Colloids and Surfaces B: Biointerfaces 101 (2013) 430–433

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles

Dhaneswar Das[a], Bikash Chandra Nath[b], Pinkee Phukon[c], Swapan Kumar Dolui[a,*]

[a] Department of Chemical Sciences, India
[b] Department of Physics, India
[c] Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Assam, India

ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form 2 July 2012
Accepted 6 July 2012
Available online 25 July 2012

Keywords:
Antioxidant
Antibacterial
Nanoparticles
CuO

ABSTRACT

CuO nanoparticles were synthesized by thermal decomposition methods and characterized by UV–visible spectroscopy, XRD and TEM analysis. The resultant particles are nearly spherical and particle size is in the range of 15–30 nm. The antioxidant behavior of synthesized CuO nanoparticles was evaluated by scavenging free radicals of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). The free radical scavenging activity of CuO nanoparticles was monitored by UV–visible spectrophotometry. The antibacterial activity of CuO nanoparticles was tested against different bacterial strains. CuO nanoparticles showed efficient antioxidant activity and bactericidal effect against *Eschericia coli* and *Pseudomonas aeruginosa*.

1. Introduction

The assessment of antioxidant activity of nano materials has become one of the important basic studies in nano science and technology. Oxidation reaction in food takes place when chemical compounds present in the food are exposed to oxygen in the air, which leads to the loss of nutritional value; creating rancidity and causing discolor in food [1,2]. Consumption of oxidized foods causes serious diseases like hepatomegaly or necrosis of epithelial tissues. Oxidative reactions produces lipid peroxides and low molecular weight compounds which are responsible for these diseases. Antioxidant plays a crucial rule in terminating the oxidative rancidity in food by scavenging the free radical which is generated during oxidation process [3,4]. Many kinds of natural and synthetic antioxidants have been investigated to inhibit these oxidation reactions to date.

Polymers are also susceptible to attack by atmospheric oxygen, especially at elevated temperatures encountered during processing of polymers. Oxidation of polymers results in break down of the polymer chain and cracks start to grow in the affected region. In case of aging of rubber oxidation inside it results the deterioration of its physical and mechanical properties. Photo-oxidation of polymer creates aldehydes, ketones and carboxylic acids at the end of the polymer chain. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and n-propyl gallate (NPG) are used as antioxidants but due to their carcinogenicity, they have limited uses [5,6].

Vitamin A, Vitamin C, Vitamin E and carotenoids are natural antioxidants. These types of antioxidants can accept an unpaired electron leading to the generation of a stable intermediate. This intermediate remains stable for a long time to interact in a controlled fashion, which prevent auto oxidation and the excess energy of surplus electron is dissipated without damage to the tissues. These dietary antioxidants can be able to recycle, which is an indication of their physiological essentiality to function as antioxidants.

CuO nanoparticles are stable, robust and have a longer shelf life compared to organic antimicrobial agents. Copper and its complexes have been increasingly used as effective materials for purification of water, textiles etc. In spite of the negligible sensitivity of human tissues to copper and its complexes, microorganisms show efficient sensitivities even in their lower concentrations. But, their actual bactericidal mechanism is yet to be known. Metallic copper nanoparticles have a tendency to fast oxidation in the presence of air leading to both chemical as well as physical instability, particularly if Cu^{2+} is formed [7,8].

Saikia et al. [9,10] reported the antioxidant activity of Fe_3O_4 and NiO nanoparticles and found 80% and 90% antioxidant activity respectively. Dolui et al. [11] synthesized copper-polystyrene nanocomposite particles using water in supercritical carbon

* Corresponding author at: Department of Chemical Sciences, Tezpur University, Tezpur 784028, Assam, India. Tel.: +91 9957198489.
E-mail address: dhan013@tezu.ernet.in (S.K. Dolui).

0927-7765/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2012.07.002

dioxide medium to study the antimicrobial activity for the synthesized particles. Cioffi et al. [12] studied the antifungal and bacteriostatic properties of Cu nanoparticles/polymer composites. Li et al. [13] successfully studied the two-level antibacterial coating with both release-killing and contact-killing capabilities. Copper oxide [8,14,15] nanoparticle have got the importance due to their applications as antimicrobials, gas sensors, catalysis, batteries, high temperature superconductors, solar energy conversion tools, etc. A recent study suggests that [16,17] oxidative stress may be the cause of the cytotoxic effect of CuO nanoparticles in human airway epithelial cells. Berntsen et al. [18] reported that in human airway smooth muscle cells the impaired cell viability and decreased in cell contractility occurred due to exposure of CuO nanoparticles.

In this paper, an attempt has been made to explore the antioxidant and antibacterial activity of CuO nanoparticles.

2. Materials and methods

2.1. Materials

Na_2CO_3, $CuSO_4{\cdot}5H_2O$ and methanol were purchased from Merk, India. 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) was purchased from Aldrich. Distilled water was used for washing purposes. All the chemicals and reagents were used as received without further purification.

2.2. Preparation of CuO nanoparticles

CuO nano particles [19] were prepared via direct thermal decomposition method with slight modification. In a typical synthesis, precursor was synthesized by adding 0.5 M Na_2CO_3 solution to 100 ml of 0.5 M $CuSO_4$ solution sonicating at 60 °C. The green precipitate, $Cu_4(SO_4)(OH)_6$ was separated by filtration and washed several times with warm deionized water to remove possible remaining ions in the final product. Resultant $Cu_4(SO_4)(OH)_6$ was transferred to a silica crucible and placed in an oven at 70 °C for 6–7 h. Finally, it was kept in an pre-heated muffle furnance at 600 °C for decomposition. After 2 h, the silica crusible was taken out from the furnance and allowed to cool to room temperature following which the collected mass was ground. The resultant brownish black powder was collected. The reaction pathway of thermal decomposition of $Cu_4(SO_4)(OH)_6$ to CuO nanoparticles is represented by the following reactions:

$$Cu_2(SO_4)(OH)_6 \cdot nH_2O \xrightarrow[70\,^\circ C]{\Delta} CuSO_4 \cdot 3Cu(OH)_2 + nH_2O$$

$$Cu_2SO_4 \cdot 2Cu(OH)_2 \xrightarrow[600\,^\circ C]{\Delta} 3CuO + 2H_2O + SO_2 + (1/2)O_2$$

2.3. Measurements of antioxidant activity

Antioxidant activity of CuO nanoparticles was measured by DPPH method, as reported by Serpen et al. [20]. In a typical process, 3.20 mL (100 μM) of DPPH was taken in a small glass bottle, and 120 mg of CuO nanoparticle sample was dispersed in to it. DPPH was used as the radical source and CuO was used as radical scavenger. DPPH radical has a deep violet color in solution, and gradually it becomes colorless or pale yellow in the presence of CuO nanoparticles. This property allows visual monitoring of the reaction, and the concentration of radicals is monitored from the change in percentage of absorption at 517 nm. The rate of the reaction was enhanced by sonicating the reaction mixture at room temperature. The supernatant containing DPPH was collected for different time intervals. The time dependent DPPH scavenging was studied at an interval of 15, 30, 45 and 60 min. DPPH scavenging activity is calculated using the following equation:

$$\text{DPPH scavenging activity } (\%) = \left[1 - \frac{As}{Ac}\right] \times 100,$$

where *Ac* and *As* are the intensity of peak at 517 nm for control (DPPH) and supernatant DPPH solvent respectively.

2.4. Antibacterial activity test

In order to investigate the antibacterial activity, CuO nanoparticles were tested against the strains: *P. aeruginosa* BS3, *Bacillus circulens* BP2, *Eschericia coli* and *Staphylococcus aureus*. Antibacterial test was done by measuring growth curve of bacterial culture incubated in the LB broth medium in presence of CuO nanoparticles with different concentrations (1 mg, 2.5 mg, and 4 mg/mL). All glassware and samples were sterilized at 120 °C for 10 min. In a typical experiment for construction of the growth curves, all the bacterial cultures with an approximate concentration of 10^6–10^7 colony forming units per milliliter (CFU/mL) were inoculated into LB broth medium containing different concentrations of CuO nanoparticles each. In order to ensure optimum contact between CuO nanoparticles and bacterial cells all experiments were performed in an incubator shaker at 37 °C and 180 rpm. Following inoculation, the optical density (OD) of the cultures was serially monitored at 600 nm with a spectrophotometer at an interval of 3 h. Cultures of nanoparticle-free medium under the same growth conditions were used as controls. In order to circumvent potential optical hindrance during optical measurements of the growing cultures caused by the light-scattering properties of the nanoparticles, the dispersion of nanoparticles without micro-organisms was used as blank control [21]. All assays were repeated three times in duplicate, to ensure uniform results. Standard deviation was calculated in terms of standard error using equation:

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

where $(x_1, x_2, x_3, \ldots x_i)$ are the observed values of the sample items and $\bar{x}$ is the mean value of these observations, $(N–1)$ is known as Bessel's correction.

2.5. Characterization

The UV–visible absorption spectra were recorded by using Shimadzu UV-2550 UV–visible spectrophotometer at room temperature. The size and morphologies of CuO nanoparticles were observed with a JEOL JEM 2100 transmission electron microscope at an acceleration voltage of 200 kV. The crystalline structure of CuO nanoparticles were studied by using X-ray diffractometry (Miniflex, Rigaku Japan) with CuKα radiation (λ = 0.15418 nm) at 30 kV and 15 mA with scanning rate of 0.005 S^{-1} in a 2θ range of 20–70°.

3. Results and discussion

3.1. Characterization of nanoparticles

UV–visible spectra of the synthesized nanoparticles are shown in the Fig. 1. In the spectra, peak at 380 nm is due to surface plasmon absorption of metal oxide. The surface plasmon absorption in the metal oxide nanoparticles is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. This type of resonance is seen when the

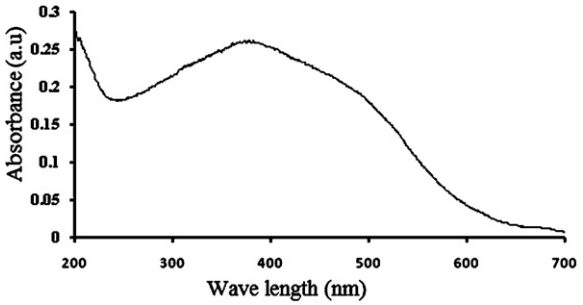

Fig. 1. UV spectra of CuO nanoparticles.

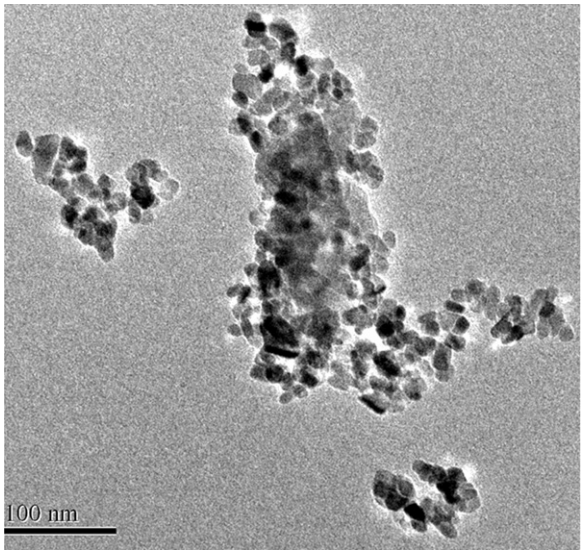

Fig. 2. TEM image of synthesized CuO nanoparticles.

wavelength of the incident light far exceeds the particle diameter. Surface plasmon absorption band with a maximum at 380 nm indicates the formation of CuO nanoparticles.

The morphology and microstructure of prepared products were further examined with TEM. Fig. 2 shows a typical TEM image of the synthesized CuO nanoparticles. From the TEM image it can be seen that the particles are nearly spherical with relatively uniform diameters and the particle size is found to be 15–30 nm.

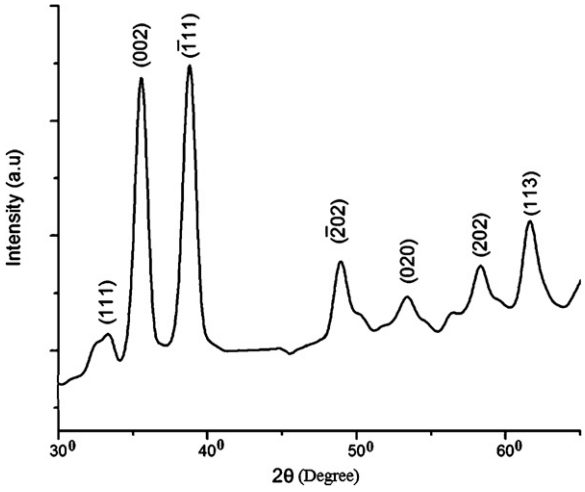

Fig. 3. XRD pattern of synthesized CuO nanoparticles.

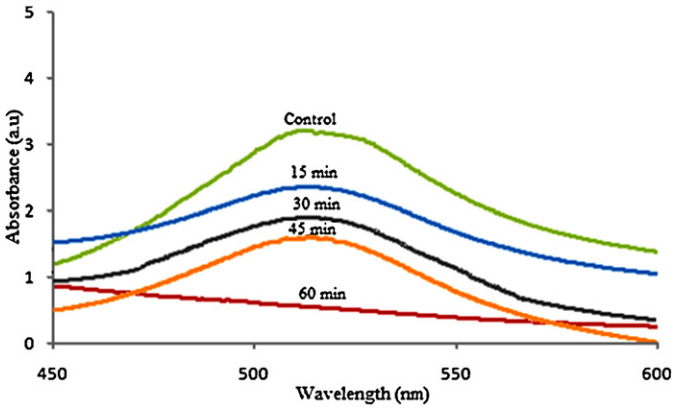

Fig. 4. Time dependent free radical scavenging by CuO nanoparticles.

Fig. 3 represents the XRD pattern of synthesized CuO nanoparticles. Different peaks were observed at $(2\theta) = 33.33^\circ$ $(1\,1\,0)$, 35.69° $(\bar{1}\,1\,1)$, 38.90° $(1\,1\,1)$, 48.91° $(\bar{2}\,0\,2)$, 53.36° $(0\,2\,0)$, 58.24° $(2\,0\,2)$ and 61.72° $(\bar{1}\,1\,3)$ corresponds to different planes of CuO nanoparticles. This confirms the formation of CuO nanoparticles. Every CuO nanoparticles has an interlayer spacing of 1.78861 Å which was calculated by using Williamson Hall plot.

3.2. Antioxidant activity

It is observed that colour of DPPH containing solution gradually changes from deep violet to pale yellow in the presence of CuO nanoparticles. Fig. 4 shows UV–visible spectrum of DPPH in the presence of CuO nanoparticles at different time intervals. The percentage of DPPH scavenging activity of CuO nanoparticles is evaluated from the decrease in percentage absorbance at 517 nm. The peak intensity at 517 nm gradually decreases with time interval, which gives the evidence of the free radical scavenging capacity of CuO nanoparticles. In this experiment, we have found that the DPPH scavenging activity of CuO nanoparticles upto 85% in 1 h. CuO nanoparticles have a capability to transfer its electron density towards the free radical located at nitrogen atom in DPPH.

3.3. Antibacterial activity test

Antibacterial activity of the CuO nanoparticles on the microorganisms *E. coli*, *S. aureus*, *P. aeruginosa* BS3 and *B. circulens* BP2 has been given in Fig. 5(a), (b), (c), (d) respectively. It shows a significant growth inhibition of bacterial culture by CuO nanoparticles with respect to the control. The antibacterial activity in case of *E. coli* shows that 1 mg/mL concentration of CuO nanoparticles inhibits bacterial growth after 9 h and in concentration of 4 mg/mL, growth inhibition starts after 3 h with respect to control culture. In case of *S. aureus*, all the concentrations of CuO nanoparticles retard the growth almost at the same time interval. But in case of *P. aeruginosa* BS3 there is a significant decrease in growth after 4 h in the highest concentration of CuO nanoparticles (4 mg/mL). Accordingly *B. cereus* shows growth inhibition approximately after 12 h in all the concentrations with respect to control experiment.

From the above experiment it can be assumed that the CuO nanoparticles are effective in killing/inhibiting a range of bacterial growth. However, higher concentration of nano CuO is significant in bactericidal effect. One of the possible reasons for this could be direct interaction between CuO nanoparticles and the external membrane surface of the bacteria. In this context a number of mechanisms have been proposed to interpret the antibacterial behavior of metal oxides. Makhluf et al. [22] explored the

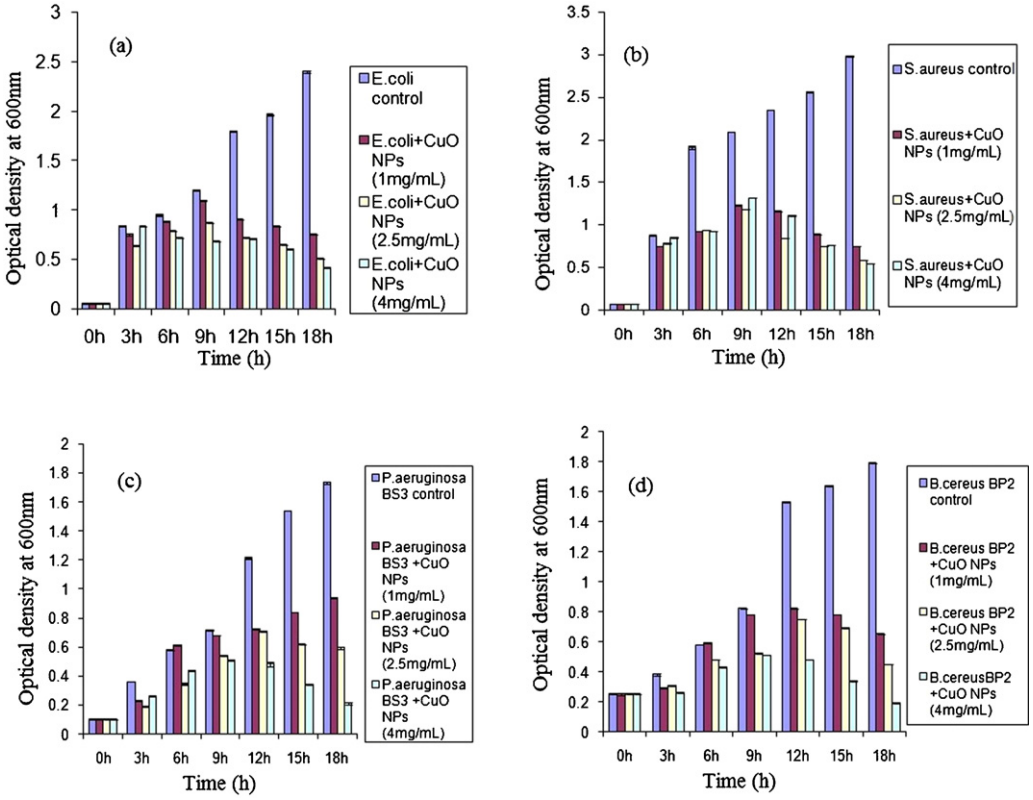

Fig. 5. The effect of CuO nanoparticles on the growth of *E. coli*, *S. aureus*, *P. aeruginosa BS3*, *B. cereus* BP2 (a), (b), (c) and (d) respectively. Cultures were set up at in LB medium containing various concentrations of nano CuO (1, 2.5 and 4 mg/mL). The standard errors were shown in the figure as calculated from 3 × 3 factorial experiments.

antibacterial behavior of MgO and proposed the production of active oxygen species due to the presence of MgO. The active oxygen species interact with bacterial membrane cell and allow penetration of individual MgO particles into the cell. Zhang et al. [23] reported that the presence of ZnO nanoparticles leads to damages to the membrane wall of *E. coli*. Such damages may be partly due to direct interactions between ZnO nanoparticles and bacterial membrane surface. The antibacterial effect of CuO nanoparticles increases with the concentration. The growth inhibition is probably due to disruptions of cell membrane by CuO nanoparticles results in malfunction of cell enzyme [8].

4. Conclusion

Thus we have successfully synthesized CuO nanoparticles and evaluated their antioxidant as well as antibacterial activity. CuO nanoparticles show free radical scavenging activity up to 85% in 1 h which is relatively higher in comparison to other metal oxide nanoparticles. CuO nanoparticles are promising antioxidants in polymer processing and non-biological systems. With the increase in CuO nanoparticles concentration, there is a significant decrease in bacterial growth. This implies the proficient antibacterial activity of CuO against *E. coli* and *P. aeruginosa.*

Acknowledgements

The authors would like to acknowledge the help and support of Tezpur University authority. The first author would also like to acknowledge receipt of the Rajiv Gandhi National fellowship from UGC, India.

References

[1] E.N. Frankel, Trends Food Sci. Technol. 4 (1993) 220.
[2] J. Fernandez, J.A. Perez-Alvarez, J.A. Fernandez-Lopez, Food Chem. 59 (1997) 345.
[3] E.A. Coppin, O.A. Pike, J. Am. Oil Chem. Soc. 13 (2001) 78.
[4] E. Beltran, R. Pla, J. Yuste, M. Mor-Mur, Meat. Sci. 66 (2004) 719.
[5] M. Ahamed, M. Karns, M. Goodson, J. Rowe, S. Hussain, J. Schlager, Y. Hong, Toxicol. Appl. Pharmacol. 233 (2008) 404.
[6] B.N. Ames, Science 221 (1983) 1256.
[7] M. Raffi, S. Mehrwan, T.M. Bhatti, J.I. Akhter, A. Hameed, W. Yawar, M.M.U. Hasan, Ann. Microbiol. 60 (2010) 75.
[8] G. Ren, D. Hu, E.W.C. Cheng, M.A.V. Reus, P. Reip, R.P. Allaker, Int. J. Antimicrob. Agents 33 (2009) 587.
[9] S. Paul, J.P. Saikia, B.K. Konwar, S.K. Samdarshi, J. Magn. Magn. Mater. 321 (2009) 3621.
[10] J.P. Saikia, S. Paul, B.K. Konwar, S.K. Samdarshi, Colloids Surf. B: Biointerfaces 78 (2010) 146.
[11] I.R. Kamrupi, S.K. Dolui, J. Appl. Polym. Sci. 120 (2011) 1027.
[12] N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M.D. Alessio, P.G. Zambonin, E. Traversa, Chem. Mater. 17 (2005) 5255.
[13] Z. Li, D. Lee, X. Sheng, R.E. Cohen, M.F. Rubner, Langmuir 33 (2006) 9820.
[14] C.T. Hsieh, J.M. Chen, H.H. Lin, H.C. Shih, Appl. Phys. Lett. 82 (2003) 3316.
[15] X. Zhang, G. Wang, X. Liu, J. Wu, M. Li, J. Gu, H. Liu, B. Fang, J. Phys. Chem. C 112 (2008) 16845.
[16] M. Ahamed, M.A. Siddiqui, M.J. Akhtar, I. Ahmad, A.B. Pant, H.A. Alhadlaq, Biochem. Biophys. Res. Commun. 396 (2010) 578.
[17] B. Fahmy, S.A. Cormier, Toxicol. In Vitro 23 (2009) 1365.
[18] P. Berntsen, C.Y. Park, B.R. Rutishauser, A. Tsuda, T.M. Sager, R.M. Molina, T.C. Donaghey, A.M. Alencar, D.I. Kasahara, T. Ericsson, E.J. Millet, J. Swenson, D.J. Tschumperlin, J.P. Butler, J.D. Brain, J.J. Fredberg, P. Gehr, E.H. Zhou, J. R. Soc. Interface (2012), http://dx.doi.org/10.1098/rsif.2010.0068.focus.
[19] E. Darezereshki, F. Bakhtiari, J. Miner. Metall. 47 (2011) 73.
[20] A. Serpen, E. Capuano, V. Fogliano, V. Gökmen, J. Agric. Food Chem. 55 (2007) 7676.
[21] N. Jones, B. Ray, K.T. Ranjit, A.C. Manna, FEMS Microbiol. Lett. 279 (2008) 71.
[22] S. Makhluf, R. Dror, Y. Nitzan, Y. Abramovich, R. Jelinek, A. Gedanken, Adv. Funct. Mater. 15 (2005) 1708.
[23] L. Zhang, Y. Jiang, Y. Ding, M. Povey, D. York, J. Nanopart. Res. 9 (2007) 479.