

# Chemically reduced and laser-ablated gold nanoparticles immobilized to silanized glass plates: Preparation, characterization and SERS spectral testing

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## Abstract

SERS-active surfaces based on gold colloidal nanoparticles attached to silanized glass plates were prepared and characterized by surface plasmon extinction (SPE) and atomic force microscopy (AFM). Aminopropyltrimethoxysilane (APTMS) was found to be more efficient in the silanization process than mercaptopropyltrimethoxysilane (MPTMS). Gold colloidal nanoparticles prepared by three different procedures, i.e. laser-ablation and chemical reduction of  $\text{HAuCl}_4$  by sodium borohydride or by sodium citrate, were immobilized to glass plates using APTMS. The laser-ablated colloids exhibited poor stability during deposition and could not be used for routine preparation of surfaces. Drying at  $100^\circ\text{C}$  after immobilization of chemically reduced nanoparticles stabilizes morphological properties of the surface and thus significantly improves the preparation procedure. Reproducibility and stability of substrates were tested by measurement of SERRS spectra of 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP). Spectra measured from different spots of a sample showed excellent reproducibility and proved high homogeneity of the Au surface. Measurement of several days aged samples revealed very good stability of the SERRS signal in time.

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## 1. Introduction

Surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS) are very sensitive methods of Raman spectroscopy due to enormous enhancement of Raman signal (up to  $10^6$ ) for molecules adsorbed on rough metal surfaces [1–3]. It is believed that two mechanisms contribute to the overall SERS enhancement. The electromagnetic mechanism is related to increase of the electromagnetic optical field in the vicinity of the roughened metal surface, particularly around the high-curvature points. The chemical (molecular) mechanism is related to increase of the adsorbate polarizability as a result of its interaction with the surface. This interaction leads to a charge transfer between the adsorbate and the surface.

The most common types of SERS-active surfaces include solid substrates (metal electrodes, island films, etc.) and metal colloidal solutions. Metal colloids are the most popular substrates due to their strong enhancement and withal cheap and simple preparation. On the other hand, metal colloids are less stable in comparison to solid substrates because of their tendency to aggregate spontaneously, which is the main source of their SERS irreproducibility. A novel preparation method of solid SERS-active surfaces that combines advantages of metal colloids and solid substrates stability has recently been developed. The substrates are prepared by attaching metal colloidal nanoparticles to silanized glass plates. First, a hydroxyl or oxide-bearing substrate (glass) is derivatized by a bifunctional organosilane. The organosilane binds through  $-\text{OH}$  groups to the glass substrate and forms a self-assembled monolayer. The silanized substrate is then immersed into colloidal solution and the metal nanoparticles bind to the terminal functional groups of the organosilane. Thiol-terminated [4,5,9–11], amine-terminated [4,6,8,9,11–13], alkyl-terminated [7,9], and phenyl-terminated [11] silanes have

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been employed for this purpose. Besides the differences in the used organosilanes, preparation procedures may differ in the organosilane concentration, in durations of the silanization and of the next immersion in the metal colloid and/or in the “drying treatment” of the surface.

In this paper, we describe optimized preparation procedure of SERS-active surfaces based on gold colloidal nanoparticles attached to silanized glass plates. We tested mercapto (MPTMS)- and amino-propyltrimethoxysilane (APTMS) for the glass plate silanization. Three types of gold colloids have been studied with respect to their adherence to the silanized substrate: laser-ablated colloid and two colloids prepared by chemical reduction of  $\text{HAuCl}_4$ : by sodium borohydride and by sodium citrate. We investigated also the influence of the surface drying on its properties. The surfaces were characterized by surface plasmon extinction (SPE) and atomic force microscopy (AFM). SERS activity of surfaces was tested by using 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP) as a model molecule.

## 2. Experimental

### 2.1. Chemicals and glassware cleaning

Deionized water of a specific resistance of  $18 \text{ M}\Omega \text{ m}$  was used for all preparations.  $\text{H}_2\text{SO}_4$  (96%),  $\text{H}_2\text{O}_2$  (30%),  $\text{HCl}$  (35%) and  $\text{HNO}_3$  (65%) were obtained from Lachema. All chemicals for nanoparticles preparation ( $\text{HAuCl}_4$ , sodium borohydride, sodium citrate), gold foil (99.99%,  $\sim 1 \text{ mm}$  thickness), methanol (99.8%), 3-mercaptopropyltrimethoxysilane (MPTMS, 97%), 3-aminopropyltrimethoxysilane (APTMS, 97%) and 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP, 97%) were purchased from Sigma–Aldrich.

All glassware was cleaned using “piranha” solution (four parts  $\text{H}_2\text{SO}_4$ , one part  $\text{H}_2\text{O}_2$ ) to remove organics and then aqua regia (three parts  $\text{HCl}$ , one part  $\text{HNO}_3$ ) to remove metal particles.

### 2.2. Gold colloids preparation

Borohydride-reduced (b.-r.) colloid was prepared by reduction of  $\text{HAuCl}_4$  by sodium borohydride according to [14]. Upon vigorous stirring, 9 ml of 2.2 mM solution of  $\text{HAuCl}_4$  was added dropwise to 3.5 mg sodium borohydride dissolved in 75 ml of deionized water precooled to  $2^\circ\text{C}$ .

Citrate-reduced (c.-r.) colloid was prepared by reduction of  $\text{HAuCl}_4$  by sodium citrate [6]. Two hundred and fifty milliliters of 1 mM solution of  $\text{HAuCl}_4$  was brought to a boil and then 25 ml of 38.8 mM solution of sodium citrate was added. Boiling continued 15 min and then the solution was left to cool.

Laser-ablated (l.-a.) colloid was prepared according to ref. [15]. A gold foil was immersed in a quartz cell irradiated by a focused beam of the 1064 nm line of a Nd-YAG pulsed laser (Continuum Surlite I, 10 Hz repetition rate, 3–6 ns laser pulse duration, energy 370 mJ/pulse) for about 5 min. The colloid was then fragmented without presence of the foil for next 15 min.

### 2.3. Preparation of SERS substrates

We used microscope slides (standard borosilicate glass) cut into  $1 \text{ cm} \times 2 \text{ cm}$  strips. Clean glass slides were immersed into 10% solution of organosilane in methanol for 30 min. After silanization, substrates were rinsed several times with methanol and then with water to remove unbound silane that could cause a colloid aggregation when the substrate is put into. Each silanized substrate was then separately dipped in a vertical position (gold surface was thus formed at both sides of the glass slide) into a vial containing 1 ml of the colloidal suspension for 3–4 h. After that they were rinsed again with water. With the purpose of finding an optimal preparation procedure, some of the substrates were left to dry in an oven at  $100^\circ\text{C}$  after the silanization, some after the immersion in the colloid, and another after both preparation steps. All manipulations of glass slides have been done using tweezers to avoid damage of the surfaces.

### 2.4. Instrumentation

SPE spectra of stock gold colloids (in 0.2 cm cells) and prepared SERS substrates were recorded on a Perkin-Elmer Lambda 12 spectrometer. The light spot at the sample was a line of about 9 mm length and 1 mm width.

AFM images of the gold-coated silanized substrates were carried out in a tapping mode in air using a MultiMode Digital Instruments NanoScope<sup>TM</sup> Dimension IIIa.

SERRS spectra of TMPyP were recorded at room temperature with a Raman spectrometer using the 514.5 nm excitation line of an  $\text{Ar}^+$  laser ILA (Carl Zeiss, power  $\sim 150 \text{ mW}$  at the sample) in reflection geometry. Laser beam was focused to a spot with a diameter of about 0.1 mm. Scattered radiation was collected at  $90^\circ$  relative to the excitation beam and detected with a nitrogen cooled CCD coupled to a 60 mm single spectrograph with a 1200 g/mm holographic grating.

## 3. Results and discussion

### 3.1. Influence of Au colloid and of organosilane

Three differently prepared gold colloids, borohydride-reduced (b.-r.), citrate-reduced (c.-r.), and laser-ablated (l.-a.) were tested. Their typical SPE spectra (Fig. 1, left) show extinction maximum at 520–530 nm (slightly down-shifted in wavelengths for b.-r. and l.-a., probably due to lower average particle size). l.-a. colloid exhibits also an extinction band at  $\sim 700 \text{ nm}$  indicating presence of aggregates. Accordingly, the l.-a. colloid has been found as the less stable in time. The five times higher extinction of c.-r. colloid over the b.-r. one is due to its higher gold content coming from the chemical preparation (1 mM versus 0.24 mM concentration of Au in c.-r. or b.-r. colloid, respectively).

Our results show that all three types of gold nanoparticles (l.-a., b.-r. and c.-r.) can be attached to the glass plates silanized by APTMS. Typical SPE spectra of such substrates are shown in Fig. 1, right. They correspond roughly to the spectra of initial colloids. The highest extinction observed for c.-r.

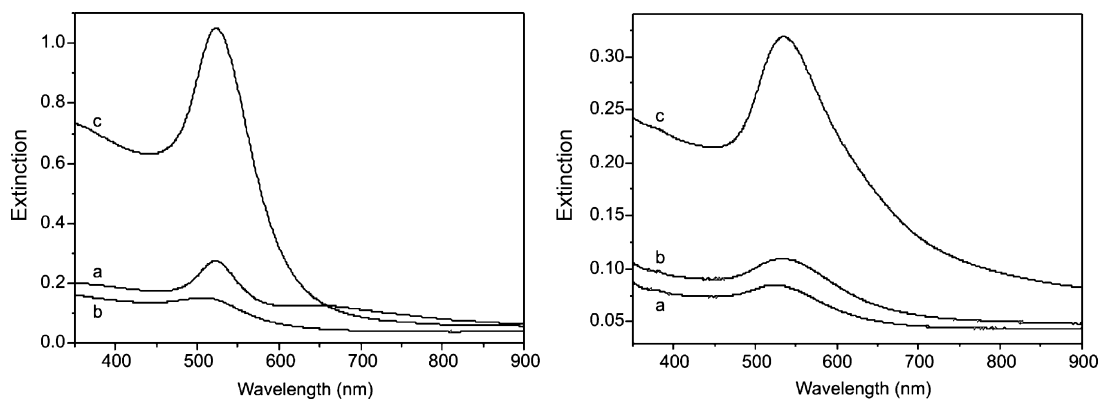


Fig. 1. Influence of Au colloid: SPE spectra of initial Au colloids (left) and typical SPE spectra of gold surfaces (right). Laser-ablated (a); borohydride-reduced (b); citrate-reduced (c) nanoparticles.

nanoparticles is due to the highest extinction of the initial colloid and probably also thanks to influence of surface chemistry of colloidal particles prepared by different reducing agent. In Ref. [11] retarded binding of b.-r. Au particles to APTMS in comparison to c.-r. ones was reported. SPE about 0.1–0.4 at maximum and surface coverage of Au nanoparticles estimated as  $(1\text{--}3) \times 10^{11}$  particles/cm<sup>2</sup> corresponds well with literature [5,6,8,11].

It was found out that while both chemically reduced colloids were very stable during soaking, l.-a. Au colloid was extremely unstable. Majority of l.-a. Au colloid samples precipitated during 10 min of soaking and only some of them show extinction comparable with surfaces prepared from b.-r. or c.-r. nanoparticles. The l.-a. colloid was therefore graded as unsuitable for routine preparation of SERS-active Au surfaces and was excluded from further study.

For tested organosilanes, MPTMS and APTMS, our results clearly show that the binding efficiency of APTMS is excellent while MPTMS binds gold nanoparticles very poorly. Fig. 2 shows five typical SPE spectra of b.-r. nanoparticles attached to glass using MPTMS (left) and APTMS (right). We can see

that only one from five surfaces prepared using MPTMS shows extinction comparable with those prepared using APTMS. On the basis of our experiences with repeated preparation of tens of surfaces we estimate that the efficiency of MPTMS is less than 20% and even long soaking times does not improve it. It is coherent with the results of other laboratories. While MPTMS was one of organosilanes used for preparation of Au substrates in pioneer works [5,11], more recent works report poor efficiency of MPTMS to bind gold nanoparticles and only APTMS is routinely used [6–8]. It can be explained by a strong tendency of –SH group to be air-oxidized [6]. In contrast to that, protonated functional –NH<sub>2</sub> group of APTMS is stable and it can bind negatively charge anions at nanoparticle surface by electrostatic (ionic) interaction [6]. For all reported below experiments, APTMS was exclusively used.

### 3.2. Stability and reproducibility of Au surfaces

Stability and reproducibility of prepared SERS-active surfaces were monitored by their SPE spectra. Very good reproducibility was found when SPE spectra were measured

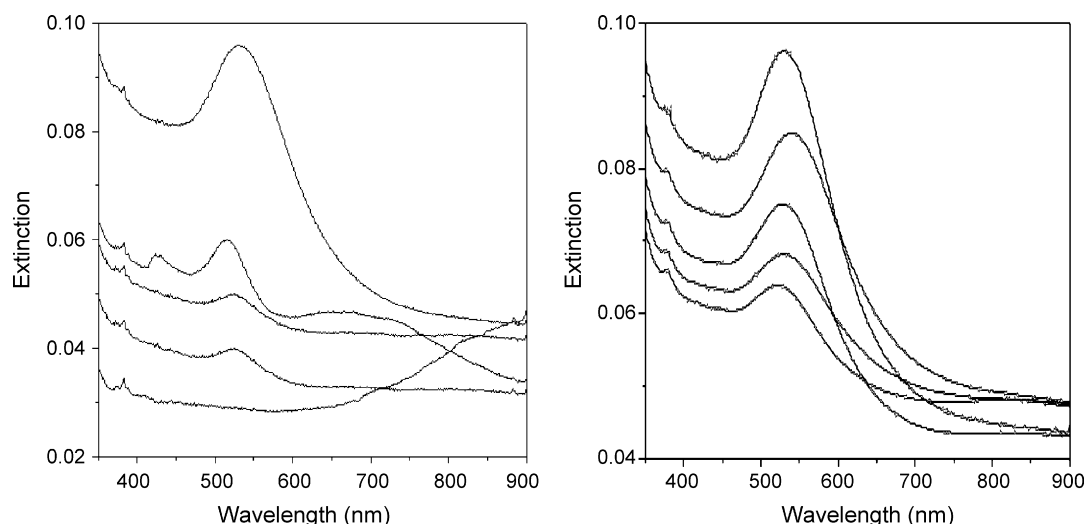


Fig. 2. Influence of organosilane: five typical SPE curves of surfaces prepared from borohydride-reduced gold nanoparticles attached to glass using MPTMS (left) and APTMS (right). Surfaces are prepared in the same conditions.

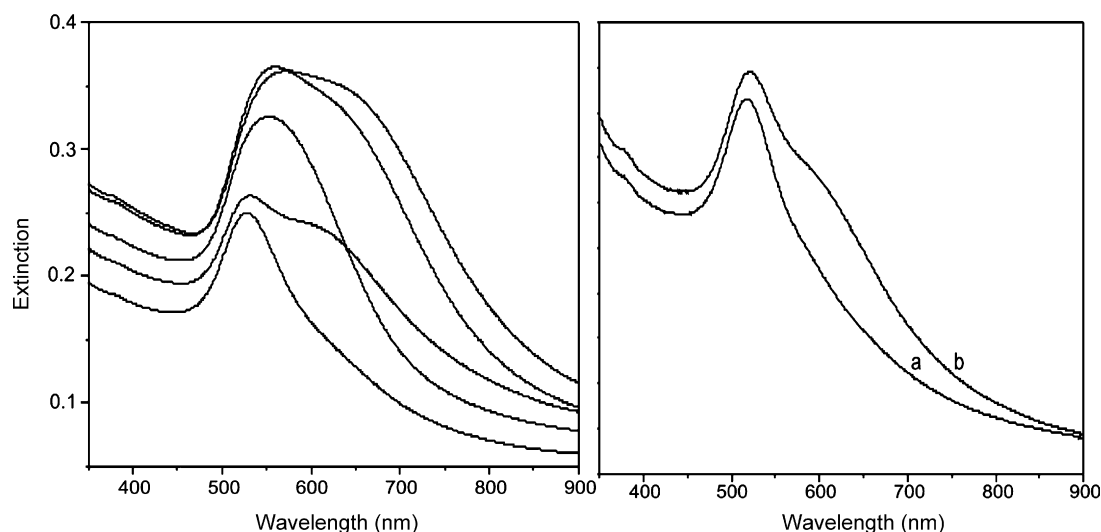


Fig. 3. Reproducibility and stability of surfaces. (Left) Five typical SPE curves of surfaces prepared from citrate-reduced gold nanoparticles attached to glass using APTMS. Surfaces are prepared in the same conditions. (Right) One surface measured immediately after preparation (a) and 1 h after preparation (b).

from different spots of the surface (spectra not shown). In contrast to that, SPE spectra of various surfaces prepared in the same conditions showed remarkable differences (Figs. 2 and 3). It is well known that this irreproducibility is caused by aggregation of nanoparticles that cannot be fully controlled and one can see similar behaviour in literature [8].

In the case of surfaces prepared from b.-r. nanoparticles (Fig. 2), the extinction spectrum contained a single dominant band situated at 520–530 nm and the differences consisted mainly in variations of the overall extinction value. On the other hand, two types of the surfaces were prepared from c.-r. colloids (Fig. 3, left). The first kind was of a red colour and their SPE spectra exhibited a single SPE maximum at 520–530 nm. The second kind of c.-r. surfaces were grey-violet and their spectra contained two maxima: at 520–530 and 600–650 nm. The extinction band at 520–530 nm is known to correspond to isolated gold nanoparticles while the one at 600–650 nm indicates interaction of close nanoparticles forming aggregates [6]. Similar differences in SPE spectra of Au surfaces have been recently reported as effect of various soaking times using drying at 100 °C as a final step of preparation procedure [8]. In our experiments without drying at 100 °C, these differences are observed even for the same soaking times. Moreover, the surfaces characterized by a single extinction band convert spontaneously to the latter ones within short periods (from minutes to hours, Fig. 3, right). This effect (spontaneous change of the surface colour) has been also reported for gold surface prepared by the same procedure in [6].

Au surfaces were also characterized by AFM (typical images of Au surfaces prepared from b.-r. and c.-r. nanoparticles are shown in Fig. 4). AFM images obtained from different sites of surfaces revealed no significant differences. This also confirms homogeneity of our Au surfaces, but in contrast to SPE spectra in a scale of micrometers. AFM shows compact cover of glass substrates by nanoparticles. As for the b.-r. (Fig. 4, left), as for the c.-r. (Fig. 4, right) surfaces, the images show also presence of small aggregates of hundred nanometers or of large particles with diameters varying from ~30 to 100 nm. In the case

of surfaces prepared from c.-r. nanoparticles the amount of the aggregates and of the larger particles is though higher. However, the most important difference is that while the b.-r. surface is formed by a single layer, the c.-r. surface seems to be a multilayer. This finding can explain the existence and spontaneous development of the additional extinction band in SPE spectra of the surfaces prepared from c.-r. colloid: a multilayer allows close arrangement of nanoparticles that can interact and form aggregates without necessity of their lateral movement.

### 3.3. Improving stability of Au surfaces prepared from citrate-reduced colloids

As mentioned above, two types of SERS-active surfaces, according to the SPE spectra, can be prepared from c.-r. Au colloids, the one characterized by a single SPE maximum being even very unstable. We have therefore concentrated our further effort to improve the preparation procedure in order to increase reproducibility and stability of the morphological and optical properties of Au surfaces. Drying in various stages of the preparation was found to be an important step of the procedure. Four preparation procedures were tested:

- without any drying;
- drying after immobilization of nanoparticles (10 min, 100 °C);
- drying after silanization (10 min, 100 °C);
- both drying steps (b and c).

Representative SPE spectra of Au surfaces prepared by particular procedures are shown in Fig. 5. Although exact positions and intensities of extinction bands differed from preparation to preparation like it is known for Au colloids themselves, drying after the immobilization of nanoparticles (procedures b and d) always led to appearance of the long-wavelength band at 600–650 nm and to a good stability of the extinction spectrum during the surface aging. On the other hand, unstable surfaces



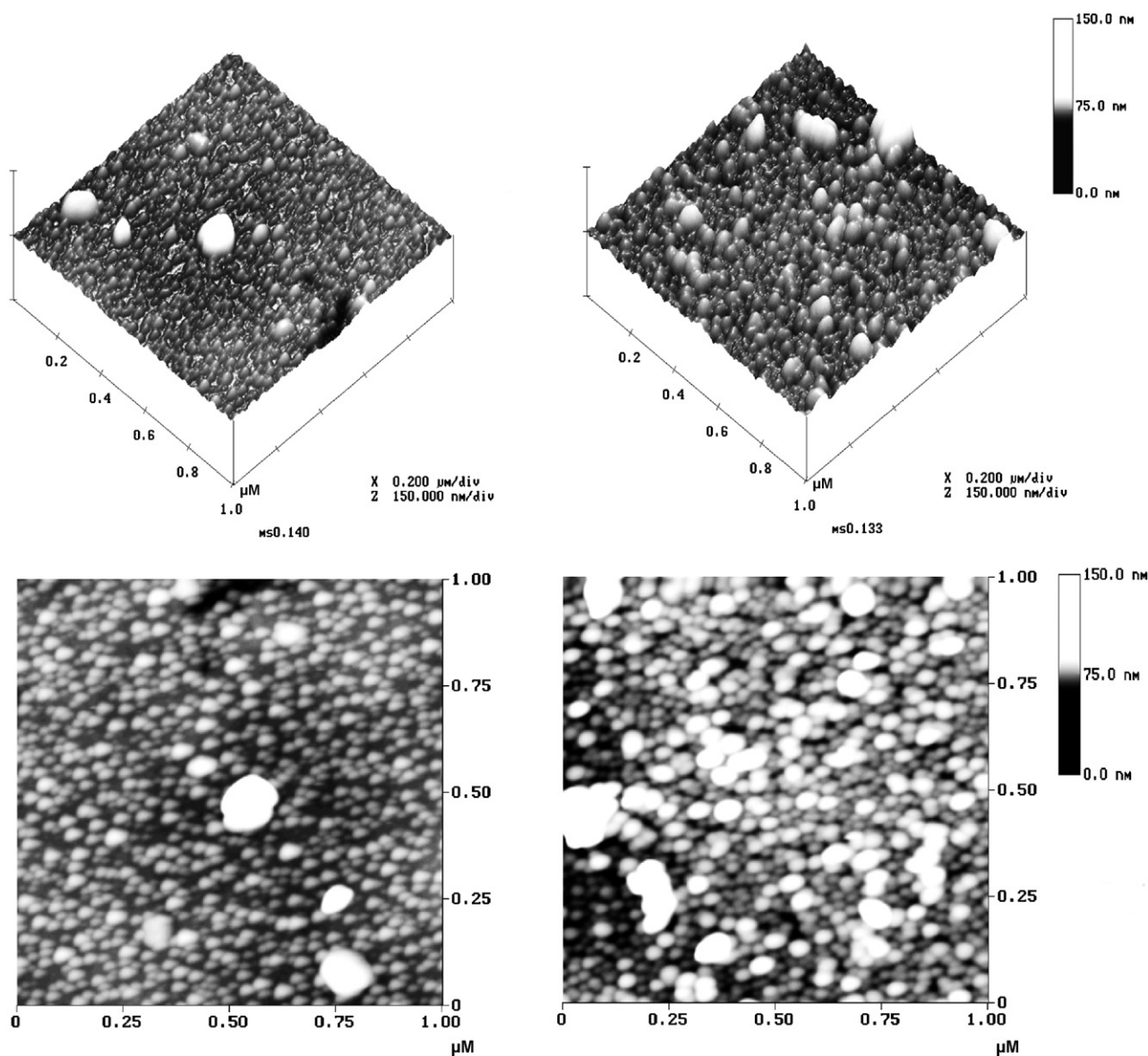


Fig. 4. Typical 3D- and 2D-AFM images of the gold surface prepared from borohydride-reduced (left) and citrate-reduced (right) nanoparticles.

characterized by a single extinction maximum were frequently prepared when drying was applied just after the silanization (procedure c). This reveals that drying after the nanoparticles immobilization is a needful step to prepare stable SERS-active surfaces from c.-r. Au colloids. Very probably this process reduces hydration of the citrate anions adsorbed at the Au surface and allows closer interaction of neighbour nanoparticles in the multilayer structure.

#### 3.4. Testing Au surfaces by SERS measurements

SERRS spectra of TMPyP (see Fig. 6 for chemical structure) were measurable when adsorbed at surface prepared from any of the three types of Au colloids (Fig. 7). The positions and the relative intensities of TMPyP bands are the same in the spectra obtained from three types of gold nanoparticles, only overall SERRS intensity is slightly different. No Raman signal

was obtained from a drop of TMPyP on a pure glass without gold surface (keeping the same TMPyP concentration of 1  $\mu\text{M}$  and experimental conditions), which demonstrates SERRS nature of obtained TMPyP spectra. They exhibit typical bands of the free-base TMPyP at  $\sim 965, 1000, 1217, 1246, 1332, 1362, 1451, 1500, 1556$  and  $1636\text{ cm}^{-1}$ . Our SERRS spectra of TMPyP are quite similar to that of tetrapyrroldiporphine (TPyP) measured from Au colloid-TPyP films [14]. Moreover, it is evident from the spectra that in contrast to silver surfaces when incorporation of a silver atom from the silver surface to the porphyrin macrocycle is an unavoidable effect [16], gold surfaces do not perturb chemical structure of the free-base TMPyP. A more detail study of TMPyP on Au surfaces prepared from c.-r. nanoparticles was done in Ref. [17]. We should note, that it was impossible at our experimental conditions to record acceptable SERRS spectra of TMPyP directly from the primary Au colloids, probably due to aggregation of colloid nanoparticles and shifting the wave-

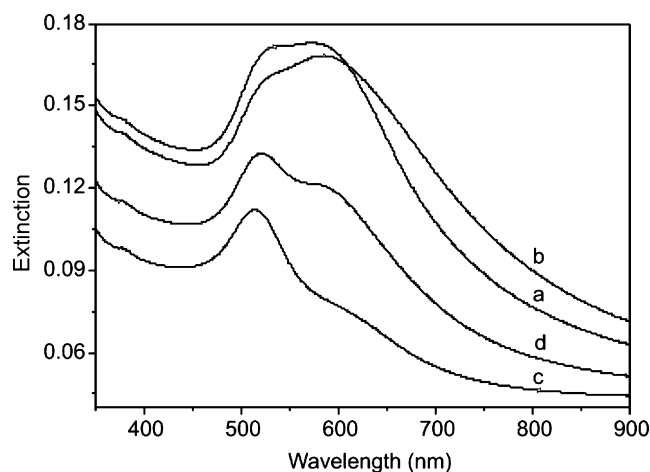


Fig. 5. SPE spectra of surfaces prepared from citrate-reduced gold nanoparticles by modified preparation procedure: (a) without any drying; (b) drying after immobilization of nanoparticles (10 min, 100 °C); (c) drying after silanization (10 min, 100 °C); (d) both drying steps (b and c).

length of the surface plasmon resonance far from the excitation conditions.

Reproducibility and stability of the SERRS signal were tested by measurement of SERRS spectra of TMPyP from different spots of a sample, measurements during its aging, and comparison of spectra obtained for various samples. SERRS intensity was characterized by an integral intensity of 1451  $\text{cm}^{-1}$  band. Spectra measured from different spots of a particular sample reveal excellent reproducibility (relative statistic deviation of about 2%) that proves very good homogeneity of Au surfaces. Measurement of aged samples (from days to 2 weeks) shows also very good time stability of the SERRS signal (relative statistic deviation about 7% and 5% in the case of b.-r. and c.-r. nanoparticles, respectively) and demonstrates that the surfaces with adsorbed TMPyP can be stored for several days without losing the SERRS signal. On the other hand, comparison of individually prepared surfaces displays occasional drops of the

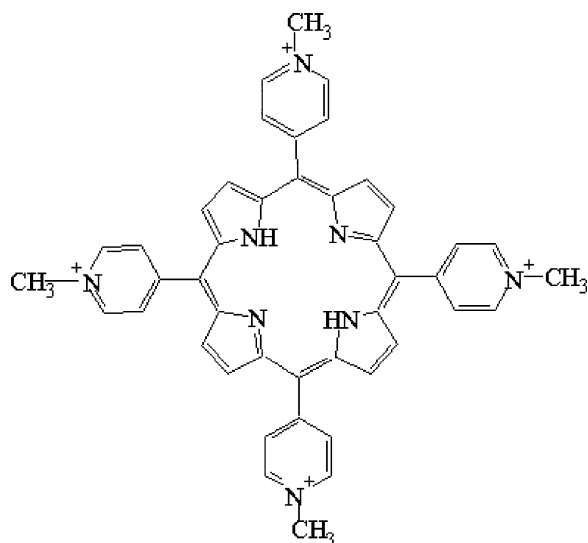


Fig. 6. Chemical structure of 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP).

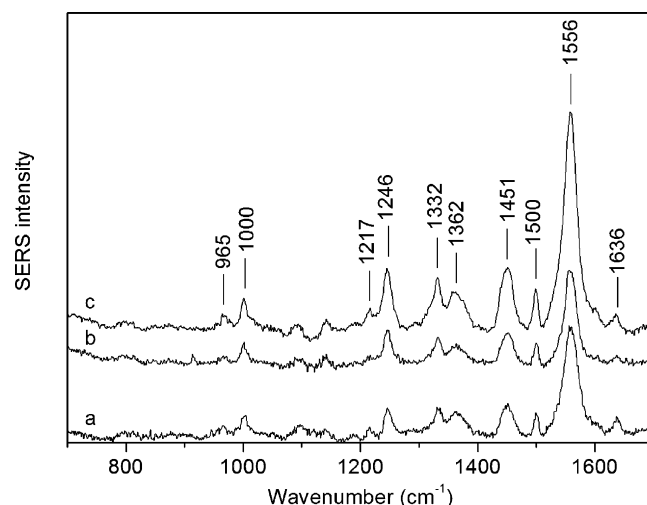


Fig. 7. SERRS spectra of TMPyP adsorbed at gold surfaces prepared from laser-ablated (a); borohydride-reduced (b); citrate-reduced (c) nanoparticles. The baseline was corrected and the Raman signal of glass subtracted.

SERRS signal level (overall statistics gives relative deviation of about 15% and 37% for the surfaces prepared from b.-r. and c.-r. Au colloids, respectively).

#### 4. Conclusions

SERS-active surfaces based on gold nanoparticles immobilized to silanized glass plates were prepared from laser-ablated, borohydride- and citrate-reduced gold colloidal nanoparticles. Aminopropyltrimethoxysilane (APTMS) was found to be more efficient in the process of silanization than mercaptopropyltrimethoxysilane (MPTMS). The laser-ablated colloids exhibited poor stability during deposition and could not be used for routine preparation of surfaces. We found out that drying at 100 °C after immobilization of citrate-reduced nanoparticles is a needful step of preparation that stabilizes morphological properties of the surface and thus significantly improves preparation procedure. Reproducibility and stability of substrates were tested by measurement of SERRS spectra of 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP). SERRS spectra of TMPyP do not show any sign of porphyrin metalation or perturbation of its native structure. Spectra measured from different spots and from aged samples showed excellent reproducibility of the Au surface and very good stability of the SERRS signal in time.

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