(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 March 2011 (24.03.2011)

PCT

(10) International Publication Number
WO 2011/033040 A2

(51) International Patent Classification:
A01P 1/00 (2006.01)

(21) International Application Number:
PCT/EP2010/063646

(22) International Filing Date:
16 September 2010 (16.09.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0916202.5 16 September 2009 (16.09.2009) GB

(71) Applicant *(for all designated States except US)*: **Centrum für Angewandte Nanotechnologie (CAN) GmbH** [DE/DE]; Grindelallee 117, 20146 Hamburg (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ALEKSANDROVIC, Vesna** [RS/DE]; Ruthsweg 10, 22307 Hamburg (DE). **SCHLUNDT, Charis Rabea** [DE/DE]; Weißenburger Str. 14, 22049 Hamburg (DE). **WERNER, Katja** [DE/DE]; Hasenwiete 5, 25336 Klein Nordende (DE). **WOOST, Marie** [FR/DE]; Struckholt 28, 22337 Hamburg (DE).

(74) Agent: **TEGETHOFF, Sebastian**; 24IP LAW GROUP, Sonnenberg Fortmann, Patent- und Rechtsanwälte, Herzogspitalstraße 10a, 80331 München (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2011/033040 A2

(54) Title: ANTIBACTERIAL PARTICLES AND THEIR SYNTHESIS

(57) Abstract: The field of the invention relates to a method for the manufacture of an antibacterial nanoparticle and its use. The present disclosure provides a method for the synthesis of antibacterial nanoparticles based on ZnO doped with copper or magnesium, and investigation of their antibacterial activity on Escherichia CoIi (*E.Coli*) DH5α as a representative of gram-negative bacteria and Staphylococcus Carnosus (*S. Carnosus*) as a representative of gram-positive bacteria.

Description

Title: Antibacterial particles and their synthesis

FIELD OF THE INVENTION

[0001] The field of the invention relates to a method for the manufacture of an antibacterial nanoparticle and its use.

BACKGROUND OF THE INVENTION

[0002] It is well known that some kind of metallic nanoparticles, such as silver, copper and zinc, have antibacterial applications. Nanoparticles as antibacterial agents are relevant for many industrial sectors like environmental, healthcare, medical care, food, synthetic textiles etc.. ZnO nanoparticles due to their antibacterial characteristics and physical stability have been used as antibacterial material in textile industry, medicine, and cosmetics. The antibacterial activity of the ZnO nanoparticles increases with decreasing nanoparticle size (Zhang, L et al., *Journal of Nanoparticle Research* 2007, *9*, 479; Yamamoto, O.; *International Journal of Inorganic Materials* 2001, *3*, 643).

[0003] Copper as an antibacterial agent has been known for a long time. Copper ions (Cu^{2+}) are soluble in water and function at low concentration as bacteriostatic substances and fungicides. The copper can be used as an anti-germ surface that can add to the anti-bacterial and antimicrobial features of buildings, such as hospitals. The advantage of the copper is the low toxicity that is useful in antibacterial treatments. The syntheses of copper nanoparticles is usually time consuming and very expensive, and often the particle agglomeration is a problem. In order to overcome these problems some groups used the deposition of the Cu on supporting substrates as, for example, silica glass or silica nanoparticles (Y.H.Kim, D.K. Lee, J Phys. Chem. B, 2006, 110, 24923; C.C. Trapalis, M. Kokkoris, G. Perdikakis, G. Kordas J. Sol-Gel Sei. Tech. 2003 26 1213), or by using chitosan (composite film - G.Cardenas, J. Diaz, M.F. Melendrez Polym Bull 2009, online 08 January ; Wen-Li Du, Ying - Lei Xu Nanotechnology, 2008, 19 085707 page 5), or sepiolite (J Mater Sei 2006 41 5208) as a carrying materials for Cu nanoparticles.

[0004] The US Patent Application Number US 2006/0222586 A1 discloses the syntheses of ZnO nanoparticles smaller or equal to 15 nm, starting with zinc chloride or zinc chloride hydrate and an inorganic alkali, both dissolved in ethylene glycol. Afterwards the precipitated ZnO particles are thermally aged.

[0005] In the US Patent Application Number US 2005/0260122 A1 a sol-gel method is described, wherein a metal oxide precursor and an alcohol based solution are mixed to form a reaction mixture that is then allowed to react in order to produce a metal oxide nanoparticle. During synthesis the pH conditions have to be carefully controlled to maintain a pH of about 7 or higher.

[0006] The antibacterial activity of the ZnO nanoparticles is described in an article by Zhang et al. (Zhang, Let al *Journal of Nanoparticle Research* 2007, *9*, 479) where the authors come to the conclusion that ZnO nanofluids as a bacteriostatic active agent against *E.coli* are suitable with a concentration above 0.25 g/1. The antibacterial activity increases with increasing nanoparticle concentrations and decreasing nanoparticle size. Additionally, the authors claim that the presence of polyethylene glycol and polyvinylpyrolidone does not affect the antibacterial activity of ZnO nanofluids.

[0007] The antibacterial activity of ZnO against *E. coli* and additionally against *S. aureus* is further described by Yamamoto (Yamamoto, O.; *International journal of Inorganic Materials* 2001, *3,* 643) .The author also describe that the antibacterial activity of ZnO increases with a decrease of particle size and increase of powder concentration.

[0008] In addition to the results of Yamamoto, Reddy et al. (K. M. Reddy, Kevin Feris, Jason Bell, Denise G. Wingett, and Cory Hanley, Alex Punnoose, *Applied physics letters* 2007, 90, 213902) report the toxicity of ZnO nanoparticles to gram-negative and gram-positive bacterial systems, *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus),* and primary human immune cells. ZnO nanoparticles with a diameter of 13 nm showed a complete inhibition of *E. coli* growth at concentrations of 3.4 mM, whereas growth of *S. aureus* was completely inhibited with 1 mM of nanoparticles.

[0009] The specific role of size scale, surface capping, and the aspect ratio of ZnO nanoparticles on toxicity toward prokaryotic and eukaryotic cells is reported by Shantikumar and colleagues (Shantikumar et al., *J.Mater.Sei.Mater.Med.* 2008). The authors investigated nanoparticles having a diameter of 40 nm, 150 nm and 350 nm. The ZnO nanoparticles that were PEG-capped were increasingly antibacterial in nature as the size was reduced from the micro-scale to the nano-scale and at increasing concentrations. The antibacterial activity was less towards Gram-positive bacteria than towards Gram-negative bacteria, but the functional dependence on particle size was the same. In contrary, starch capping of the nanoparticles appeared to provide greater protection to bacteria, possibly due to the OH-related quenching of positive charges on the ZnO nanoparticle surface.

[0010] The antibacterial activity of MgO nanoparticles synthesised by microwave-assisted synthesis were tested against *E. coli* and *S. aureus* by Makhluf and colleagues (Makhluf et al., *Adv. Funct.lMater.* 2005, *15*, 1708). The authors used a solution with a concentration of about 1 mg/ml and demonstrated that the antibacterial activity of the MgO nanoparticles depends strongly on their particle size.

[0011] The preparation of ZnO/Mg nanoparticles is described by Huan-Ming et al. (Huan-Ming et al, *Angewandte Chemie International Edition*, 2009, 48, 15, 2727 starting from the previously synthesized ZnO nanoparticles and afterwards applying sonication procedure in the presence of magnesium acetate tetra hydrate. The particles were not synthesized starting from precursor for Zn and Mg.

[0012] International Patent Application WO2008043396 discloses all materials based on colloidal silica, titanium dioxide, zirconium dioxide, stannic dioxide and zinc oxide, connected trough organic ligands with metal ions as antibacterial and antimycotic materials. WO 2008/043396 does not describe incorporation of metal ions in the nanoparticle or directly on the nanoparticle surface.

[0013] The WO 2006/019008 A1 discloses polymer-modified nanoparticles, which are stably present over long time without aggregating. This document refers to metal sulphide

nanoparticles with particles stabilised with polymers that start to grow directly from the nanoparticle surface.

[0014] None of the prior art discloses a simple method for the manufacture of ZnO particles doped with copper or magnesium as disclosed herein for the manufacture of new antibacterial material with low toxicity and higher antibacterial effect than ZnO particles.

BRIEF SUMMARY OF THE INVENTION

[0015] The present disclosure provides a method for the synthesis of antibacterial nanoparticles based on ZnO doped with copper or magnesium, and investigation of their antibacterial activity on Escherichia Coli (*E.Coli*) DH5α and *Pseudomonas aeruginosa (P aeruginosa)* as a representative of gram-negative bacteria and Staphylococcus Carnosus (*S. Carnosus*), staphylococcus aureus (S. aureus) and bacillus subtilis (B. subtilis) as a representative of gram-positive bacteria.

[0016] The present disclosure teaches the synthesis of ZnO particles by a wet procedure in methanol as a reaction media, and during the syntheses, the ZnO particles were doped with various amount of copper or magnesium. The shape of the particles was varied from spherical via rice-shape to elongated, rod like particles. The particle size varied from a few nanometres up to 100 nm.

[0017] Antibacterial tests with Escherichia Coli (*E.Coli*) DH5α and Staphylococcus Carnosus (*S. Carnosus*) bacteria confirmed the higher antibacterial activity of the doped ZnO particles than the pure ZnO nanoparticles. The antibacterial activity was demonstrated for both types of nanoparticles, doped with Cu or Mg. Additionally, it was found that antibacterial activity is strongly dependent on concentration of doped elements in the ZnO nanoparticles.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The invention provides a method for the manufacture of colloidal ZnO particles doped with Cu or Mg comprising:

a. dissolving salts of Zn and Cu or Mg in an alcoholic solution to form a reaction mixture;
b. heating and stirring the reaction mixture until a clear solution is formed
c. adding a basic solution at room temperature drop by drop under vigorous stirring
d. heating the reaction mixture.

[0019] As precursor $Zn(Ac)_2 \times 2H_2O$ and $Cu(Ac)_2 \times H_2O$ or $Mg(NO_3)_2 \times 6H_2O$ can be used. Prior to heating the reaction mixture a solution of $Cu(Ac)_2 \times H_2O$ dissolved in an alcohol at room temperature can be injected. It is further intended to use 0.5 to 15 mol % of copper precursor calculated on the amount of $Zn(Ac)_2$. Finally it is within the scope of the present disclosure that the temperature of the reaction mixture is heated to 64°C followed by cooling to 40°C for 10 to 30 min before injecting the solution of $Cu(Ac)_2 \times H_2O$.

[0020] The alcoholic solution used in the disclosed method comprises methanol or ethanol as solvent. It is also within the scope of the invention that the alcoholic solution comprises other solvents, for instance water. The basic solution comprises a solution of an alkali in an alcoholic solution, for example methanol. Within the meaning of the disclosed method the term "alkali" shall be understood as alkali metal or earth alkali metal hydroxide and a basic solution shall be a solution comprising an alkali, like the basic solution of KOH or $Ba(OH)_2$.

[0021] The reaction mixture of the Zn and Cu or Mg salts may be heated to a temperature in the range of 60 to 65°C and the final heating of the reaction mixture can be done for at least 22 h at 64°C.

[0022] The size and/or shape of the resulting particles may be adjusted by the amount of copper or magnesium precursor, since there is a dependency between these parameters.

[0023] It is further intended that he ZnO particles may be doped with Cu or Mg that is bound to a polymer.

[0024] A further object of the invention is a particle consisting of ZnO doped with copper. The size of such a particle may be in the range of 3 to 10 nm for spherical particles and 3 mm in one dimension and up to 100 nm in the other dimension for rod like shaped particles.

[0025] The disclosed particle shall comprise up to 10 % of incorporated copper.

[0026] Another object of the disclosure of this invention is an antibacterial composition comprising ZnO/Cu and /or ZnO/Mg particles manufactured according to one of the disclosed methods above. Such a composition may be a pharmaceutical, cosmetic or medicine product.

[0027] The disclosed particles and the composition may be further used as disinfectant, antibacterial coating or as an additive in cosmetic, medicine or pharmaceutical products.

[0028] It is also intended that the disclosed particles may be used for the manufacture of textiles or fibres for the manufacture of textiles with antibacterial properties.

[0029] The syntheses of the nanoparticles based on ZnO doped with copper or magnesium is performed by a wet procedure in methanol as reaction media, starting form different salts of Zn and Cu or Mg as a precursor. Mixture of the different salts added in certain ratios was stirred at 64°C until a clear solution was formed. Then a solution of alkali in methanol was added drop by drop at room temperature under vigorous stirring. Formed nanoparticles were heated for a minimum of 22 hours at 64°C. Particle size was varied from few nanometres up to 100 nm. Additionally, shape of the particles was varied from spherical via rise-shape until elongated, rod like particles by changing a precursor ratio in reaction mixture.

[0030] The present disclosure describes a method for the manufacture of antibacterial nanoparticles and its use. The disclosed method comprises a defined sequence of procedural steps, which is critical for obtaining nanoparticles with the described properties as described below.

[0031] The US 2005/260122 A1 discloses reaction conditions indicating broad ranges for reaction, for example a reaction temperature from at least about 30° C to about 80° C. It is obvious for a person skilled in the art, that the indicated range is very general and is not suitable to specify new and inventive reaction conditions. Although this document mentions copper as a dopant, the antibacterial properties of nanoparticals doped with copper is not disclosed. Besides this, the description of the US 2005/260122 A1 does not support the use of other metal oxides as zinc oxide, since the described method is limited to zinc oxides.

[0032] The claimed process of the US 2005/260122 A1 differs from the process of the present disclosure, because it claims the formation of an alcohol-based solution and maintaining the pH greater than 7 before adding the metal oxide precursor. The method claimed within the present disclosure starts with dissolving the metal oxide precursor in an alcohol solution followed by heating and stirring of the reaction, before a basic solution is added drop wise. It seems to be important according to paragraphs 67 and 68 process of the US 2005/260122 A1 that the metal oxide precursors are added to a solution with a pH above 7, while this is in contrast no prerequisite for performing the method described within the context of the present disclosure.

[0033] As can be taken from the description of example 6 of the US 2005/260122 A1 it is explicitly stated that a turbid solution was obtained without keeping the pH above 7, resulting in the presence of ZnO particles of much larger diameter. Thus, a person skilled in the art would not consider adding the metal oxide precursor to an alcohol solution without keeping the pH above 7. The disclosure of the US 2005/260122 A1 is leading away from the specific sequence of procedural steps as claimed below.

BRIEF DESCRIPTION OF THE FIGURES

[0034] The invention will be further described by figures and examples without being limited to the described embodiments:

Fig. 1 Influence of Cu concentration in starting reaction mixture on nanoparticle size

Fig. 2 TEM images of ZnO/Cu nanoparticles synthesized a) with 10% $Cu(Ac)_2$; b) with 0.5% $Cu(Ac)_2$ (after 22 h heating at 64 °C)

Fig. 3 XRD patterns of ZnO/Cu nanoparticles synthesized with A - 10 % $Cu(Ac)_2$ and B with 0.5 % $Cu(Ac)_2$

Fig.4 Emission bands of ZnO/Cu nanoparticles synthesized with A - 10 % $Cu(Ac)_2$ and B -0.5 %$Cu(Ac)_2$

Fig. 5 Growth curves of E.Coli in LB medium inoculated in the presence of ZnO/Cu (Cu-3%) nanoparticles at different concentrations and of ZnO (ZnO-RZO1) nanoparticles.

Fig. 6 Growth curves of S.Carnosus in LB medium inoculated in the presence of ZnO/Cu (Cu-3%) nanoparticles at different concentrations and of ZnO (ZnO-RZO1) nanoparticles.

Fig. 7 Growth curves of E.Coli in LB medium inoculated in the presence of ZnO/Mg (Mg-1%)) nanoparticles at different concentrations and in the presence of ZnO/Cu (Cu-2%) nanoparticles.

Fig.8 Growth curves of E.Coli in LB medium inoculated in the presence of ZnO/Cu nanoparticles with different copper percentages and in the presence of ZnO nanoparticles

Fig. 9 SEM images of E.Coli: (a) E.Coli before treatment; (b) E.Coli after treatment with ZnO/Cu (Cu–3%) at 1.5 mg/mL for 4h).

Fig. 10 Growth test with S. carnosus in flasks coated with nanoparticle-polmer

Fig. 11 Growth test with S. carnosus in medium supplemented with doped nanoparticles

Fig. 12 Growth test with B. subtilis in medium supplemented with doped nanoparticles

Fig. 13 Growth test with P aeruginosa in medium supplemented with doped nanoparticles

Fig. 14 Growth test with E. coli in medium supplemented with doped nanoparticles

Fig. 15 Growth test with S. aureus in medium supplemented with doped nanoparticles

Fig. 16 Growth test with C. albicans in medium supplemented with doped nanoparticles

DETAILED DESCRIPTION OF THE FIGURES

Synthesis of ZnO/Cu colloid nanoparticles

[0035] High quality crystalline copper doped ZnO nanoparticles were synthesized by procedures as described in the examples below. The shape of the synthesized ZnO/Cu nanoparticles can be controlled from spheres to rods, depending on the copper precursor concentration and on the time of a copper precursor addition. The formation of ZnO/Cu rods is based on an oriented attachment of preformed quasi-spherical nanoparticles. The length of nanoparticles synthesized with different copper concentrations is shown in Figure 1. The nanoparticles were heated at 64 °C for 22 hours.

[0036] The length of the rods decreases with the copper concentration until a concentration limit (1.5 mol %) is reached, where the oriented attachment of semi-spherical particles formed at the beginning is avoided. If the $Cu(Ac)_2$ amount exceeds 1.5 mol % calculated on amount of $Zn(Ac)_2$, the formed the ZnO/Cu nanoparticles have a spherical shape. By usage of higher amount of copper precursors than the 1.5 mol % the nanoparticles became smaller and spherical. Two typical samples of nanoparticles synthesized starting from 10 and 0.5 mol % of copper precursor are shown in the TEM images in Fig 2.

[0037] The crystallinity of the synthesized nanoparticles was checked using X-ray diffractometry. The typical powder X-ray diffractogram is shown at Figure 3. The

measurements show that the nanoparticles have a crystalline structure that is typical for Wurtzite type of ZnO. Using Scherer line it was calculated for the nanoparticles synthesised with 10 % $Cu(Ac)_2$ that their average nanoparticle diameter is around 5 nm. This calculation is in very good agreement with the TEM images (Figure 2.)

[0038] A decrease of the copper concentration leads to an increase of the elongation of the nanoparticles along the c axis. With a 0.5 % copper precursor, the nanoparticles are rods with a length of 30 nm and a diameter of 8 nm - calculated based on TEM image (Figure 2 (b)). The rod formation along the c axis was confirmed by XRD diffractogram (Fig. 3 (b)) that shows much sharper 002 reflection than the other reflections. Characteristic peaks for CuO was not found at XRD patterns, thus it can be supposed that copper atoms replace zinc in the hexagonal lattice and/or copper segregate to the non-crystalline region probably on the nanoparticle surface.

[0039] Conformation of Cu atoms incorporation into the nanoparticles was obtained by measurement of the room temperature photoluminescence (PL). The room temperature photoluminescence (PL) of the ZnO nanostructures typically exhibits a sharp emission band in near UV range (originating from the exciton recombination ER) and a broad emission band in the visible region of the spectrum (so called green emission GE) that is attributed to defects in ZnO like vacancies of zinc or oxygen. Since the ZnO nanoparticles exhibit different types of defects, the emission in the visible region remains difficult to identify.

[0040] The photoluminescence spectrum of the ZnO/Cu nanoparticles in chloroform synthesized with 0.5 and 10 % of the copper precursors are shown in Figure 4. The PL spectrum exhibits the typically sharp emission in near UV of ZnO material. However the intensity of the green emission depends strongly on the copper precursor concentration used during the synthesis of the ZnO/Cu nanostructures. The intensity ratio (Intensity of ER / Intensity of GE) obtained form the spectra is presented in table 1.

[0041] The more copper precursors used for the synthesis, the weaker the intensity of the peak. For a concentration of copper precursor larger than 5 %, the green emission was

quenched. Therefore it can be supposed that the copper atoms fill the states corresponding to the defects in ZnO.

[0042] Amount of Cu atoms present in synthesized nanoparticles was determined by energy-dispersive X-ray analysis (EDX). The obtained results are summarized in table 1.

Synthesis	Amount of Cu in reaction mixture %	Shape of nanoparticles	Size diameter / length (nm)	PL Intensity ratio	%Cu (EDX)
Cu-a	0,50	rods	8 / 30	1/7	___
Cu-b	1	rods	8 / 20	1/4	___
Cu-c	1.5	rods	8 / 11	1/1	___
Cu-d	2	rice	5 / 9	1/0,5	0,50%
Cu-e	3	dots	8	1/0,4	0,80%
Cu-2%	5	dots	7	no green emission	2%
Cu-3%	10	dots	5	no green emission	3%
Cu-4%	15	dots	5	no green emission	4%

Table 1: Influence of the amount of Cu precursor in reaction mixture on ZnO/Cu nanoparticle shape, size and photoluminescence (PL).

[0043] In all of the samples Cu was detected except in the sample Cu-a synthesized with 0.5 % copper precursor. However the intensity of the green emission of Cu-a measured by PL remained lower than the intensity of the pure ZnO nanoparticles, which implies that some of the copper ions are incorporated into the nanoparticles.

Antibacterial study

[0044] The bacteria used for the antibacterial study were *E. Coli* DH5α, Gram-negative and *S.Carnosus* TM300. *S. Carnosus* as a typical gram-positive bacterium. Antibacterial tests were performed with a solution of nanoparticles in distilled water.

[0045] The ZnO/Cu nanoparticles synthesized with 15 %, 10 % and 5 % of the copper precursor were used for the antibacterial study. The average size of the ZnO/Cu nanoparticles was from 7 to 5 nm and the fraction of the copper (in mol) ranges from 2 to 4 %. The ZnO nanoparticles synthesized according to the method of Pacholsky et al. (C. Pacholski, A. Kornowski, H. Weller *Angew. Chem* 2002 114 7 1234). was used as

comparison. Their average size was 7 nm that is in the same range as investigated ZnO/Cu nanoparticles.

Effect of the nanoparticle concentration on antibacterial properties

[0046] *E. Coli* and *S. Carnosus* bacteria were tested with different concentrations of the nanoparticles in order to observe the effect of nanoparticle concentration on bacterial growth. The final concentration of the ZnO and ZnO/Cu nanoparticles in the bacterial cultures ranged from 0.300 to 0.070 mg/ml. As the control, samples were used the same solvent as for the nanoparticles and LB medium.

[0047] The growth curves of the *E.Coli* DH5α and *S. Carnosus* TM300 bacteria are shown on Figure 5 and 6, respectively. The bacteria growth was determined by measuring the time evolution of the optical density (OD) of the sample at 600 nm. As the value of the OD at 600 nm represents the absorbance of the bacteria, an increase of the number of the bacteria implies more light being absorbed by the bacteria.

[0048] As expected the control sample containing the solvent showed no antibacterial activity. The results shows in Fig.6 indicate an increase of the antibacterial activity with increase of the nanoparticle concentration in the medium. The ZnO/Cu nanoparticles containing 3% copper are antibacterial against the *E.Coli* bacteria above a concentration of 0.09 mg/ml while the ZnO nanoparticles show the antibacterial effect only above 0.13 mg/ml. similar results was obtained with *S. Carnosus* (Figure 6.*)*. Thus the results demonstrate that the ZnO/Cu nanoparticles have a higher antibacterial activity than the ZnO nanoparticles.

[0049] The same investigations were done with the ZnO nanoparticles doped with Mg. The growth curves of *E.Coli* DH5α inoculated with the ZnO/Mg nanoparticles that contain 1% of Mg were shown in Figure 7. The ZnO/Mg nanoparticles with 1% of Mg show the antibacterial activity against *E.Coli* bacteria above a concentration of 0.07 mg/ml, that indicates higher antibacterial effect of ZnO/Mg nanoparticles than pure ZnO nanoparticles.

Effect of the copper percentage in the ZnO/Cu nanoparticles on antibacterial properties

[0050] A series of experiments was done with the ZnO/Cu nanoparticles that contain different percentages of Cu. The nanoparticles were investigated in the same way as was previously described. The growth curve of *E.Coli* bacteria inoculated with the ZnO/Cu nanoparticles containing copper percentages from 2, 3 and 4 % are shown in Figure 8.

[0051] The results indicate a small increase in the antibacterial activity of the ZnO/Cu nanoparticles with a decrease of the copper percentage. All of the ZnO/Cu nanoparticles show the antibacterial activity against the *E.Coli* bacteria above a concentration of 0.09 mg/ml, which remains higher than the antibacterial activity of the ZnO nanoparticles.

SEM analysis of the *E.Coli* bacteria

[0052] The antibacterial effect of the ZnO/Cu nanoparticles against the *E.Coli* bacteria was directly confirmed by scanning electron miscopy (SEM) of the *E.Coli* bacteria before and after exposure to the nanoparticles (Figure 9). Figure 9 shows normal *E.Coli* bacteria and *E.Coli* bacteria after a treatment with the ZnO/Cu nanoparticles containing 3% copper at a concentration of 1.5 mg/ml for 4 hours, respectively. The images indicate that the presence of the ZnO/Cu nanoparticles leads to the damages of membrane wall of the *E.Coli* bacteria.

[0053] Investigations of the synthesized ZnO/Cu nanoparticles and the synthesized ZnO/Mg nanoparticles on polystyrol plates also showed a higher antibacterial effect than the pure ZnO nanoparticles. These results indicate possible future application of synthesized nanoparticles not only in cosmetic or medicine but additionally in all areas where antibacterial coating are required. Any other commercial polymer surface may be suitable like for example polyethylene, polypropylene, PVC etc.

Growth test with S. carnosus

[0054] Figure 10 shows the results of a growth test using S. carnosus with nanoparticle/polymer coated flasks, wherein the nanoparticles were doped with ZnO or ZnO:Cu. For both kinds of doped nanoparticles different concentrations were used as indicated in order to determine whether an effect is depending on the nanoparticle concentration. The growth of the bacteria in the solution was determined by measuring the optical density (OD) of the solution at 600 nm at the beginning and after 2, 4, 6 and 8 h after incubation. The results of the negative controls are depicted on the left and right side of figure 10.

[0055] The negative control comprising medium only with bacteria (left side of fig. 10) shows the typical growth curve for cultured bacteria. The same applies for the negative control with added polymer but without nanoparticles (right side of fig. 10).

[0056] In contrast to the bacteria growth in the negative controls, the use of a nanoparticle-covered polymer results in a clear inhibition of bacteria proliferation. The growth of the bacteria is more or less static, while the use of a higher concentration of doped nanoparticles results in a slightly better inhibition of bacteria growth as can be observed in the results using 1 mg/ml with more or less in a constant amount of bacteria in the medium. The observed effects can be traced back to the nanoparticle/polymer coating of the flask, which is quite surprising and demonstrates that the coating of material with a doped nanoparticle-polymer according to the disclosure is appropriate for producing a bactericide surface.

[0057] Figure 11 shows the results of a growth test with S. carnosus, wherein the nanoparticles were directly added to the medium. The control experiment (results depicted on the left side) shows the expected exponential growth of the bacteria within 24 hours. Again, in contrast to this, the addition of nanoparticles doped with ZnO in a concentration of 0.2 mg/ml or 0.1 mg/ml resulted in a clear inhibition of any bacteria growth. The addition of the doped nanoparticles to the medium seems to cause a slightly stronger effect than coating a surface with a nanoparticle-coated polymer. However, the results depicted in

fig. 11 demonstrate that adding nanoparticles according to the disclosure is very efficient with regard to the inhibition of bacteria growth in a solution.

Growth tests with B. subtitlis, P aeruginosa, E. coli, S aureaus and C. albicans

[0058] Figures 12 to 16 show further experiments performed with different bacteria or yeasts as indicated on top of each figure. The nanoparticles were added directly into the growth medium in a concentraion of 0.5 mg/ml and at the beginning, after 3, 16 and 24 h the optical density (OD) was determined at a wavelength of 600 nm in order to measure the bacteria concentration. Table 2 summarizes the particles, which were added to the respective sample.

[0059] Table 2: Overview of samples and added substances.

Sample	Addition of
Cl14, CH239	ZnO:Cu doped nanoparticle
Cl15, CH240	ZnO:Mg doped nanoparticle
Cl16, CH241	ZnO doped nanoparticle
PK	positve control using Sagrotan®
NK	negative control using H_2O

[0060] Figure 12 shows the results of growth experiments with B. subtilis. The bacteria growths is clearly inhibited by the addition of doped nanoparticles according to the present disclosure, independently whether they were coated with ZnO, ZnO:Mg or ZnO:Cu.

[0061] In figure 13 P. aeruginosa was used and an anti-bacterial effect can be observed, although the added nanoparticles are less efficient in reducing or preventing growth of P. aeruginosa. The same applies for the effect on the growth of E. coli (figure 14), whereas the growth of S. aureus (figure 15) as well as of C. albicans (figure 16) is obviously suppressed by the added nanoparticles in a considerable degree.

EXAMPLES

Syntheses of nanoparticles

[0062] Colloidal ZnO/Cu nanoparticles and ZnO/Mg nanoparticles were synthesized from zinc acetate dehydrate ($Zn(Ac)_2$ x $2H_2O$) with copper acetate monohydrate ($Cu(Ac)_2$ x H_2O) and magnesium chloride or nitrate ($Mg(NO_3)_2$ x 6 H_2O) hexahydrate, respectively, in an alcohol solution under basic conditions. In order to improve solubility of $Cu(Ac)_2$ it will be solved in diluted hydrochlorid acid. The synthesis is a modification of the method developed by Pacholsky et al. (C. Pacholski, A. Kornowski, H. Weller *Angew. Chem.* 2002 114 7 1234). The shape and size of the colloidal nanoparticles was varied from spheres to rods depending on the concentration of copper precursor in reaction mixture, wherein copper can be used in an amount of up to 15 % w/v.

[0063] Example 1: 3.0 g of $Zn(Ac)_2$ x $2H_2O$ and 0.27 g of $Cu(Ac)_2$ x H_2O were dissolved in 18 ml of methanol to form a reaction mixture. The reaction mixture was heated at 64°C in a three-neck flask until a clear solution was formed. To produce the nanoparticles, a solution of 1.5 g of KOH dissolved in 6.5 ml of methanol at room temperature was added drop by drop into the three-neck flask under vigorous stirring. After the addition the reaction mixture was heated for minimum 22 hours at 64°C.

[0064] Example 2: 3.0 g of $Zn(Ac)_2$ x $2H_2O$ and 0.4 g of $Cu(Ac)_2$ x H_2O were dissolved in 18 ml of methanol to form a reaction mixture. The reaction mixture was heated to 64°C in a three-neck flask until a clear solution was formed. To produce the nanoparticles, a solution of 1.5 g of KOH dissolved in 6.5 ml of methanol at room temperature was added drop by drop into the three-neck flask under vigorous stirring. After the addition the reaction mixture was heated for minimum 22 hours at 64°C.

[0065] Example 3: 3.0 g of $Zn(Ac)_2$ x $2H_2O$ was dissolved in 18 ml of methanol to form a reaction mixture. The reaction mixture was heated to 64°C in a three-neck flask until a clear solution was formed. A solution of 1.5 g of KOH dissolved in 6.5 ml of methanol at room temperature was added drop by drop into the three-neck flask under vigorous stirring. After 1.5 hours a Solution of 0.27 g of $Cu(Ac)_2$ x H_2O dissolved in 7.5 ml methanol at

room temperature was injected into the three-neck flask. After the injection the reaction mixture was heated for minimum 22 hours at 64°C.

[0066] Example 4: 3.0 g of $Zn(Ac)_2$ x $2H_2O$ and 0.36 g of $Mg(NO_3)_2$ x $6H_2O$ were dissolved in 18 mL of methanol to form a reaction mixture. The reaction mixture was heated to 64°C in a three-neck flask until a clear solution was formed. To produce the nanoparticless, a solution of 1.5 g of KOH dissolved in 6.5 mL of methanol at room temperature was added drop by drop into the three-neck flask under vigorous stirring. After the addition the reaction mixture was heated for minimum 22 hours at 64°C.

[0067] Example 5: 3.0 g of $Zn(Ac)_2$ x $2H_2O$ and 0.14 g of $MgCl_2$ were dissolved in 18 mL of methanol to form a reaction mixture. The reaction mixture was heated to 64°C in a three-neck flask until a clear solution was formed. To produce the nanoparticles, a solution of 1.5 g of KOH dissolved in 6.5 mL of methanol at room temperature was added drop by drop into the three-neck flask under vigorous stirring. After the addition the reaction mixture was heated for minimum 22 hours at 64°C.

[0068] US Patent US 6710091 Bl discloses the synthesis of ZnO nanoparticles having an average particle diameter of less than or equal to 15 nm, which are redispersible in organic solvents and/or water by basic hydrolysis of at least one Zn-compound in alcohol or an alcohol/water mixture. US 6,710,091 does not disclose the synthesis of larger particles, or rods, and no doping of the ZnO nanoparticles with other metals.

Characterization of nanoparticles

[0069] The synthesized nanoparticles were characterized by low and high-resolution transmission electron microscopy (TEM), electron diffraction, and X-ray diffraction. TEM samples were prepared by dropping diluted aqueous solutions of the ZnO/Cu nanoparticles onto 400-mesh carbon-coated copper grids with excess solvent immediately evaporated.

[0070] The optical properties of the nanoparticles have been characterized by electronic absorption and fluorescence spectroscopy. Absorption spectra were obtained using a Cary

50 spectrophotometer. Photoluminescence measurements were performed at room temperature using a Cary 50 spectrofluorimeter.

Tests of antibacterial activity

[0071] Escherichia Coli *(E.Coli)* DH5a and Staphylococcus Carnosus *(S. Carnosus)* TM300 bacteria were used for the antibacterial tests. The bacteria were cultured in an LB medium at 37°C on a shaker. The bacterial culture was suspended in a sterile LB medium at a final concentration of approximately 10^7 CFU cm^{-3} (CFU:Colony Forming Unit) and used for the bacterial assays.

[0072] The antibacterial activity of the nanoparticles was assessed by measuring the growth curve of the bacteria. 1.5 ml of the ZnO/Cu nanoparticles solution was diluted in 14.7 ml LB medium with 300 µl bacteria. The bacterial cultures incubated with the nanoparticles were grown at 37°C under an agitation condition. The growth curve was determined by measuring the time evolution of the optical density (OD) of the sample at 600 nm. A blank LB broth medium culture under the same conditions was used as a control.

Claims

1. A method for the manufacture of colloidal ZnO particles doped with Cu or Mg comprising:
   - dissolving salts of Zn and Cu or Mg in an alcoholic solution to form a reaction mixture;
   - heating and stirring the reaction mixture until a clear solution is formed
   - adding a basic solution at room temperature drop by drop under vigorous stirring
   - heating the reaction mixture.

2. The method according to claim 1, wherein $Zn(Ac)_2 \times 2H_2O$ and $Cu(Ac)_2 \times H_2O$ or $Mg(NO_3)_2 \times 6H_2O$ are used as a precursor.

3. The method according to one of the above claims, further comprising for the manufacture of ZnO particles doped with Cu injecting a solution of $Cu(Ac)_2 \times H_20$ dissolved in an alcohol at room temperature prior to heating the reaction mixture.

4. The method according to one of the above claims, wherein 0.5 to 15 mol % of copper precursor calculated on the amount of $Zn(Ac)_2$ are used.

5. The method according to one of the above claims, wherein the alcoholic solution comprises methanol or ethanol as solvent

6. The method according to one of the above claims, wherein the basic solution comprises the solution of an alkali in methanol.

7. The method according to one of the above claims, wherein the alkali in the basic solution is KOH.

8. The method according to one of the above claims, wherein the reaction mixture of the Zn and Cu or Mg salts is heated to a temperature in the range of 60 to 65°C.

9. The method according to one of the above claims, wherein the final heating of the reaction mixture is done for at least 22 h in the range of 60 to 65°C.

10. The method according to one of the above claims, wherein the size and/or shape of the resulting particles is dependent on the amount of copper or magnesium precursor.

11. The method according to one of the above claims, wherein the ZnO particles doped with Cu or Mg are bound to a polymer.

12. A particle consisting of ZnO doped with copper.

13. The particle of claim 12, with a size of 3 to 10 nm for spherical particles and 3 mm in one dimension and up to 100 nm in the other dimension for rod like shaped particles.

14. The particle of claim 12 or 13, comprising up to 10% incorporated copper or 1% incorporated magnesium.

15. An antibacterial composition comprising ZnO/Cu and /or ZnO/Mg particles manufactured according to one of the methods of claims 1 to 11.

16. The composition according to claim 15, wherein the composition is a pharmaceutical, cosmetic or medicine product.

17. Use of the particle according to any of the claims 12 to 14, or the composition according to claim 14 or 15 as disinfectant.

18. Use of the particle according to any of the claims 12 to 14 or the composition according to claim 14 or 15 as antibacterial coating.

19. Use of the particle according to any of the claims 12 to 14 or the composition according to claim 14 or 15 as additive in cosmetic, medicine, pharmaceutical products.

20. Use of the particle according to any of the claims 12 to 14 or the composition according to claim 14 or 15 for the manufacture of textiles or fibres for the manufacture of textiles with antibacterial properties.

Drawings

Figure 1

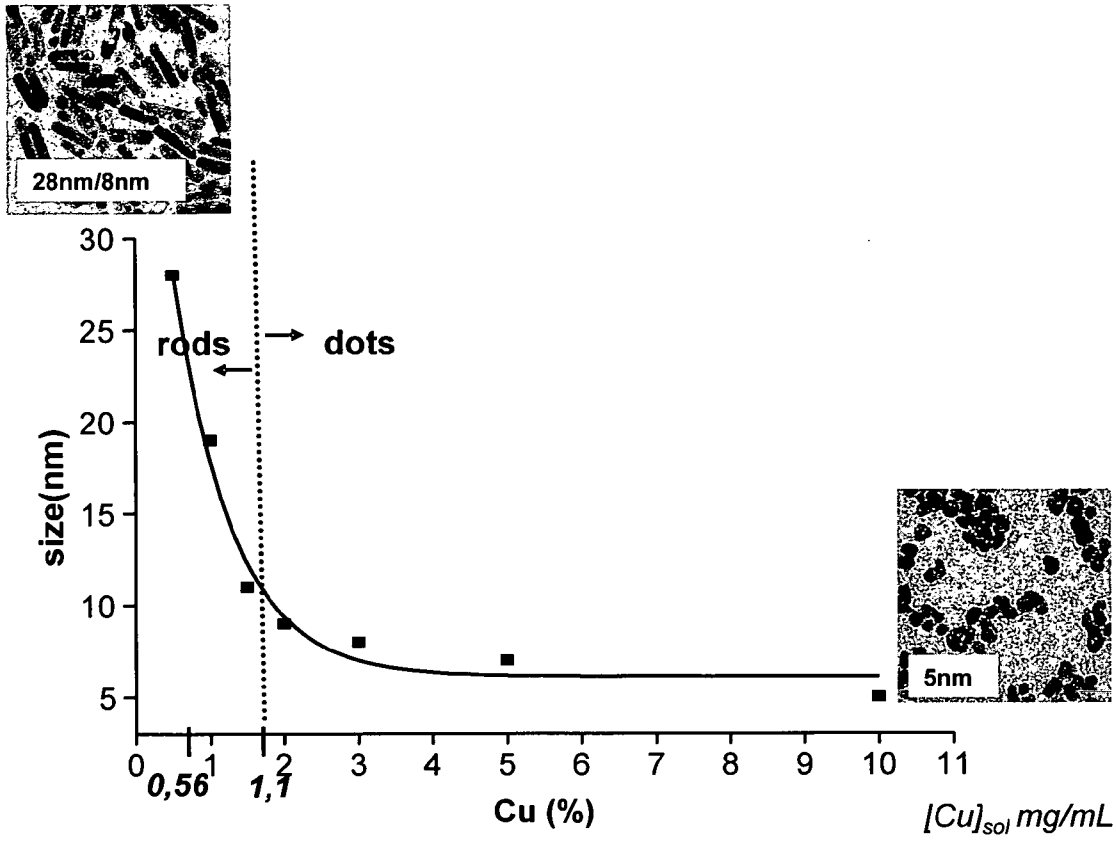


Figure 2

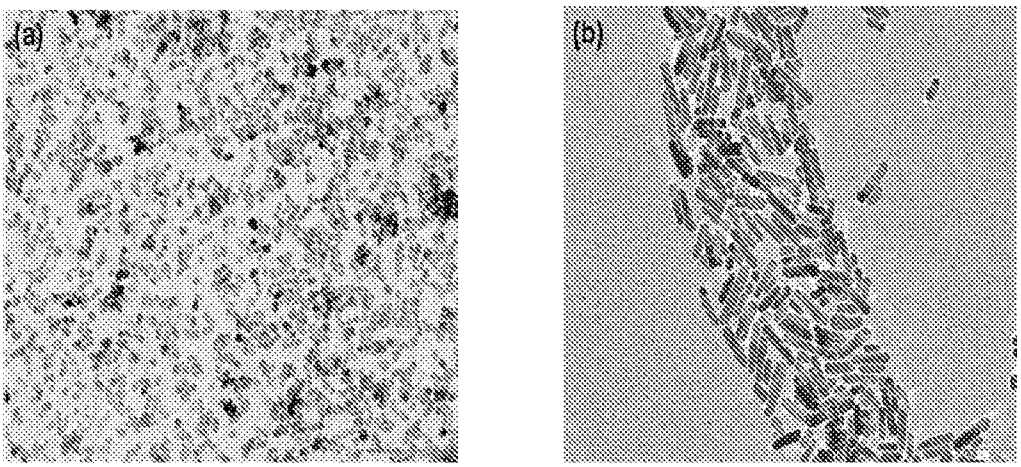


Figure 3

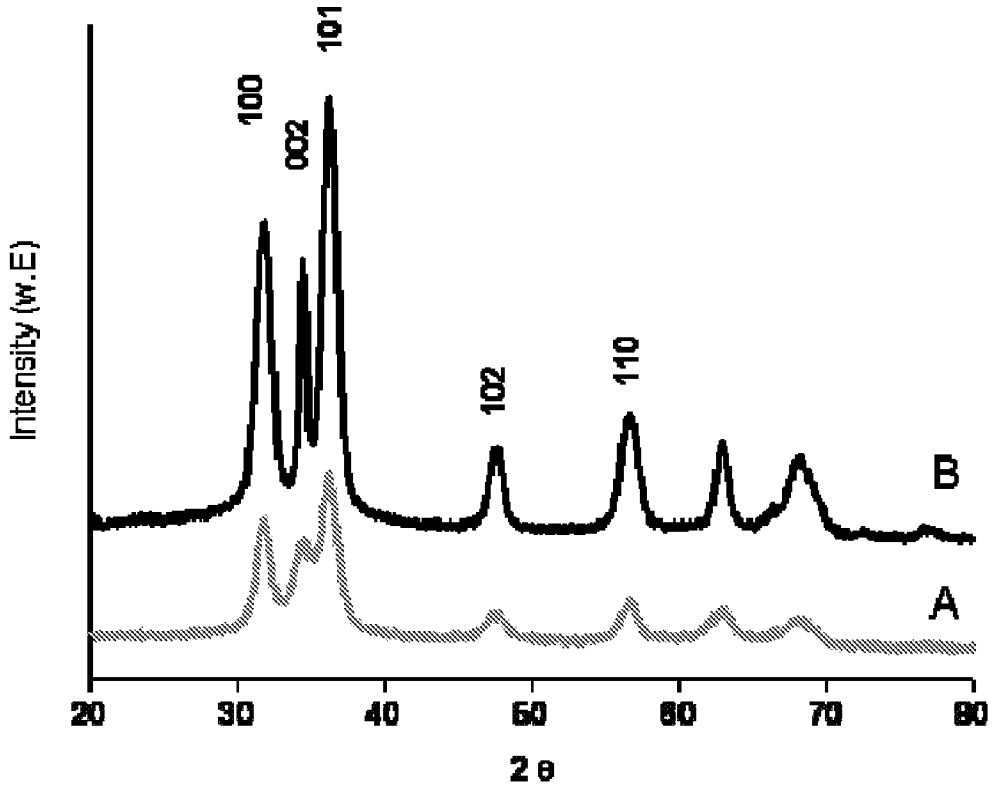


Figure 4

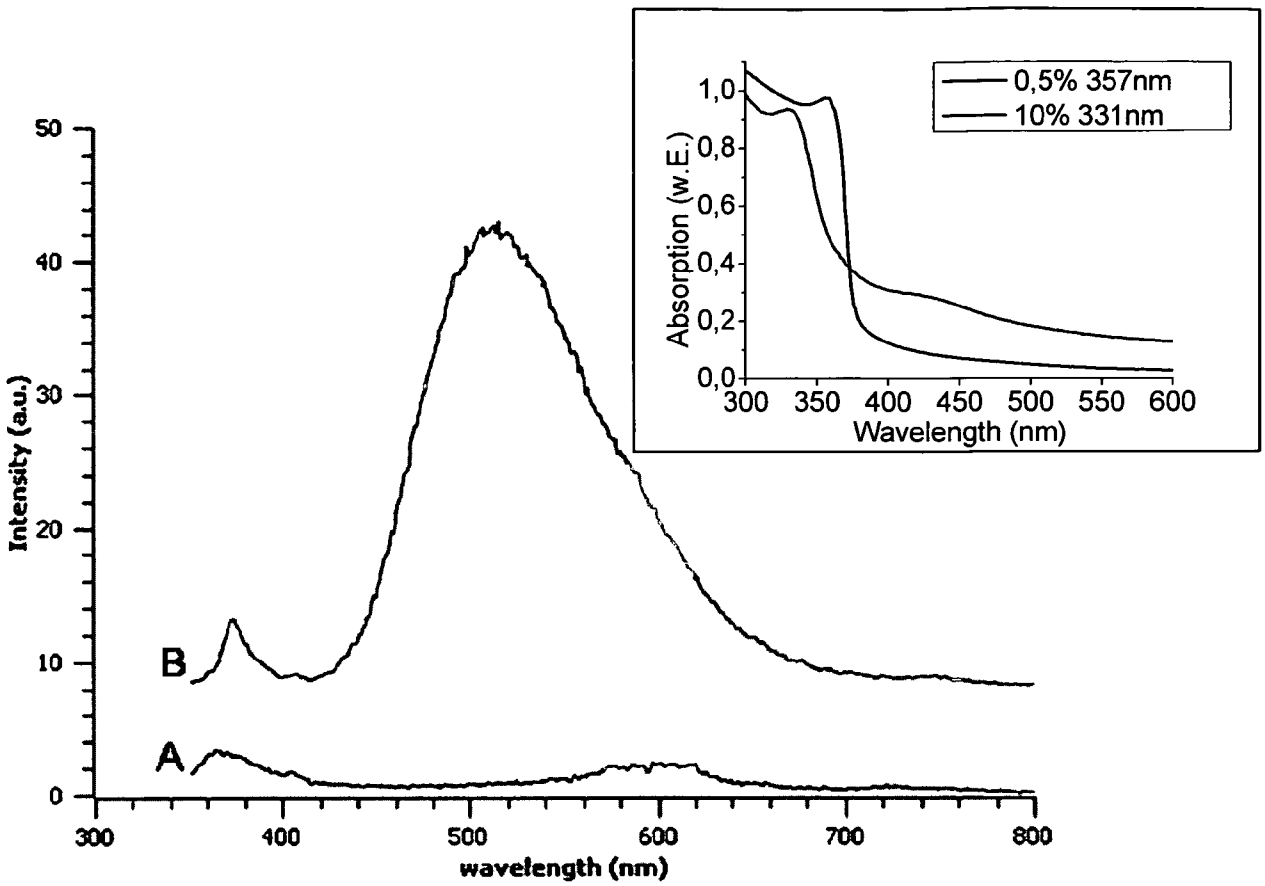

Intensity (a.u.)
wavelength (nm)
B
A
Absorption (w.E.)
Wavelength (nm)
0,5% 357nm
10% 331nm

Figure 5

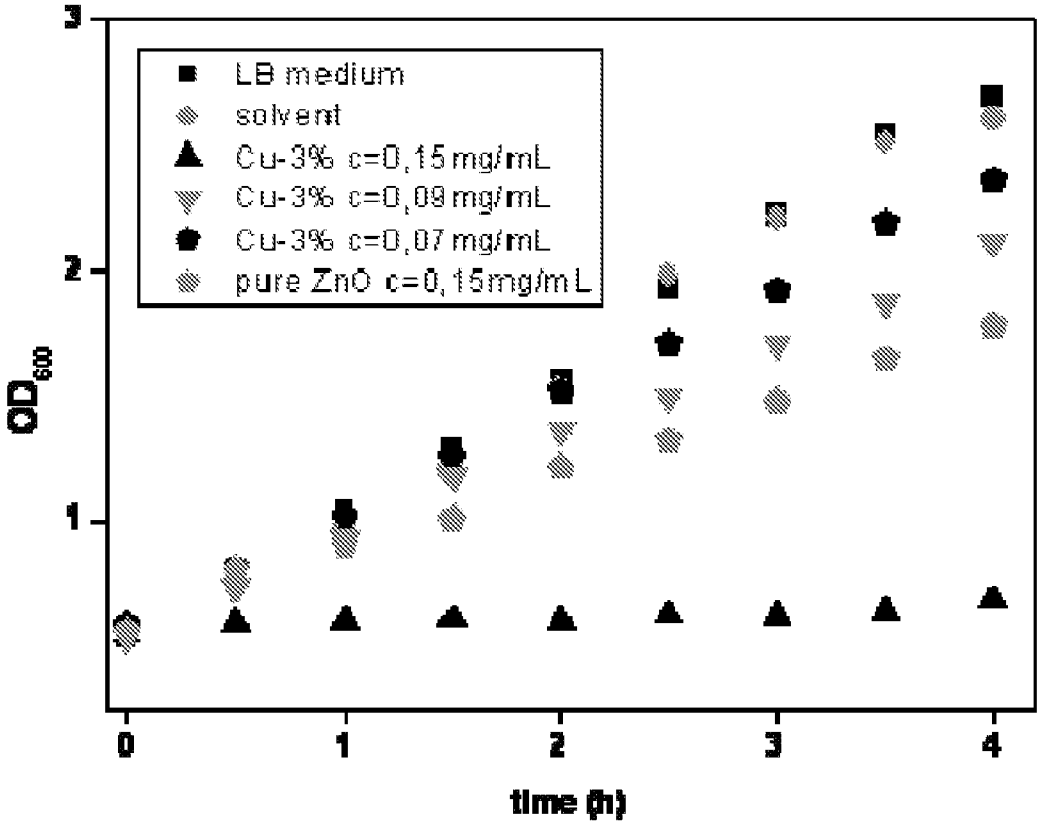


Figure 6

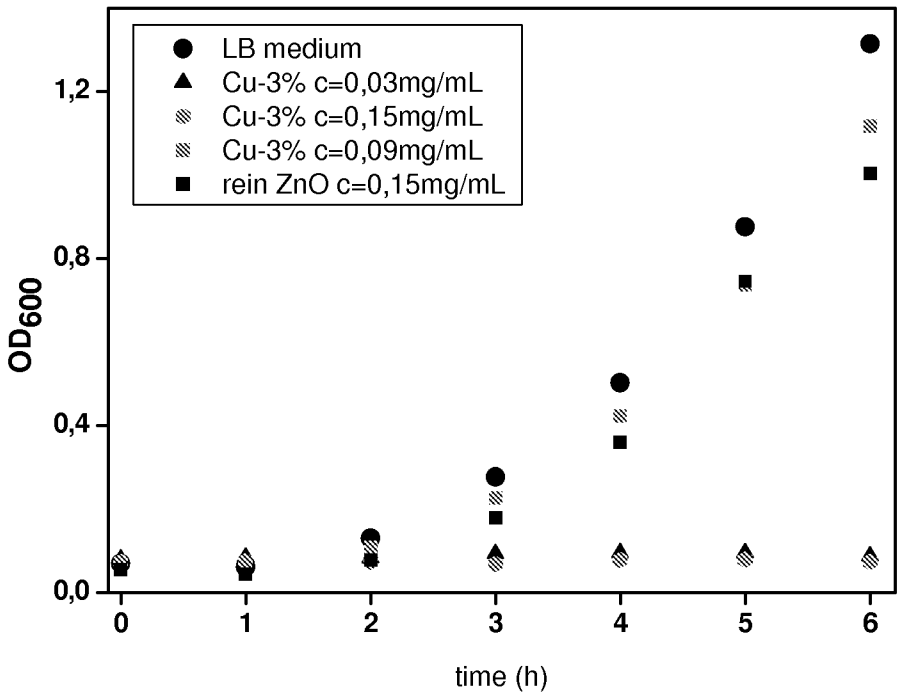


Figure 7

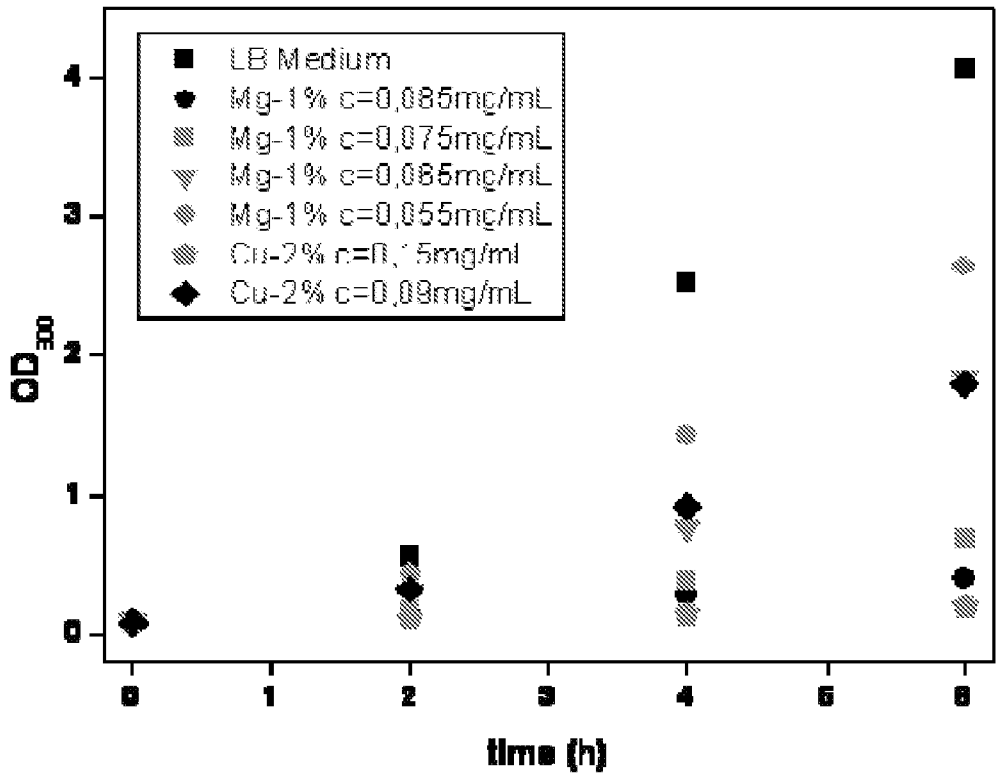


Figure 8

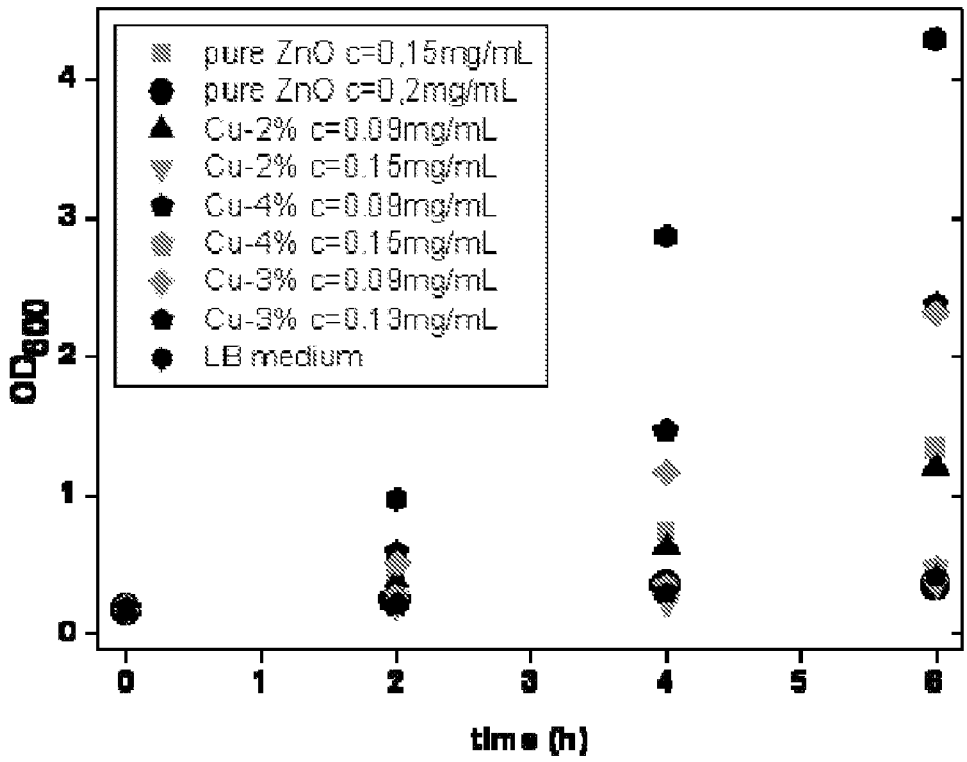


Figure 9

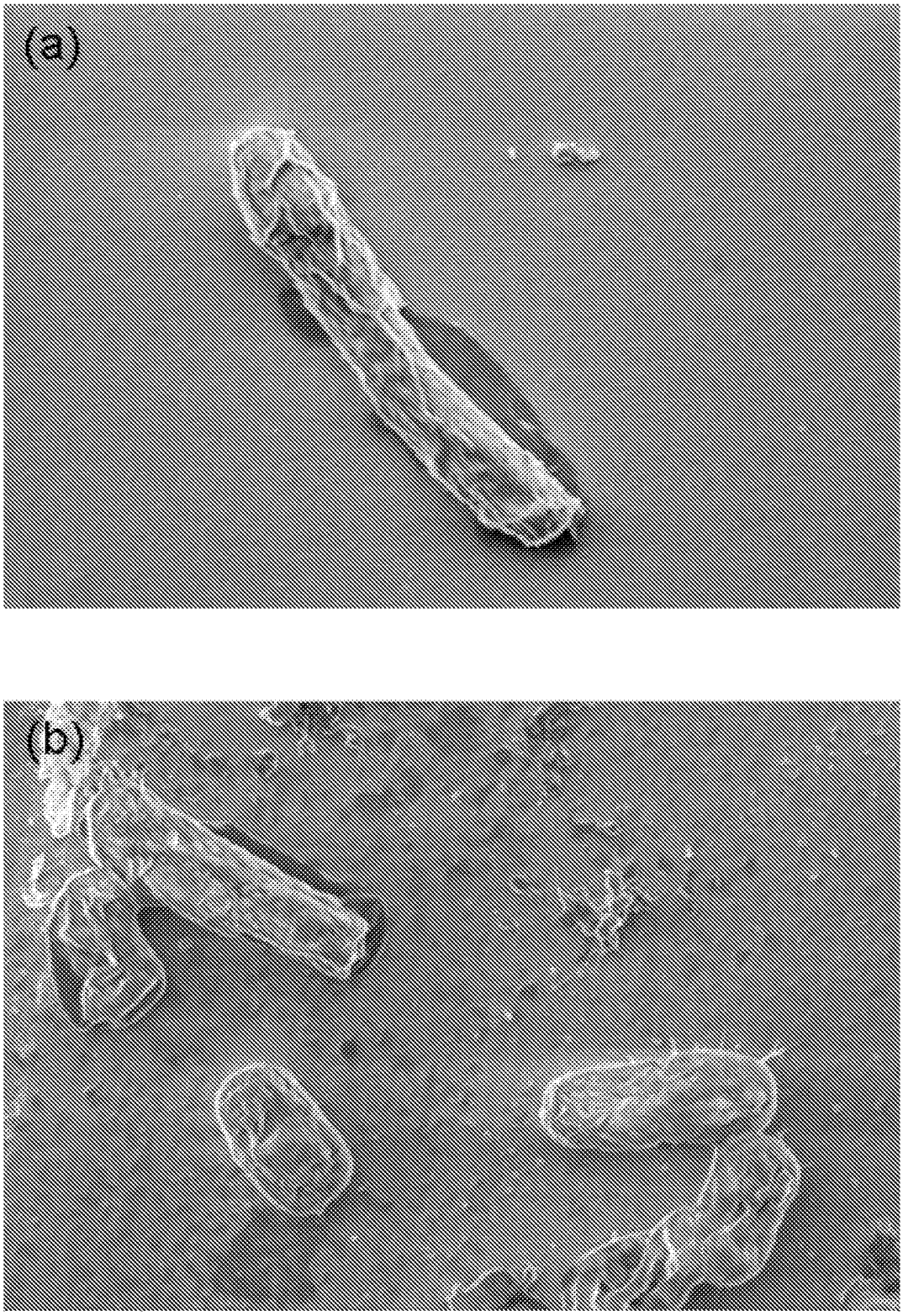


Figure 10

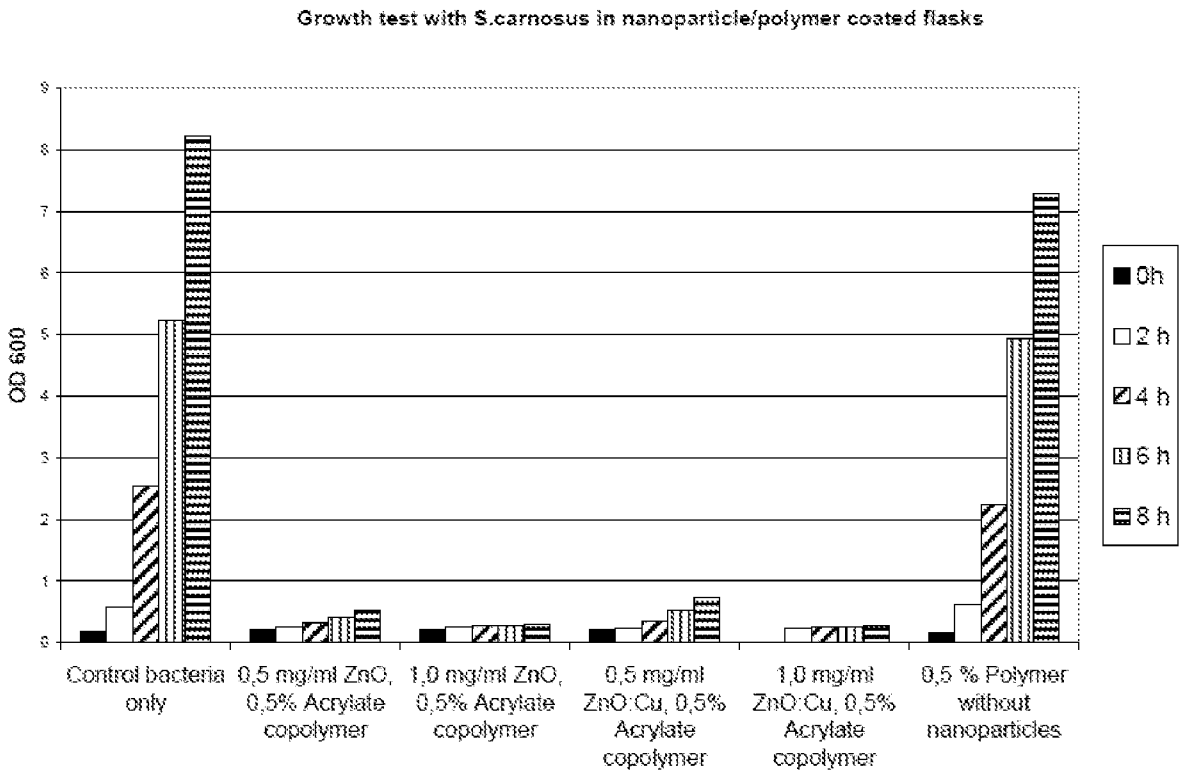


Figure 11

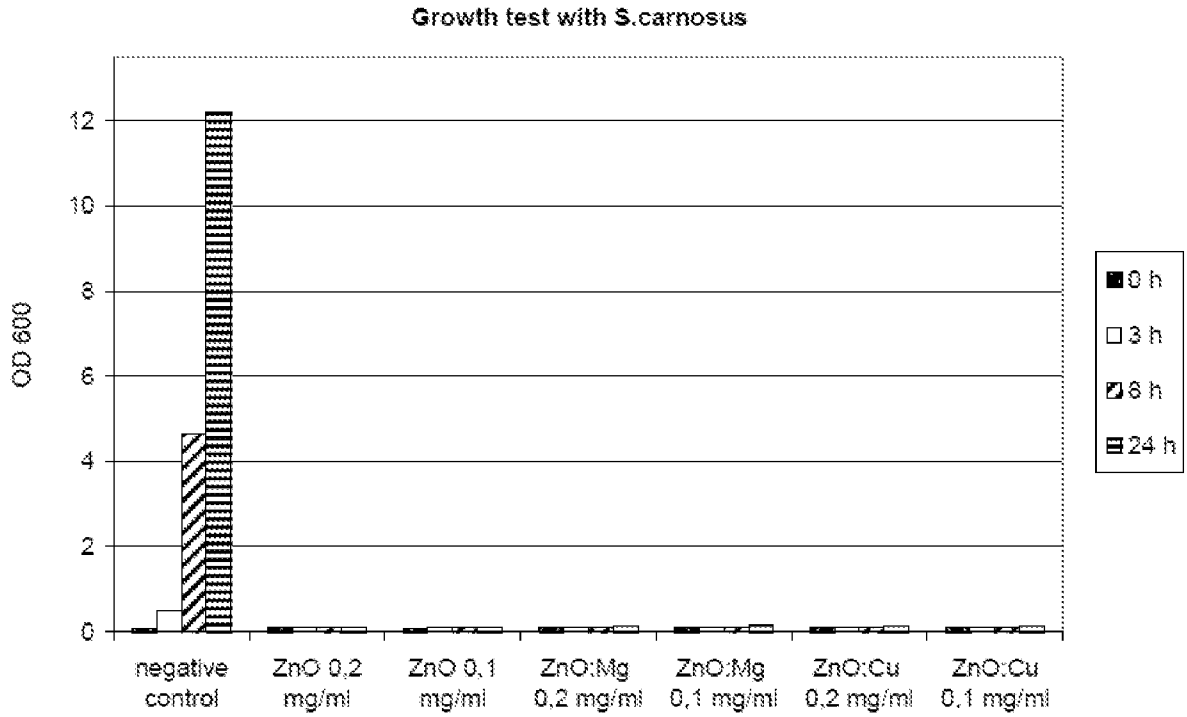


Figure 12

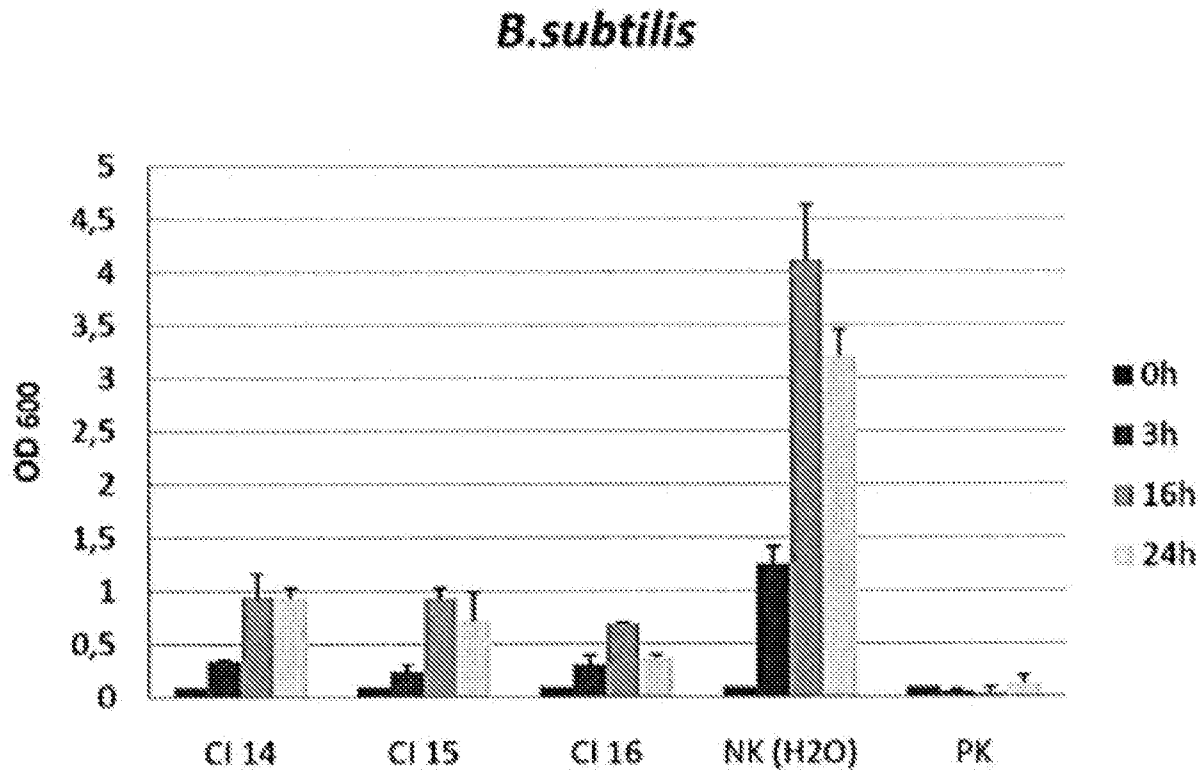


Figure 13

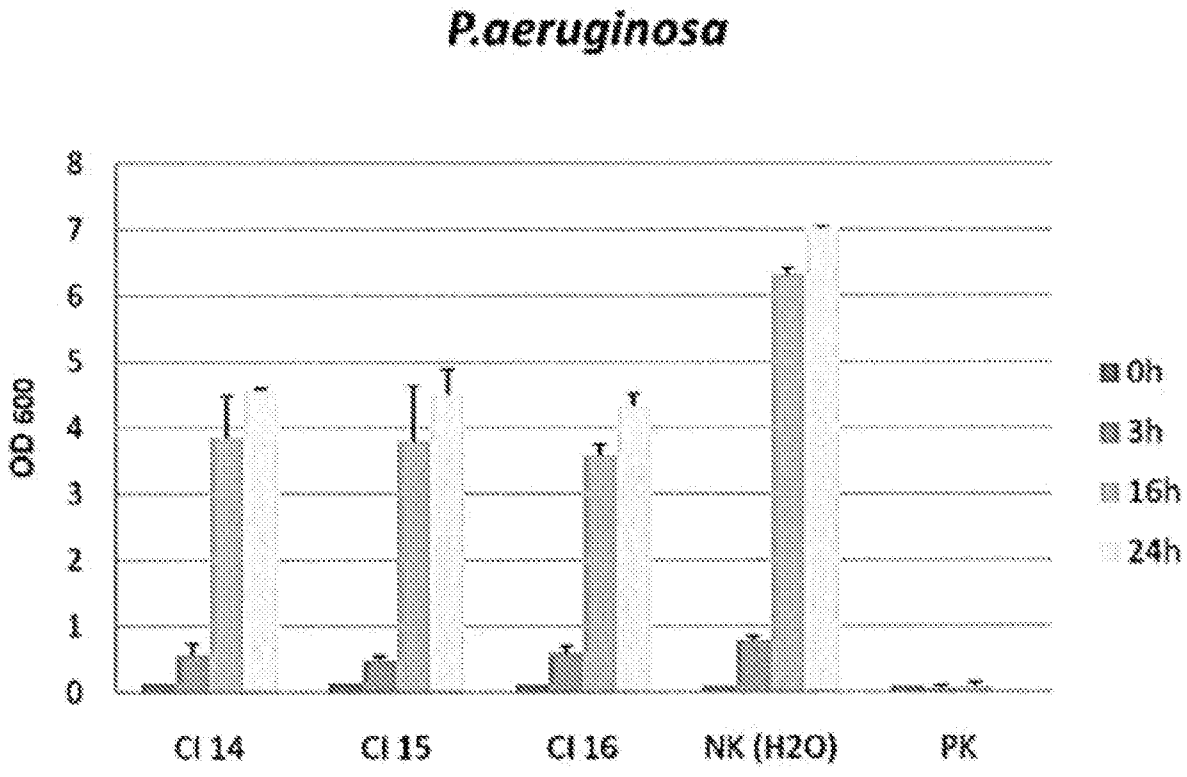


Figure 14

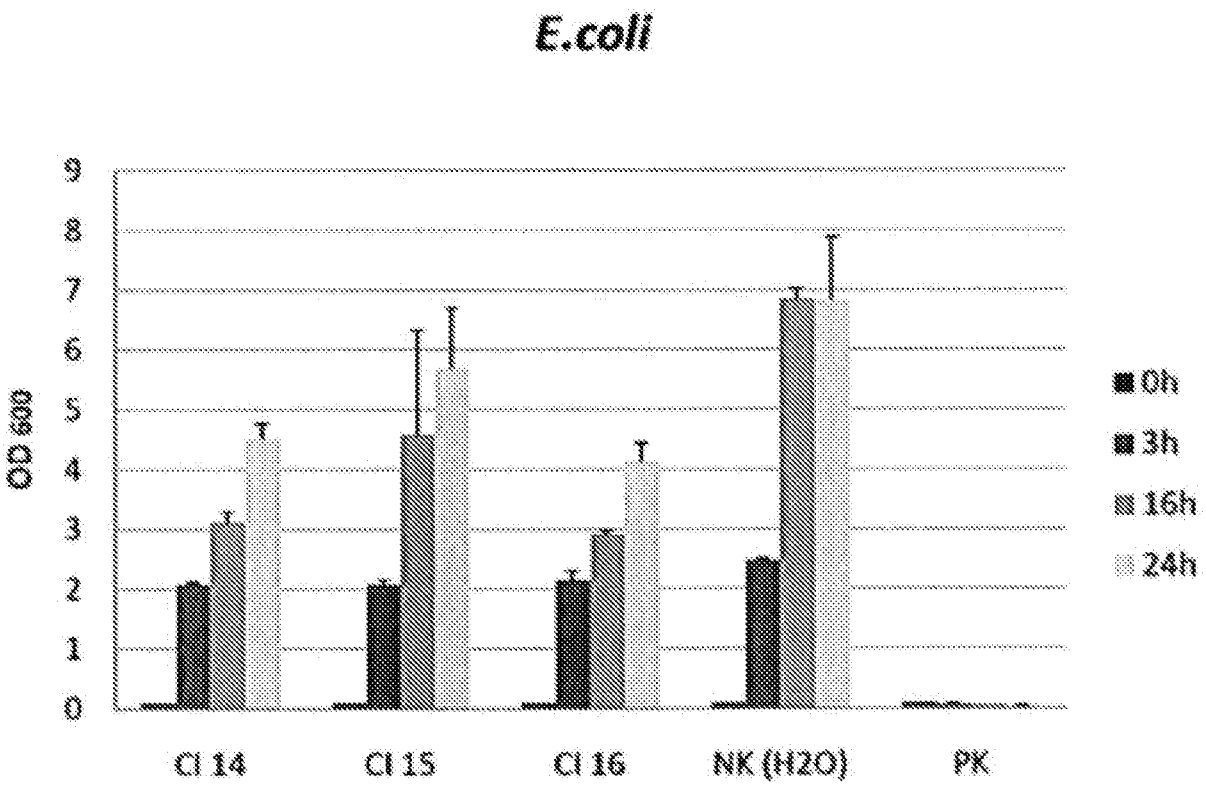


Figure 15

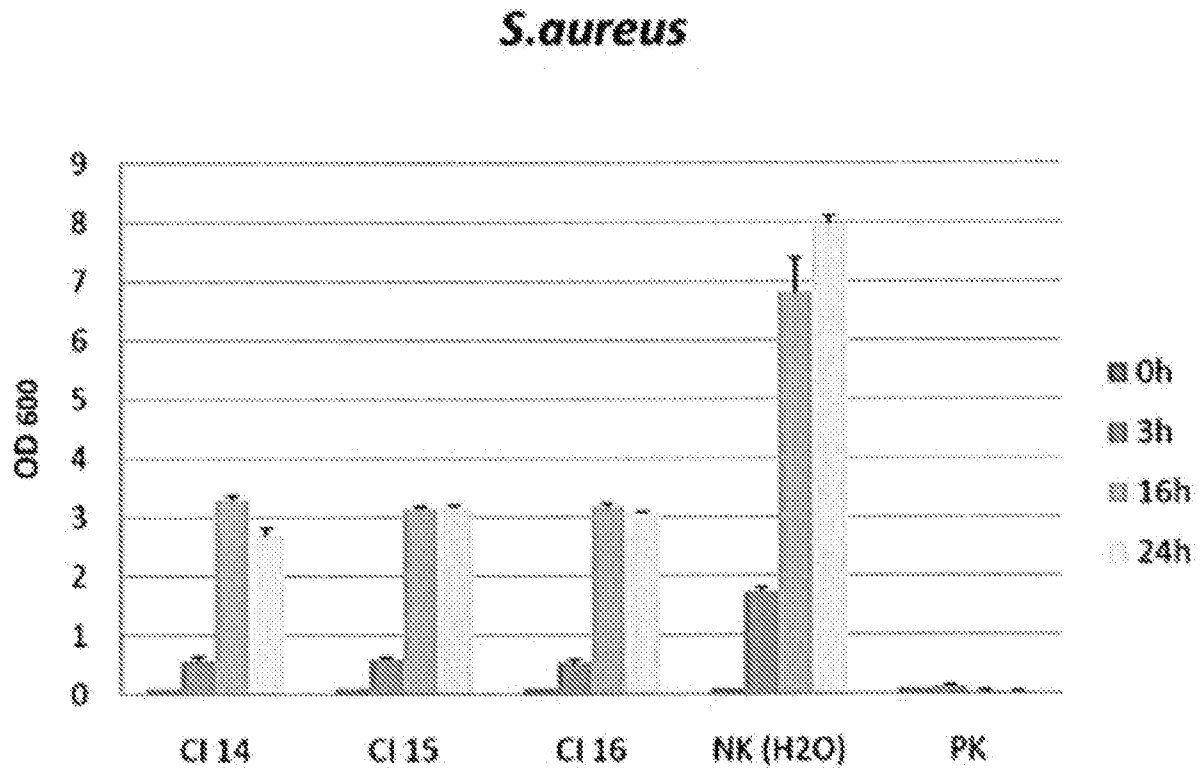
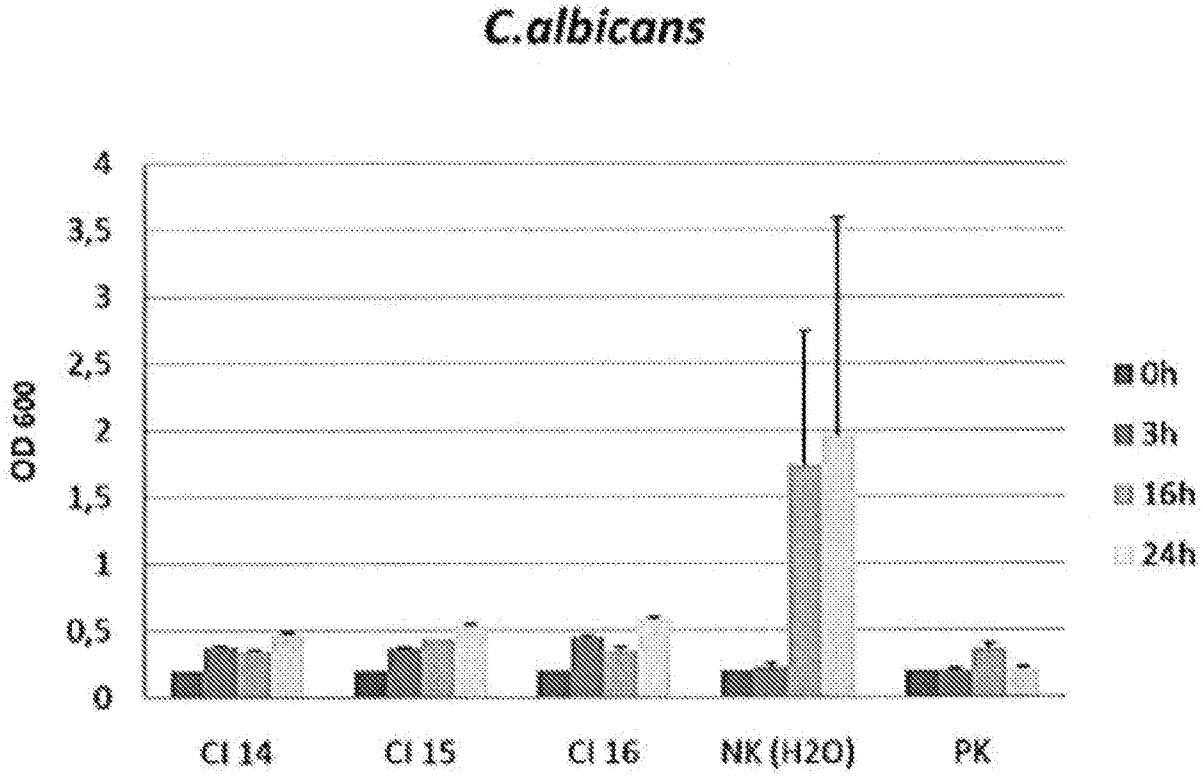


Figure 16


C.albicans
OD 600
4
3,5
3
2,5
2
1,5
1
0,5
0
Cl 14
Cl 15
Cl 16
NK (H2O)
PK
0h
3h
16h
24h