Evaluation of Antibacterial Activities of Zinc Oxide-Titanium Dioxide Nanocomposites Prepared by Sol-Gel Method

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ABSTRACT

The antibacterial activity of zinc oxide nanoparticles embedded at 10, 20 and 30 wt% in titanium dioxide glass matrix synthesized via sol-gel route was studied via conductimetry assay against Staphylococcus aureus and Escherichia coli bacterial. The zinc oxide-titanium dioxide nanocomposite was characterized by Field Emission Scanning Electron Microscope and X-Ray Diffractometer to observe the microstructure morphology and crystallinity phase of the synthesized nanocomposite. The nanocomposite granules microstructure has been analyzed using FESEM and show a very high surface area qualitatively and the x-ray spectrum does not show TiO_2 crystal phase suggesting that TiO_2 is in amorphous glass phase. Nanocomposites with 20wt% and 30wt% of ZnO nanoparticles in TiO_2 solgel matrix (will be refer as TiO_2-ZnO20 and TiO_2-ZnO30 respectively) inhibited 40% to 95% of both antibacterial proliferation from different batch of nanocomposite products. Both nanocomposites selectively inhibit towards *E.Coli* compare with *S. Aureus*. A clear dose-dependent response between TiO2-ZnO20 and TiO2-ZnO30 was recorded in S. *Aureus* assay.

1 INTRODUCTION

ZnO powders applications have been found for varistors and other functional devices and also can be used as enforcement phase in wear resistant phase, anti-sliding phase, and antistatic phase in composites in consequence of its high elastic modulus and strength properties and current characteristic as an n-type semiconductor material [1-3].

Recently, antibacterial activity of ceramic powders has attracted attention as a new technique that can substitute for conventional methods using organic agents. Ceramic powders of zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show marked antibacterial activity [4–6]

The use of ZnO has the following advantages: It contains mineral elements essential to humans and exhibit strong antibacterial activity in small amounts without the presence of light. It was found that ZnO exhibits antibacterial activity at Ph values in the range from 7 to 8 [7], and these values are suitable for use in water used for washing. The antibacterial activity of ZnO is considered to be due to the generation of hydrogen peroxide (H_2O_2) from its surface [8]. However, the use of ZnO in powder form for antibacterial water treatment is limited as it will cause water to be turbid and the nano particles will also flow with water stream and contaminated to locations where clear water is required. Thus, ZnO powder has to find a way in a form composite for many applications in water purification, antibacterial coatings and etc.

Thin films or nanoscale coating of ZnO nanoparticles on suitable substrates is also potential as substrates for functional coatings, printing, UV inks, e-print, optical communications (securitypapers), protection, barriers, portable energy, sensors, photocatalytic wallpaper with antibacterial activity etc.[9,11–24]. The nanocomposite coating of ZnO nano particles with biomolecules, oil, pigments (calcium carbonate, clay, talc, silicates, TiO2, etc.), polymers, plastics etc. has been reported with the help of suitable binders and cobinders [10]. However, to the best of our knowledge till now no paper has reported on the synthesis of nanocomposites of Zinc Oxide nanoparticles with the TiO_2 amorphous glass as matrix via sol gel method.

In the present work, nanocomposites containing nano particle zinc oxide (average size 20 nm) in titania sol gel matrix has been synthesized with various loading weight percentage of nano zinc oxide powder with respect to titania sol and dried at 40˚C for 8 hours. After the nanocomposites were dried into flakes, they were characterized using electron microscopy and x-ray diffractometer to analyse the structure and morphology of the nanocomposites. In this study, the antibacterial activities of the nanocomposites with various nano particles zinc oxide weight percentage were also studied against Staphylococcus aureus and Escherichia coli by conductimetric assay.

2 EXPERIMENTAL

2.1 Chemicals and materials

A commercially available reagents grade alkoxide solution of Titanium (IV) Isopropoxide (TTIP) (purity > 98%, Sinopharm Chemical Reagent Co. Ltd), Isopropyl alcohol (IPA), deionised water with resistivity of 18.3 MΩ and Hydrochloric acid 3 Molar concentration were used to make the TiO_2 solgel. ZnO nanoparticles (purity > 99.7%, Sinopharm Chemical Reagent Co. Ltd) with an average particles size (nm) of 20 and specific surface area of more than 90 m2/g. All the chemicals and materials mentioned were used as purchased without further purification.

2.2 Nanocomposite synthesis technique

Firstly, the TiO2 sol was prepared by mixing the TTIP ($Ti(OC_3H_7)_4$) with solvent, acid catalyst and deionised water that will lead to series reactions of hydrolysis and condensation. The TTIP alkoxide solution was mixed and stirred using magnetic stirrer in isopropyl alcohol at a molar ratio of 1:14.7 for 30 minutes before four drops of hydrochloric acid (3M) was added into this solution and followed by another four drops of deionised water into the above solution. The reaction mixture was keep stirred and aged for 1 hour before various weight percentages of ZnO nano particles (10, 20 and 30 wt %) were added into the TiO_2 sol. The resultant mixture of the ZnO nanoparticles in TiO_2 sol was stirred for 1 hour and followed by sonication process in ultrasonic bath for 10 minutes. After the sonication, the TiO_2 sol containing ZnO nano particles were transfer into petri dish and covered by laboratory film (Parafilm) with few holes for the evaporation of volatile solvent during heat treatment in oven at 40˚C for around 6 hours until the nanocomposites solution dried and form granules prior to characterization follow by heat treatment at 400ºC for 1 hour.

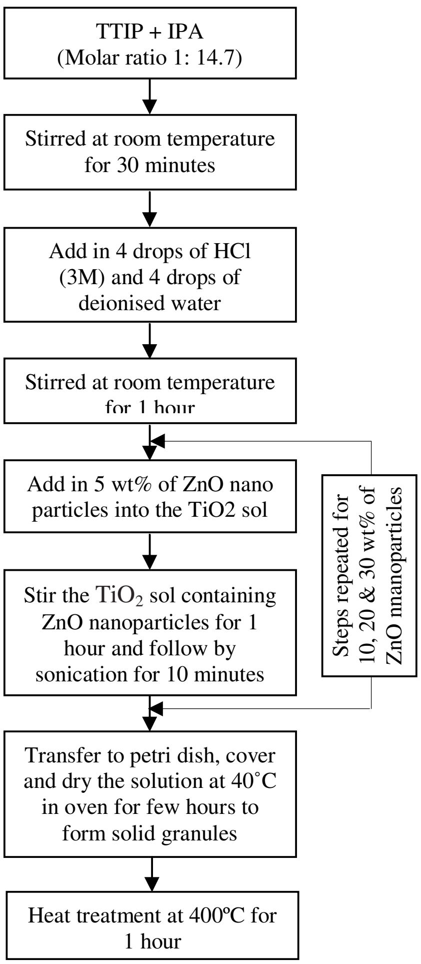

Fig. 1 Flow chart for the preparation of TiO_2 glass – ZnO nanoparticles nanocomposites granules by the sol-gel technique.

2.3 Nanocomposites materials characterization

The ZnO nanoparticles and the prepared nanocomposites granules surface morphology and elemental composition was characterized using FESEM (JEOL-JSM7500F) with built-in EDS. XRD patterns were obtained using PANAlytical X'Pert Pro MPD advanced powder X-ray diffractometer (using Cu Kα = 1.54056 A° radiation) with scanning range of 2 theta from 15º to 85º.

2.4 Antibacterial analysis technique

A collection strain of *E. coli* and *S. aureus* (American Type Culture Collection, Rockville, MD) has been used in this study. Bacteria from frozen stock cultures were grown aerobically to late logarithmic or early stationary phase in LB broth (Oxoid Ltd, Basingstoke, UK) at 37.8C. Cells were harvested by centrifugation and re-suspended in fresh medium. Inocula were prepared by adjusting the cell suspension to predetermined optical densities (OD) corresponding to 10^8 CFU/ml.

The antibacterial analysis has been performed following the method of Weiss et al. with minor modifications. 2 ml of the bacterial inoculums (approximately 10^6 bacteria) were placed at each well on 24 well culture plate against different concentration/weight of test material for 24hr.

Bacterial growth at 24hr was assay by colorimetric method (Drummond et al, 2000) 50ul of 10% resazurin solution (Sigma, St. Louis) was added to each well and the plate was then incubate at 37°C for 1hr. The OD in each well was measured at excitation 485nm and emission 530nm in a microplate reader (Tecan, Switzerland). All experiments, carried out under aseptic conditions, were repeated three times to ensure reproducibility.

3 RESULTS AND DISCUSSION

3.1 Nanocomposites materials characterization

The synthesized ZnO-TiO2 nanocomposite granules microstructure were characterized by FESEM and shown as follows:

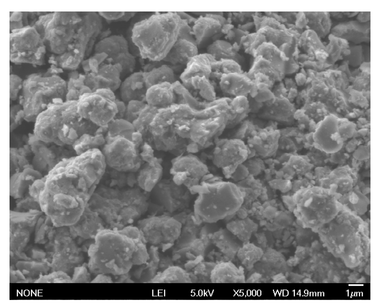

Fig. 2 SEM micrograph of ZnO-TiO210 (10 wt% ZnO in TiO2 sol-gel matrix)

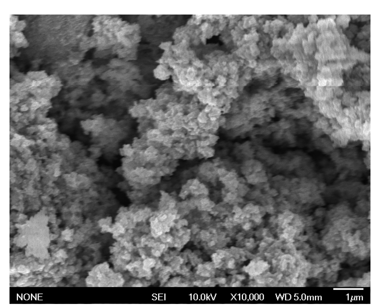

Fig. 3 SEM micrograph of ZnO-TiO20 (20 wt% ZnO in TiO2 sol-gel matrix)

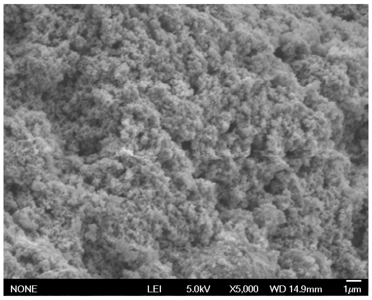

Fig. 4 SEM micrograph of ZnO-TiO30 (30 wt% ZnO in TiO2 sol-gel matrix)

It was observed that the higher the ZnO nanoparticles content, the higher the surface area as shown in figure 2 to 4. The higher surface area size on the surface of the nanocomposite granules is hypothesized to cause higher effectiveness in bacterial killing effect.

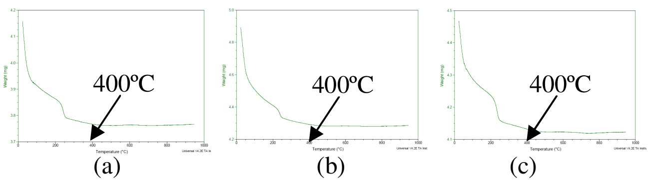

Fig. 5 Thermogravitometry (TGA) analysis of TiO2-ZnO nanocomposites (a) TiO2-ZnO10, (b) TiO2-ZnO20, (c) TiO2-ZnO30

Thermogravitometry analysis was performed on the TiO2-ZnO10, TiO2-ZnO20 and TiO2-ZnO30 nanocomposites and shown that after 400ºC, all the organic and volatile components from the alkoxide precursors have been removed totally. Thus, heat treatment temperature parameter was set at this temperature for all the samples

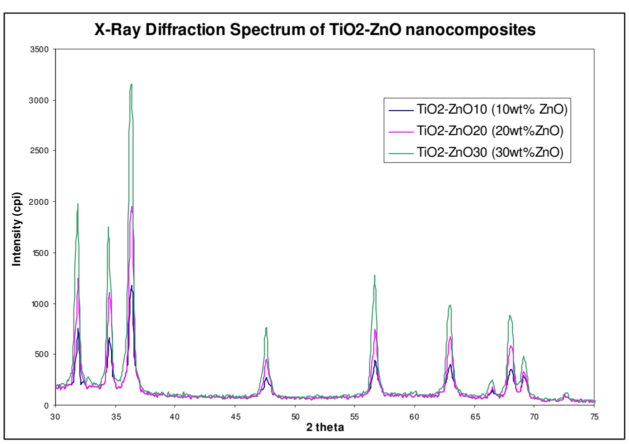

Fig. 6 X-Ray diffractometry spectrum of TiO2-ZnO10, TiO2-ZnO20 and TiO2-ZnO30

As shown in X-Ray Diffractometry spectrum, only ZnO crystals were shown and no TiO2 spectrum indicating that the TiO2 is in amorphous glass matrix. Also shown that the higher the ZnO nanoparticle weight percentage, the intensity peaks was also higher.

3.2 Antibacterial analysis

The antibacterial activity of TiO2-ZnO20 and TiO2-ZnO30 nanocomposites materials was tested using *E. coli* and *S. aureus* in comparison with ZnO power as positive controls, the results of which are presented in Fig. 6. TiO2-ZnO10 nanocomposite was not tested due to inability to form granules with required size for the antibacterial test. Data were represented as % survival of control against weight of ZnO powder present after 24hr incubation. Bacterial survival rate drops as the ZnO power concentration increased for both *E. coli* and *S. aureus*. The bacteria growth reduced by half at 15mg/ml and 18mg/ml respectively. Similar results were observed in both TiO2-ZnO20 and TiO2-ZnO30 nanocomposites materials. However, ZnO powder present in TiO2-ZnO20 and TiO2-ZnO30 nanocomposites contain 20% and 30% w/w of ZnO powder respectively. Our current result suggested that ZnO nanocomposites materials have better antibacterial activity compared with ZnO powder alone.

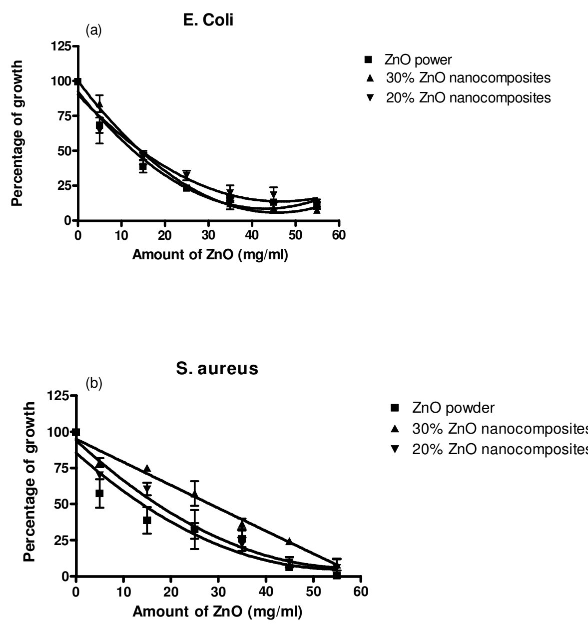

Fig. 7 Effect of ZnO nanocomposites on the growth of *E. coli* and *S. aureus*. Plots present mean % of growth of *E. Coli* (a) and *S. aureus* (b) exposed to different concentration of ZnO present for 24 hr incubation (n=4).

4 CONCLUSION

This antibacterial activity studies of ZnO nanopowder embedded in TiO_2 amourphous matrix approach has shown excellent results against E. Coli and S.Aureus bacteria and proven the concept of creating nanocomposites of ZnO and TiO_2 which can be used in granules flakes form as an antibacterial material or can be coated to any surfaces when the nanocomposite is in the sol form. Thus, the problem of turbidity caused by ZnO nanopowder in water or ZnO nanoparticle ashes in dry application such as air purification will be solved by this nanocomposite. The TiO2-ZnO nanocompsoite material has been characterized using FESEM, EDS, XRD. Considering the potential implication of this TiO2-ZnO nanocomposite material, it may potentially prove useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimes and coated layers can be varied using suitable chemistry for desired applications.

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