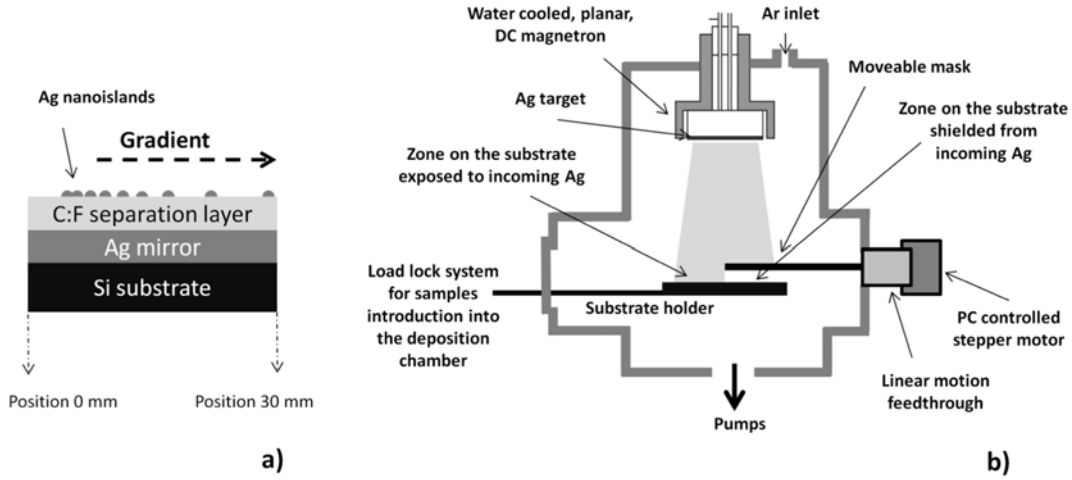

Fig. 1. a) Schematic illustration of the substrate, b) schematic representation of the setup used for production of gradient nanoislands.

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