

SERRS of free-base porphyrins on immobilized metal gold and silver nanoparticles

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Abstract

SERRS of three differently charged free-base porphyrins, namely cationic 5,10,15,20-tetrakis(1-methyl-4-pyridyl) porphyrin (TMPyP), anionic 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TSPP), and neutral 5,10,15,20-tetrakis(tetraphenylporphyrin) (TPP), was measured on SERS-active substrates prepared by immobilization of Ag or Au nanoparticles to silanized glass plates. Au surfaces were suitable for TMPyP and TSPP detection while Ag surfaces for TMPyP and TPP. SERRS spectra exhibited excellent reproducibility, very good stability in time (relative standard deviation lower than 10%) and did not contain any sign of the porphyrin metalation or perturbation of its native structure. Even SERRS spectra of water-insoluble porphyrin (TPP) were obtained without any interference of solvent Raman signal. Estimated limits of detection (LOD) were between 3×10^{-9} and 8×10^{-8} M porphyrin concentrations in soaking solution. SERRS on immobilized Ag and Au nanoparticles was found a suitable analytical method for detection of free-base (including water-insoluble) porphyrins, advantageous considerably with respect to routinely used metal sols.

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Keywords: SERS; Porphyrin; Gold and silver; Immobilized nanoparticles; Limit of detection

1. Introduction

Porphyrins are important biomolecules and their derivatives are or can potentially be applied in photodynamic therapy of cancer, antiviral treatments, molecular biology, specific sensing of DNA sequences, selective cleavage of nucleic acids, transport of oligonucleotides into the cell, etc.

Porphyrins have been extensively studied by using surface-enhanced (resonance) Raman scattering – SE(R)RS – spectroscopy. SERRS is a very sensitive method of Raman spectroscopy due to enormous enhancement of Raman signal (up to 10^6) for molecules adsorbed on rough metal surfaces [1,2]. In SERS applications, metal colloidal nanoparticles are the most frequently used substrates due to their cheap and simple preparation. Serious drawback for analytical applications is though their chemical instability and irreproducibility of spectral measurements. Colloidal solutions are unsuitable for study of water-insoluble porphyrins due to a strong spectral

interference of solvent. Moreover, in the case of free-base porphyrins adsorbed onto Ag colloids, the porphyrin metalation (incorporation of a metal ion from the surface into the porphyrin core) has been observed [3–6]. The metalation substantially decreases reproducibility of porphyrin SERRS spectra and even makes obtaining of SERRS spectra of unperturbed free-base porphyrin impossible. Although considerable attention has been paid to discovering how to prevent free-base porphyrins from metalation (e.g. by using an efficient molecular spacer) it seems to be extremely difficult in some cases [7–8]. Thus, developing of a new analytical method for detection of porphyrins is highly desirable.

Above-mentioned disadvantages of metal colloids can be overcome by immobilization of metal colloidal nanoparticles onto solid plate. A novel preparation method of SERS-active surfaces that combines advantages of metal colloids (homogeneous particle size distribution, high SERS enhancement) and of solid substrates (stability and spectral reproducibility) has recently been developed. The substrates are prepared by attaching metal colloidal nanoparticles to silanized glass slides [9–11]. This method is in principle suitable for preparation of stable and inexpensive SERS-active substrates with reproducible

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signals. We improved preparation of Au substrates [12] and employed them to study concentration dependence of porphyrin SERS spectra [13]. Our first results also showed that the substrates prepared from Ag nanoparticles that are not being used frequently [10], are suitable for porphyrin study [14].

This contribution deals with SERS study of three kinds of free-base porphyrins, cationic 5,10,15,20-tetrakis(1-methyl-4-pyridyl) porphyrin (TMPyP), anionic 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TSPP) and neutral 5,10,15,20-tetrakis(tetraphenylporphyrin) (TPP), on a silver and gold colloidal nanoparticles attached to glass slides silanized by 3-aminopropyltrimethoxysilane (APTMS). We propose here this technique as a new promising way of porphyrin detection and analytical study, applicable even to free-base and/or water-insoluble porphyrins.

2. Experimental

2.1. Chemicals

Deionized water of a specific resistance of 18 MΩ cm was used for all preparations. H₂SO₄ (96%), H₂O₂ (30%), HCl

(35%) and HNO₃ (65%) and tetrahydrofuran were obtained from Lachema. HAuCl₄, AgNO₃, sodium citrate, sodium borohydride, methanol (99.8%), 3-aminopropyltrimethoxysilane (APTMS, 97%), 5,10,15,20-tetrakis(1-methyl-4-pyridyl) porphyrin (TMPyP) and 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (TSPP), 5,10,15,20-tetrakis(tetraphenyl) porphyrin (TPP) were purchased from Sigma–Aldrich (see Fig. 1 for chemical structures of porphyrins).

2.2. Preparation of SERS-active systems

Au and Ag colloid were prepared by reduction of HAuCl₄ by sodium citrate [12] and of AgNO₃ by sodium borohydride [7], respectively.

All glassware was cleaned using “piranha” solution (4 parts H₂SO₄, 1 part H₂O₂) to remove organics and then *aqua regia* (3 parts HCl, 1 part HNO₃) for removal of metals. Clean glass slides (1 cm × 2 cm strips) were derivatized in 10% and 20% solution (for Au and Ag substrates, respectively) of APTMS in methanol for 30 min. After the silanization, substrates were rinsed several times with methanol and then with water to remove any physisorbed organosilane, which could cause

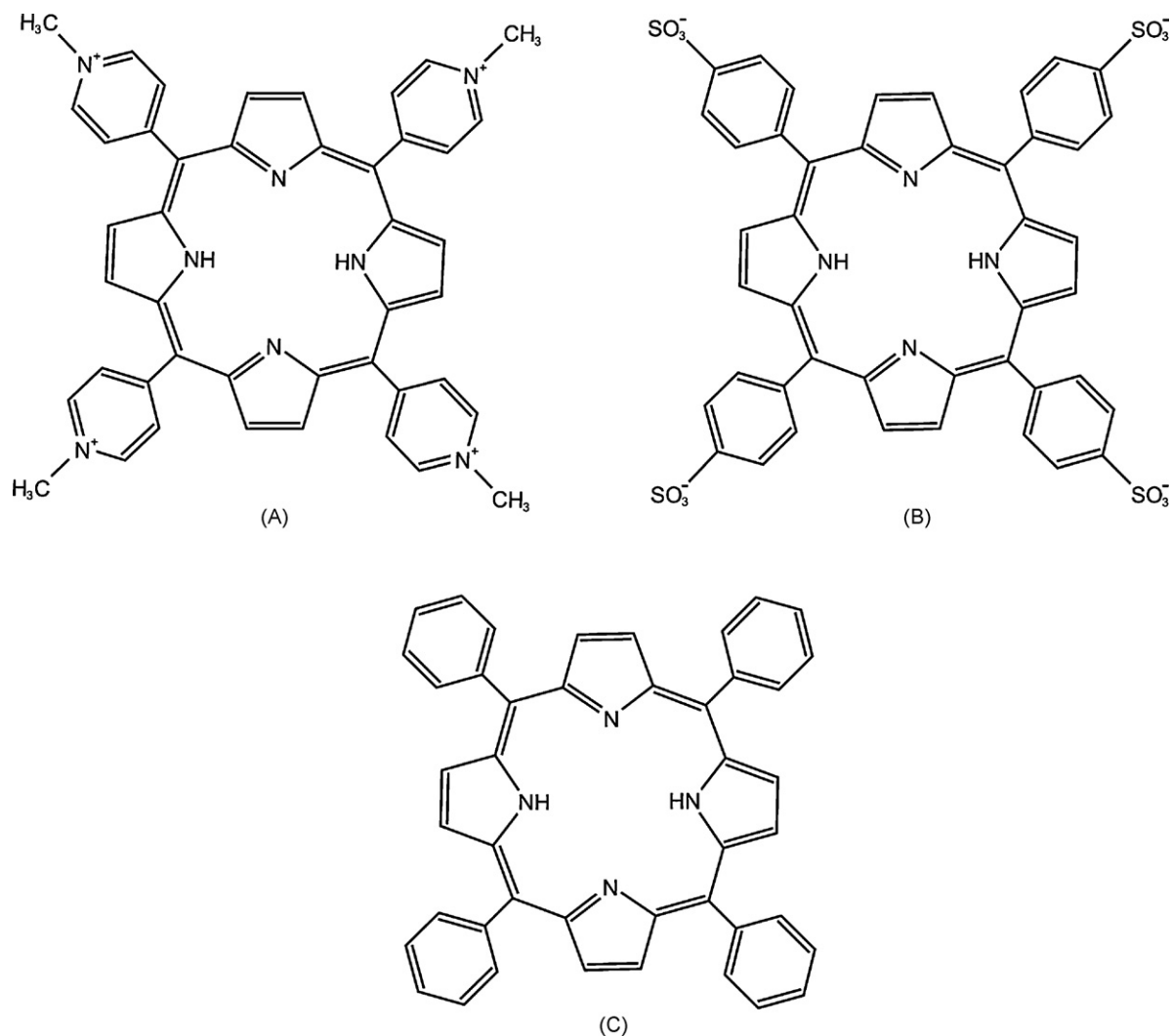


Fig. 1. Chemical structure of TMPyP (A), TSPP (B) and TPP (C).

aggregation of colloidal particles in suspension during next step. Each silanized glass plate was dipped in vertical position (metal surface is thus formed on both sides of the glass slide) into a tube containing 1 ml of colloidal suspension for 3–4 and 6 h, for Au and Ag, respectively. Then the plates were rinsed with water. Au substrates were after left to dry at 100 °C for 10 min. Preparation of Au surfaces is described in detail in our previous paper [12].

TMPyP and TSPP were dissolved in water and TPP in tetrahydrofuran. For SERS measurements, the plate with metal substrate was placed into the porphyrin solution for 20 min, then it was rinsed by deionized water and SERRS spectrum was measured.

2.3. Instrumentation

SERRS spectra were recorded at room temperature with a Raman spectrometer using the 514.5 nm excitation line of an Ar⁺ laser Innova 305 (Coherent, power ~150 mW at the sample) in reflection geometry. Excitation light was collected by a long-focus lens to achieve about 0.1 mm diameter of the spot at the surface. Scattered radiation was collected at 90° relative to the excitation beam and detected with a nitrogen cooled CCD (100 × 1340 pixels, Princeton Instruments) coupled to a spectrograph with a 1600 grooves/mm grating (Jobin Yvon-Spex 270M Instruments S.A., Inc.). Accumulation time was 1 × 60 s and 5 × 120 s for measurement from Ag and Au surfaces, respectively.

Surface-plasmon extinction (SPE) spectra of metal substrates were recorded on a Lambda 12 (PerkinElmer) absorption spectrometer. The light spot at the sample was a line of about 9 mm length and 1 mm width.

Scanning electron microscopy (SEM) images were obtained by using Quanta 200 FEG (FEI) and Hitachi S5000 devices.

3. Results and discussion

3.1. Characterization of Au and Ag surfaces

Both borohydride- and citrate-reduced Au nanoparticles can in principle be used for SERS measurements, the latter are

though more convenient because of their higher surface coverage and broader region of suitable excitation wavelengths [12]. Our experience has revealed that in the case of Ag, only immobilized borohydride-reduced nanoparticles are usable as the citrate-reduced ones bring about a strong signal of graphitic carbon after laser excitation and thus make the SERS measurement impossible. The graphitic carbon is produced by burning of citrate anions on the surface. Its Raman signal was often observed in the case of citrate-reduced Ag sols [15], but this effect is unfortunately much stronger in the case of immobilized Ag nanoparticles. On that account the citrate-reduced Au nanoparticles and the borohydride-reduced Ag nanoparticles were chosen for our study.

Au and Ag surfaces were characterized by surface plasmon extinction (SPE) spectra and SEM images. Fig. 2 shows SPE spectra of typical Ag and Au surfaces. Ag surfaces were yellowish with the extinction maximum at about 400 nm corresponding to the spectrum of initial Ag colloid (Fig. 2 left). Au surfaces were grey-violet and their spectra contained two overlapping bands with maxima at 520–530 nm and 600–650 nm (Fig. 2 right). The former extinction band is known to correspond to isolated gold nanoparticles while the latter indicates interaction of close nanoparticles in aggregates.

SEM images obtained from different sites at both sides of the plate revealed no significant differences, which confirmed good homogeneity of the nanoparticle layers. Typical SEM images are shown in Fig. 3. Ag surfaces contained isolated Ag nanoparticles of 6–20 nm and small aggregates of 50–100 nm, but the plates were not completely covered (Fig. 3 left). Even higher concentration of APTMS or use of other silan type (3-mercaptopropyltrimethoxysilane) did not lead to higher covering by Ag nanoparticles. On the other hand, Au surfaces showed a compact cover by nanoparticles of diameters varying from ~30 to 100 nm and by small aggregates of hundred nanometer size (Fig. 3 right).

Although Ag surfaces were found stable for several months, Au surfaces had to be stabilized by drying after the immobilization [12]. Good Au surfaces with SPE between 0.08 and 0.2 (see Fig. 2) were obtained from almost each preparation while the yield of usable Ag substrates was only 60–70%.

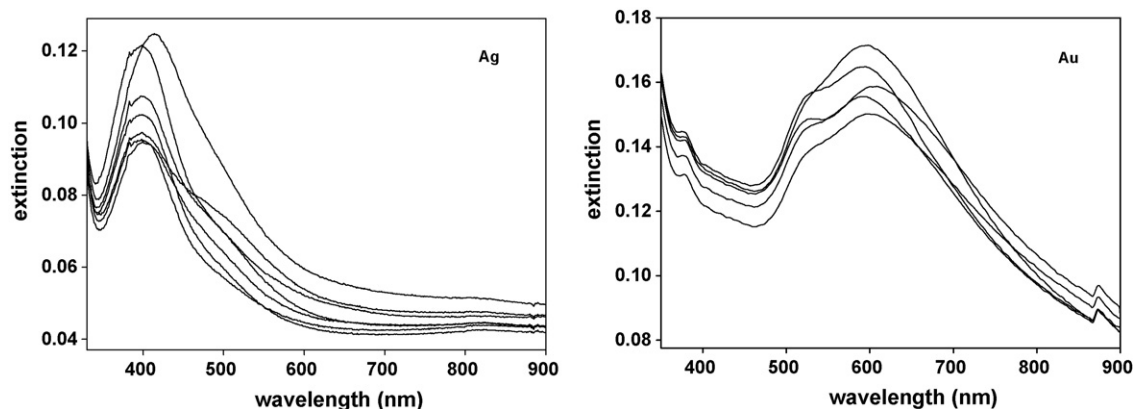


Fig. 2. Typical SPE spectra of silver (left) and gold surfaces (right).

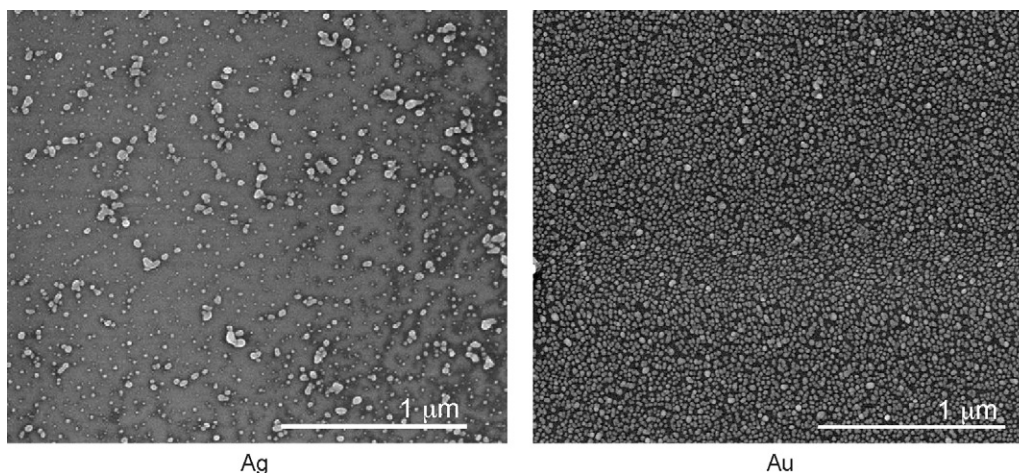


Fig. 3. Typical SEM images of silver (left) and gold surfaces (right).

3.2. SERS spectra of porphyrins

Both Ag and Au surfaces were tested by using the three kinds of free-base porphyrins (see Fig. 1 for chemical structures). Raman signal of the glass plate appearing at ~ 550 and 1100 cm^{-1} in porphyrin spectra was eliminated by subtraction of a blank plate spectrum measured at the same conditions. Spectral baseline correction was applied if necessary. The effect of surface enhancement was verified by an experiment when a drop of the soaking porphyrin solution was dried out at a pure glass surface. No Raman signal was obtained from this spot if the same conditions as for the SERS measurements were kept.

Fig. 4 shows SERS spectra of anionic TSPP adsorbed onto the Au and Ag surfaces. Spectra obtained from Au surfaces are of high intensity and good signal-to-noise ratio (Fig. 4A). These surfaces allowed us to obtain well-resolved TSPP spectrum in 1×10^{-5} to 1×10^{-7} M range of soaking concentrations. In contrary, spectra from Ag surfaces were weaker and of less

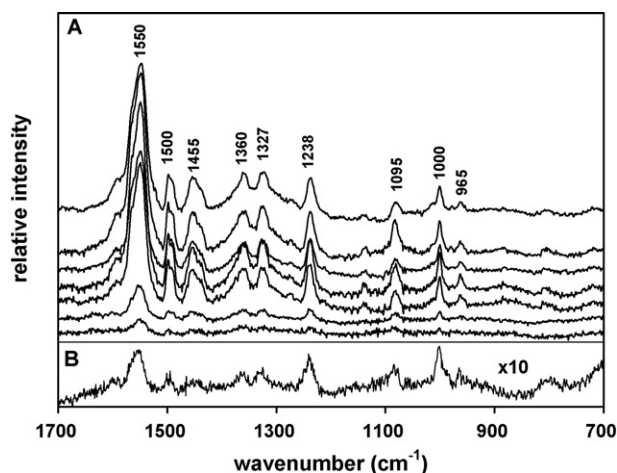


Fig. 4. (A) SERS spectra of TSPP obtained from Au surfaces, soaking concentrations from bottom to top: 1×10^{-7} M, 5×10^{-7} M, 1×10^{-6} M, 2×10^{-6} M, 4×10^{-6} M, 6×10^{-6} M and 1×10^{-5} M. (B) SERS spectrum of TSPP adsorbed from 1×10^{-5} M solution and measured from Ag surface. The baseline was corrected and the Raman signal of glass subtracted.

quality. Only the highest soaking concentration of 1×10^{-5} M provided satisfactory TSPP spectrum (Fig. 4B).

Fig. 5 shows SERS spectra of cationic TMPyP adsorbed onto the Au and Ag surfaces. Excellent spectra were obtained

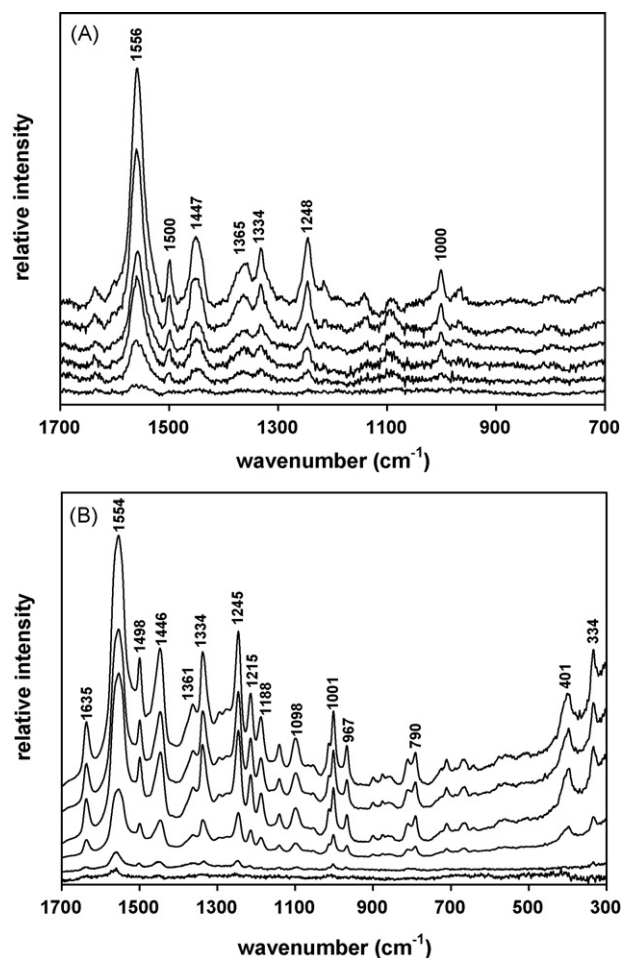


Fig. 5. (A) SERS spectra of TMPyP obtained from Au surfaces, soaking concentrations from bottom to top: 1×10^{-7} M, 5×10^{-7} M, 1×10^{-6} M, 2×10^{-6} M, 6×10^{-6} M and 1×10^{-5} M. (B) SERS spectra of TMPyP obtained from Ag surfaces, soaking concentrations from bottom to top: 1×10^{-8} M, 5×10^{-8} M, 1×10^{-7} M, 3×10^{-7} M, 6×10^{-7} M and 1×10^{-6} M. The Raman signal of glass was subtracted.

Table 1

Limits of detection, reproducibility and stability in time of porphyrin SERRS signal measured on immobilized metal nanoparticles

Metal surface	Porphyrin	LOD (M)	Reproducibility of signal (%) ^a	Time stability of signal ^a
Gold	TSPP	3×10^{-8}	4.2	6.9% (3 days)
	TMPyP	5×10^{-8}		
	TPP	–		
Silver	TSPP	–	5.5	8.5% (2 days)
	TMPyP	3×10^{-9}		
	TPP	8×10^{-8}		

^a Relative standard deviation (RSD) of signal from tens of experiments.

from both surfaces in wide concentration range; Ag substrates seemed to be more efficient as good SERRS spectra were obtained even from 1×10^{-8} M TMPyP soaking solution (Fig. 5B).

Fig. 6 shows SERRS spectra of neutral TPP adsorbed onto the Ag surfaces from 1×10^{-7} to 6×10^{-6} M soaking TPP solution in tetrahydrofuran. No spectral contribution from the solvent is however present in the spectra. We did not obtain any spectrum of TPP by using Au surfaces even for longer soaking times and higher TPP soaking concentrations.

Although good SERRS spectra of all three porphyrins were obtained, our results indicated certain “selectivity” of Au and Ag surfaces in respect to particular porphyrins. Cationic TMPyP is known to adsorb easily on both citrate- and borohydride-reduced colloids [5], which corresponds with good spectra of TMPyP recorded from both types of surfaces. Better signal obtained from Ag surfaces is due to well known higher enhancement effect of silver in comparison to gold. Adsorption efficiency of anionic or neutral molecules is known to be more sensitive to the surface properties of nanoparticles (namely size, charge, concentration of ions, surface potential, etc.) coming from chemical reduction [16]. Thus we suppose that the reason why we obtained good spectra of TSPP only from Au surfaces and of TPP only from Ag surfaces is a different type of nanoparticle preparation (Au surfaces

prepared from citrate-reduced and Ag surfaces from borohydride-reduced nanoparticles).

SERRS spectra of all studied porphyrins are similar showing typical bands corresponding to vibrations of the porphyrin macrocycle. Comparison of our SERRS spectra with the well known free-base ($\sim 330, 960 + 1000, 1330 + 1360, 1550 \text{ cm}^{-1}$) and metalated form ($\sim 395, 1010, 1340, 1540 \text{ cm}^{-1}$) markers [3–6] clearly proves that we obtained pure SERRS spectra of the free-base porphyrin forms. Series of SERRS spectra measured for various time delays (from 5 to 30 min) between the system preparation and spectral acquisition were treated by factor analysis that did not reveal any trace of the metalated form. The same results were obtained for tens of substrates when measured several days after the preparation or when SERRS spectra from various spots at the substrate were compared. This means that both our gold and silver surfaces do not change chemical structure of free-base porphyrins and are thus suitable for their SERRS spectroscopy. It is not surprising in the case of Au surface as free-base porphyrins do not metalate even when adsorb onto Au particles in sol (due to large size of Au atoms). In Ag colloids, on the other hand, free-base porphyrins metalate rapidly [4,5,14]. Preservation of the free-base form in the case of immobilized Ag nanoparticles is then unforeseen. We propose that the silane used for nanoparticle immobilization may or act as a molecular spacer [7,8] or modify surface properties by picking up Ag^+ adsorption sites necessary for the porphyrin metalation.

Comparing SERRS spectra obtained from tens of measurements we have found out very good reproducibility, uniformity over the surface (in 10^{-2} mm scale) and stability in time (Table 1). This indicates potential analytical applicability of the surfaces. Limits of detection (LOD), estimated by extrapolation to concentration when intensity of the strongest porphyrin band at $\sim 1550 \text{ cm}^{-1}$ exceeds triple of the blank signal standard deviation [17]) range from 8×10^{-8} M concentration in soaking solution for TPP on Ag surface to 3×10^{-9} M concentration for TMPyP on Ag surface (Table 1). These values of LOD are comparable or even better than those obtained for same porphyrins by using Ag sols [5,18,19].

4. Conclusions

We have developed new technique of porphyrin detection. Up to now, silver sols were the most frequently used substrates for SERS studies of porphyrins. Serious drawbacks for analytical applications were though the colloid aggregation

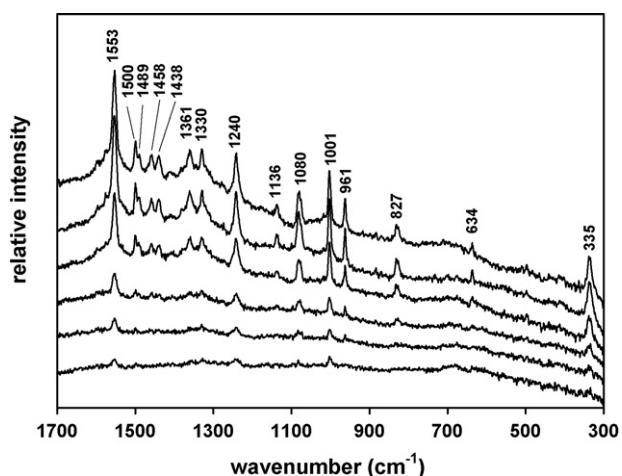


Fig. 6. SERRS spectra of TPP measured from Ag surfaces, soaking concentrations from bottom to top: 1×10^{-7} M, 4×10^{-7} M, 8×10^{-7} M, 2×10^{-6} M, 3×10^{-6} M and 6×10^{-6} M. The Raman signal of glass was subtracted.

caused by the adsorbate that led to irreproducibility of spectral measurements and the metalation of free-base porphyrins in the case of Ag colloids. Moreover, colloidal solutions are unsuitable for water-insoluble porphyrins. Our technique based on immobilized colloidal nanoparticles provides detection limits comparable with those obtained by using Ag sols but have crucial advantages in comparison with colloidal solutions:

1. Excellent spectral reproducibility and time stability of signal.
2. Direct detection of unperturbed free-base porphyrins, even from silver surfaces.
3. Detection of water-insoluble porphyrins without interference of solvent.

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