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Synthesis, characterization and antibacterial investigation of silver–copper nanoalloys

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Ag–Cu nanoalloys were synthesized by chemical co-reduction of their metal salts in aqueous solution with hydrazine hydrate, in the presence of complexing agent and stabilizer, preventing the oxidation of copper, as revealed by XPS. Their antibacterial behavior was tested against *Escherichia coli* strains, attesting far better ability of the Ag–Cu compared to Ag-only nanoparticles.

Single metal nanoparticles have recently been in focus for their particle characteristics, size dependent properties, easy synthesis and chemical modifications. They also exhibit increased photochemical activity, modified chemical, biomedical and biological properties due to their high surface to volume ratios and unique properties that are different from their bulk.[1–5] Preparation and characterization of the monometallic nanoparticles using different synthesis methods have been investigated for many years, whereas metal nanoalloy syntheses have not been as widespread due to handling problems and difficulties in preparation.

Copper-only and silver-only nanoparticles (NPs) can be synthesized by the chemical reduction of copper and silver ions, which are produced by dissolving the salts of copper and silver in aqueous media, with the help of a reducing agent according to different procedures.[1,6] Silver and copper alloy nanoparticles can also be prepared by co-reduction of their salts in aqueous solutions. The aqueous chemical reduction method is preferred over other nanoparticle preparation techniques because of its ease of application, variation in particle characteristics and different experimental parameters such as concentration, temperature and pH.[7] For the synthesis of the silver and copper nanoalloys (NAs) the main problems encountered are their high air sensitivity, low stability, and reactivity of the copper nanoparticles that cause the formation of copper oxide in the end product.[8,9] To suppress the oxidation problem of copper in the nanoalloy synthesis, purging of the reaction media with inert gases, use of non-aqueous media, very strong reducing agents, complexing agents, surfactants and different kinds of stabilizers are the commonly employed procedures to prevent oxide formation both during preparation and storage.[7,8,10–13]

The nanoparticles can be stabilized by capping with a suitable stabilizer or complexing agent, by virtue of the steric effects of different kinds of polymers, and by keeping nucleation sites of stabilizers and nanoparticles with the help of electrostatic interactions as far as possible.[12,14–16] Thiol group (–SH) containing stabilizers dominate in terms of protection, because metal nanoparticles have higher tendency towards carbon bonded sulfhydryl groups.[12,16–19] For the protection of AgCu NAs against oxidation, cysteine which also contains the –SH group at the terminal position is preferred over other weaker binding complexing agents or stabilizers, such as oleylamines, electrostatically protecting agents or ligands containing deprotonated negatively charged carboxylic acid groups in order to suppress the oxidation of Cu^0 to Cu^{2+}.

For many years materials containing particles of metals such as zinc, silver and copper have been used for antibacterial applications because they have the ability to penetrate into bacteria causing cell death or interact with microbial cell membranes that cause deactivation of their activity, and in general antimicrobial activity of metals decreases with an increase in their size.[14,16,18,20–24] Nanoparticles have the advantage of a high surface to volume ratio and their small size allows them to exhibit higher bactericidal activity, over their larger colloidal forms and/or their bulk metals. In addition, silver and copper metal nanoparticles have the advantage of closely interacting with cell membranes, which is not dependent on the release of metal ions into the solution.[20,25] Furthermore it was reported that combination of silver-only and copper-only NPs increased the antibacterial effect against different combinations of bacteria populations with an initial bacteria concentration in the range of 10^2 to 10^4 CFU ml^{-1} where it was also claimed that the bactericidal effects of nanoparticles depended on both the initial bacteria concentration and the concentration of nanoparticles.[21] Antibacterial activity of the NAs can be tested against different kinds of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* species by various common techniques such as counting the number of bacteria after a certain incubation time, antibacterial drop test or disk-diffusion (Kirby–Bauer) test as a function of the NP inserted film thickness as well as the amount of NPs/NAs and the concentration of metal nanoparticles against Gram positive and Gram negative bacteria strains.[26–29]

Silver nitrate, copper acetate salts and cysteine were obtained from Fluka; formaldehyde was from Carlo Erba; sodium borohydride, sodium hydroxide and hydrazine hydrate were obtained from Merck and BDH. Sodium citrate and sodium chloride were obtained from

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Aldrich, and the water used in all experiments was obtained from a three-stage Millipore Milli-Q Synergy 185 purification system. Since metal nanoparticles exhibit a strong surface plasmon resonance band in the visible spectrum UV-vis spectroscopy is the most commonly used optical technique.[1] The metal particles of silver and gold show distinct and well-defined plasmon absorption in the visible region whereas a distinct band is not observable for copper.[4] X-Ray Photoelectron Spectroscopy (XPS) which is mostly used for surface analysis is also one of the most commonly utilized techniques for characterization of nanoparticles.[30] XPS can also be used to determine the chemical states of atoms.[31,32] Therefore, UV-visible spectroscopy (Thermo Scientific, Evolution 160), XPS (Thermo Scientific, K-alpha) and X-ray Diffraction (Rigaku, Miniflex) were employed for characterization of the nanoalloys prepared.

Antibacterial investigations on the AgCu nanoalloy and Ag-only nanoparticles were performed against *Escherichia coli* DH5alpha strains. *E. coli* DH5α cells were grown to optical densities of 0.2, 0.1 and 0.05 (at 600 nm) at the beginning of the experiment and the bacterial growth was monitored by the absorbance increase before, during, and after incubation periods. *E. coli* DH5α colonies were grown in 10 ml liquid LB medium at 37 °C with constant agitation at 225 rpm to minimize any possible settlement and aggregation during incubation. For each set of experiments the same amount of bacteria were incubated in different test tubes with the same amount of NP or NA solutions (1 ml of different concentrations) in order to prevent any dilution or contamination of nutrients in media. Also the controls were prepared under same conditions, *i.e.* bacteria, LB medium and water, but without supplements of nanoalloy or nanoparticles. The LB medium was used as a carrier to dilute the nanoalloy and nanoparticle solutions to different concentrations. The change in the optical density at the wavelength of 600 nm was determined by using a Beckman DU 640 spectrophotometer. Quantification of the antimicrobial effects of Ag NPs and AgCu NAs was performed by determining the minimum growth inhibitory concentration (MIC) defined as the lowest concentration of NP or NA solution that inhibits the growth of bacteria. Briefly, the same amount of bacteria were grown overnight in serial dilutions of NPs and NAs (from 30 μg ml^{-1} to 58 ng ml^{-1}) and the minimum concentration at which no bacterial growth was observed (as determined by OD measurements) was selected to be the MIC. Similarly, the minimum bactericidal concentration (MBC) was determined by spreading 100 μl of the mixture incubated overnight but with no observable growth of LB+bacteria from MIC analysis.

To determine the colony forming abilities of bacteria on NP/NA covered agar plates 100 μl of *E. coli* DH5α of 7×10^8 CFU ml^{-1} with different dilutions in the range of 10^0 to 10^7 was introduced. These LB agar plates supplemented with different concentrations of silver-only and silver–copper nanoparticles, starting from 30 μg ml^{-1} of Ag NPs and AgCu NAs solutions and after 10^1 to 10^5 dilutions, were incubated for 18 h at 37 °C and the growth of colonies was observed directly. For the control group, LB-agar plates were prepared exactly in the same manner as described, except for addition of nanoparticles.

Silver nanoparticles were synthesized by the reduction of $AgNO_3$ salt with the help of a strong reducing agent, sodium borohydride, under ambient conditions without any oxidation or precipitation problems, which can easily be detected by the change of color of the solution to yellow after reduction of the silver ions.[33] Several different synthesis routes were employed for obtaining stable AgCu NAs, most of which failed due to unpreventable oxidation of Cu in the end-product, till we had established a route which yielded stable nanoalloy particles. In this route, one-step synthesis of AgCu NAs was performed using 0.01 M silver nitrate and copper acetate solution by mixing it with a 4 ml mixture of 0.01 M cysteine and sodium hydroxide solutions. This metal mixture solution was slowly added dropwise to another solution mixture, containing 0.01 M sodium citrate complexing agent and 5 ml of 4×10^{-4} M hydrazine hydrate (HH) reducing agent, and the reaction medium was stirred vigorously. The reaction was allowed to continue for 30 min as the color of the solution slowly changed into dark wine-brown indicating the formation of AgCu NAs. The thin-films of silver-only nanoparticles and the silver copper nanoalloy were obtained by the evaporation of the nanoparticle and nanoalloy solution drops on microscope slides for XPS characterization, as well as for anti-bacterial testing. Although HH is a highly toxic reducing agent it is very unlikely that it can impart additional antibacterial effect to the nanoalloy solutions, since HH decomposes very quickly into nontoxic products during the reduction process. In addition, the concentration of HH used in our study is very low as compared to the previous HH concentrations which were used for other antibacterial applications.[34–38]

Ag NPs do not have oxidation problems during synthesis, but Cu NPs' spontaneous oxidation, uncontrollable reaction kinetics, and stability are problematic due to the low (0.34 V) reduction potential of Cu^0/Cu^{2+}, as compared to that of silver (0.80 V).[39] Therefore a strong reducing agent hydrazine hydrate, which leads to immediate reduction of copper ions before being oxidized, was employed. In order to bring the Ag and Cu reduction potentials closer, a suitable complexing agent sodium citrate was added to the reaction solutions during synthesis of nanoalloys.[40] As a protecting agent, cysteine was introduced rather than the commonly used polyvinylpyrrolidone (PVP) to prevent the oxidation of copper.[41,42] Furthermore purging with inert nitrogen gas during the nanoalloy synthesis was also employed.

In Fig. 1, we present the UV-visible spectra of the nanoparticles in solution. Ag-only NPs have a strong surface plasmon resonance peak at 390 nm, while that of the AgCu NAs is red-shifted to 420 nm and broadened.[43] It is also well established that Ag and Cu alloys phase-separate easily and obtaining a good X-ray diffraction (XRD) pattern is difficult.[27,28] Nevertheless, as shown in Fig. 2, for the AgCu nanoalloys, we always obtain a weak diffraction peak at a slightly

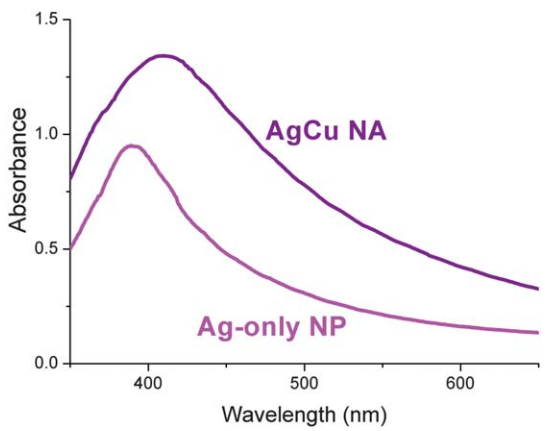

Fig. 1 UV-vis absorption spectra of aqueous solutions of Ag-only and AgCu alloy nanoparticles.

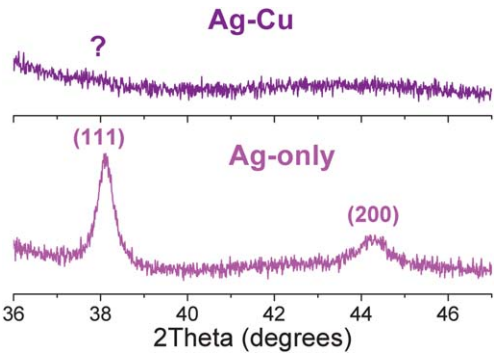

Fig. 2 XRD patterns of Ag-only and AgCu alloy nanoparticles.

lower value than that of the Ag(111) peak at $2\theta = 38°$, which might be considered as some indication of alloy formation. This point was also discussed by Yun *et al.*, who advocated that due to the radius mismatch between Cu and Ag as well as the random distribution of the two atoms within the alloy-nanoparticles, the XRD cross-section might be severely decreased.[3] We also used XPS to characterize the particles after drop-casting on glass slides and drying, for determining the stoichiometry as well as the chemical states of the metals, especially that of Cu. One representative survey spectrum for the AgCu alloy particles is given in Fig. 3, together with Cu2p and Ag3d regions recorded separately to reveal the surface composition to be *ca.* 2 : 1 with respect to Cu : Ag, and more importantly that both atoms are in their metallic state (Cu^0 and Ag^0). We note in passing that all the other synthesis routes we had tried yielded little or no Cu in their XPS spectra, and when present it was mostly found in its oxidized state (Cu^+ or Cu^{2+}) with a characteristic shake-up satellite structure in the Cu2p region.[11,27,28]

In order to establish the difference in the antibacterial properties of Ag NP and AgCu NA, the MIC and MBC were determined as given in Table 1. AgCu NAs were inhibitory at concentrations even as low as 0.5 μg ml^{-1}, whereas Ag NPs were not even inhibitory at a concentration of 150 μg ml^{-1}, and the AgCu NAs were able to kill 99% of the initial *E. Coli* present.

Further, antibacterial tests were performed against *E. coli* DH5α strains on LB agar plates containing different concentrations of

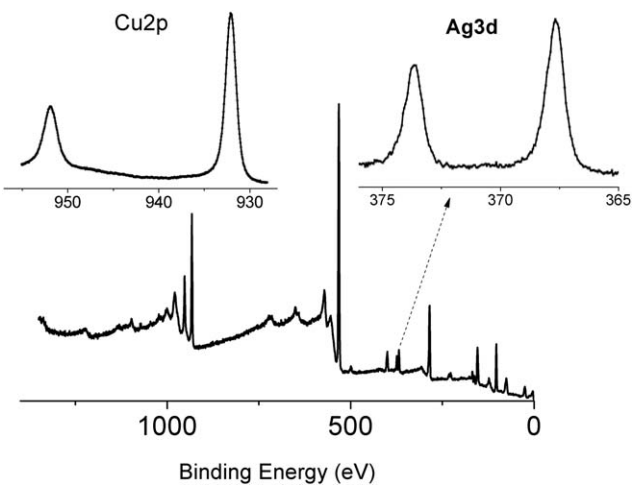

Fig. 3 XPS spectrum of AgCu alloy nanoparticles.

Table 1 MIC (μg ml^{-1}) and MBC (μg ml^{-1}) of silver nanoparticles and silver–copper nanoalloys for *E. coli* DH5α

MIC/μg ml^{-1}		MBC/μg ml^{-1}	
Ag NP	AgCu NA	Ag NP	AgCu NA
>150 μg ml^{-1}	0.5 μg ml^{-1}	N/A	0.5 μg ml^{-1}

silver-only and silver copper solutions with different dilutions of *E. coli* DH5α. After 18 hours of incubation time, colony growth was directly observable on silver-only nanoparticle inserted agar plates, where silver-copper nanoalloy which was spread on agar completely inhibited the bacterial growth at a concentration of 3–30 μg ml^{-1}, as shown in Fig. 4(a) and (b) and in Table 2. All controllable parameters such as concentration, incubation time, incubation and application conditions, and bacteria suspensions were exactly the same for the two cases. Accordingly, silver–copper nanoalloy is totally effective for bacteria crucial applications and prevents microbial colony growth. In contrast, even higher amounts of silver-only nanoparticles cannot prevent bacterial growth. In separate sets of experiments, the bacterial

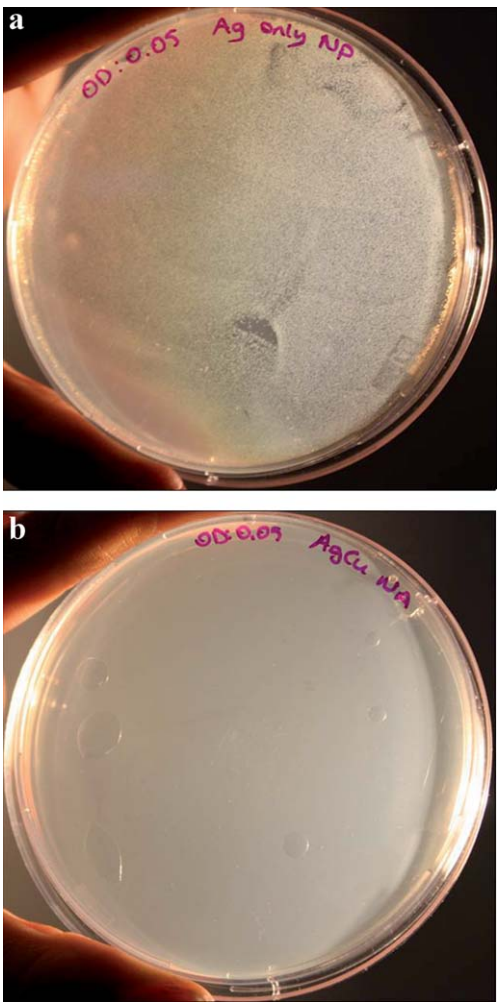

Fig. 4 Representative images of agar plates containing an equal amount of (a) Ag-only nanoparticles with *E. coli* DH5α colony formation, and (b) AgCu nanoalloys without colonies.

Table 2 Effects of different concentrations of NP and NAs on the dilution series of *E. coli* DH5α

	Number of colonies for given dilution factors (DF)							
NP/NA	1	10^1	10^2	10^3	10^4	10^5	10^6	10^7
AgCu (30 μg ml^{-1})	0	0	0	0	0	0	0	0
AgCu (3 μg ml^{-1})	0	0	0	0	0	0	0	0
AgCu (0.3 μg ml^{-1})	UNC[a]	UNC	UNC	316	542	42	42	0
AgCu (30 ng ml^{-1})	UNC	UNC	UNC	3040	388	372	192	14
Ag (150 μg ml^{-1})	UNC	UNC	UNC	3376	960	482	304	32
Ag (15 μg ml^{-1})	UNC	UNC	UNC	3208	1496	860	236	32
No NP/NA	UNC	UNC	UNC	UNC	1313	703	445	18

[a] UNC = uncountable (large number of colonies).

growth was also monitored in the liquid LB medium supplemented with *E. coli* cells having initial optical densities of 0.05 (1.7×10^8 CFU ml^{-1}), 0.1 (3.5×10^8 CFU ml^{-1}), and 0.2 (7.0×10^8 CFU ml^{-1}) OD for both Ag-only NPs and AgCu NAs, and the dynamics of the bacterial growth was monitored by the change in the optical density at a constant wavelength of 600 nm as depicted in Fig. 5. Initial concentrations as high as 0.2 OD are rarely found in real-systems for *E. coli* DH5α, but were preferred for our research in order to emphasize the superior antibacterial property of AgCu NAs even at much higher bacterial concentrations, with the assumption that the concentration which is enough to kill a high number of bacteria should also be able to kill the lower numbers, as found in nature. Ag-nanoparticles show a decrease in the growth of *E. coli* DH5α for all the three optical densities with respect to the negative control, but the AgCu nanoalloys have far better antibacterial activity, since the optical density of the bacteria did not change with in time. In contrast, for the group with OD 0.2 (Fig. 5b), the addition of AgCu nanoalloys resulted in bacterial death, as interpreted from the decrease in the OD values. Even after 24 hours, the bacteria could not grow in AgCu nanoalloys including the culture, while in the Ag-only nanoparticle supplemented culture cells grew to the degree of the stationary phase (Fig. 5a and b). To eliminate the possibility that the addition of NP/NA solutions results in dilution of media and alters bacterial growth profiles, the same amount of water was added to the control tube, which resulted in exactly the same growth profile of bacteria after 18 hours (data not shown).

When we compare the effectiveness of the 75 day old NA solution with that of the silver NP solution, the antibacterial efficiency of the NA solution was similar to that of silver-only NPs which is not effective for inhibition of the bacterial growth. The decrease in the effectiveness of the nanoalloy solution with time can be explained by formation of copper oxide during the storage as can be seen from the Cu2p region in the XPS spectrum in Fig. 6.

In conclusion, successful preparation of the silver and copper nanoalloy was demonstrated by co-reduction of silver and copper salts in the presence of a strong reducing agent, hydrazine hydrate, a complexing agent and a stabilizer without any copper oxide formation under ambient conditions. The prepared AgCu nanoalloy particles were characterized by UV-Vis, XPS spectroscopic and XRD diffraction techniques. The antibacterial behaviors of the AgCu NAs and Ag NPs were inspected against *E. coli* DH5α strains and it was observed that colonies were not developed in the presence of the AgCu nanoalloys indicating the excellent antibacterial activity of AgCu NAs as compared to the equal amount of Ag NPs. However, these particles undergo oxidation during storage; hence a decrease in their antibacterial efficiency is observed. Therefore, techniques for storing under oxygen-free environment and/or more efficient protective coatings are highly desirable for longer storage and use.

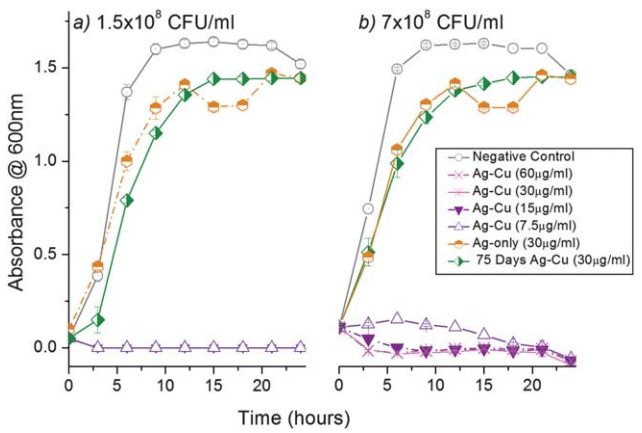

Fig. 5 Representative batch growth profiles in the presence of Ag-only nanoparticles and AgCu nanoalloys for initial concentrations of *E. Coli* DH5α: (a) 0.05 OD and (b) 0.2 OD.

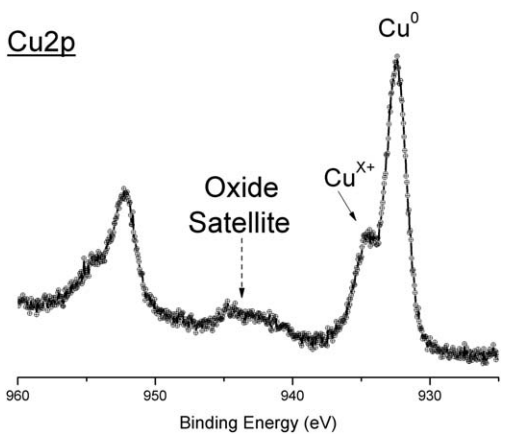

Fig. 6 Cu2p region of the XPS spectrum of AgCu nanoalloys after storing for 75 days, showing an additional CuO_x satellite structure.

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References

1 D. L. Feldheim and C. A. Foss, *Metal Nanoparticles: Synthesis, Characterization, and Applications*, CRC, 2002.
2 J. A. Lichter, K. J. Van Vliet and M. F. Rubner, *Macromolecules*, 2009, **42**, 8573.
3 J. Yun, K. Cho, H. C. Kang, B. K. Ju and S. Kim, *Jpn. J. Appl. Phys.*, 2008, **47**, 5070.
4 P. V. Kamat, *J. Phys. Chem. B*, 2002, **106**, 7729.
5 O. Siiman, E. Matijevic and I. Sondi, *J. Colloid Interface Sci.*, 2004, **275**, 503.
6 M. Valodkar, S. Modi, A. Pal and S. Thakore, *Mater. Res. Bull.*, 2011, **46**, 384.
7 M. Grouchko, A. Kamyshny, K. Ben-Ami and S. Magdassi, *J. Nanopart. Res.*, 2009, **11**, 713.
8 P. K. Khanna, S. Gaikwad, P. V. Adhyapak, N. Singh and R. Marimuthu, *Mater. Lett.*, 2007, **61**, 4711.
9 C. Sangregorio, M. Galeotti, U. Bardi and P. Baglioni, *Langmuir*, 1996, **12**, 5800.
10 H. Wang, Y. Huang, Z. Tan and X. Hu, *Anal. Chim. Acta*, 2004, **526**, 13.
11 Y. H. Kim, D. K. Lee, H. G. Cha, C. W. Kim, Y. C. Kang and Y. S. Kang, *J. Phys. Chem. B*, 2006, **110**, 24923.
12 Y. Wei, S. Chen, B. Kowalczyk, S. Huda, T. P. Gray and B. A. Grzybowski, *J. Phys. Chem. C*, 2010, **114**, 15612.
13 N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M. D'Alessio, P. G. Zambonin and E. Traversa, *Chem. Mater.*, 2005, **17**, 5255.
14 E. Falletta, M. Bonini, E. Fratini, A. Lo Nostro, G. Pesavento, A. Becheri, P. Lo Nostro, P. Canton and P. Baglioni, *J. Phys. Chem. C*, 2008, **112**, 11758.
15 M. S. Bootharaju and T. Pradeep, *J. Phys. Chem. C*, 2010, **114**, 8328.
16 V. Sambhy, M. M. MacBride, B. R. Peterson and A. Sen, *J. Am. Chem. Soc.*, 2006, **128**, 9798.
17 S. Aryal, R. Bkc, N. Dharmaraj, N. Bhattarai, C. H. Kim and H. Y. Kim, *Spectrochim. Acta, Part A*, 2006, **63**, 160.
18 M. V. Varghese, R. S. Dhumal, S. S. Patil, A. R. Paradkar and P. K. Khanna, *Synth. React. Inorg., Met.-Org., Nano-Met. Chem.*, 2009, **39**, 554.
19 J. L. Elechiguerra, J. L. Burt, J. R. Morones, A. Camacho-Bragado, X. Gao, H. H. Lara and M. J. Yacaman, *J. Nanobiotechnol.*, 2005, **3**, 1.
20 J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez and M. J. Yacaman, *Nanotechnology*, 2005, **16**, 2346.
21 J. P. Ruparelia, A. K. Chatterjee, S. P. Duttagupta and S. Mukherji, *Acta Biomater.*, 2008, **4**, 707.
22 S. Pal, Y. K. Tak and J. M. Song, *Appl. Environ. Microbiol.*, 2007, **73**, 1712.
23 R. D. Deshmukh and R. J. Composto, *Chem. Mater.*, 2007, **19**, 745.
24 S. K. Smoukov, K. J. M. Bishop, B. Kowalczyk, A. M. Kalsin and B. A. Grzybowski, *J. Am. Chem. Soc.*, 2007, **129**, 15623.
25 K. H. Cho, J. E. Park, T. Osaka and S. G. Park, *Electrochim. Acta*, 2005, **51**, 956.
26 D. Lee, R. E. Cohen and M. F. Rubner, *Langmuir*, 2005, **21**, 9651.
27 Z. Li, D. Lee, X. Sheng, R. E. Cohen and M. F. Rubner, *Langmuir*, 2006, **22**, 9820.
28 S. T. Dubas, P. Kumlangdudsana and P. Potiyaraj, *Colloids Surf., A*, 2006, **289**, 105.
29 I. Sondi and B. Salopek-Sondi, *J. Colloid Interface Sci.*, 2004, **275**, 177.
30 I. Tunc, S. Suzer, M. A. Correa-Duarte and L. M. Liz-Marzán, *J. Phys. Chem. B*, 2005, **109**, 7597.
31 E. Ozkaraoglu, I. Tunc and S. Suzer, *Polymer*, 2009, **50**, 462.
32 F. Karadas, G. Ertas, E. Ozkaraoglu and S. Suzer, *Langmuir*, 2005, **21**, 437.
33 W. C. Bell and M. L. Myrick, *J. Colloid Interface Sci.*, 2001, **242**, 300.
34 E. Dujardin, C. Peet, G. Stubbs, J. N. Culver and S. Mann, *Nano Lett.*, 2003, **3**, 413.
35 M. G. Guzmán, J. Dille and S. Godet, *Int. J. Chem. Biomol. Eng.*, 2009, **2**, 104.
36 M. S. A. S. Shah, M. Nag, T. Kalagara, S. Singh and S. V. Manorama, *Chem. Mater.*, 2008, **20**, 2455.
37 W. Zhang, X. Qiao and J. Chen, *Mater. Chem. Phys.*, 2008, **109**, 411.
38 W. P. Xu, L. C. Zhang, J. P. Li, Y. Lu, H. H. Li, Y. N. Ma, W. D. Wang and S. H. Yu, *J. Mater. Chem.*, 2011, **21**, 4593.
39 C. Han, Q. Liu and D. G. Ivey, *Mater. Sci. Eng., B*, 2009, **164**, 172.
40 D. Radziuk, D. G. Shchukin, A. Skirtach, H. Möhwald and G. Sukhorukov, *Langmuir*, 2007, **23**, 4612.
41 S. Ayyappan, R. S. Gopalan, G. N. Subbanna and C. N. R. Rao, *J. Mater. Res.*, 1997, **12**, 3981.
42 H. N. Vasan and C. N. R. Rao, *J. Mater. Chem.*, 1995, **5**, 1755.
43 P. Mulvaney, *Langmuir*, 1996, **12**, 788.

