Biomimetic Synthesis and Antibacterial Characteristics of Magnesium Oxide–Germanium dioxide Nanocomposite Powders

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ABSTRACT: The efficiency of growth of nanocrystalline magnesium oxide–germanium dioxide nanocomposite powders at room temperature was investigated in the presence of the amino acids histidine, aspartic acid, and the biopolymer poly-L-lysine under varying conditions. It was observed that of the three, poly-L-lysine and histidine were more efficient and formed higher yields of the products. The growth of the nanocomposites was found to be pH-sensitive as indicated by zeta potential as well as TEM, and dynamic light scattering analyses. Furthermore, the nanocomposite powders were found to have significant antibacterial activity against Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria.

KEY WORDS: magnesium oxide, nanocomposites, germania, amino acids.

INTRODUCTION

OVER THE PAST decade, metal oxide nanocomposites have been attracting significant attention due to their various potential applications in catalysis, electronics, photonics, and sensors [1–8]. In addition to alumina, silica, and titania, germania has been gaining tremendous importance mainly due to its enhanced reactivity and optical properties [9,10]. Germania–silica hybrid materials, Nd$^{3+}$-doped germaina glasses, and erbium-doped germania have been prepared for optical device fabrications [11–13]. Most methods employed for the synthesis of germanium oxide-based materials, however,
involve the use of high-temperature conditions [14,15], which sometimes may lead to sintering. Thus, milder methods for the synthesis of such nanomaterials would be beneficial. Recently, germanium oxide was synthesized under mild conditions using bioinspired methods [16–18]. The methods involved the use of specific peptide sequences derived from the combinatorial phage-library or amino acids that helped in the efficient mineralization of the materials. Nanocomposite powders of germanium oxide integrating gold, palladium, tin oxide nanoparticles have also been prepared using biomimetic methods [19,20]. Although the catalytic and optical applications of germanium oxide nanocomposite powders are well known, research related to the antimicrobial activities of germanium oxide nanocomposite powders is relatively sparse.

In this work we have explored the development of a new material, by preparing nanocomposite powders of magnesium oxide–germania using amino acid catalysts. In the past, magnesium oxide nanoparticles and microparticles have been used as a reinforcing reagent, as well as a component in super conductors [21]. In addition, their high surface reactivity, chemical, and thermal stability is desirable for applications in sensors, catalysis, and additives [22,23]. Recently, the antimicrobial properties of MgO nanoparticles were studied and they were found to be highly effective against bacteria. They have also been found to be potent in toxic waste remediation [24–28]. In order to prepare new materials with enhanced properties, composites of MgO such as alumina–magnesia, silica–magnesia mixed oxides have been prepared by sol–gel methods and have been found to function as efficient supports for various catalytic applications [29,30]. It has also been suggested that magnesia or mixtures of MgO with other refractory inorganic oxides may have improved properties for various applications [31]. Since both magnesium oxide and germanium compounds possess antimicrobial properties [32], it would be interesting to study those properties by preparing nanocomposite powders of germanium oxide–magnesium oxide.

In this article, the efficacy of growth of the magnesium oxide–germanium oxide nanocomposite powders using biomimetic methods was studied in the presence of aspartic acid, histidine, and poly-L-lysine. The biological approach used in this work is a mild method of preparation of such nanocomposite powders, which upon calcination lead to highly crystalline materials. Further, we examined the antibacterial effects of nanocomposites of magnesia–germania against both Gram-negative and Gram-positive bacteria. To our knowledge, this is the first time that nanocomposite powders of magnesium oxide–germanium oxide have been prepared by biomimetic methods and tested for antimicrobial properties. Such new materials may not only be potentially useful for catalysis, sensors, and optics, but can also be used as antimicrobials for environmental remediation.

EXPERIMENTAL

Materials

Germanium (IV)–methoxide, magnesium methoxide, histidine, aspartic acid, and poly-L-lysine were purchased from Sigma-Aldrich and used as received. Buffer solutions of various pH were purchased from Fisher Scientific. Bacterial strains *Escherichia coli* (XL-1 blue strain) and *Staphylococcus aureus* were a gift from the laboratory stock of the Microbiology Laboratory at Queens College, CUNY. Other materials for bacteria cultivation such as agar, yeast extract, Luria broth, sodium chloride, tryptone, petri dishes were bought from Gibco or Sigma Aldrich.
Methods

PREPARATION OF MAGNESIA–GERMANIA

The nanocomposite powders were prepared by adding 20 μL (0.1 M) magnesium methoxide and germanium methoxide (10 μL) in methanol (2:1 molar ratio) to the solutions of the amino acids (0.1 M) in respective buffer solutions (pH 4–9) (500 μL) every 15 min for 2 h under constant stirring. The magnesium oxide–germania nanocomposite powders were thus prepared by co-gelling the precursors in the presence of the amino acids and the biopolymer poly-L-lysine. The samples were vortexed slowly for 3 h, centrifuged at 4500 rpm, and washed twice with nanopure water. Finally, the resultant products obtained were calcined in an inert atmosphere at 500°C for 3 h. All experiments were done in triplicates.

ANTIBACTERIAL ACTIVITY STUDIES

A single colony of *E. coli* (XL-1 blue strain) or *S. aureus* was grown overnight in Luria Broth (LB) at 37°C with shaking until late log phase. Then 100 μL of bacteria was transferred into 30 mL of LB and then 2 mL of that were placed in separate sterile tubes and 100, 200, or 300 μL of various nanocomposite powders that were 50 mg/mL in double-distilled water were added. As diluent controls, to a series of LB tubes we added 100, 200, or 300 μL of double-distilled water. All tubes were then incubated at 37°C with shaking for approximately 16 h. We determined the relative number of bacteria in the cultures by measuring the turbidity of three separate 100 μL aliquots in 96-well plates at 630 nm light in a BioTek plate reader.

Because LB contains many components we also tested the effects of incubating nanocomposite powders with bacteria in water. We grew overnight cultures of *E. coli* and *S. aureus* as before, but 100 μL was pelleted and re-suspended in 30 mL. We added 100, 200, or 300 μL solutions of various nanocomposite powders that were 50 mg/mL in double-distilled water and then incubated at 37°C with shaking for approximately 16 h. To assay for the viability of the bacteria, 50 μL was spread onto the surface of LB agar plates and incubated at 23°C and tested. The number of viable cells in the sample was determined by choosing the appropriate dilution of the sample onto LB agar plates and counting colonies that appeared on the plates under a microscope. The average number of viable cells was obtained by averaging the numbers in three replicate plates.

CHARACTERIZATION

Transmission Electron Microscopy (TEM): The particle sizes and morphology of the samples were analyzed by TEM (JEOL 120 EX operated at 100 kV). The samples were vortexed and 5 μL of the samples were pipetted on to a carbon-coated copper grid, dried over night, and analyzed at various magnifications.

X-ray Diffraction: Powder X-ray diffraction studies were carried out using a PANalytical X’Pert Pro diffractometer with the CuKα radiation 180 (λ = 1.55 Å) source and equipped with a diffractometer beam monochromator at 25°C.

EDS (Electron Dispersive X-ray Analysis): The composition of the nanocomposite powders was analyzed by an SEM (Amray 1000), equipped with EDS. For sample preparation, for SEM–EDS, a few drops of the sample solution (in ethanol) were pipetted out of each sample onto MCE filter paper (5 mm) with Whatman filter paper underneath to aid in absorbing the fluids. The powders quickly built up on top of the MCE filter paper, which was air-dried. Portions of the stained MCE filter paper were then cut out and mounted on SEM sample
stubs with double-sided tape and carbon coated. The samples were then examined under the SEM by EDS. The SEM was set at 20 keV, at a 33° sample tilt.

**Dynamic Light Scattering (DLS) and Zeta Potential Measurement:** A NICOMP 380 ZLS zeta potential/particle sizer system (Santa Barbara, California, USA) was used to confirm the charge and particle sizes of the samples. The samples were diluted with nanopure water at room temperature, before measurements for the DLS analysis. Measurements of ξ-potential of the nanocomposite powders were done at 25°C and at different pH values. The concentrations of the suspensions were adjusted within the operational limits of the instrument and the suspension pH was adjusted by the addition of standard buffer solutions. The reported ξ-potential values are the average of at least five measurements (spread of values ±5% of the reported mean values).

**Infrared Spectroscopy:** Fourier transform IR (FT-IR) spectra of the nanocomposite powders were recorded using Mattson Infinity FTIR infrared spectrophotometer in the range of 4000–400 cm⁻¹. Samples were dried at room temperature and then KBr pellets were prepared. The transmittance of the samples was measured.

**RESULTS AND DISCUSSION**

The preparation of magnesia–germania nanocomposite powders was carried out by biomimetic methods in the presence of a basic amino acid (histidine), an acidic amino acid (aspartic acid), and a biopolymer (poly-L-lysine). In addition to being pH-sensitive, poly(l-lysine) can form α-helix, β-sheet, or random coil conformation, though it was recently shown that the transformation of the conformations is relatively inhibited within silica matrices [33,34]. Since the amino acids as well as poly-L-lysine are all pH-sensitive, the growth of the nanomaterials was examined at different pH. The magnesium oxide–germanium dioxide nanocomposite powders were prepared by co-gelling germanium methoxide and magnesium methoxide in the presence of the amino acids. The pH was varied from 4 to 9. In general, in order to prepare metal oxide nanoparticles, one of the common methods to make the solution basic is by the addition of ammonium hydroxide, or other reagents but this method, does not necessarily allow for significant control on the growth of the nanoparticles [35]. Our method is a mild biological method, which allows for the efficient formation of crystalline nanocomposite powders with controlled size without the addition of external additives such as ammonium hydroxide or sodium hydroxide. It was observed that highest yields of product (Figure 1), was obtained in the presence of poly-L-lysine after 2 h of reaction time. Overall, it was observed that higher yields were obtained under basic pH conditions for poly-L-lysine and histidine, while aspartic acid gave relatively higher yields under acidic conditions. Even though the mechanism of the amino acid catalysis may not be clear yet, the amino acids appear be involved in a two-step bio-mineralization process and most likely influence the hydrolysis and polycondensation that provide the initial nuclei for precipitation. Therefore the relative rates of hydrolysis and condensation also influence the size of nuclei, and consequently the final precipitate morphology [36]. The solution became instantly turbid under basic conditions in the presence of both histidine and poly-L-lysine and a white precipitate progressively formed. Thus, the amino acids may be increasing condensation rates at higher pH, leading to the formation of polycondensed species. The imidazole ring of histidine has two nitrogens. One of the nitrogens is bound to hydrogen and donates its lone pair to the aromatic ring and as such is slightly acidic, while the other donates only one electron to the ring, hence has a free lone pair and is basic.
In the case of poly-L-lysine, additional $\text{NH}_3^+$ groups are present instead of the imidazole ring system. Although protonated amino groups are generally not considered highly catalytic but as the pH is increased and deprotonation occurs, electrostatic interactions increase due to the availability of the lone pairs of electrons from nitrogen, leading to more polycondensation.

The mechanism for gelation in the presence of aspartic acid seems to be different due to the presence of the extra carboxyl group. Under acidic conditions, it can donate a proton to the alkoxide group thus, the electron density is withdrawn from the Mg or Ge atom, making it more electrophilic. Previous studies with propionic acid and acetic acid, have also revealed similar interactions of the organic acids with germanium oxide [37]. The mechanism may be similar, and it is likely that the species goes through a short-lived transition state, and then releases the alcohol group. In all probability the next step involves the formation of networks of MgO–GeO$_2$. Under basic conditions, even though the amino group is deprotonated, the presence of two negatively charged $-\text{COO}^-$ groups most likely repels the negatively charged MgO and GeO$_2$ groups thus causing less interactions and consequently less product. Detailed kinetic studies would need to be performed to further elucidate the exact mechanism. For silicates, it has been reported that in the presence of amines, Si expands its coordination sphere to a penta-coordinate intermediate (a structure similar to that found in silatranes) [38,39] and the mechanism includes a penta-coordinate intermediate and a hexa-coordinated transition state. However, it is yet to be determined whether the process is similar for Ge-based materials. Further studies exploring the exact mechanism of the formation are being conducted.

The size and morphology of the MgO–GeO$_2$ nanocomposite powders formed were analyzed via TEM and AFM. Figure 2 shows the TEM images obtained in the presence of poly-L-lysine. It was observed that pH played a significant role in the shapes and sizes of the nanocomposite powders obtained in the case of poly-L-lysine catalyzed products. At low pH, the particles were larger in size, (in the range of 100–150 nm) lower in yield and had varied shapes such as rhombic or cubic shapes. However, at high pH, smaller (30–50 nm) spherical particulates, which formed networks were observed. The electron
diffraction patterns of the nanocomposite powders indicate that they are polycrystalline. The trend was similar in terms of yields of nanocomposite powders obtained in the presence of histidine, except that we did not observe cubic-shaped particles, and most of the nanocomposites were spherical and did not vary significantly in size or in shape irrespective of pH (data not shown). In the presence of aspartic acid, yields were relatively low under basic conditions, but slightly higher in the case of acidic conditions and all nanocomposites were spherical in shape. While the exact mechanism for the shape control of the nanocomposite powders at low pH in the case of poly-L-lysine is not known at this point, it appears that poly-L-lysine at low pH, acts as a template and wraps around the nuclei, allowing for the reaction to go slower and growth on the 111 face of the nanocomposites, leading to the cubic shapes. Further, it is not likely that the poly-L-lysine itself aggregates at low pH.

**DLS and Zeta Potential Analysis**

The size range of the nanocomposite particulates obtained was further confirmed by DLS analyses. Figure 3(a) shows the size range of magnesia–germania nanocomposite powders formed at pH 4 in the presence of histidine. In general, particulates whose mean diameters ranged from 125 to 135 nm were observed at low pH and at high pH a size range of 35–65 nm were observed in the presence of histidine and poly-L-Lysine. For aspartic acid, the mean diameter of particulates obtained irrespective of pH was found to be around 80 nm. The effect of pH on the amino acid catalyzed growth of the nanocomposite powders was also examined by the results obtained from measurements of the ζ-potential. The electrostatic interactions exerted between the negative sites of the metal oxide surfaces and the positively charged protonated amino groups of the amino acids were found to be largely dependent upon the pKₐ values of the amino acid side chains. The variation of

**Figure 2.** TEM image of magnesia-germania formed in the presence of poly-L-lysine at (a) pH 4; (b) at pH 9.
\[ \xi \text{-potential with pH (Figure 3(b)) indicates that by and large, the zeta potentials are positive in acidic conditions but negative in basic conditions. The point of zero zeta potential was obtained at about pH 6.3 for the histidine samples, which is close to the pK}_a \text{ value of 6.1–6.3 for the imidazole group in histidine. Below pH 7, the zeta potentials of the nanocomposites increased almost linearly with the decrease of solution pH values from 7 to 4 (further lower pH was not tested as it may decrease the stability). This can be attributed to the protonation of the amino groups (i.e., from } -\text{NH}_2 \text{ to } -\text{NH}_3^+ \text{). From pH 7 to 10, the negative zeta potentials of the nanocomposite powders, however, did not appear to decrease significantly with the increase of pH and seemed to level off after pH 8. This may indicate that the amino groups were not completely deprotonated under these pH conditions (from } -\text{NH}_2 \text{ to } -\text{NH}^- \text{). After pH 7, in all cases the zeta potential values were found to be negative.} \]

**Figure 3.** (a) Dynamic light scattering analysis of nanocomposite powders obtained in the presence of histidine at pH 4; (b) comparison of zeta potentials of amino acids before and after formation of nanocomposites at various pH.
XRD Analysis

The overall crystallinity of the nanocomposite powders was studied by X-ray diffraction analysis. Figure 4 shows the diffraction pattern of a nanocomposite sample, obtained using poly-L-lysine at pH 9. In general, crystallization of germanium dioxide sets in at higher temperatures [14] and this phenomenon was observed in the nanocomposite samples as well. The peaks observed, combined with the intensity of the 101 peak at 90,000 counts/s, indicates that the particles are of high purity. The characteristic peaks for MgO \((2\theta = 39.5)\); \((2\theta = 441.8)\) for (111) and (200), respectively and that of GeO\(_2\) at \((2\theta = 20.5)\); \((2\theta = 23.4)\) for (100) and (101) are observed confirming the formation of the nanocomposite powders [40,41]. Further, we utilized the Debye–Scherrer formula [42]

![Figure 4. (a) XRD analysis of calcined nanocomposite powder obtained in the presence of poly-L-Lysine at pH 9; (b) EDS analysis of calcined nanocomposite powder obtained in the presence of poly-L-Lysine at pH 9.](image)
for crystallite size determination of the nanocomposite crystallite particles obtained using poly-L-Lysine as the catalyst (since the materials obtained in the presence of poly-L-Lysine had maximum yield and a significant size and shape control). The Scherrer formula is given by $D = \frac{(K\lambda)}{\omega \cos \theta}$ where $D$ is the crystallite size, $\lambda$ is the wavelength of X-ray, $\omega$ is the width on a 2$\theta$ scale, and $K$ is a constant close to unity. Values for the coefficient ‘$K$’ depend on the geometry of the crystallites and may not always be consistent in the literature, $\theta$ is the Bragg’s angle. Initially, Scherrer developed the formula for cubic crystallites and obtained a value of 0.94 for $K$. Klug and co-workers [43] further simplified the derivation of the Scherrer equation leading to a value of 0.89 for $K$. As shown by Nanda et al. [44,45], a modified form of the equation, which utilizes a value of 0.9 for $K$, can be used to estimate the diameter of spherical particles from the width of a given Bragg reflection. For the purposes of our calculations, we have used ‘$K=0.94’ for cubic crystallites obtained at low pH and ‘$K=0.9’ for spherical particles obtained at higher pH. The (101) peak was used for crystallite size determination. Table 1 shows a comparison of the sizes of the nanocomposite crystallites obtained using poly-L-lysine by various methods. The results indicate that the particle sizes of the samples obtained by TEM and DLS analyses are fairly consistent with the average crystallite size determined from the XRD data.

The composition of the nanocomposite powders was confirmed by EDS analysis and is shown in Figure 4(b). The elemental ratio for Mg/Ge/O was found to be 24.2/15.6/60.2 when grown in the presence of poly-L-lysine. In general, the ratios were fairly consistent with the molar ratios of the precursors used.

**Infra-red Spectroscopy**

The formation of the magnesia–germania nanocomposite powders was also confirmed by IR spectroscopy. Figure 5 shows a comparison of IR spectra of non-calcined and calcined nanocomposite powders. The non-calcined sample (Figure 5(b)) shows broad peaks in the region of 860–1100 and 520–550 cm$^{-1}$. The peaks at 520 and 550 cm$^{-1}$ correspond respectively to the bending vibrational mode and the stretching vibrational modes for MgO [46]. Bands centered at $\sim$1639 and $\sim$1460 cm$^{-1}$ are assigned to water and the amide II peak most likely from the left over amino acids, respectively. The broad peaks in the range of 860–1150 cm$^{-1}$ are assigned to the bending mode of different types of surface hydroxyl groups. There is a decrease in their intensity after calcination, which overlaps with the Ge–O–Ge peak. The calcined material (Figure 5(a)) shows a sharp peak around 960 cm$^{-1}$ and a short peak at 1100 cm$^{-1}$, which correspond to Ge–O–Ge [47].

<table>
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<tr>
<th>pH</th>
<th>Mean diameter (nm) calculated using Scherrer formula</th>
<th>Mean diameter (nm) using TEM analysis</th>
<th>Mean diameter (nm) using dynamic light scattering analysis</th>
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<tr>
<td>4</td>
<td>129.6</td>
<td>128.2</td>
<td>129.3</td>
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<tr>
<td>9</td>
<td>42.1</td>
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**Table 1. Comparison of the Mean Particle Diameter of Magnesia-Germania Obtained using poly-L-lysine.**
Antibacterial Activity

The antibacterial activity of the nanocomposite powders was evaluated by examining the inhibition of bacterial growth of *E. coli* (XL-1 blue strain) (Gram negative) and *S. aureus* (Gram positive). In preliminary studies (data not shown), we did not observe significant size effects of nanocomposite powders (between 50 and 100 nm) on the growth of bacteria, hence all the samples used for antibacterial studies were those nanocomposite powders obtained using poly-L-lysine as catalyst at pH 9. The samples were washed, centrifuged, and calcined before testing for antibacterial activity. Various concentrations of nanocomposite powders were examined for inhibition studies to determine the minimal inhibitory concentration (MIC). The effect of varying concentrations of nanocomposite powders on the percent of bacterial growth is shown in Figure 6(a). On comparing *S. aureus* with *E. coli*, we observed that the nanocomposite powders were more effective against *S. aureus* rather than *E. coli* at lower concentrations. At higher concentrations (>5 mg/mL), the growth of bacteria was almost completely inhibited (>95%) in both cases. The MIC for *S. aureus* was found to be 0.05 mg/mL where as that of *E. coli* was found to be 0.25 mg/mL. This may be due to the fact that the Gram-positive bacteria are encased in a plasma membrane covered with a thick wall of peptidoglycan, while Gram-negative bacteria are encased in a triple layer, the outermost layer being a lipopolysaccharide. In the past it has been shown in some cases that Gram-negative bacteria may be more resistant to chemical agents than Gram-positive bacteria [48] and it appears to be the case for the MgO–GeO₂ nanocomposite powders as well. Another set of bactericidal tests were conducted after washing the cultures from their growth medium in water. We observed that the bactericidal activity was slightly higher (~10% higher) in water rather than in the growth medium. This may due to the fact that the growth medium contains proteins, which may inhibit the attachment of nanocomposite powders to the

*Figure 5. FTIR analysis of (a) calcined vs. (b) noncalcined nanocomposite powder.*
bacterial cell walls. In general, all samples were incubated overnight (~10 h) before examining bactericidal effects. Figure 6(b) shows the growth/no growth of *E. coli* bacteria that were treated with different concentrations of nanocomposite powders in water and then spread on agar plates. The growth of bacteria is completely inhibited when higher concentrations (>5 mg/mL) of nanocomposite powders were added as seen in (iii) and (iv), while at 3 mg/mL (ii), 10 CFUs were observed. Therefore from the above studies conducted, it can be inferred that the MgO–GeO₂ nanocomposite powders have significant bactericidal activity, and overall may be more effective against Gram-positive bacteria.
CONCLUSIONS

In summary, we have developed a simple biological method for the preparation of magnesia–germania nanocomposite powders at room temperature in aqueous medium by biomimetic methods. Of the amino acids used, poly-L-lysine and histidine were found to be the most efficient for the formation of the nanocomposite powders. Calcination of the products led to crystalline materials. The prepared materials were analyzed by several individual methods. The nanocomposite powders were found to be bactericidal toward both Gram-negative as well as Gram-positive bacteria, though they were more efficient against Gram-positive bacteria.

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