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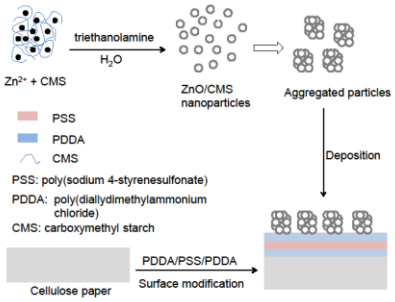
Table of contents entry

A coating of CMS-stabilized ZnO nanoparticles has been deposited on a cellulose paper surface by a layer-by-layer approach. The CMS-stabilized ZnO-coated paper shows a higher brightness, whiteness and UV-stability compared with standard blank paper. The CMS-stabilised, ZnO-nanoparticle-coated paper also shows good antibacterial activity against MRSA and *A. baumannii*.



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ARTICLE TYPE

Whiter, brighter, and more stable cellulose paper coated with antibacterial carboxymethyl starch stabilized ZnO nanoparticles

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Small, carboxymethyl-starch-stabilised zinc oxide nanoparticles with a defined shape, size and morphology were prepared *in situ* in water at relatively low reaction temperatures using soluble carboxymethyl starch (CMS) as a combined crystallising, stabilising and solubilising agent and triethanolamine as the reducing agent. Aqueous colloidal solutions of these CMS-stabilised ZnO nanoparticles were used to deposit a coating of ZnO nanoparticles on cellulose paper by a wet-chemistry, polyelectrolyte, layer-by-layer approach using water as the only solvent. Such cellulose paper samples, coated with these CMS-stabilised ZnO nanoparticles, show higher brightness and whiteness than that of blank reference paper and are more stable to UV-radiation than the paper reference as well as demonstrating good antibacterial activity against MRSA and *A. baumannii*.

1. Introduction

Zinc oxide (ZnO), an n-type semiconductor with wide band gap (3.37 eV)[1] and binding energy (60 meV) [2], has attracted significant interest because of its excellent electrical, optical and chemical properties[3]. Nanosized ZnO can be utilized in electrodes [4], optics [5], optoelectronics[6], sensors[7], and light-emitting diodes [8]. ZnO nanoparticles also exhibit excellent UV protection and antibacterial activity[9 – 12]. It has been reported that ZnO nanoparticles have selective toxicity to bacteria, but exhibit a minimal effect on human cells, which is important taking into account the increasing presence of nanoparticles in the environment.[13 – 16] Studies have also shown that the nanoparticle size and morphology play pivotal roles in determining the antibacterial activities of ZnO powders – antibacterial activity increases with increasing surface area and decreasing nanoparticle size[13, 17, 18].

Many different physical and chemical synthetic approaches have been developed to prepare ZnO nanoparticles, with regard to controlling the morphology, size and shape.[19 – 21] Compared with physical methods, chemical methods, such as precipitation and sol-gel approaches, have shown some distinct advantages for the synthesis of ZnO nanoparticles, including easy scale-up, low reaction temperature and inexpensive equipment.[22] The synthesis is generally carried out in alcohol using zinc salts such as $Zn(ClO_4)_2$, $Zn(NO_3)_2$ or $Zn(Ac)_2 \cdot H_2O$ as starting materials in the presence of a base such as NaOH and LiOH. However, alkali ions (Li+ or Na+) doped in ZnO affects electrical and luminescent properties dramatically. Thus, much effort has been expended in an attempt to purify the final materials by removing the alkali ions through washing.[23] At the same time, many new processes, without addition of base, have also been developed to avoid this problem.[22, 24, 25] Another problem generally occurring with the chemical synthetic methods is the aggregation of nanoparticles in solution in order to minimize surface energy. This aggregation can be prevented or inhibited by the formation of self-assembled monolayers with synthetic polymers and surfactants on the nanoparticle surface during the synthesis.[26, 27, 28]

Recently, cellulose derivatives, including starch, have been developed as a crystallising and stabilising agent to control the shape, size and size distributions of ZnO nanoparticles.[25, 29, 30, 31] Along with their traditional applications in paper and cotton textiles, cellulose derivatives are also very important, environmentally friendly, biocompatible, sustainable and cost-effective, sources of carbon-based polymers and substrates for the development of sophisticated nanocomposite materials.[32] Cellulose is a extensive, linear, mainchain polysaccharide consisting of repeating β-D-glucopyranose moieties covalently linked through acetal functions between the equatorial OH groups. The presence of this very large number of hydrophilic hydroxyl groups [33, 34] promotes the nucleation and growth of inorganic phases at the cellulose fibre surface, which in turn can facilitate the production of cost-effective organic/inorganic nanocomposites.[35, 36, 37]

Protection of cellulose-based materials against different kinds of degradation, such as photochemical and bacterial degradation, and the creation of functional surfaces could be realised by the presence of a functional nanoparticle coating. Small ZnO nanoparticles are much less photocatalytically active than the corresponding TiO_2 nanoparticles of a similar size and shape and so do not photo-degrade the polysaccharide coating or cellulose

substrate, such as textiles, bandages, etc, as similar TiO_2 nanoparticles might well do. Small, spherical ZnO nanoparticles are very efficient absorbers of UV light and can exhibit pronounced antibacterial activity.[9, 10] Surprisingly, there appears to be a limited number of reports of functional ZnO nanoparticle/cellulose nanocomposites. Ling et al reported the deposition of commercial ZnO nanoparticles (d ~ 20 nm) on the surface of paper, by an ultrasound-assisted method, which possesses antibacterial activity against *Escherichia Coli* 11634. [38] Rod-shaped ZnO particles were reported to grow *in situ* on cellulose fibres using a two-step process, i.e., formation of ZnO seeds on a fibre surface and then growth of the seeds into larger particles by controlled hydrolysis of Zn(II)-amine complexes. [30] ZnO-nanoparticle coated papers, with good anti-fungal and UV-protecting properties, were also prepared using a pigment slurry containing a dispersant, china clay and starch-stabilized ZnO nanoparticles (prepared using zinc nitrate and sodium hydroxide as precursors).[29]

Carboxymethyl starch (CMS) is a cellulose derivative with carboxymethyl groups (-CH_2-COOH) bound to the polysaccharide backbone. The polar carboxyl groups render CMS more resistant to heat and bacteria and also more hydrophilic, i.e., more water soluble, than starch. CMS is a cheap, renewable resource that is used commercially in food science as a viscosity modifier or thickener and to stabilize emulsions in various products. Therefore, we decided to investigate the use of CMS to prepare novel, water-soluble CMS-stabilised ZnO nanoparticles. We wished to evaluate them as a functional, protective coating for cellulose fibres deposited using a combination of a simple, dip-coating technique *and* an efficient wet-chemistry polyelectrolyte layer-by-layer paper coating process,[39, 40, 41] both of which use water as the solvent. No impact or spray coating techniques, chemical binders, surfactants, dispersants or a post-treatment curing step are required. The CMS-stabilised ZnO nanoparticles are prepared in a simple fashion using water as the solvent at relatively low temperatures using triethanolamine as the reducing agent and CMS as a combined crystallising, stabilising and solubilising agents. A much lower loading of nanoparticles is required using this surface-based approach than dispersing nanoparticles in the bulk fibre mixtures used to prepare paper, fabrics, textiles, etc., which is advantageous in terms of minimising contamination of the environment with nanoparticles.

This approach could facilitate the mass manufacture of lighter sheets of printer paper, for example, which would represent a significant saving in the enormous amounts of pulp used in paper manufacture, be much more energy efficient and produce less contaminated waste water.[41] It could also have a significant impact on minimising the transfer of multidrug resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is a constituent part of the natural flora of the human body, between patients in a hospital environment. *Staphylococcus aureus* is as an opportunistic pathogen responsible for serious infections, which can often be treated with broad-spectrum antibiotics, such as oxacillin.[30] However, some bacteria are becoming ever more resistant to many types of organic antibiotics, particularly β-lactams, which can lead to much more extensive hospital stays and even, in some cases, to death.[31] Therefore, the prevention of infections caused by multidrug resistant bacteria, such as MRSA, could reduce patient mortality and associated treatment costs. Antibacterial coatings of small ZnO nanoparticles with a large active surface area on cellulose bandages, uniforms and bed linen, for example could make a significant contribution to reducing bacterial transfer and also preventing injury- or elective surgery-related wound infections.

2. Experimental

2.1. Materials and characterization methods

The experiments were performed using the following chemical substances: zinc nitrate hexahydrate $Zn(NO_3)_2{\cdot}6H_2O$, triethanolamine, poly(diallydimethylammonium chloride) (PDDA, 20 wt% in water, MW 100,000 – 200,000) and poly(sodium 4-styrenesulfonate) (PSS, MW 70,000) were supplied by Aldrich and used as received. Carboxymethyl starch (CMS, MW 5,000,000) was provided by TiTk, Germany. Ultrapure water with the specific resistance of 18.2 MΩ·cm was obtained by reversed osmosis followed by ion-exchange and filtration (UPQ PS system, ELGA, USA). The Mondi cellulose papers which are 1 mm thick were provided by Mondi group.

Scanning electron microscopy (SEM) images were obtained using Carl Zeiss SMT 'EVO60' SEM microscope operating at 20 kV and EDX data were obtained using an Oxford Instruments 'INCA' Energy Dispersive X-ray Spectrometer. Transmission electron microscopy (TEM) was collected using a Jeol 2010 TEM running at 200 kV. Images were obtained with a Gatan Ultrascan 4000 digital camera. Solid samples were prepared by suspension in distilled water and 5 μL aliquots of a suitable dilution dropped onto carbon coated copper grids. Thermogravimetric analyses (TGA) were performed on a Netzsch TGA TG209 thermal balance. The amounts of zinc were determined by an inductively coupled Perkin Elmer plasma 40 emission ICP instrument. X-ray powder diffraction (XRD) analysis was carried out using a SIEMENS D5000 Instrument. The nanoparticle size was estimated by using the Scherrer equation, [42] $D_{h,k,l} = 0.89\lambda/\beta cos\theta$, where D is the crystallites size, λ the X–ray wavelength used, 0.154 nm, β the broadening of the diffraction line measured at half of its maximum intensity (FWHM) and θ, the corresponding angle. The whiteness was measured with a standard whiteness tester (Lorentzen & Wettre, Elrepho). Brightness was determined using a Suntest XLS^+ instrument from ATLAS Material Testing Solutions (90 min at 500 W and at 2700 kJ m^{-2}).

2.2. Aqueous colloidal solutions of CMS-stabilised ZnO nanoparticles

The synthesis of ZnO nanoparticles in the presence of carboxymethyl starch was performed as follows. $Zn(NO_3)_2{\cdot}6H_2O$ (0.44 g) was first dissolved in water (10 mL), CMS solution (0.2%, 90 mL) was added slowly under stirring to the reaction mixture, which was then heated at 90 °C for 3 h. Triethanolamine was then added and the reaction mixture was heated at 90 °C for a period of either 3 or 20 h. After cooling in ice, in order to stop the reaction quickly, the reaction mixture was centrifuged at 10,000 rpm for 15 min. The isolated CMS-stabilised ZnO

nanoparticles were washed three times with distilled water, in order to remove by-products and excess soluble CMS, and then dried under vacuum overnight.

2.3. Coating of cellulose paper with CMS-stabilised ZnO nanoparticles

Firstly, two aqueous solutions of polyelectrolytes were prepared, i.e., 1% (wt/v) of PDDA in 0.5 M NaCl and 1% (wt/v) of PSS in 0.5 M NaCl. Samples of cellulose paper were then immersed (10 min) first in the PDDA solution, then in the PSS solution and finally again in the PDDA solution. After each immersion step, the cellulose paper samples were washed with water and dried under vacuum. Aqueous colloidal solutions of CMS-stabilised ZnO nanoparticles were prepared by adding ZnO/CMS (100 mg) nanoparticles to distilled water (12 mL) and the resultant mixtures sonicated for 15 min. Then, the PDDA/PSS/PDDA-treated cellulose paper samples (~ 0.6 g) were immersed in the colloidal solutions of CMS-stabilised ZnO nanoparticles at room temperature and then sonicated for a further 15 min. The ZnO/CMS-coated paper samples were then washed with water and dried under vacuum overnight.

2.4. Antibacterial properties of cellulose paper coated with CMS-stabilised ZnO nanoparticles

The antibacterial properties of colloidal solutions of CMS-stabilised ZnO nanoparticles and those of the corresponding cellulose paper samples, with a coating of identical CMS-stabilised ZnO nanoparticles, were assessed using diffusion and microtitre assays against bacterial isolates. Isolates of *Pseudomonas aeruginosa* (Clinical isolate PA3 from blood) and *Burkholderia cenocepacia* (Clinical isolate BCC1 from cerebral spinal fluid) were identified *via* biochemical profiling with API-20NE (BioMerieux, La Balme Les Grottes, France) and MALDI-TOF mass-spectrometry (Bruker, Coventry, UK).and obtained from the Health Protection Agency laboratories, Colindale, UK. Type strains of methicillin resistant *Staphylococcus aureus* (NCTC 12493), *Acinetobacter baumannii* (NCTC 12156) purchased from Pro-lab diagnostics, Wirral, UK and *Stenotrophomonas maltophilia* (NCTC 10258) purchased from the Health Protection Agency laboratories, Porton Down, UK. These isolates were chosen due to their resistance to antibiotics and their ability to cause serious skin and wound infections and infections associated with invasive devices such as catheters and intravenous lines (IV). All media was purchased from Oxoid, Basingstoke, UK and autoclaved prior to its use.

The antibacterial activity of aqueous colloidal solutions of the CMS-stabilised ZnO nanoparticles against all bacterial isolates was determined using a microtitre assay. Stock aqueous solutions of the CMS-stabilised ZnO nanoparticles were prepared by the addition of 4 mg of ZnO powder to sterile distilled water (10 mL). Solutions were shaken and then sonicated for 15 mins to form stable, aqueous colloidal solutions. Double dilutions of the stock solutions were performed in IsoSensitest broth before samples (100 µL) of each concentration were pipetted into individual microtitre wells. A 100 µL sample of broth, containing the bacterial inoculums (10^6 colony forming units [CFU]/mL) taken and diluted from 6 h cultures (Stationary phase of growth), was then added to each well. The final well concentration of the CMS-stabilised ZnO nanoparticles ranged from between 200 and 0.195 µg/mL. All the microtitre plates were incubated at 37 °C for 24 h before being checked for the presence of bacterial growth. A solution of 0.2% CMS in water was used as a control.

Iso-Sensitest agar plates were individually inoculated with isolates of MRSA (NCTC 12493) and *A. baumannii* (NCTC 12156) using the standardized method by Moosdeen *et al.*[43] Paper samples coated with the ZnO nanoparticles or PDDA/PSS/PDDA coated control papers were added to individual inoculated agar plates (10^6 CFU/mL), which were then incubated at 37 °C for 24 hours. Three replicate experiments were performed for each agar plate in order to confirm the results.

The antibacterial activity of the cellulose paper samples coated with or CMS-stabilised ZnO nanoparticles were evaluated using a method based on that by Pollini *et al.* [44] Antibacterial action was rated "good" (zone of inhibition > 1 mm), "fairly good" (zone of inhibition ≤ 1 mm), "sufficient" (growth up to, but not on, the paper sample), "limited" (limited growth on the paper sample) or "poor" (paper sample is overgrown with bacteria ≥ 50 %).

3. Results and discussions

3.1. Preparation of CMS-stabilised ZnO nanoparticles

Dissolving CMS (0.2%) in hot water under stirring gives a solution with a high viscosity. No colloidal solution forms after heating of the $Zn(NO_3)_2 \cdot 6H_2O$ – CMS solution at 90 °C for 3 h. The solution only then turns into a colloidal solution after the addition of triethanolamine. A white, milky colloidal solution is obtained after reaction at 90 °C for 3 h. However, the colloidal solution is not very stable as evidenced by the formation of precipitates upon standing at 0 °C over night. After centrifuging, washing and drying, a white sponge (0.084 g) was obtained. XRD analysis indicates the formation of cubic ZnO, with an average nanoparticle size of about 10 nm (Figure 1). However, reaction at 20 h gives a white powder, which has a similar XRD pattern to that of the sample prepared by heating for 3 h. The IR spectra of both samples are the same as that of CMS itself, indicating the formation of ZnO/CMS nanocomposites (Figure 2). TGA curves of both samples show that the main reduction in mass occurs at 300 – 440 °C due to the decomposition of CMS. The amount of residue at 800 ˚C is 74.7 and 83.6% for the CMS-stabilised ZnO nanoparticles prepared by heating for 3 h or 20 h, respectively, suggesting that the content of carboxymethyl starch present in the composite decreases with increasing reaction time (Figure 3). TEM images also show the formation of large (d ~500 nm) ZnO/CMS granules (Figure 4) composed of numerous small, spherical CMS-stabilised ZnO nanoparticles (d ~ 10 nm). It can also be noticed from the TEM images that the particles of ZnO/CMS-20h are more compact than those of ZnO/CMS-3h due to less of CMS presence in the particles. The large granules with particle size of 500 ± 50 nm for ZnO – 20h and 550 ± 50 nm for ZnO – 3h are also observed in the corresponding SEM images (Figure 5). The formation of aggregated large particles in polysaccharides solution, such as starch solution, has also been reported because polysaccharides have high number of coordinating functional groups.[25] When $Zn(NO_3)_2$ was added to the CMS solution and heated at 90 ˚C for 3 h, the zinc ions are probably closely associated with the CMS molecules. Thus,

nucleation and initial crystal growth may preferentially occur within regions of both high CMS and Zn^{2+} concentration, leading to the formation of about 10 nm nanoparticles in order to reduce the high surface area as is often the case for nanoparticles. In a further step, they aggregate and form larger spherical CMS-stabilised ZnO nanoparticles. In this system, the CMS probably acts both as a flocculant and facilitator of nanoparticle aggregation.[25]

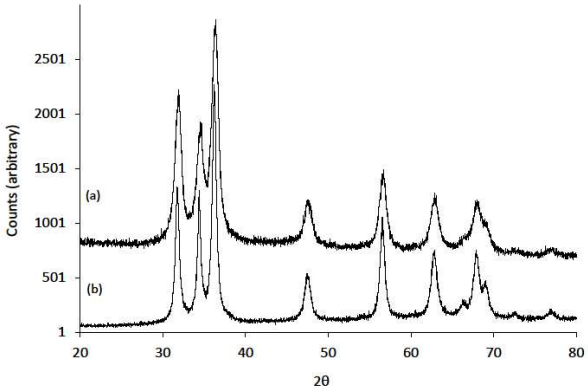


Fig. 1 XRD patterns of ZnO/CMS prepared at 90 °C for 3 h (a) and 20 h (b).

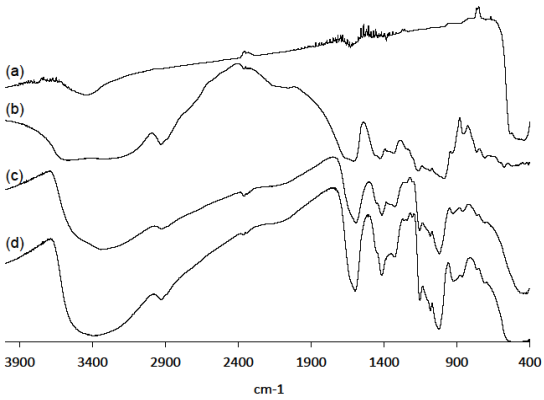


Fig. 2 IR spectra of (a) ZnO, (b) CMS and ZnO/CMS nanoparticles prepared at 90 °C for 3 h (c) and 20 h (d).

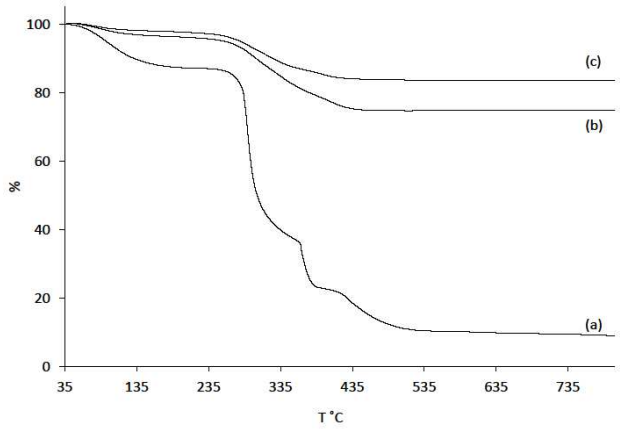


Fig. 3 TGA of (a) CMS and (b & c) ZnO/CMS nanoparticles prepared at 90 °C at different times (b) 3h and (c) 20 h.

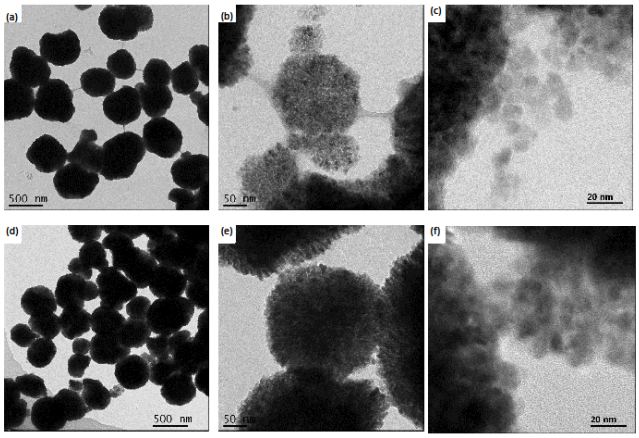


Fig. 4 TEM image of the CMS-stabilised ZnO nanoparticles and aggregates prepared at 90 °C for 3 h (a, b and c) and 20 h (d, h and f).

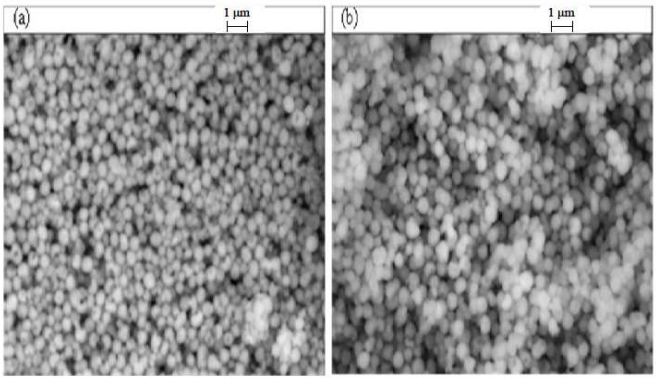


Fig. 5 SEM image of the CMS-stabilised ZnO nanoparticles and aggregates prepared at 90 °C for 20 h (a) and 3 h (b).

3.3. Coating of cellulose paper with CMS-stabilised ZnO nanoparticles

Cellulose fibres are normally negatively charged over a wide pH range, due to the presence of ionisable moieties, such as carboxyl or hydroxyl groups. CMS-stabilised ZnO nanoparticles, in contact with neutral aqueous solutions, are also negatively charged. Therefore, we have developed a Layer-by-Layer procedure to induce a positive charge on the surface of cellulose paper samples in order to facilitate the coating and fixing of these samples with CMS-stabilised ZnO nanoparticles making use of strong coulombic interactions.

The XRD spectra of the cellulose paper samples coated with ZnO/CMS nanoparticles (Figure 6) show peaks attributable to cubic ZnO in addition to a strong peak at 23 ° attributable to cellulose itself. SEM images of the ZnO/CMS coated cellulose papers clearly show that nearly no CMS-stabilised ZnO particles have been deposited on the cellulose fibres, if the paper surfaces have not been treated first using the Layer-by-Layer procedure (Figure 7). In contrast CMS-stabilised ZnO nanoparticles are seen to have been deposited and fixed on LBL-treated paper surfaces and are not removed by repeated washing with copious amounts of water. A relatively homogenous coating of CMS-stabilized ZnO nanoparticles can be observed on the LBL-treated paper surfaces in the SEM images (Figure 7). The loading of ZnO on the paper samples is 2.73 and 3.83% for the cellulose papers coated with CMS-stabilized ZnO nanoparticles prepared at 90 °C for 3 and 20 h, respectively.

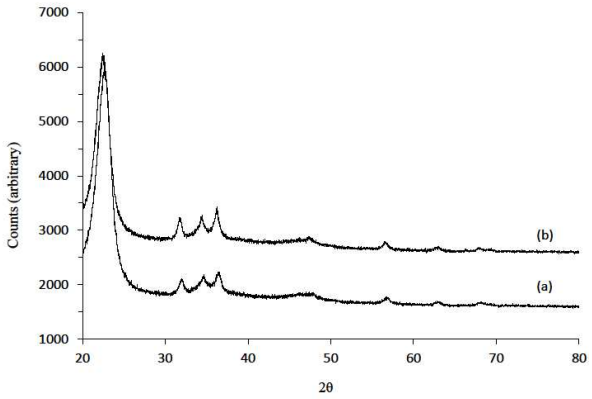


Fig. 6 XRD patterns of the cellulose paper samples coated with ZnO/CMS nanoparticles prepared at different reaction times (a) 3 h and (b) 20 h.

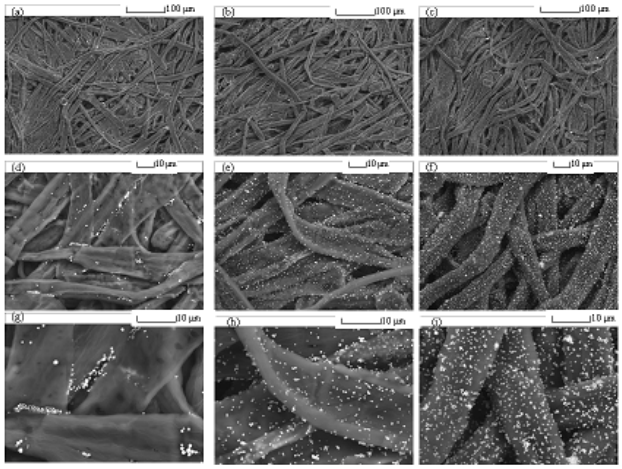
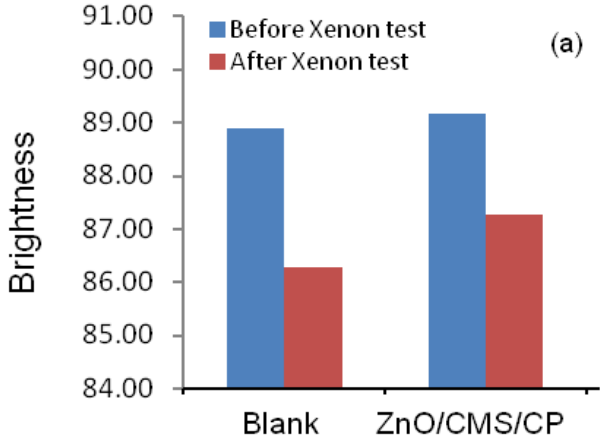
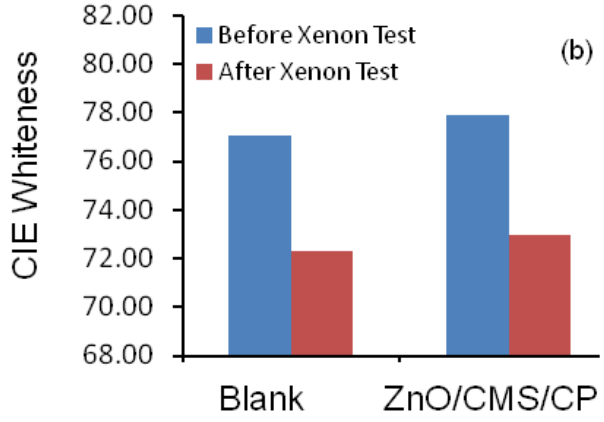


Fig. 7 SEM images of ZnO/CMS coated cellulose papers. (a, d, g) samples without an LBL treatment, but with immersion in a colloidal solution of CMS-stabilized ZnO nanoparticles prepared at 90 °C for 20 h. All the other samples have been treated using a LBL approach followed by immersion in colloidal solutions of CMS-stabilized ZnO nanoparticles prepared at different reaction times. (b, e, h) 3 h and (c, f, i) 20 h.





The ISO-brightness and CIE whiteness of blank reference paper and the paper samples coated with a thin layer of ZnO/CMS nanoparticles, before and after the xenon UV-stability test, are shown in Figure 8. It can be seen that both the brightness and whiteness of the samples of cellulose paper coated with CMS-stabilised ZnO nanoparticles are both higher than the corresponding values determined for the blank paper reference.

3.4 Antibacterial activity of the or CMS-stabilised ZnO coated cellulose paper samples and colloid solutions

Results from the microtitre assays for the two CMS-stabilised ZnO nanoparticle samples, ZnO/CMS-3 and ZnO/CMS-20, indicate that each of these aqueous colloidal solutions demonstrate an antibacterial activity against all of the bacterial isolates tested (Table 1), with the exception of ZnO/CMS-3 against *S. maltophilia* (NCTC 10258), where concentrations ≥200 µg/mL of both ZnO/CMS samples were required to induce inhibition of bacterial growth. The highest activity was observed against *P. aeruginosa*, where inhibition is seen at concentrations of 25 – 50 µg/mL. As expected the CMS control solution was found to have no antibacterial effect and actually resulted in increased bacterial growth at 0.2% compared with lower concentrated dilutions. The antibacterial activity of the cellulose paper samples coated with CMS-stabilised ZnO nanoparticles against MRSA and *A. baumannii* are shown in Table 2.

Table 1. Minimum inhibitory concentrations of aqueous colloidal solutions of CMS-stabilised ZnO nanoparticle against bacterial isolates (µg/mL)

Bacterial Isolate	ZnO/CMS-3	ZnO/CMS-20	CMS solution
P. aeruginosa (PA3)	50	50	No effect
B. cenocepacia (BCC1)	50	100	No effect
MRSA (NCTC 12493)	100	100	No effect
S. maltophilia (NCTC 10258)	>200	200	No effect
A. baumannii (NCTC 12156)	100	200	No effect

ZnO/CMS-3 and -20 samples are paper samples coated with CMS-stabilized ZnO nanoparticles prepared at 90 ˚C for 3 and 20 h, respectively. It can be been seen that ZnO/CMS/CP-20 exhibits a more significant antibacterial effect against MRSA than that of ZnO/CMS/CP-3 (Table 2). There is a clear antibacterial effect against MRSA for the paper sample treated with ZnO/CMS/CP-20 with a clear zone of inhibition (Figure 9). Against *A. baumannii* there appeared to be no significant difference in antibacterial action between ZnO/CMS/CP-3 and -20. Control papers coated only with PDDA/PSS/PDDA were found to have no antibacterial effect with bacterial growth occurring up to and on the papers.

Table 2. Antibacterial activity and zones of inhibition of cellulose paper samples coated with CMS-stabilised ZnO nanoparticles against isolates of meticillin resistant *Staphylococcus aureus* (MRSA) NCTC 12493 and *Acinetobacter baumannii* NCTC 12156.

ZnO Sample	*Bacterial isolate*	
	MRSA	*A. baumannii*
ZnO/CMS/CP-3	Fairly good (1 mm ± 0.5)	Fairly good (1 mm ± 0.5)
ZnO/CMS/CP-20	Good (5 mm ± 0.5)	Fairly good (1 mm ± 0.5)
PDDA/PSS/PDDA Control	Poor (0 mm ± 0)	Poor (0 mm ± 0)

Zones of inhibition are presented as the diameter of the area of no bacterial growth, minus the diameter of the paper itself.

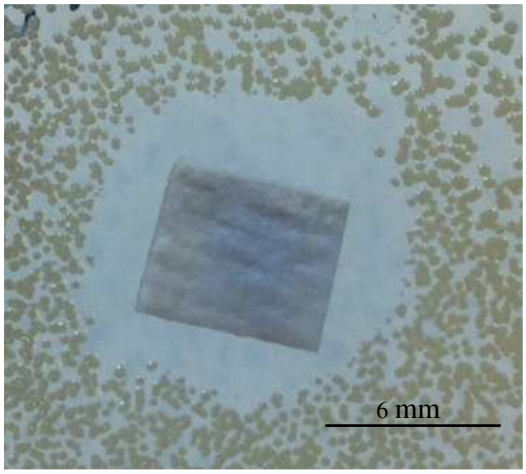


Fig. 9 Antibacterial effect of ZnO/CMS/CP-20 paper against MRSA NCTC 12493.

The ZnO/CMS-20 paper was washed with ethanol after the antibacterial test to remove bacteria (dead or living) and then checked again using SEM analysis. No significant difference in ZnO nanoparticle size can be observed when comparing the SEM images before and after the antibacterial test (see support information). That means that the ZnO/CMS particles are quite stable during the antibacterial test and after the ethanol washing process.

Potential mechanisms involved in the antibacterial activity of the CMS-stabilised ZnO nanoparticles include production of reactive oxygen species (ROS), which are highly reactive chemical agents, and which contain oxygen, e.g., H_2O_2. These agents are formed in the presence of ZnO nanoparticles and they can result in damage to bacterial DNA and cell membranes (lipid peroxidation) and protein dysfunction. The release of zinc ions is proposed as a mechanism of the antimicrobial action of nanoparticles in this work. Zinc ions released from nanoparticles can interact with thiol groups on essential bacterial enzymes, resulting in their inactivation and consequently leading to cell death. Another potential mode of action is through impaired membrane function, which is thought to be due to electrostatic

interaction of ZnO nanoparticles and the surface of the bacteria resulting in ZnO nanoparticle aggregation on the cell surface and changes in cell morphology, leading to significant cell growth inhibition.[45] All the proposed mechanisms have greater potency in ZnO nanoparticles than ZnO macroparticles/solid zinc due to the nanoparticles increased surface area and reactivity, resulting in greater interactions with cells and the release of higher concentrations of zinc ions. The mechanism behind the resistance, or lower susceptibility, to ZnO nanoparticles of *S. maltophilia* (NCTC 10258) could include the active efflux of the ZnO nanoparticles from the bacterium and a reduced uptake due to a reduced number of membrane transporters.[46]

Several explanations could account for the lower antimicrobial effect of ZnO/CMS-20 than ZnO/CMS-3 against *B. cenocepacia* and *A. baumannii*. Although ZnO/CMS-20 solutions have a greater number of nanoparticles, the amount of CMS per nanoparticle is less as shown by TGA analysis, resulting in more compact groups of nanoparticles and reduced interaction with the surface if the bacterial cells. Another explanation could be that the increased number of nanoparticles in ZnO/CMS-20 could trigger increased active efflux in these bacteria resulting in lower concentrations of ZnO within the bacterial cells. Future work should investigate the actual mechanisms behind the variances in antimicrobial effect of ZnO nanoparticles between different bacterial species

The concentrations of CMS-stabilised ZnO nanoparticles required to inhibit bacterial growth are relatively high, when compared to that of most conventional antibiotics. However, the potential for using aqueous colloidal solutions of or CMS-stabilised ZnO nanoparticles in a medical setting is high, as they could be used topically or as coatings for bandages, beddings, uniforms and medical devices, for example, at a low overall concentration.

It should be noted that although ZnO is an effective antimicrobial agent, that they would not be suitable to use against bacterial isolates, which produce the metallo-β-lactamase enzymes and which sequester zinc for use in degrading beta-lactam antibiotics. The use of ZnO would increase the likelihood of antibiotic treatment failure. In this case an alternative metal nanoparticle, such as silver, would be more appropriate.

4. Conclusions

Spherical, carboxymethyl-starch-stabilised ZnO nanoparticles with a cubic crystal structure were prepared *in situ* in water at relatively low reaction temperatures using water-soluble carboxymethyl starch (CMS) as a combined crystallising, stabilising and solubilising agent and triethanolamine as the reducing agent. The CMS-stabilised ZnO nanoparticles can be re-dispersed in water to create stable aqueous colloidal solutions, which can then be used to deposit a coating of CMS-stabilised ZnO nanoparticles on the surface of samples of cellulose paper by a wet-chemistry polyelectrolyte layer-by-layer approach. Compared with a blank paper reference, the ZnO/CMS-coated paper samples show higher brightness and whiteness and a greater stability under UV-illumination than those of the reference and also good show antibacterial activity against MRSA and *A. baumannii*.

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