Antibacterial Activity of Plasma-Treated Cu-Bentonite Nanocomposites

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Abstract. Copper (Cu) ions were successfully loaded in a bentonite matrix via ion-exchange method producing the Cu-Bentonite nanocomposites. Samples were treated using 13.56 MHz radio frequency (RF) plasma system with argon (Ar) and oxygen (O₂) gases at varying input power (30 and 80 W) and constant treatment time of 10 min. X-ray diffraction patterns revealed the presence of Cu metal after the plasma treatment of samples. SEM images confirmed the changes on the surface of the nanocomposites after plasma treatment. The untreated and treated Cu-bentonite nanocomposites showed effective antibacterial activity against *E. coli* and *S. aureus*. The nanocomposite can be used for biological as well as biomedical applications due to its antibacterial capabilities.

1. Introduction

Clay minerals are commonly used in various applications because of its high cationic exchange capacity and surface area. Bentonite clays, belonging to the smectite group, are formed mainly by montmorillonite, particularly sodium (Na)–montmorillonite [1]. Because of its low cost and availability as well as its stable mechanical and chemical property, bentonites are being used for ion exchange and adsorption applications [2, 3]. The interlayer, formed by one octahedral sheet sandwiched between two silica tetrahedral sheets, between its units is composed of positive cations and water molecules making them excellent supporting materials for nanoparticles [4].

Metal and metal oxide nanoparticles have attracted much attention because of their high surface area to volume ratio. Among others, Cu [5, 6] and Ag [7, 8] metals are considered to be an efficient antibacterial agent against well-known bacteria. Comparing the two metals, Cu nanoparticles are best alternative to Ag because of its low cost [9]. Recent studies have shown that Cu ions interact freely with bacteria protein. The oxidation of Cu ions generates hydroxyl radicals breaking down the bacterial proteins and DNA [10]. Inorganic antibacterial agents are usually in the form of nanocomposites, in which the metallic nanoparticles are impregnated in the carrier [11].

Considering that some nanomaterials are hazardous, combining these materials with environment friendly, inert and stable material is a solution to take advantage of the properties of these materials in various applications. Clay minerals are effective excellent fillers for metal nanocomposites [12, 13]. Their swelling and adsorption capacities are effective in attracting contaminants to the surface enhancing the efficiency of the dispersed nanoparticles [14]. Different methods have already been
employed to incorporate metal-based compounds in the clay matrix. Others have done gamma irradiation [15], polyol [16], chemical reduction [17, 18], hydrothermal synthesis [19] and electroless plating [20]. Some of these methods require the use of additional chemicals and the use of high temperatures which is non-ideal in some cases. Recently, the use of plasma has received special attention in terms of surface modification applications owing to its high efficiency, simple operation and being environment friendly [21, 22]. Depending on the gases used, it is possible to activate a surface in the presence of active species such as hydroxyl and carbonyl which react on the surface with specific chemical functions [23-25]. As a result, the surface is being functionalized with desirable properties for different technologically important applications without affecting its bulk properties.

Studies have shown that argon (Ar) and oxygen (O₂) plasmas can directly reduced supported metal ions into their metallic state. This dry process is independent on the amount of loading and the type of supporting material indicating that plasma driven reduction may be universal for metal ions [26]. This is different from the conventional reduction method which requires reducing agent. In this work, the antibacterial activity of pristine and plasma treated Cu-bentonite nanocomposites prepared via ion-exchange method against *E. coli* and *S. aureus* was evaluated.

2. Experimental Methods

2.1. Sample preparation and treatment

Bentonite samples were gathered from Mangatarem, Pangasinan, Philippines (Saile Industries, Inc.).

For the ion-exchange method, 1 g of bentonite powders were stirred for 5 h in 0.2 M CuCl₂ solution. Powders were then collected and washed via centrifugation at 2500 rpm for 5 min. The as-prepared samples were dried at 60°C for 1 h. The samples were pelletized having a constant diameter of 8 mm.

For the plasma treatment, a 13.56 MHz radio frequency (RF) plasma system defined elsewhere [27] was used to treat the prepared samples. Gases used were Ar and O₂ with a constant operating pressure of 100 Pa. Exposure time was set at a constant of 10 min while the RF power was varied at 30 and 80 W.

2.2. Materials characterization

The prepared samples were characterized using X-Ray diffraction (XRD) and Scanning Electron Microscopy (SEM). XRD patterns were recorded using Rigaku RINT2100 CMJ diffractometer at 20 Bragg angle range from 3° to 80°. The morphologies of the nanocomposites were analyzed using Hitachi, S-3400N.

2.3. Antibacterial assay

After the plasma treatment, the antibacterial activity of the samples was tested. The antibacterial property of the composite was tested against *S. aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria) using the inhibition zone method. The sides treated were exposed to the agar surface. Agar plates were incubated at 35°C and observed after 24 h. The experiments were done in the Natural Science Research Institute (NSRI), University of the Philippines Diliman. The clearing zones were observed below the sample and around the sample. The presence of clearing zones or zones of bacterial growth inhibition indicates antibacterial activity.

3. Results and Discussion

Bentonites were typically stable in nature and this was proven even after the reaction with plasma gases at varying power. Figure 1 shows the XRD spectra of the bentonite samples before and after plasma treatment. The XRD spectrum of the bentonite samples showed peaks at 2θ = 5° and 10°, reflecting montmorillonite peaks (δ(Na,Ca)|ρ:|1:AlMg)-(Si4O10)(OH)²⁻·nH₂O indicating its main mineral component [29]. Plasma reduction occurs when high energy electrons from the plasma recombine with the metal ions present in the template. Based from figure 1, peak at 2θ = 50° for the samples after plasma treatment can be attributed to pure Cu metal (JCPDS 04-0836). These results suggest that the Cu ion in the bentonite was reduced to Cu metal under plasma treatment [28]. However, the peaks
related to Cu showed the amorphous phase of Cu impregnated in the bentonite framework. It is further suggested that increasing the exposure time could have resulted to a more crystalline phase incorporated in the matrix.

Surface morphologies of the bentonite before and after plasma treatment were shown in figure 2. Micrographs showed the roughening of the bentonite surface after the plasma treatment. There were considerably small cracks and fine particles attached on the surface of the bentonite after the plasma treatment. Exposure to plasma could have an effect on the external surface structure. This can be attributed to etching where active species generated in the plasma induced changes in the upper layer of the nanocomposites during the treatment. The etching effect also increased the active area of the bentonite thus increasing reactivity.

The antibacterial activities of pristine and modified bentonite were tested against two strains of bacteria. Theoretically, bentonite clays have no antibacterial activity. This is proven in the antibacterial index that even after the plasma treatment, no significant effects were observed. Results have shown that Cu was successfully diffused in the matrix of material due to an increase in the index even before plasma treatment. An increase in the antibacterial index was observed after the plasma treatment which can be attributed to the reduced Cu ions incorporated in the matrix. Higher antibacterial index was observed for S. aureus compared to E. coli due to structural differences of the two bacteria. Gram-negative bacteria (E. coli) are more resistant to the inhibiting effect of Cu-based compounds [30]. The cell walls of S. aureus contain peptidoglycan making it highly susceptible for penetration. Exact mechanisms regarding the antibacterial property of Cu based compounds were not yet established. Others have proposed the formation of reactive oxygen species that penetrates the cell wall of the bacteria producing free radicals which causes the disintegration of vital enzymes leading to cell death [10]. This could possibly explain the reason behind higher antibacterial index values for nanocomposites treated with oxygen gas. On the other hand, it also suggested that the death of these bacteria is due to the charges acting on the bacteria itself and the antibacterial agent. Under certain conditions, bacterial cell walls are negatively charged due to the functional groups present at the surface. Thus, the Cu\(^{2+}\) ions dispersed in the matrix binds with the functional groups of the bacteria causing the inactivation and inhibition in cell processes [30].

![Figure 1. XRD patterns of Cu- Bentonite nanocomposites (a) before plasma treatment and after treatment with (b) Ar – 30 W, (c) O\(_2\) – 30 W, (d) Ar – 80 W and (e) O\(_2\) – 80 W.](image-url)
Figure 2. SEM images of Cu- Bentonite nanocomposites (a) before plasma treatment and after treatment with (b) Ar – 80 W, (c) Ar – 30 W, (d) O₂ – 80 W and (e) O₂ – 30 W.

Table 1. Antibacterial indices of the as-synthesized samples

| Treatment   | E. coli | S. aureus | E. coli | S. aureus |
|-------------|---------|-----------|---------|-----------|
| No treatment| -       | -         | 1.4     | 1.5       |
| Ar – 30 W   | -       | -         | 1.6     | 1.6       |
| O₂ – 30 W   | -       | -         | 1.5     | 1.6       |
| Ar – 80 W   | -       | -         | 1.5     | 1.8       |
| O₂ – 80 W   | -       | -         | 1.5     | 1.8       |

4. Conclusions
This study showed the effective incorporation of Cu in a bentonite matrix by facile ion-exchange method. Followed by plasma treatment using different gases (Ar and O₂) and varying incident powers (30 W and 80 W), the Cu²⁺ ion was reduced to an amorphous Cu in the matrix. It was revealed that after the plasma treatment, the surface of the material became rough. All of the Cu-Bentonite samples showed antibacterial activity against E.coli and S. aureus. This indicates the applicability these composites in biomedical-related applications.

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