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Silica microsphere decoration with silver nanoparticles by an impregnation and reduction technique

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A simple impregnation–reduction technique is suggested to prepare a nanocomposite of silica microspheres and silver nanoparticles demonstrating plasmonic resonance for possible applications in surface enhanced Raman spectroscopy for biomedical studies.

Surface enhanced Raman spectroscopy (SERS) is important for monitoring biological, organic and nanoparticle objects in biology, materials science and medicine.[1,2] The underlying phenomena causing SERS are plasmon resonance and charge transfer allowing to amplify a Raman signal by five or even six orders of magnitude. These effects give a chance to study a single molecule conformation or to detect analytes in extremely diluted solutions below ppm concentrations.[3] An additional benefit of SERS is a possibility of yielding a signal even without a direct physical contact with the test molecule since surface enhanced Raman signal remains detectable within a distance of 10–20 nm between a nanoparticle and the analyte.[1,2,4] This effect gives a unique and powerful ability to study the conformation of molecules in different compartments of an intact alive cell.[4] Thus, it provides a new outstanding approach for noninvasive fundamental studies of cell properties and for the sensitive medical diagnostics of cell pathologies and control of physiological fluids.

Inspite of the known advantages of SERS, there are only a few works with a detailed study of living cells.[4,5] Partly the difficulty for using SERS in biological studies relates to the possible negative effect of nanoparticles on cells and feeble cell or protein contact with nanoparticles. Therefore, a particular task to monitor the properties of the living cell with SERS would benefit from the usage of special substrates possessing micro- and nanostructured surfaces decorated with noble metal nanoparticles. Such substrates can provide better conditions for the cell attachment and, therefore, a more stable enhancement factor of the SERS signal. At present, there are no SERS studies of living cells and only a few studies of biomacromolecules that deal with such nanostructured substrates.[6]

A simple chemical tactics to produce nanostructured substrates is naturally connected to the formation of nanocomposite building blocks followed by their self-assembly into a planar structure. An ideal building block for such a route is represented by a silica microsphere decorated with noble metal nanoparticles. This nanocomposite block[7–13] is cheap and robust to produce and there is a plenty of known methods to assemble silica microspheres into an ordered microporous structure (photonic crystal) including layers of a given thickness, from a single layer to a much thicker coating transporting test solutions by capillary forces in a hypothetic microfluidic optical sensor device. At the same time, metal–silica nanocomposite microspheres are produced using the only way of the separate formation of silver nanoparticles, their superficial modification and then chemical bonding the nanoparticles with the outer surface of the microspheres.[8–11] This approach works effectively; however, it has some disadvantages due to an increase in the number of processing stages and also silver covering with organic molecules would cause disturbances of a SERS signal from target biomolecules. Thus, naked silver nanoparticles immobilized on silica seem preferable.

Experimentally, silica microspheres in this work were prepared according to the Stöber method.[7,†]

The hydrolysis of TEOS in the Stöber method normally gives nearly monodisperse microspheres consisting of hydrated amorphous silica. The reaction pathway leads to microchannels providing reaction product exchange with the environment. The final

† 100 ml of ethanol, 2 ml of water and 6.7 ml of ammonia solution were stirred at 40 °C for 2 h followed by admixing 6 ml of tetraethoxysilane (TEOS). The resulting solution was kept overnight under stirring. Silica microparticles were collected by centrifugation at 10000 rpm (Sartorius Sigma 3–30 K) for 10 min and washed repeatedly with ethanol and water. The separated particles were dried at room temperature and then further desiccated at 110 or 300 °C for 1 h. For the synthesis of silica microspheres decorated with silver nanoparticles, 2 ml of 1 M aqueous solution of silver nitrate was added to 50 mg of as-prepared silica particles suspended in deionized water. After sonication (Elmasonic S30H) for 1 h, the suspensions were kept overnight. The impregnated silica particles were washed several times with water without their complete separation from the supernatant liquid. 2 ml of a nontoxic biocompatible reducing agent [100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution with pH 7.4 or 1 M ascorbic acid] was then injected to cause silver nanoparticle formation on the surface of silica microspheres still containing absorbed silver nitrate solution in their pore structure. This resulted in a microsphere color change from snow-white to olive-green in a couple of minutes. After ageing the dispersion for 24 h under the supernatant layer at room temperature, the silver–silica nanocomposite microspheres were filtered off, washed with deionized water and dried at room temperature.

Thermogravimetric analysis (TGA) of silica microspheres was performed using a NETZSCH STA 409 PC/PG thermal analyzer at a heating rate of 3 K min^{-1} in air in the temperature range of 30–900 °C. The data collected were analyzed after a baseline has been subtracted. The silica particles were characterized by the capillary adsorption of nitrogen at 77 K. Surface areas and total pore volumes were calculated using BET and BJH techniques, respectively. Infrared spectra of the samples were recorded using a Spectrum One FTIR spectrometer (Perkin–Elmer) while the UV-VIS absorption spectra of silica–silver composites were measured by means of a Lambda 950 UV-VIS spectrophotometer (Perkin–Elmer). In both cases, a diffuse reflectance accessory was attached to analyze the finally dried powdered product. Transmission electron microscopy (TEM) images were obtained with an LEO912 AB OMEGA electron microscope.

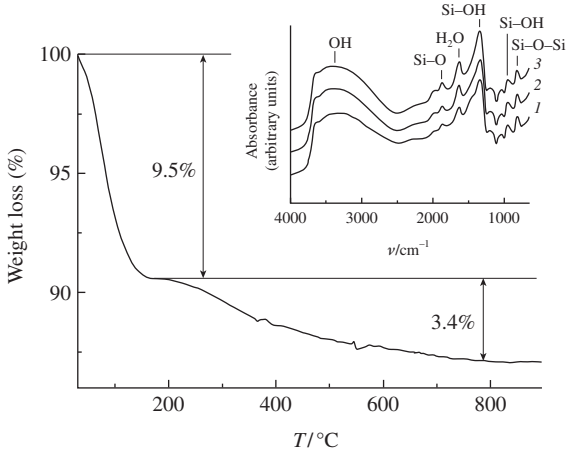

Figure 1 TGA of silica particles. Inset: FTIR spectra of silica particles: (*1*) as-prepared; desiccated at (*2*) 110 and (*3*) 300 °C.

product would remain porous, but it is impossible to exclude that some residual liquid remains in the pores. In order to open up the pores and thus optimize the preparation temperature of a set of microcontainers for impregnation with silver nitrate solution, thermal analysis was performed (Figure 1). The TGA data demonstrate that there is a major weight lost around 100 °C reflecting the evaporation of physically absorbed water. The amount of this water is about 9.5 wt%. This value suggests the presence of microchannels in the microspheres filled with water or solution. The next stage would correspond to amorphous silica structure deterioration and its crystallization since weight decreases gradually by another 3.4 wt% approaching a plateau at above 800 °C. A half of this chemically bound water releases in the range of 100–350 °C. FTIR spectra of silica samples annealed at several temperatures corresponding the above stages of water liberation (Figure 1, inset) are significantly related each other and prove the presence of regular silica–oxygen bond vibrations of a different kind and also the existence of different hydroxyl groups making the silica microsphere surface hydrophilic and readily prepared to impregnation with aqueous solutions.

Annealing at medium temperatures changes the porous structure of silica microspheres (Table 1). The overall surface area was determined using the capillary adsorption of nitrogen. It is easy to estimate that the geometrically expected surface for microspheres with a mean diameter of 240 nm (Figure 2) does not exceed 3 m^2 g^{-1}. At the same time, experimental value is higher than the estimated one by a factor of ~7 (Table 1). The reason of such a discrepancy is to be related to an increased in the pore volume of the microspheres, which is 3.5–7.5×10^{-8} m^3 g^{-1} depending on annealing temperature (Table 1). A surface area and total pore volume decrease observed in the case of silica microspheres desiccated at 300 °C is caused by the overall shrinkage of micro-

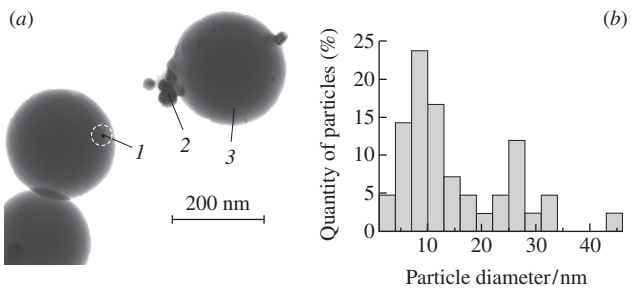

Figure 2 (*a*) TEM photograph of silica microspheres (*3*) decorated with (*1*) 5–10 and (*2*) 20–40 nm silver nanoparticles. (*b*) Particle size distribution of the colloidal Ag nanoparticles presented in the composite silica–silver microspheres.

Table 1 Surface areas and total pore volumes of silica microspheres.

Sample	Surface area/ m^2 g^{-1}	Total pore volume/ 10^{-8} m^3 g^{-1}
SiO_2	13.8	3.8
SiO_2 desiccated at 110 °C	21.2	7.5
SiO_2 desiccated at 300 °C	10.9	3.5

spheres loosing residual water. Considering such a porous microsphere as a container, it is possible to estimate that this pore volume filled with 1 M of silver nitrate could result in about 5 mg of reduced silver per gram of microspheres. Visually, it would correspond to a 1:1000 volumetric ratio of reduced (metallic) silver spherical particles and the silica microspheres.

This estimation is close to the real microstructural observation of silica–silver nanocomposite. Figure 2 exhibits 240 nm silica microspheres and 5–40 nm silver nanoparticles attached to the surface of silica. The size distribution of silver nanoparticle seems to be broad enough evidencing for both homogeneous and heterogeneous silver nucleation. The first mechanism is probably related to silver nuclei formation in the homogeneous solution near the surface of silver nitrate impregnated silica microspheres; the size of such nanoparticles is larger due to their free growth in the solution and a constant supply of silver ion from neighboring microspheres. After growth, such nanocrystals are attached to the surface of silica electrostatically or due to interaction with superficial groups of silica microspheres. Another mechanism is heterogeneous nucleation on the surface of silica, probably, near the microchannel outlets. Such nanoparticles are limited in their size because of constraint grow conditions caused by superficial interactions and exhausting nutrient solution due to its diffusion from the microsphere to surrounding liquid.

The finally dried silver–silica nanocomposite demonstrates peaks of plasmonic resonance at ~450 nm (Figure 3) caused by silver nanoparticles observed microstructurally (Figure 2) and visually because of the sample color change. Two visible maxima can be caused by the presence of silver nanoparticles with different sizes, as seen from a bimodal distribution of nanoparticles in Figure 2(*b*). These peaks are absent when measuring pure silica microspheres. Thus, we conclude that the suggested approach is able to produce semiproduct for SERS-active substrate self-assembly in a simple and robust way.

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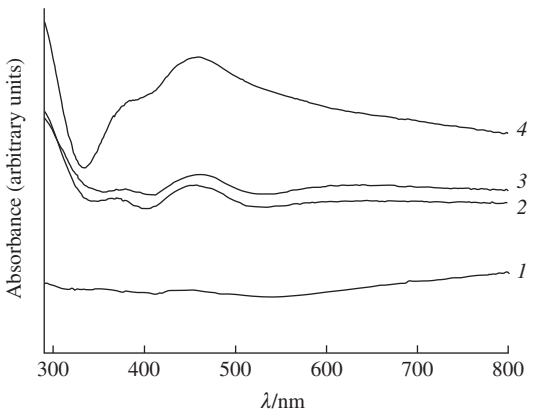

Figure 3 UV-VIS absorption spectra of (*1*) silica microspheres, and silica–silver composites, prepared from (*2*) original and heat-treated particles desiccated at (*3*) 110 or (*4*) 300 °C.

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