Modification of egg shell powder with in situ generated copper and cuprous oxide nanoparticles by hydrothermal method

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Abstract

The poultry waste of egg shells was cleaned and finely powdered which was subsequently modified with in situ generated copper nanoparticles (CuNPs) and cuprous oxide nanoparticles (Cu₂ONPs) by one step hydrothermal method. The modified egg shell powder (MESP) was characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, x-ray diffraction, thermogravimetric analysis and antibacterial tests. The MESP had in situ generated spherical CuNPs and Cu₂ONPs in the size range of 50 nm to 120 nm. The spectral analysis indicated lowering of intensity of the peaks of MESP indicating the involvement of the functional groups of the protein component of ESP in the generation and stabilization of the nanoparticles. X-ray analysis indicated the generation of both CuNPs and Cu₂ONPs in MSEP. The thermal analysis indicated lowering of the thermal stability for MESP. The MESP exhibited excellent antibacterial activity against both the Gram negative and Gram positive bacteria. Hence the low cost MESP with in situ generated CuNPs and Cu₂ONPs can be used as antibacterial cleaning powder for houseware and also as antibacterial filler in the preparation of polymer composites.

1. Introduction

Though polymers in general and polymer composites in particular are serving the society in many fields, unfortunately, they are non-biodegradable and hence pose many environmental problems [1]. Hence, the present trend is shifting to develop biodegradable polymers and their composites [2, 3]. Though some biodegradable polymers such as cellulose, polyactic acid, polypropylene carbonate, Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) etc are available, they lack sufficient strength and thermal stability. Further, most of them are expensive when compared to the synthetic polymers such as polyethylene and polypropylene. In order to overcome these problems, some waste materials such as tamarind nut powder [4], egg shell powder [5, 6], spent tea leaf powder [7, 8], waste leather buff powder [9] etc were used as fillers in making biocomposites with the above listed biodegradable matrix materials. Among these, the egg shells form poultry and kitchen waste which is generated in large quantities all over the world. These egg shells can pose many environmental and health problems if not properly disposed [10]. In this direction, the biodegradable films using polyactic acid [5] and polypropylene carbonate [6] as matrices and egg shell powder (ESP) as filler were made for packaging applications. In order to widen the usage of this abundantly generated poultry waste of egg shells, some researchers also identified the other uses. In this direction, in one study, ESP was utilized to immobilize cadmium and lead in a contaminated soil [11]. Similarly, Haroon et al [12] proposed to utilize ESP as an effective

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cleaning powder for house ware. However, in all these cases, the ESP used does not possess antibacterial activity and hence limits its applications. In order to identify new and environmentally friendly and medical applications, in the present work, we made an attempt to impart antibacterial activity to ESP by modifying it with \textit{in situ} generated copper nanoparticles (CuNPs) and cuprous oxide nanoparticles (Cu2ONPs) by simple one step hydrothermal (thermal assisted) method. ESP is a natural bioceramic composite that contains \(~95\%\) calcium carbonate (in the form of calcite) and \(~5\%\) organic component (X collagen and polysaccharides form)\cite{13}. The main functional groups responsible for the reduction of metal ions are carbonyl, hydroxyl, amino and methoxide which by electrostatic interaction bind the metal ions\cite{14}. The functional groups present in the protein component of ESP are expected to generate copper based nanoparticles by hydrothermal method. The prepared modified egg shell powder (MESP) was characterized by physical appearance, scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, x-ray diffraction (XRD), thermogravimetric analysis (TGA) and antibacterial tests. We aimed to prepare MESP with \textit{in situ} generated copper based nanoparticles that can be used as cheaper filler in the preparation of antibacterial polymer nanocomposites for packaging and medical applications and also to utilize it as a cheaper antibacterial cleaning powder for kitchenware.

2. Materials and methods

2.1. Materials

In the present work, the waste egg shells were collected from the local restaurants and households. Copper sulfate pentahydrate supplied by M/s SDFCL, Mumbai, India was used as received. In all the present experiments, deionized water was used.

2.2. Preparation of ESP

Initially, the procured chicken egg shells were thoroughly cleaned with deionized water and dried at ambient conditions in the open. Using a cleaned kitchen mixer, the dried egg shells were made into fine powder. The egg shell powder (ESP) was sieved and in the present work, ESP with a particle size <25 \(\mu\)m was used.

2.3. Modification of ESP with \textit{in situ} generated copper nanoparticles (CuNPs)

For modification of ESP, 3 g of sieved ESP was added to 100 ml of 250 mM aq.\(\text{CuSO}_4\cdot5\text{H}_2\text{O}\) solution and the mixture was maintained at 80 °C for 24 h. The color of the ESP changed to greenish blue which preliminarily indicates the \textit{in situ} generation of copper based nanoparticles in ESP. The obtained MESP was filtered, washed well with water and subsequently dried. The prepared MESP was stored in the desiccators till tested.

2.4. Characterization

2.4.1. Visual observation

For preliminarily indicating the modification of ESP with \textit{in situ} generated copper based nanoparticles, the unmodified and MESP were observed visually and their digital images were recorded.

2.4.2. Scanning electron microscopy (SEM) analysis

The SEM images of the MESP were recorded on Zeiss EVO scanning electron microscope operated at 10 KV. For confirming the presence of copper element in the MESP, the energy dispersive x-ray (EDX) spectra were recorded using the same scanning electron microscope.

2.4.3. Fourier transform infrared (FTIR) spectral analysis

In order to examine the interactions between the generated CuNPs and the ESP in the MESP, their FTIR spectra were recorded using RXI Perkin Elmer FTIR spectrometer in the wavenumber region of 4000–400 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\).

2.4.4. x-ray analysis

In order to examine the influence of generated copper based nanoparticles on the crystallinity of MESP, the x-ray diffractograms of ESP and MESP were recorded employing Rigaku Ultimo IV x-ray diffractometer operated at 40 kV and 30 mA. In the present case, the diffractograms in the \(2\theta = 10^\circ—80^\circ\) range were recorded at a scanning rate of 4 ° min\(^{-1}\).

2.4.5. Thermogravimetric analysis

In order to understand the effect of generated nanoparticles on the thermal properties of MESP, the thermograms of both ESP and MESP were recorded employing a Perkin Elmer TGA-7 instrument in the temperature range of 80 °C to 800 °C at a heating rate of 10 °C min\(^{-1}\) in nitrogen atmosphere.
2.4.6. Antibacterial activity analysis
As the copper based nanoparticles are known to exhibit antibacterial activity, in order to examine whether the MESP with in situ generated copper based nanoparticles also possesses antibacterial activity or not, the antibacterial test by standard well method [13] was conducted. In the present investigation, the antibacterial test was conducted against both Gram negative (E. coli and P. aeruginosa) and Gram positive (S. aureus and B. licheniformis) bacteria. In each case, the clear zones formed which indicate the inhibition to the growth of bacteria were photographed and the zone diameter was measured. For comparison, the test was also conducted for ESP, CuSO₄·5H₂O and Cu₂O. In each case 10 mg of sample was used.

3. Results and discussion

3.1. Visual observation
In order to examine the appearance of the MESP with in situ generated copper based nanoparticles, the digital images of egg shells, the ESP and MESP were recorded and are presented in figure 1(a)–(c) respectively.

From figure 1, it can be seen that the color of the ESP changed to greenish blue for the MESP. Though the source solution (aq.CuSO₄·5H₂O) was blue in color, the MESP was Greenish blue. The color change preliminarily indicates the in situ generation of copper based nanoparticles. Further, the color of the MESP remained unchanged even after several washings with water during preparation indicating the generation of permanent copper based nanoparticles in it.

3.2. SEM analysis
In order to observe the in situ generated copper based nanoparticles on the surface of the MESP, the SEM analysis was carried out. The SEM image, EDX spectrum and the histogram indicating the particle size distribution of the generated copper based nanoparticles are presented in figures 2(a)–(c) respectively.

From figure 2(a), it is evident that nanoparticles were generated on the surface of the calcite crystals of ESP. The element copper in the MESP was confirmed by the corresponding EDX spectrum (figure 2(b)) which indicates the peak corresponding to copper element. From figure 2(c), it is evident that the size of the generated copper based nanoparticles in the MESP varied between 50 nm and 120 nm with majority of them in the size
range of 80 nm to 120 nm. The average size of these particles was found to be 90 nm. As ESP has 5 wt% organic matter in addition to 95 wt% of calcium carbonate (calcite), the functional groups present in the organic component might have reduced the copper salt source solution into copper based nanoparticles.

3.3. FTIR spectral analysis

In order to probe the structural changes due to the in situ generation of copper based nanoparticles in ESP, the FTIR spectral analysis was carried out. The FTIR spectra of ESP and MESP are shown in figure 3.

From figure 3, it is evident that the spectrum of ESP was broad while that of MESP with in situ generated CuNPs yielded sharp peaks. In the case of ESP, the broad peak observed at around 3400 cm\(^{-1}\) corresponds to the moisture. The other peaks observed at 712 cm\(^{-1}\), 874 cm\(^{-1}\), 1460 cm\(^{-1}\) and 2521 cm\(^{-1}\) are characteristic peaks of CaCO\(_3\). From figure 3, it can also be observed that the bands of MESP became sharp and shifted towards lower frequency. Of these, the broad and strong peak observed around 1460 cm\(^{-1}\) corresponds to the vibrations of carbonate C–O bonds. The other peaks observed at 1803 cm\(^{-1}\), 2874 cm\(^{-1}\) and 2981 cm\(^{-1}\) were attributed to the vibrations of C–O from the carbonate ion (CO\(_3^{2-}\)) [15, 16]. These observations confirm the presence of CaCO\(_3\) in ESP. Further, as ESP contains 95 wt% CaCO\(_3\) and 5 wt% X collagen as binding protein, the peaks at 1640 cm\(^{-1}\), 1540 cm\(^{-1}\) and 1250 cm\(^{-1}\) corresponding to amide I, II and III groups [17] are expected. However, these bands could not be observed in figure 3 as the broad peak at 1460 cm\(^{-1}\) (ranging from 1100 cm\(^{-1}\) to 1700 cm\(^{-1}\)) obscured them. The observed shift of the peaks in MESP indicates the lowering of vibrational frequencies due to the electrostatic interactions between the CaCO\(_3\) of ESP and the generated CuNPs and Cu\(_2\)ONPS. Further, the lowering of intensity in the case of MESP indicates the interactions between the generated nanoparticles and ESP.

3.4. X-ray analysis

In order to study the influence of the generated copper based nanoparticles on the morphology of MSEP, the x-ray analysis was carried out. The x-ray diffractograms of CuSO\(_4\)·5H\(_2\)O, ESP and MSEP are presented in figure 4(a).

From figure 4(a), it is evident that in the case of the diffractogram of ESP, an intense peak at 2\(\theta\) = 29.2° arose due to the reflections from (211) plane of the major component of CaCO\(_3\) with calcite structure [18, 19]. In the case of CuSO\(_4\)·5H\(_2\)O, the peaks at 2\(\theta\) = 15.9°, 1.9°, 25.4°, 27.2°, 38.6°, 49.9° and 51.3° arose due to the reflections from the (100), (011), (111), (130), (222), (202) and (123) planes respectively. This indicates the triclinic structure of CuSO\(_4\)·5H\(_2\)O used in the present work. As the intensity of the ESP is dominating, the additional peaks corresponding to the generated nanoparticles could not be seen in the overlapped diffractograms (figure 4(a)). In order to observe the peaks corresponding to the nanoparticles and also to establish their oxidative state, the diffractogram of MESP in the 2\(\theta\) = 20° to 80° range in expanded form is presented in figure 4(b). From figure 4(b), additional peaks at 2\(\theta\) = 32°, 36.7°, 42.4°, 43.6°, 50.4°, 60.4°, 61.2°, 73.6°, 74° and 77.4° can be observed. Of these, the peaks at 2\(\theta\) = 43.6°, 50.4° and 74° arose due to the reflections from (111), (200) and (220) planes respectively of CuNPs whereas those observed at 2\(\theta\) = 32°, 36.7°, 42.4°, 61.2°, 73.6° and 77.4° arose due to the reflections from (002), (111), (200), (220), (311) and (222)
planes respectively of Cu₂O nanoparticles [20]. Thus x-ray analysis indicates the in situ generation of both CuNPs and Cu₂ONPs in MESP.

3.5. Mechanism of in situ generated CuNPs and Cu₂ONPs in MESP
As the x-ray analysis indicated the generation of both CuNPs and Cu₂ONPs and as ESP has both CaCO₃ as major component and collagen protein as the binding material in MESP, there are two possibilities of the reactions. The first possibility is by the participation of CaCO₃ and CaO in the reaction by which the nanoparticles are generated. However such reactions are possible only at very high temperature [21]. The formation of CuNPs and Cu₂ONPs in aqueous phase is reported in the literature [22]. In the present work, the MESP was prepared by hydrothermal method and hence the other possibility is through the protein component route. Collagen protein in egg shell is composed of mainly three amino acids —Glycine, Proline and Hydroxy proline [23]. The hydroxyl proline can react with aqueous copper sulfate solution to undergo reduction of Cu²⁺ to Cu° to form proline. The formed Cu metal undergoes complexation with amino group in glycine amino acid present in collagen [24]. The possible reactions involving the three amino acids of collagen component of ESP are presented in figure 5: Thus all the three amino acids of collagen component of ESP were involved in the formation and capping of both the CuNPs and Cu₂ONPs.

3.6. Thermal analysis
In order to study the influence of the in situ generated CuNPs and Cu₂ONPs on the thermal stability of the MESP under study, the thermogravimetric analysis was carried out. For comparison, the experiment was also conducted on the ESP. The primary thermograms of ESP and MESP are presented in figure 6.

From figure 6, it is evident that the in situ generated copper based nanoparticles decreased the thermal stability of the MESP. It is understandable as the generated copper nanoparticles act as catalyst to increase the thermal degradation. However, even at 700 °C the MESP retained 70 wt% of its initial mass (for ESP, the weight at 700 °C was 87%) indicating that the thermal stability of the MESP is still good. Further, the weight variation for the MESP from initial weight in the 200 °C to 400 °C was 92% to 80%. This indicates that in the usage temperature (room temperature to 80 °C cleaning temperature range), the MESP did not thermally degrade.

3.7. Antibacterial activity
In order to study the antibacterial activity of the MSEP, the well method was used. For comparison, the test was also conducted for ESP (Matrix), CuSO₄.5H₂O and Cu₂O powders. The zones of clearance formed in each case were photographed and the images are presented in figure 7(a).

From figure 7(a), it is evident that both Cu₂O and CuSO₄.5H₂O exhibited significant antibacterial activity as evidenced by the formation of zones of clearance. Similar observations were made in the case of filter paper embedded with Cu₂O and CuSO₄.5H₂O [25] and CuSO₄.5H₂O [26]. To examine whether the MESP also exhibits the antibacterial activity or not against the bacteria, the antibacterial test for both ESP and MESP was carried out. The zones of clearance in each case were photographed and the images are shown in figure 7(b).

From figure 7(b) it is evident that ESP did not form any zone of clearance and hence failed to exhibit antibacterial activity against both the Gram negative and Gram positive bacteria. However, the MSEP formed significant zones of clearance and hence exhibited excellent antibacterial activity. In order to quantify the antibacterial activity, the diameters of the zones of clearance were measured and are presented in table 1.
From Table 1, it is clearly evident that in the case of both CuSO$_4$·5H$_2$O and Cu$_2$O powders, the diameter of the zone of clearance varied between 16 mm and 25 mm. On the other hand, the MESP formed the clearance zones with diameters ranging from 36 mm to 40 mm. The higher zones of the clearance in the case of MSEP can

Figure 5. Reactions involving the three amino acids of collagen component of ESP.

Figure 6. Primary thermograms of ESP and MESP.

From Table 1, it is clearly evident that in the case of both CuSO$_4$·5H$_2$O and Cu$_2$O powders, the diameter of the zone of clearance varied between 16 mm and 25 mm. On the other hand, the MESP formed the clearance zones with diameters ranging from 36 mm to 40 mm. The higher zones of the clearance in the case of MSEP can
Figure 7. (a) Antibacterial activity of Cu$_2$O (A) and CuSO$_4$·5H$_2$O (B) against both the Gram negative bacteria—E. coli (a) and P. aeruginosa (b) and Gram positive bacteria—S. aureus (c) and B. licheniformis (d). (b) Images of clear zones indicating the inhibition of growth of bacteria by MESP with *in situ* generated CuNPs (Y) and ESP (O) against E. coli (a) and P. aeruginosa (b) and Gram positive bacteria—S. aureus (c) and B. licheniformis (d).
be attributed to the higher surface energy of the CuNPs and CuONPs generated in it. Thus, the MESP exhibited excellent antibacterial activity and hence can find applications as antibacterial filler in the preparation of nanocomposites and also as antibacterial low cost cleaning powder for house wares.

4. Conclusions

The cleaned and dried egg shells were finely powdered and the resulting egg shell powder (ESP) was modified by in situ generated copper nanoparticles (CuNPs) and cuprous oxide nanoparticles (Cu2ONPs) by simple one step hydrothermal method. The modified ESP (MESP) had spherical copper based nanoparticles in the size range of 50 nm to 120 nm with an average of 90 nm. Both CuNPs and Cu2O nanoparticles were in situ generated in MESP as revealed by the x-ray analysis. The MESP exhibited antibacterial activity against both Gram negative and Gram positive bacteria. The MSEP can be used as a low cost antibacterial filler in the preparation of polymer nanocomposites and also as antibacterial cleaning powder for houseware.

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Conflict of interest

None.

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