

Assessing the antibacterial properties of eggshell-titanium powder

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SC Onwubu¹, MU Mokgobole², PS Mdluli³, TH Mokhothu⁴

ABSTRACT

Introduction

The global distribution of oral diseases caused by bacterial and the severity of their consequences constitute a pandemic condition.

Aims and Objectives

The present paper reports on the antibacterial properties of a modified eggshell and titanium dioxide using the mechanochemical method.

Methods

EB@TiO₂ was modified and characterized using X-ray diffraction (XRD) while the degradation condition was studied using Thermogravimetric analysis (TGA). The inhibitory properties of EB@TiO₂ at different concentrations (2:1; 3:1 and 4: 1) against both Gram-positive (*E. coli*) and negative bacterial (*B. Cereus*) strain were studied the using disk diffusion method.

Results and conclusions

The XRD result confirmed the presence of a thermodynamically stable calcite structure, which is indicative of calcium carbonate. The diffraction peak of the XRD at $2\theta = 29.5^\circ$ suggests the deposition of TiO₂ on the surface of CaCo₃. TGA curves shows the decomposition of anatase form of titanium dioxide and calcium carbonate. The study evidently shows the

antibacterial activities of EB@TiO₂ against *Escherichia coli* and *B. Cereus*. The salient feature of the study finding is that modifying eggshells with titanium dioxide improves the antibacterial properties, and thus offers a promising role for the development of potent dental materials in the management of oral diseases.

Keywords: Antibacterial; eggshell; Mechanochemical; titanium dioxide

INTRODUCTION

Although the oral status of the general population globally has improved, oral disease such as dental caries and periodontitis remains a significant public concern posing a grave challenge for the oral health care provider in mitigation and prevention¹. From an epidemiological perspective, the World Health Organisation (WHO) underlines that oral diseases like dental caries affect around 60 to 90% of the general population^{2,3}. The aforementioned oral disease is mostly caused by bacterial activities in the mouth. Bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, and lactobacilli are the main oral cariogenic pathogens due to their ability to produce high levels of lactic acid following sugar fermentation and their resistance to the adverse effects of low pH⁴. Modern molecular analyses and microbial culture techniques have also demonstrated that an entire range of bacteria can contribute to the caries process at different stages⁵. Besides the aforementioned bacteria species, some classes of fungi like the candida albicans significantly enhance the cariogenic virulence of plaque biofilms⁶.

To address and help prevent oral diseases, published literature suggests that brushing regularly with well-formulated fluoride toothpaste can prevent the onset of this disease⁷. Concerning, however, young children may accidentally swallow enough amount of fluoridated dentifrice to produce levels of fluoride consumption associated with a risk of developing dental fluorosis⁸. More so, fluoride is an incessant non-biodegradable, hazardous, and persistent pollutant, which is of concern to the environment. Lacson et al.⁹ note that the uncontrolled concentration of fluoride can subsequently cause fluorosis, which is a common fluoride- and water-borne disease. To counteract this looming concern, providing safe, effective, environmentally friendly and affordable material in toothpaste formulation becomes highly important.

Author affiliations:

1. Stanley C Onwubu: *PhD*, Chemistry, Durban University of Technology (DUT), Durban, South Africa, ORCID: 0000-0002-4499-1534
2. Mokgadi Ursula Mokgobole: *MHSc*, Somatology, Durban University of Technology (DUT), Durban, South Africa, ORCID: 0000-0001-6530-7079
3. Phumlane Selby Mdluli: *PhD*, Chemistry, Durban University of Technology (DUT), Durban, South Africa
4. Thabang Hendrica Mokhothu: *PhD*, Chemistry, Durban University of Technology (DUT), Durban, South Africa.

Corresponding author:

Onwubu Stanley Chibuzor
Dental Sciences Department, Dental Technology Programme,
Durban University of Technology (DUT),
Durban, South Africa
Email: stanleyo@dut.ac.za

Author contributions:

1. Stanley Chibuzor Onwubu 50%
2. Mokgadi Ursula Mokgobole 20%
3. Phumlane Selby Mdluli: 15%
4. Thabang Hendrica Mokhothu 15%

Ultimately, eggshells could be an effective environmental alternative in toothpaste formulation. Xia and his team, for example, recently developed a simple defluoridation by boiling eggshell with the addition of acetic acid and sodium phosphate intended for household application¹⁰. This suggests that eggshells could be a viable alternative to fluoride as it is safe for household use. Moreover, eggshells are utilised in various industries such as dentistry, the pharmaceutical industry and medicine¹¹. In dentistry, various studies have illustrated the effective use of eggshells such as Onwubu et al.¹² found that eggshell powder has been successful in reducing the surface roughness of acrylic dentures and Mony et al.¹³ concluded that eggshells are capable of remineralizing enamel carious lesions. Furthermore, recent studies by Onwubu et al.¹⁴ demonstrate that eggshells modified with titanium dioxide provide robust resistance to enamel demineralization against erosive acids.

Besides this, other studies have reported that titanium dioxide (TiO₂) is an effective antimicrobial agent, and it is compatible with the human body or environment^{15, 16}. Given the usefulness of eggshells as a biomaterial, this study envisages that assessing the antibacterial properties of eggshell-modified titanium dioxide (EB@TiO₂) will help in validating the material as an alternative dental material in the prevention of oral diseases. To our knowledge, there is limited research that has investigated the antibacterial properties of a modified eggshell and titanium dioxide. The purpose of this study was to assess in vitro the antibacterial properties of a modified eggshell and titanium dioxide against *Escherichia coli* and *B. Cereus* in comparison with commercially available toothpaste.

MATERIALS AND METHODS

Preparation of Eggshell-Titanium dioxide composite (EB -TiO₂)

Modification of eggshell with titanium dioxide was achieved in two steps in accordance with the techniques reported by Onwubu et al.¹⁷. In the first step, eggshells were ball-milled by placing 30g of the eggshell in a 500ml stainless jar (inner diameter of 100 mm), together with 10 stainless steel balls of 10 mm diameter and dry-milled in a planetary ball mill (Retsch® PM 100) at 400 rpm for 20 min. The collected powder was sieved to a particle size of ≤ 25µm using a mechanical sieving shaker (Retsch AS 200, Germany). The eggshell powder and titanium dioxide mixing ratio were optimized following the procedure reported by 18, 20g of the fine eggshell powder obtained in step 1 were modified by adding 5g of anatase titanium dioxide (≤ 15µm). The mixture was subsequently ball-milled for 150 min to obtain eggshell-titanium dioxide composite. The prepared powder was subsequently characterised to determine the physico-chemical properties.

Characterization of EB@TiO₂

- Fourier Transform Infrared Spectroscopy Analysis
- The infrared spectra were measured using a Perkin Elmer Universal ATR spectrometer to identify the functional group constituents of EB@TiO₂.

- Transmission Electron Microscopic Analysis
- A Transmission Electron Microscope (TEM) was used to observe the particle size, shape and distribution of EB@TiO₂. Very small quantities of EB@TiO₂ were dispersed in 10ml ethanol and sonicated at 10kv for 10 min. Subsequently, thin cross-sections of cryo-microtomed specimens were prepared using a Leica microtome (South Africa) and placed on carbon copper grids. Analysis was conducted using a transmission electron microscope (TEM-Philips CM 120 model) at 120 kV.
- Thermogravimetric analysis
- Thermal degradation and stability of the modified EB-TiO₂ were studied using thermo-gravimetric analysis (TGA). The thermal stability was measured using a TA instrument (Thermal Universal Analyser V4.5A). The test was performed under a dry nitrogen gas flow at the rate of 100mL/min from 20°C, at a heating run of 10°C/min.

Antibacterial Assessment of EB@TiO₂

The inhibitory properties of EB@TiO₂ against both Gram-positive and negative bacterial strains were studied the using disk diffusion method. The Bacterial strain was obtained from Anatech supplies, South Africa. Thereafter, pure colonies from freshly grown strain *E.coli* (ATC 25922), and *B. Cereus* (ATC 10876) were isolated and subsequently transferred from the plates into a sterile normal saline solution and vortexed to form bacterial homogenous suspensions. The turbidity was then adjusted to 0.5 McFarland standard units, and the suspensions were poured over Mueller-Hinton agar (MHA) plates.

Sterile filter paper disks with a diameter of 6 mm were placed over these plates. The sterile disks were impregnated with 20 µL of the tested compounds (10 mg/mL dissolved in DMSO). Positive control (Ciprofloxacin) and negative control (sterile distilled water) were used. The impregnated plates were incubated at 37°C for 24 hours, in line with the procedure described by Wiegand et al.¹⁹, the inhibition zones were calculated and the value estimated in millimetres.

RESULTS

Characterization

The FT-IR spectra of eggshell powder, titanium dioxide, and EB@TiO₂ is presented in Figure 1. The band aspect of the FT-IR spectra shows the difference between the eggshell powder (Figure 1 (A)), EB@TiO₂ (Figure 1(B)), and titanium dioxide (Figure 1(C)). The prominent absorption peak of carbonate at 1411 cm⁻¹ was observed for both eggshell powder (Figure 1A) and EB@TiO₂ (Figure 1B) suggesting that both materials contain a thermodynamically stable form of calcium carbonate. In addition, the broad stretching displayed for EB@TiO₂ above 1000 cm⁻¹ suggests the surface medication of calcium carbonate structure in eggshell powder with titanium dioxide (Figure 1B).

From Figure 2 the TGA curves show the incidence of two thermal events within the temperature range of 50-900°C. The first phase (600°C) is attributed to

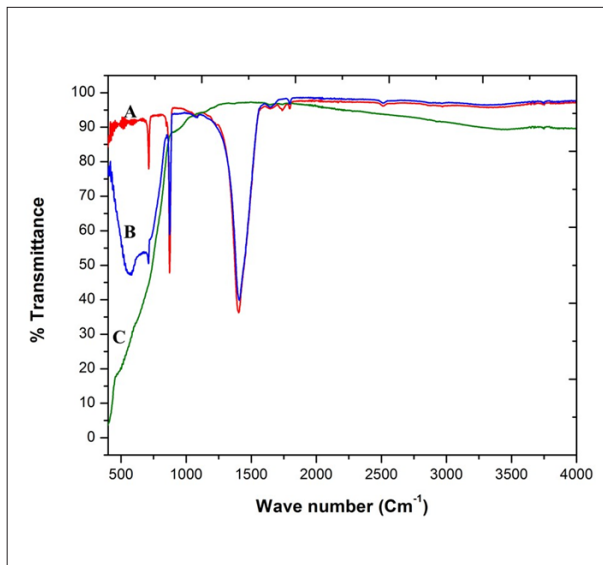


Figure 1: FTIR spectra showing (A) eggshell powder; (B) EB@TiO₂; (C) titanium dioxide

the decomposition of the anatase form of titanium dioxide, which caused a small weight loss (~ 1.061%). The second phase (699.14°C) is endothermic and is linked to the decomposition of calcium carbonate into carbon dioxide and calcium oxide ²⁰.

This weight loss equated to approximately 30.41% of the total mass.

The TEM image of EB@TiO₂ is shown in Figure 3. The image revealed a nonhomogeneous structure of spherical shape particles and irregular particles with different sizes distribution. The irregular shape particles are indicative of eggshell powder while the spherical particles typified the titanium dioxide particles. It can be observed that the pure titanium dioxide particles were scattered on the surface of the composite.

Antibacterial properties

The antibacterial screening of the EB@TiO₂

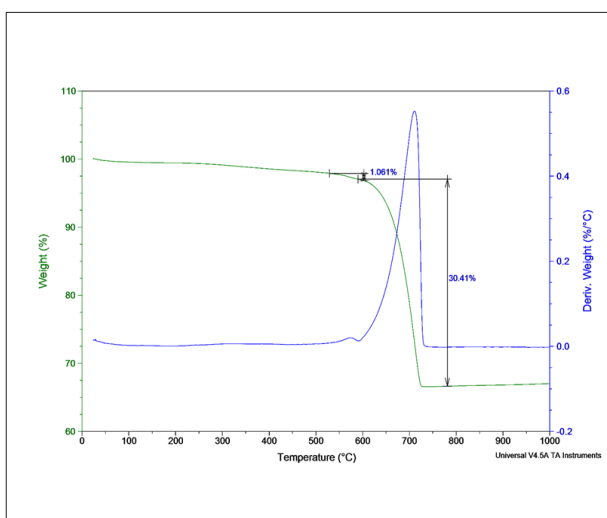


Figure 2: TGA curves of EB@TiO₂

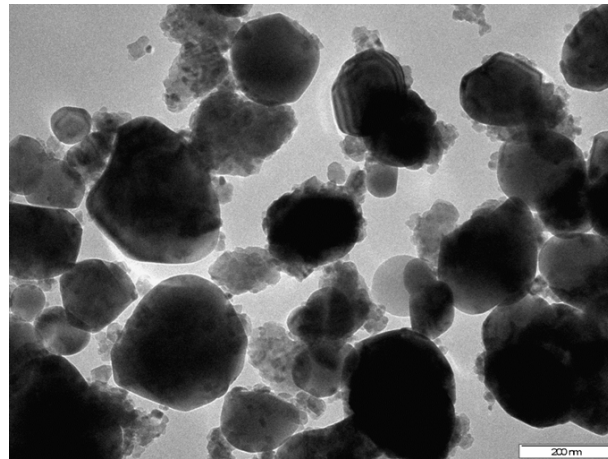


Figure 3: TEM image EB@TiO₂

compounds at different ratios of eggshell and titanium dioxide modification (2:1, 3: 1, and 4:1) was determined using the disc diffusion method against *E. coli* and *B. Cereus*. The results obtained have shown that these compounds displayed a slight inhibition against *E. coli* and *B. Cereus* (Table I). It was also found that irrespective of the ratio of eggshell and titanium dioxide used, the inhibitory properties observed for EB@TiO₂ were more or less the same. On the contrary, tested toothpaste (Colgate Pro-relief, Sensodyne Repair, Sensodyne rapid relief), and eggshell alone didn't display any inhibition against the bacteria used.

The inhibitory zone observed for EB@TiO₂ at different ratio compositions is given in Figure 4. The image visibly confirmed the inhibitory properties of EB@TiO₂ against *E. coli* and *B. Cereus*. The inhibitory properties of Ciprofloxacin (Control) demonstrated the maximum zones of inhibition with a mean diameter of 18.6 and 22.5 mm for *Escherichia coli* and *B. Cereus*, respectively when compared to EB@TiO₂ which showed maximum zones of 6 mm.

DISCUSSION

Oral disease such as dental caries continues to pose a significant public health concern due to the associated negative impacts on the quality of life for individual sufferers. Fluoride containing toothpaste has largely been the most common effective oral hygiene practice that aids in the prevention of oral disease ⁷, particularly those caused by bacterial. However, the risks of dental fluorosis, as well as the environmental concern posed by fluoride has necessitated an alternative ingredient in toothpaste formulation. The purpose of this study was to assess in vitro the antibacterial properties of a modified eggshell and titanium dioxide (EB@TiO₂) against *Escherichia coli* and *B. Cereus*. As reported in the literature, some common oral bacteria responsible for dental caries formation include *Staphylococcus aureus* ²¹, *Escherichia coli* ²², *Streptococcus mutans* ²³, *Streptococcus sobrinus* ²⁴, *Lactobacilli sp.* ²⁵, and *Porphyromonas gingivalis* ²². The finding of this study showed the EB@TiO₂ at different concentrations tested in the study exhibited antimicrobial activity against both *Escherichia coli* and *B. Cereus* (Table 1).

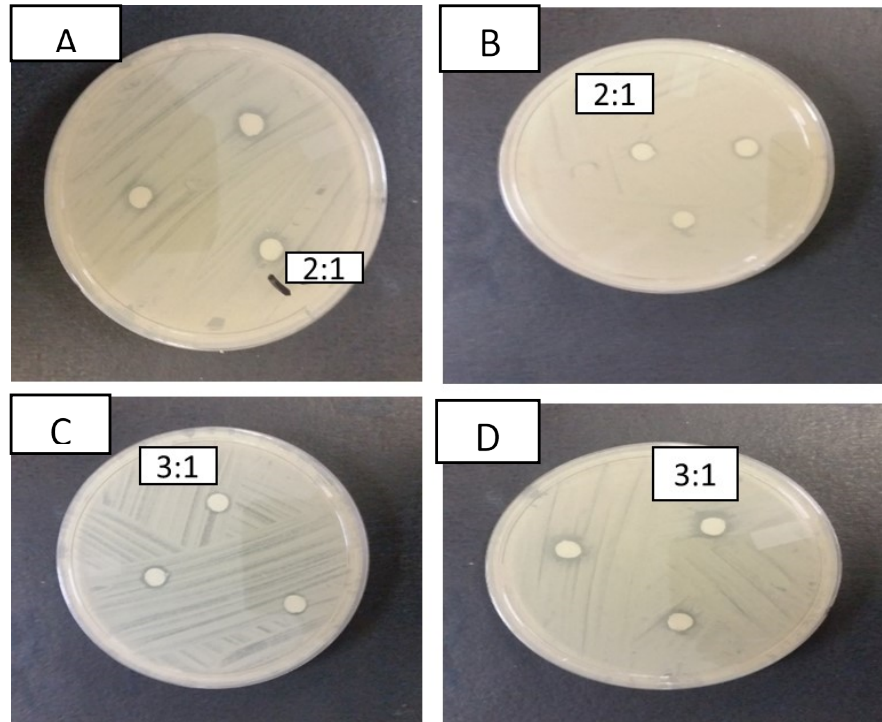


Figure 4: Inhibitory zone (mm) of compounds 2:1 against *E. coli* (A) and compounds 3:1 against *B. Cereus* (B), Compounds 3:1 against *E. coli* (C), compound 3:1 against B (D) against *B. cereus*

Comparison of the zones of inhibition formed showed evidence of antibacterial activities (Figure 4). This may be attributed to the modification of eggshells with titanium oxide.

Further to the above, TiO_2 has been reported to be an effective antimicrobial agent that is compatible with the human body or environment^{15,16}. In particular, and in a more recent study, it was demonstrated that titania reduces the ability of bacterial such as *Staphylococcus aureus* (*S. aureus*) to adhere to surfaces by rupturing their cell membrane²⁶.

As such, one could attribute the inhibitory properties of EB@TiO_2 to the attachment of titanium oxide (TiO_2) on the surface of the eggshell powder. This is can be supported by the fact eggshell powder alone showed no antibacterial activities whilst the antibacterial activities of TiO_2 was comparable to EB@TiO_2 (Table 1). The plausible explanation for this may be attributed to the

fact that the calcium carbonate which is a constituent of eggshells is a poor source of nutrients for microbes to colonise. This is corroborated by Sepehrnia et al.²⁷, that calcium carbonate inhibits the colonisation of bacteria.

While the slimy extracellular polymeric substances (EPS) produced by dental caries causing bacteria offers it protection and mechanical supports²⁸, it can be said the abrasiveness of eggshells (since it is mainly calcium carbonates) and the antibacterial properties of TiO_2 could offer robust protection through mechanical removal of biofilms, as well as, rupturing of the cell walls of the bacterial. This is relevant as the calcium carbonates in eggshells will aid in providing the mechanical removal of the biofilms.

Although the inhibitory zones found for EB@TiO_2 against both *Escherichia coli* and *B. Cereus* were lower than that measured Ciprofloxacin (Control), it is, however, higher when compared to the commercially available toothpaste which showed no antimicrobial activities (Table I). The probable reason for this could be that the main active ingredients in the tested toothpaste function mainly act as anti-sensitivity. In reviewing the literature on antibacterial properties of commercially available toothpaste, it was found that the inhibitory zones measured for EB@TiO_2 against *Escherichia coli*, for example, was higher when compared to the 2.22mm reported for Colgate Salt (Neem) by Dhakal et al.²⁹. This thus indicates the antibacterial properties of EB@TiO_2 .

While this study provides insight into the antimicrobial activity of the tested EB@TiO_2 , certain limitations exist

Table I: Inhibitory properties of the samples		
Compounds	<i>E. coli</i> 25922 (mm)	<i>B. Cereus</i> 10876 (mm)
EB@TiO ₂ (2:1)	6.1±0.5	6.0±0.0
EB@TiO ₂ (3:1)	6.0±0.0	6.0±0.0
EB@TiO ₂ (4:1)	6.0±0.0	6.0±0.0
Eggshell	0.0±0.0	0.0±0.0
Titanium dioxide	6.0±0.0	7±0.0
Colgate Pro-relief	0.0±0.0	0.0±0.0
Sensodyne repair	0.0±0.0	0.0±0.0
Sensodyne rapid relief	0.0±0.0	0.0±0.0
Ciprofloxacin (Control)	18.6±0.5	22.5±1.0

such as the limited number of tested microorganisms selected for this study. Hence, it may be premature to dismiss the antibacterial properties of commercially available toothpaste. Future study needs to comprehensively assess the antibacterial properties of EB@TiO₂ in conjunction with other ingredients in toothpaste formulations.

CONCLUSION

Oral diseases caused by bacterial is a serious public health concern to the general public. The study showed that modifying eggshell with titanium dioxide increases its antibacterial properties. This is vital towards the use of eggshells as an ingredient in toothpaste formulation. From a public health perspective and environmental sustainability, the study envisages that using eggshell-titanium dioxide in toothpaste formulation will offer a robust strategy in the prevention of oral diseases caused by bacterial and at the same time, acting as a catalyst towards a greener environment.

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