Synthesis, Raman enhancement, and chemical stability of CaCO₃ microparticles with copper coating

N E Markina¹, A I Danchuk¹, A M Zakharevich², A V Markin¹,²
¹Institute of Chemistry, Saratov State University, Saratov 410012, Russia
²Education and Research Institute of Nanostructures and Biosystems, Saratov State University, Saratov 410012, Russia

Abstract. The aim of the work is the microwave-assisted (MW) synthesis of SERS-active copper nanoparticles (CuNPs) and the core-shell composite based on nanostructured copper layer deposited over CaCO₃ microspheres (CaCO₃@Cu; 1.4±0.4 μm). The comparison of MW synthesized CuNPs and gold nanoparticles showed the equal orders of magnitude of the Raman enhancement. The temporal stability of CaCO₃@Cu samples was found significantly better than that of CuNPs: 4 days vs. 30 min. Stabilizer- and organic solvent-free synthesis of CaCO₃@Cu enables to minimize background SERS signal. The applicability of CaCO₃@Cu for chemical analysis was showed by detection of antibiotic drug (ceftriaxone) in a range of concentrations which is relevant for practical purposes (5–500 μg/mL).

1. Introduction
Generally, plasmonic gold and silver nanostructures are used as materials for surface-enhanced Raman spectroscopy (SERS) [1]. However, the results of our recent review shows that copper nanostructures can also be successfully used as reliable and cost-effective SERS-active materials (Cu-SERS substrates) [2]. Nevertheless, the information about Cu-SERS substrates is still very limited and additional studies are on demand. In particularly, low chemical stability of copper is the main challenge which restricts widespread application of Cu-SERS substrates. Although an application of organic solvents and strongly bonded stabilizers enables to minimize corrosive action of the oxygen of air and aqueous environment, they also lead to appearance of significant background signal that diminishes reliability of SERS based analysis. Earlier we proposed incorporation of copper nanoparticles (CuNPs) to the matrix of CaCO₃ microparticles to improve their temporal stability during storage [3]. However, despite the advantage of extremely long stability (> 4 months), this approach requires dissolution of the CaCO₃ matrix before SERS measurements that can be not optimal in some cases, e.g., instability of the analyte in acidic media.
Here we report on fabrication of Cu-SERS substrates (CaCO₃@Cu) using microwave-assisted synthesis (MW synthesis). Although the MW synthesis enables significant reaction acceleration and homogeneous heating, there is only one report it was used to prepare Cu-SERS substrate [4]. The authors synthesized SERS-active CuNPs by decomposition of acetylacetone in benzyl alcohol, but the final substrate has low Raman enhancement and requires removing of the organic solvent before the use in analysis. Thus, we developed the synthesis in water and studied Raman enhancement and temporal stability of the substrates in aqueous environment. Additionally, the performance of the Cu-SERS substrates was verified in comparison with gold nanoparticles (AuNPs). The practical applicability of the substrates was tested by detection of antimicrobial drugs used as a model analyte.
2. Experimental part

2.1. Materials
Copper(II) sulfate (CuSO₄·5H₂O), hydrogen tetrachloroaurate(III) (HAuCl₄·3H₂O), sodium citrate (Na₃C₆H₅O₇·2H₂O), hydrazine hydrate (N₂H₄·H₂O), and 4-aminophenol (4-ATP) were purchased from Sigma-Aldrich. Ethanol, calcium chloride (CaCl₂·2H₂O), and sodium carbonate (Na₂CO₃) were purchased from Vekton (Russia). Ceftriaxone (CEF) was used from Ceftriaxone powder for preparation of injections (Biosintez, Russia). Milli-Q (18 MΩ cm) water was used for preparation of all solutions.

2.2. Syntheses
The protocol of CaCO₃@Cu preparation includes two steps: (i) the synthesis of CaCO₃ microspheres and (ii) coating of the microspheres with SERS-active layer of nanostructured copper. The CaCO₃ microspheres were prepared by fast addition of Na₂CO₃ (3 mL, 0.167 М) to CaCl₂ (3 mL, 0.167 М) under continuous ultrasound (US) treatment for 100 s in US bath (0.5 L, 35 kHz, 50 W). Then the CaCO₃ microspheres were rinsed two times by water and one time by ethanol with further drying at 60°C for 3 h. The deposition of nanostructured copper coating onto the surface of CaCO₃ microspheres was performed as follows. A portion (5 mL) of the mixture containing CaCO₃ (10 mg), CuSO₄ (1 mM), and hydrazine hydrate (150 μL) was heated in the domestic MW oven at the maximal power (700 W) for 20 s. The mixture of CaCO₃ and CuSO₄ was shaken for 3 min before addition of hydrazine and heating in order to saturate CaCO₃ surface with Cu²⁺ ions. Finally, CaCO₃@Cu was allowed to sediment, 3 mL of the supernatant solution was removed, and the residue was stored in closed plastic container without any rinsing.

The solutions of citrate stabilized CuNPs and AuNPs were synthesized also using MW heating. The CuNP sample was prepared by hydrazine reduction of CuSO₄ (0.5 mM) in the presence of sodium citrate (1 mM). The AuNPs were synthesized by citrate reduction of HAuCl₄ according to protocol described by Frens [5]; AuNPs were concentrated by centrifugation to achieve the concentration comparable with CuNPs.

2.3. Methods of characterization and SERS measurements
The morphology of CaCO₃@Cu and its EDX spectra were studied using scanning electron microscopy (SEM: Mira II LMU, Tescan, Czech Republic). In order to remove byproducts, the samples were rinsed by ethanol (one time) before SEM and EDX measurements. Probing nanolaboratory Ntegra Spectra (NT-MDT, Russia) with integrated Raman spectrometer (Solar) and He-Ne laser (632.8 nm) was used for SERS measurements. Raman spectrometer was calibrated using Raman band of crystalline silicon at 520 cm⁻¹. Preliminary SERS studies were performed using the mixtures of 4-ATP (10 μL of ethanolic solution) with the suspension of CuNPs or AuNPs (1 mL). SERS spectra of CEF were collected using the mixture of the analyte and CaCO₃@Cu at 1:1 v/v ratio. All SERS spectra were collected from 10 μL of the analyte mixed with SERS substrate placed on cover slide. SERS signal was generated using 9 mW laser power and 1 sec signal acquisition time; at least 15 spectra were registered to obtain averaged signal.

3. Results and discussions

3.1. SERS-activity of CuNPs and AuNPs
It is well known that MW heating enables to minimize the influence of the walls of reaction vessel, to avoid formation of “metallic mirror” on the walls, and to improve homogeneity of the produced nanostructured coating. These advantages are of particular importance for fabrication of SERS substrates with reproducible enhancement properties. At the first step, the capability of MW heating to produce SERS-active materials was checked by synthesizing citrate stabilized CuNPs and AuNPs. A
The model analyte (4-ATP) was used to test enhancement ability due to its high affinity to the surface of both metals. The results of SERS measurements show that both CuNPs and AuNPs generate strong SERS signal (Figure 1a). Although AuNPs provide around 2 times larger SERS signal, they significantly lose in cost and availability compared to CuNPs. Additionally, the orders of magnitude of both SERS signals are the equal meaning comparable enhancement capabilities of CuNPs relative to AuNPs. The dependences of SERS signal on the ATP concentration (Figure 1b) have a linear form in logarithmic coordinates that is typical for adsorption isotherms on energetically heterogeneous surfaces (Freundlich isotherm).

![Figure 1(a, b).](image)

Figure 1(a, b). (a) SERS spectra of 4-ATP (0.05 μg/mL) obtained using copper (CuNPs) and gold (AuNPs) nanoparticles; (b) Dependence of SERS signal intensity on concentration of 4-ATP (band at 1074 cm⁻¹).

3.2. Preparation of CaCO₃@Cu

Unfortunately, colloidal CuNPs have very low chemical stability (discussed in the next section). This disadvantage was partially overcome by fabrication of SERS-active core-shell structure consisted of rough copper surface deposited over CaCO₃ microspheres. Additionally, in the previous work, we demonstrated that the deposition of SERS-active coating over the calcium carbonate positively influences Raman enhancement due to contribution of rough surface of CaCO₃ to the formation of hot spots [6].

The pure CaCO₃ microspheres were prepared using modified protocol described earlier [7]. The original protocol yields 4–6 μm particles which sediment fast and cannot be coated by the copper coating properly: CuNPs are reduced in the bulk of the solution instead of the CaCO₃ surface. Thus, we synthesized CaCO₃ under constant ultrasound treatment that enabled to get ~2.5 times smaller particles with lower sedimentation rate.

Besides of appropriate size of CaCO₃, successful deposition of the copper coating onto CaCO₃ surface requires preliminary adsorption of copper ions to the pores of CaCO₃ for several minutes. This step enables to initiate copper reduction at the CaCO₃ surface instead of the solvent volume (e.g. formation of CuNPs suspension). Besides of cost-efficiency and availability of CuSO₄, it was also used because sulfate ions have very low affinity to the copper surface enabling to minimize its influence on the analyte adsorption and Raman enhancement.

According to SEM studies, the size of final CaCO₃@Cu is 1.4±0.4 μm (N=200) (Figure 2a,b). Although SEM results do not enable to distinguish between CuNPs and CaCO₃, CaCO₃@Cu have dark-red color and EDX measurements prove the presence of copper in CaCO₃@Cu (Figure 2c).

The rinsing of the samples was used only before SEM and EDX studies, but not before SERS measurements because it reduces Raman enhancement of the composite. We explain this fact by partial oxidation of the copper surface at neutral pH that is in agreement with earlier published results regarding to aggregates of CuNPs [3]. Additionally, the rinsing is not needed because precursors and byproducts do not influence SERS background. In contrast, the residue of N₂H₄ removes the oxygen...
from the vessel and increases temporal stability of the sample during the storage. Also, the synthesis does not require stabilizers resulting in the minimal background signal.

![Figure 2(a-c)](image)

**Figure 2(a-c).** SEM images (a, b) and EDX spectrum (c) of CaCO$_3$@Cu microspheres.

### 3.3. Temporal stability and analytical performance of CaCO$_3$@Cu

As discussed in Introduction, temporal stability of Cu-SERS substrates is the most important challenge which has to be overcome in order to achieve widespread usage of the substrates in SERS based analysis. Because we are interested in the application of SERS for analysis of biofluids, the temporal stability of Raman enhancement capability of CuNPs and CaCO$_3$@Cu was studied in aqueous environment. The results show that the enhancement of CuNPs is drastically reduced during 30 min (Figure 3a). In contrast, CaCO$_3$@Cu produce stable SERS signal during at least 4 days after preparation. We explain this improvement by reduction of specific surface area compared to CuNPs. Finally, the potential of CaCO$_3$@Cu for SERS based therapeutic drug monitoring was estimated using ceftriaxone, a widely used antibiotic of cephalosporin class. Analyte concentrations were selected at the level of 5–500 μg/mL that corresponds to therapeutically relevant antibiotic content in the urine of the patients [8]. The obtained SERS spectra (Figure 3b) have the appropriate intensity and are in agreement with the previously reported spectra obtained using silver nanoparticles [9]. Although CaCO$_3$@Cu have small background signal, the signal is weak and does not overlap with Raman bands of the analyte making quantitative analysis possible and reliable.

![Figure 3(a, b)](image)

**Figure 3(a, b).** (a) Influence of CuNPs and CaCO$_3$@Cu storage time on SERS signal intensity of ceftriaxone at constant concentration (10 μg/mL); (b) SERS spectra of blank and ceftriaxone solutions obtained using CaCO$_3$@Cu and dependence of SERS signal intensity on ceftriaxone concentration (according to Raman band at 1358 cm$^{-1}$).
4. Conclusions
The main result of this paper is the copper based SERS-active composite whit improved temporal stability and strong Raman enhancement. Because the synthesis was performed without organic reducing agents, solvents, and stabilizer, it yields the composite with minimal background SERS signal making it reliable enough for analytical application. The high Raman enhancement and simplicity of preparation make the composite really competitive with noble metal based SERS substrates. Finally, recorded SERS spectra of the antibiotic drug within wide range of concentrations prove general applicability of the composite for therapeutic drug monitoring. However, further studies on selectivity and stability of the composite in biofluids are on demand.

5. Acknowledgments
The study was funded by RFBR according to the research project № 17-03-00537.

6. References
[1] Cialla D, März A, Böhme R, Theil F, Weber K, Schmitt M, Popp J 2012 Anal. Bioanal. Chem. 403 27
[2] Markin A V, Markina N E, Popp J, Cialla-May D 2018 Trends Anal. Chem. 108 247
[3] Markina N E, Volkova E K, Zakharevich A M, Goryacheva I Y, Markin A V 2018 Microchim. Acta 185 481
[4] Dar M I, Sampath S, Shivashankar S A 2012 J. Mater. Chem. 22 22418
[5] Frens G 1973 Nature Phys. Sci. 241 20
[6] Stetcuira I Y, Markin A V, Ponomarev A N, Yakimansky A V, Demina T S, Grandfils C, Volodkin D V, Gorin D A 2013 Langmuir 29 4140
[7] Volodkin D V, Larionova N I, Sukhorukov G B 2004 Biomacromol. 5 1962
[8] Patel I H, Chen S, Parsonnet M, Hackman M R, Brooks M A, Konikoff J, Kaplan S A 1981 Antimicrob. Agents Chemother. 20 634
[9] Markina N E, Goryacheva I Y, Markin A V 2018 Anal. Bioanal. Chem. 410 2221