Scientific paper

Silver Nanoparticles in SiO_2 Microspheres – Preparation by Spray Drying and Use as Antimicrobial Agent

Boris Mahltig,[1,*] Helfried Haufe,[1] Kerstin Muschter,[1] Anja Fischer,[1] Young Hwan Kim[1,2] Emanuel Gutmann,[3] Marianne Reibold,[3] Dirk Carl Meyer,[3,4] Torsten Textor,[5] Chang Woo Kim[6] and Young Soo Kang[6]

[1] Gesellschaft zur Förderung von Medizin-, Bio- und Umwelttechnologien e.V., GMBU e.V., Postfach 520165, 01317 Dresden, Germany

[2] Pukyong National University, 599-1, Daeyeon3-Dong, Nam-Gu, Pusan 608-737, Korea

[3] Technische Universität Dresden, Institut für Strukturphysik, 01062 Dresden, Germany

[4] Technische Universität Bergakademie Freiberg, Institut für Experimentelle Physik, Silbermannstrasse 1, 09599 Freiberg, Germany

[5] Deutsches Textilforschungszentrum Nord-West e.V., DTNW e.V., Adlerstrasse 1, 47798 Krefeld, Germany

[6] Sogang University, Department of Chemistry, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, Korea

** Corresponding author: E-mail: mahltig@gmbu.de; Tel: ++49-351-2695343 / Fax: ++49-351-2695341*

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Abstract

Silver nanoparticles embedded in SiO_2 particles of micrometer size are prepared using spray drying. The spray drying is performed with a SiO_2 sol (solvent water:ethanol 4 : 1) containing SiO_2 and silver particles of nanometer size. During spray drying the SiO_2 nanoparticles aggregate to SiO_2 microspheres whereas the silver particles exhibit only a small tendency of aggregation and keep their nanometer size. However under special conditions also the formation of crystalline silver rods is observed.

The antibacterial activity of the resulting Ag/SiO_2 powders is determined against the bacteria *Escherichia coli* and *Bacillus subtilis*. Because of this antibacterial acitivity and the fact that the powder of SiO_2 microspheres exhibits a good dispersibility, such materials have an immense potential to be used as antimicrobial additive in processes like master batch or fiber production.

Keywords: Sol-gel technology, antimicrobial, antibacterial, silver, nanoparticular, spray drying

1. Introduction

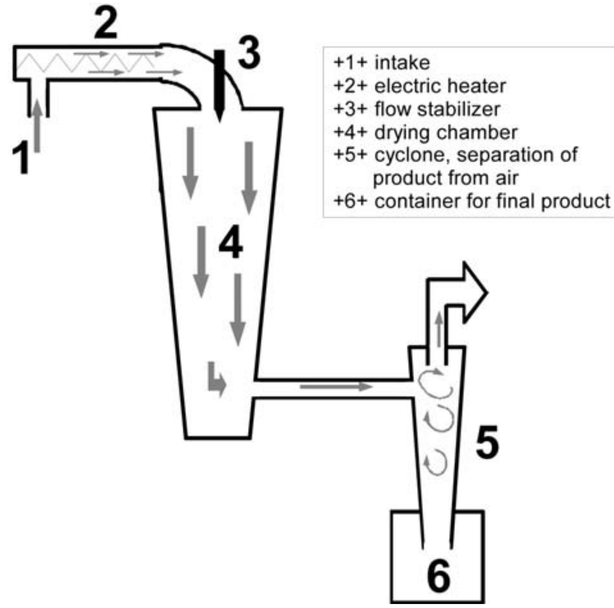
Nanoparticular silver is one of the most effective antimicrobial agents strongly acting against bacteria and mostly without any effect on human health.[1–4] Silver particles are already used in a large variety of application for example for antimicrobial modification of organic polymers, glass slides, catheters or textile fabrics.[5–10] Other interesting material combinations reported recently are silver-doped silica composite membranes or silver nanoparticles in mesoporous silica nanoparticles.[11, 12] Due to the high ratio of surface to volume of nanometer sized particles, nanoparticular silver exhibits the tendency of aggregation that is in most cases undesirable but can be prevented in the presence of SiO_2 particles in a sol-gel approach.[13] However, such nanosol solutions only have limited stability, because of an early start of gelation or precipitation. Furthermore, for many applications like master batch it is of interest to have a solid powder as additive instead of a liquid. An appropriate method to prepare a solid powder from a liquid inorganic nanosol is the spray drying process.[14–16] Using this method, dry inorganic particles of micrometer size, interesting for several applications, can be prepared.

Regarding this background, the aim of the work presented here is the application of a well described nanosol, containing silver and SiO_2 nanoparticles, in a spray drying process in order to prepare SiO_2 powder with embedded silver nanoparticles.[13] Related to this topic, several groups reported on the preparation of silver nanoparticles on silica microspheres.[17, 18] However, they prepared these silica microspheres separately and deposited the silver particles afterwards by reduction of Ag^+. In contrast, the approach described here starts with a sol containing both silver and silica nanoparticles, in which the silver particles are obtained in the presence of silica nanoparticles by reduction of $AgNO_3$ with triethanolamine.[13] During spray drying of this mixture only the SiO_2 particles show a distinct tendency of aggregation to microspheres whereas the silver nanoparticles mainly keep their nanometer size. Therefore, in the following an appropriate method to prepare powders of micrometer sized SiO_2 spheres containing regularly dispersed and stabilized particles of crystalline and nanometer sized silver is reported.

2. Experiments

2. 1. Preparation

Two types of nanoparticular solutions of SiO_2 (pure and modified with silver) are used for powder preparation by spray drying. Both solutions are prepared by an alkaline sol-gel process as described in the following. The pure SiO_2 sol is prepared by stirring of a mixture of 19 ml tetraethoxysilane (TEOS), 77 ml water and 3.8 ml triethanolamine for duration of at least 2 days until the solution becomes homogeneous and clear. Due to the fact that the SiO_2 particles formed cannot be separated from the liquid by centrifugation or filtration the prepared liquid mixture is better described by the term solution instead of dispersion of SiO_2 particles. The solid content of the resulting SiO_2 sol is 9.6 wt-%. For preparation of a silver modified SiO_2 sol, a solution of 0.1 g $AgNO_3$ in 5 ml of water is added to 100 ml of a SiO_2 sol. Afterwards this solution is thermally treated under reflux for 5 hours, as reported earlier.[13] The resulting sol is directly used for spray drying. The sols are stable for at least one month and do not show any kind of precipitation. The spray drying of both sols is performed with a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Switzerland). The inlet temperature for spray drying is set to 210 °C. The resulting powder is collected at two different places in the device – at the drying chamber and the collecting container (see Scheme 1).



Scheme 1: Schematical drawing of the used spray dryer.

2. 2. Analytical Methods

X-ray diffraction (XRD) is applied to investigate the overall crystallinity of the composite microspheres and to determine the mean crystallite size of the silver nanoparticles. Therefore XRD patterns are recorded using Cu-Kα radiation on an X-ray diffractometer D8 (Bruker AXS) and URD6 (Seifert FPM). For determination of the mean crystallite size a quantitative analysis of the diffraction patterns is conducted using the commercial computer program TOPAS (Bruker AXS) as it is described in detail in earlier publications.[13,19] XRD investigations are done on dried powder samples obtained from spray drying or from the liquid sols by evaporation of the solvent at room temperature for a minimum duration of 24 hours. For determination of particle size distribution of SiO_2 sols dynamic light scattering (DLS) is used. The DLS measurements are performed with a commercially available device Zetasizer 1000 Has (Malvern Instruments). Prior to DLS measurements the liquid samples have been diluted with water in a ratio of 1:10. A dilution with ethanol is not suitable, because fast gelation of the sol occurs in case of dilution with organic solvents.

Transmission electron microscopy (TEM) is used to determine the size of silver and silica particles and moreover High-resolution transmission electron microscopy (HRTEM) is used to determine the crystalline phase of silver particles. These investigations are performed with a Philips CM30 transmission microscope as in detail described in earlier publications.[13,19] Scanning electron microscopy (SEM) is performed to determine the particle size and shape of the spray dried powders. For all measurements a commercial device Topcon-microscope ATB55 is used. For SEM the powder samples are pre-treated by sputtering with gold. The antibacterial activity of the silver containing powder is determined with *Escherichia coli* (gram-negative bacteria) and *Bacillus subtilis* (gram-positive bacteria). For this purpose the powder obtained from spray drying is dispensed with bacteria suspension and inoculated for 24 hours at a temperature of 30 °C. Afterwards the powder is leached in phosphate buffer (pH=7) and the buffer is inoculated on agar plates. After one days the colony forming units are counted.

3. Results and Discussion

3. 1. Properties of Nanoparticular Solutions Used for Spray Drying

Before discussing the characteristics of particles obtained by spray drying, a description of the properties of the sol, used as educt for the spray drying process, is given (Figures 1 and 2). The size distribution in the sols is determined by DLS (Figure 1). With this method mainly the size of SiO_2 particles in solution is determined, because compared with silica the amount of silver in the solution is significantly smaller. The pure SiO_2 sol contains mainly particles smaller than 10 nm with a maximum of particle size distribution of around 2 nm. The silver containing Ag/SiO_2 sol exhibits only slightly larger particles. Therefore, in both starting solutions mainly SiO_2 particles of sizes less than 10 nm are present.

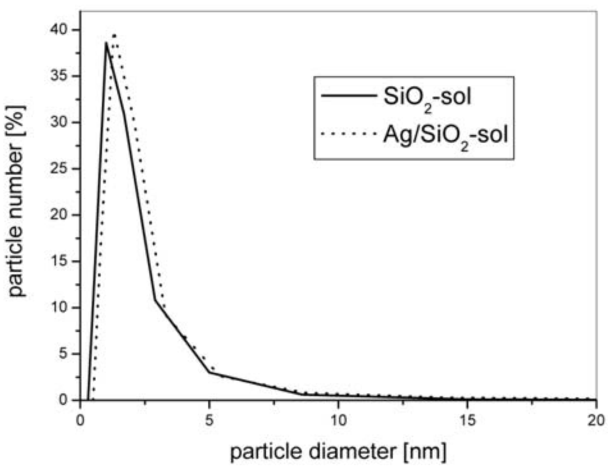


Figure 1: Particle size distribution of SiO_2 sols and SiO_2 in silver containing Ag/SiO_2 sols as used for spray drying.

Because the size of silver particles cannot be determined by DLS, TEM is used to determine the silver particle size (Figure 2). For this investigation the liquid Ag/SiO_2 sol is coated onto a graphite membrane and dried afterwards. The TEM image of this coating indicates clearly that the silver particles have a size of around 5 nm. Additional investigations by XRD indicate that the mean size of silver crystallites in this system is 3 nm or smaller.[13] Altogether it can be stated that the starting solution for the spray drying process contains only nanometer sized components of silver and silica.

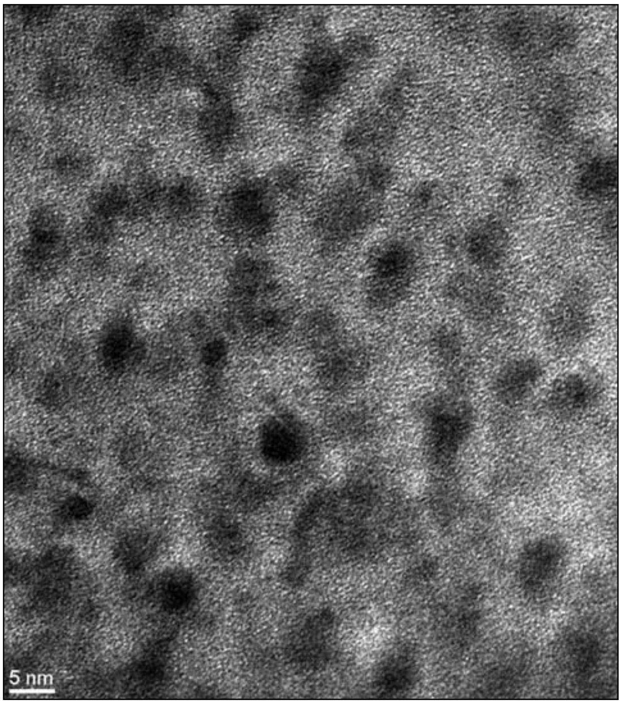


Figure 2: HRTEM image of a coating prepared from Ag/SiO_2 sol, as used for spray drying.

3. 2. Size and Shape of Silica Microspheres

After spraying of the pure SiO_2 sol, particles with diameters of 0.5 µm up to 5 µm are obtained (Figure 3). Most of these silica microspheres contain pin-holes and especially the larger particles exhibit a kind of donut structure. Those structures are quite typical for inorganic spray-dried particles.[15,20] The formation of those structures is theoretically described for drying droplets of solutions containing polymer lattices.[21–23] Due to the solvent evaporation during the spray drying process, the SiO_2 particles probably aggregate near the air/liquid interface and form a kind of skin at the evaporating droplet. As a result a further shrinkage of the droplet is not possible by solvent evaporation. In case of a collapse of the skin, due to low mechanical stability, the formation of holes in the particles can be observed.

3. 3. Size and Shape of Silver Nanoparticles

In case of spray drying Ag/SiO_2 sols the size and shape of silica microspheres formed is similar and not affected by the additional silver component in the sol (Figu-

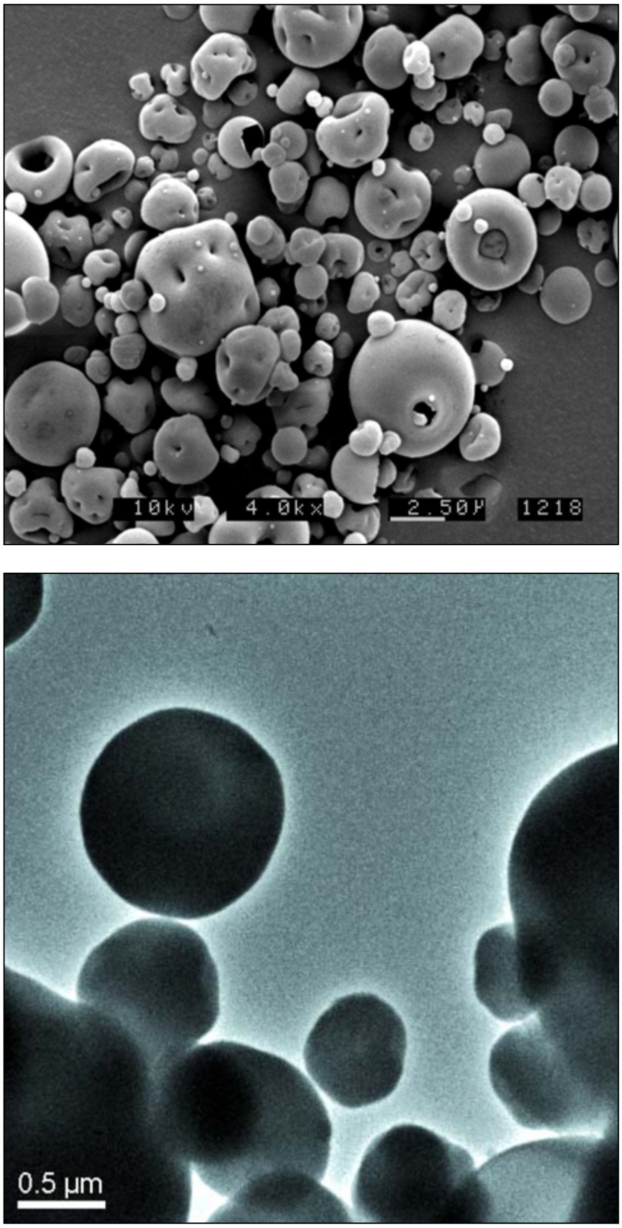


Figure 3: SEM image (above) and TEM image (below) of particles gained by spray drying of a SiO_2 sol.

re 4). From TEM images it can be gathered that small silver particles are embedded in the silica microspheres (Figure 5). As indicated also by crystallite size evaluation from XRD measurements (Figure 6) these silver particles are still nanocrystalline. Only a small growth of crystallites up to a size of 9 nm has taken place, referring to the evaluation of line broadening of silver reflections in the XRD patterns. This is only a negligible particle growth compared to that of SiO_2 particles starting from a nanoscale size and ending at a microscale size. Thus it can be clearly stated that in the sol the nanometer sized SiO_2 component aggregates to particles of micrometer size wheres the nanometer sized silver component only exhibits a small tendency of aggregation and keeps its nanometer size. This concept of growth of only one component, while the size of the other remains, is the key for the preparation of microspheres with embedded nanoparticles. The reason for the different aggregation behaviour of the two species of nanoparticles is probably the significantly higher concentration of silica in the sol compared to silver. During solvent evaporation the SiO_2 particles get in contact with each other and form a xerogel network. The less concentrated silver particles are fixed in this silica network as nanoparticles and are not able to diffuse to other silver particles to aggregate. The SiO_2 is in this case essential to stabilize the nanometer sized silver particles and to prevent them from aggregation.

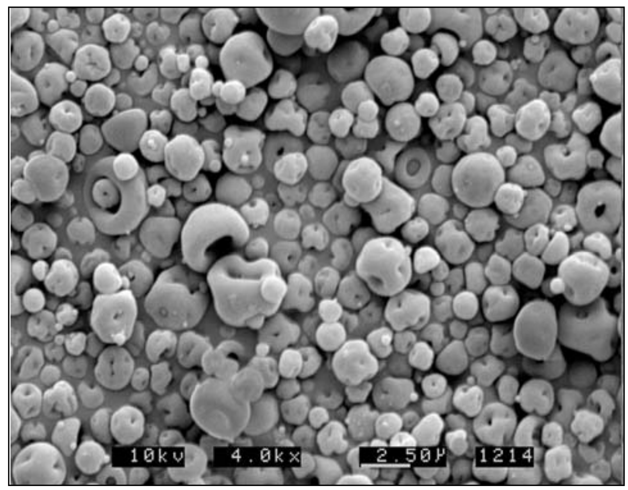


Figure 4: SEM image of particles gained by spray drying of an Ag/SiO_2 sol.

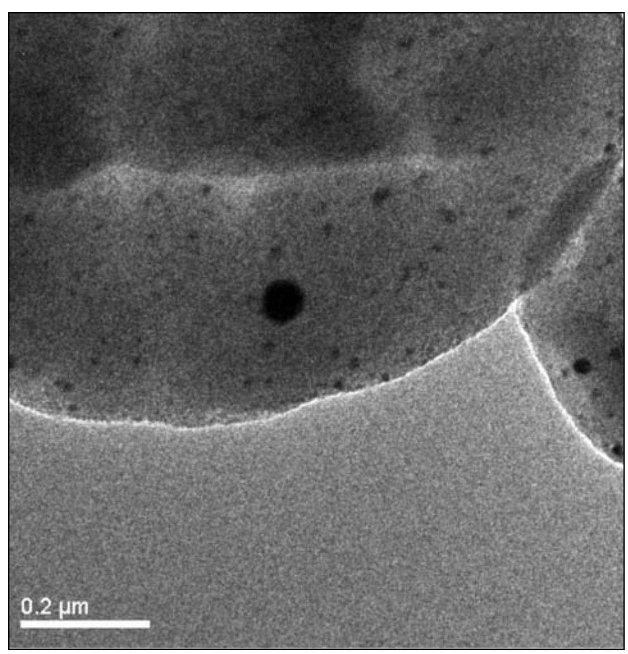


Figure 5: TEM image of particles gained by spray drying of an Ag/SiO_2 sol, product deposited in the collecting container.

However it is observed that this kind of stabilisation does not work in all cases. From TEM images it can be gathered that in some cases beside small silver nanoparticles also larger rod-like structures are formed (Figure 7). Apparently these structures grow out of the silica microspheres. By analysing the lattice distances of these rod-like structures in the HRTEM images it can be clearly stated that they consist of crystalline silver (Figure 8). Rod-like structures are only found in the spray dried product deposited in the collecting container of the spray dryer (Scheme 1). Samples collected from the walls of the drying chamber of the spray dryer exhibit mainly silica microspheres and silver nanoparticles (Figure 9). An explanation for this difference could be that the larger silver rods are due to their higher weight more favourable to be deposited at the collecting container. In contrast, smaller and thus lighter particles should have a longer retention time and are therefore more favourable to be deposited at the drying chamber.

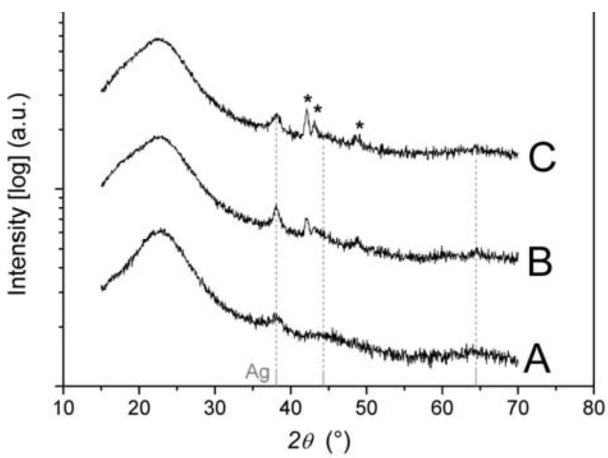


Figure 6: XRD patterns from the liquid sol (pattern A) and of dried powder samples gained from spray drying (pattern B for sample from collecting container and pattern C for sample from the cyclone). Reflections marked with an asterix (*) belong to the sample holder.

3. 4. Antimicrobial Properties of the Silver Containing Spray Dried Materials

The antimicrobial properties of the silver containing spray dried Ag/SiO_2 powders are tested against *Escherichia coli* and *Bacillus subtilis*. For this, the Ag/SiO_2 powders are dispensed into a solution containing bacteria and the amount of dispensed powder is increased from 0.33 wt-% up to 1.67 wt-% (Table 1). Compared to a reference containing no Ag/SiO_2 powder, the growth of bacteria is significantly decreased. Especially against *Escherichia coli* the antimicrobial activity is excellent and no growth of bacteria is observed even with the lowest Ag/SiO_2 powder concentration. The antimicrobial effect against *Bacillus subtilis* is also significant, however at lower concentration of Ag/SiO_2 powder a small bacteria growth can be observed. Altogether it can be stated that the as-obtained powders of SiO_2 microspheres with embedded nanoscaled silver particles are highly effective agents for suppressing the growth of bacteria.

4. Conclusions

A solution containing nanoscaled SiO_2 and Ag particles can be easily used for spray drying to prepare SiO_2 microspheres with embedded silver nanoparticles. The underlying concept is that during the spray drying process

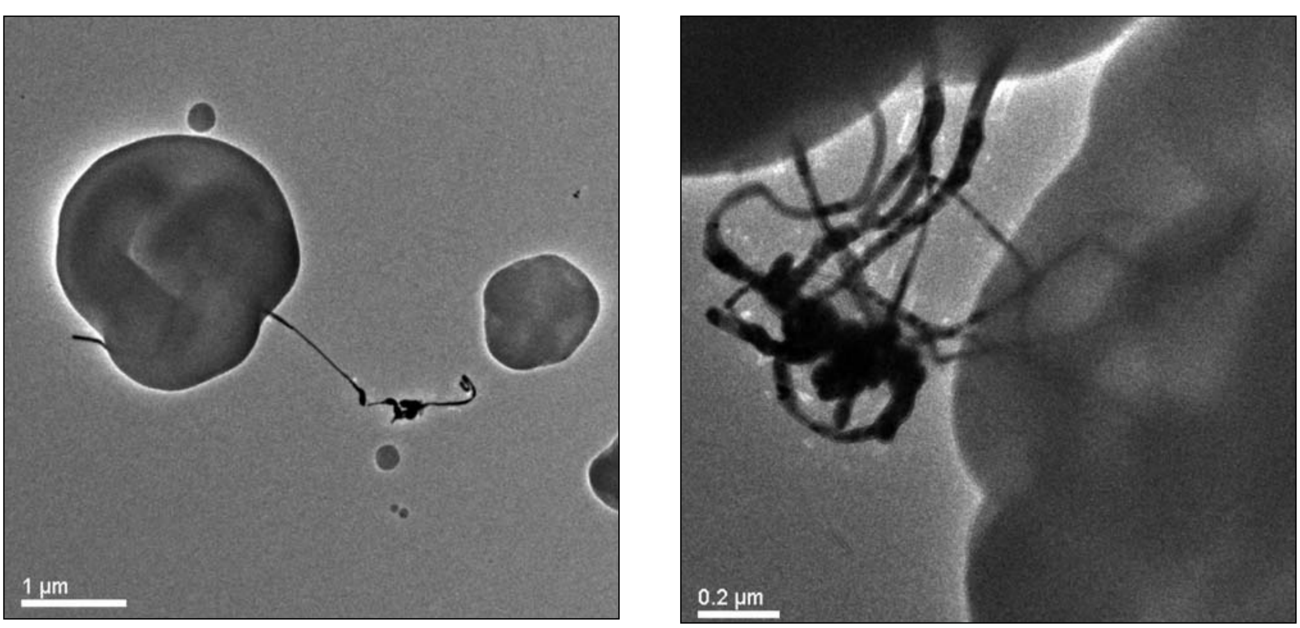


Figure 7: TEM images of particles gained by spray drying of an Ag/SiO_2 sol, product deposited in collecting container.

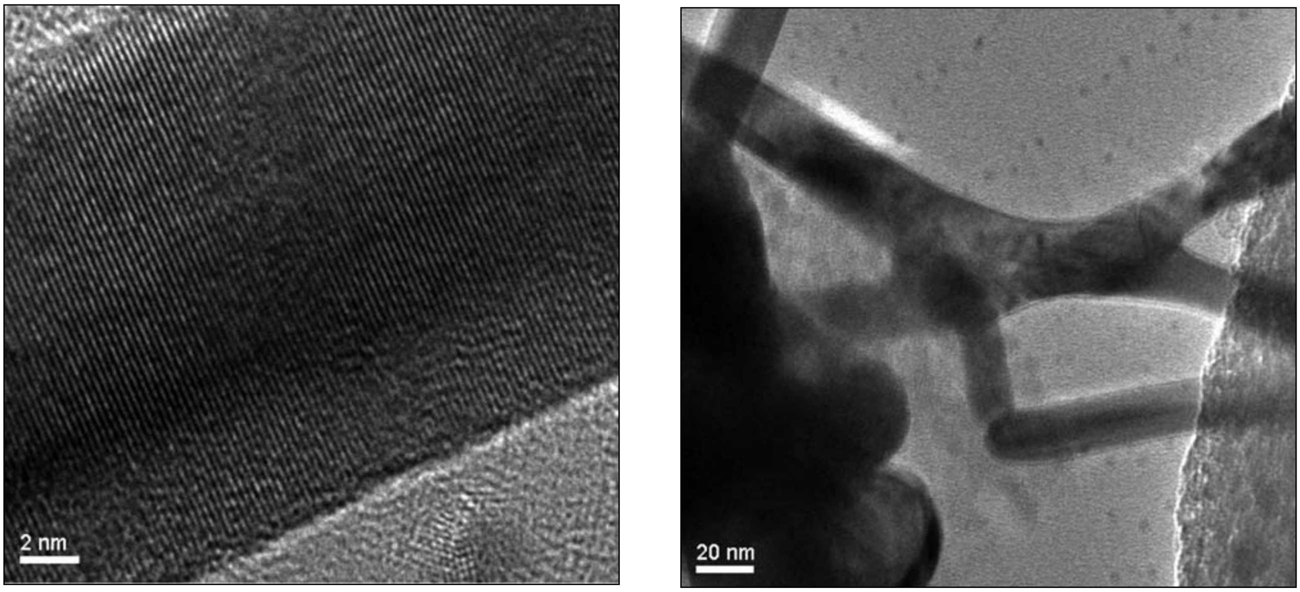


Figure 8: HRTEM images of particles gained by spray drying of an Ag/SiO_2 sol, product deposited in collecting container.

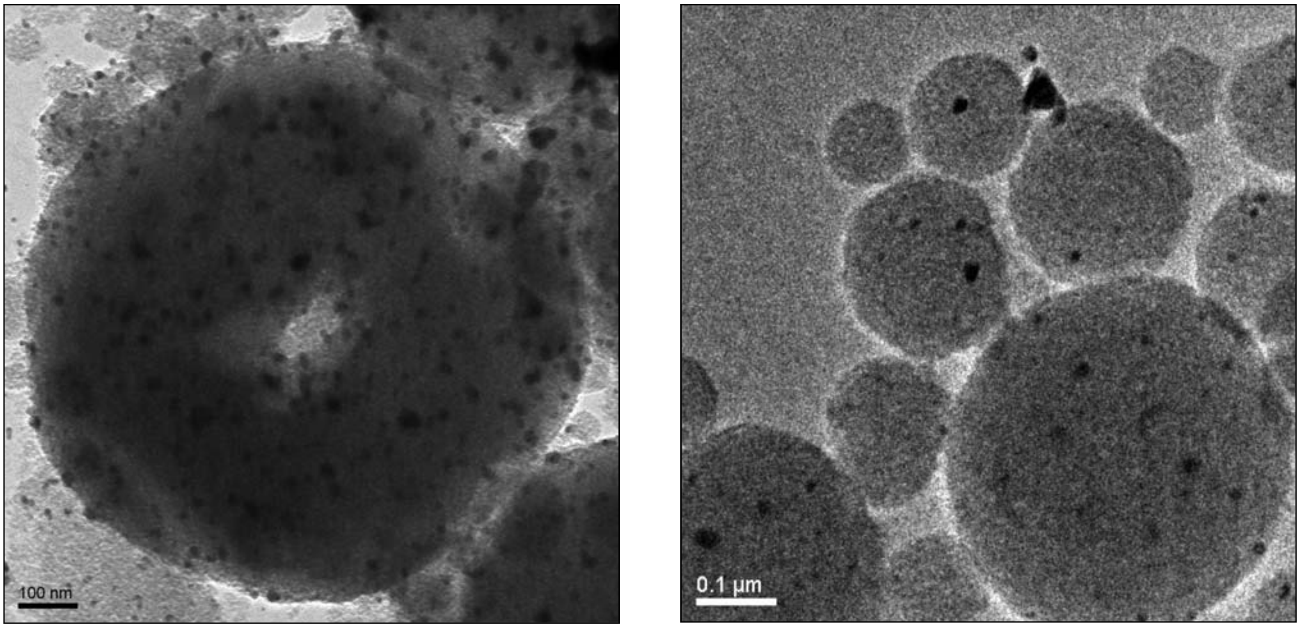


Figure 9: TEM images of particles gained by spray drying of an Ag/SiO_2 sol, product deposited in drying chamber.

Table 1: Results of bactericidal tests with *Escherichia coli* and *Bacillus subtilis* for suspensions containing increasing amount of spray dried Ag/SiO_2 powder. The tests are performed with bacteria suspensions of increasing dilution in grades from 10^2 up to 10^5.

Amount of spray dried Ag/SiO_2 powder	**Results of bacteria count after 24h *Escherichia coli***				**Results of bacteria count after 24h *Bacillus subtilis***			
	10^5	**10^4**	**10^3**	**10^2**	**10^5**	**10^4**	**10^3**	**10^2**
Control with 0 wt-%	14	>200	>500	>500	42	98	>500	>500
0.33 wt-%	0	0	0	0	0	4	15	40
0.83 wt-%	0	0	0	0	0	4	11	44
1.67 wt-%	0	0	0	0	0	0	0	0

the SiO_2 nanoparticles grow and aggregate to microscale particles whereas the silver particles mainly maintain their nanoscaled size. The resulting silver containing powders are effective antimicrobial agents and are particularly suitable to be used as additives for coating agents or in master batch processes. Under special preparation conditions also the formation of silver nanorods can be observed. The analysis of process parameters affecting the development of these rods will be in the focus of following investigations.

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Povzetek

Nanodelce srebra, vgrajene v delce silicijevega oksida mikrometrske velikosti, smo pripravili z razpršilnim sušenjem sola, ki je vseboval nanodelce silicijevega oksida in srebra. Kot topilo smo uporabili vodo in etanol v razmerju 4 : 1. Med razpršilnim sušenjem so nanodelci silicijevega oksida agregirali v delce mikronske velikosti, medtem ko so nanodelci srebra ohranili prvotno velikost. Pod posebnimi pogoji je srebro kristaliziralo v obliki paličic.
Antibakterijsko aktivnost prahov Ag/SiO_2 smo preverili z bakterijami Escherichia coli in Bacillus subtilis. Materiali imajo zaradi opisanih lastnosti velike možnosti uporabe kot antimikrobni dodatki v različnih kemijskih procesih.