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Original Research Article

SYNTHESIS OF ZEOLITE A FROM KAOLIN LOADED WITH CARBON NANOTUBES AND THE EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

This study evaluates the antimicrobial activity of zeolite A loaded with Carbon Nanotubes (CNTs) against bacterial isolates usually common in water (especially wastewater). The zeolite was synthesised using the hydrothermal synthesis method while incorporation of CNTs into it was via the sol gel method. The sample was characterised using X-ray Diffraction (XRD) Fourier Transform Infrared spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). The results showed well-defined cubic crystals of Zeolite A were formed and confirms the homogeneous distribution of CNT around them. The antimicrobial activity of Zeolite A/CNT was tested against three gram-negative bacteria (Salmonella typhi, Escherichia coli and Pseudomonas Aaeruginosa), using the agar dilution method at concentrations of 12, 16 and 20 mg/ml. Results showed no significant activity irrespective of the concentration. Only delayed growth was noticed in P. aeruginosa.

1. INTRODUCTION

Over the years, disease causing microorganisms have developed resistance to conventional antibiotics leading to serious health issues. This resistance is greatly attributed to vast and inappropriate use of large particle sized antimicrobial agents (Dizaj et al., 2015). In response to this, experts developed interest in studying better drugs and more effective ways to deliver them. One very important material that has been studied as drug host or carrier is zeolite, due to its unique properties which include molecular sieving, shape selectivity, high adsorption capacities, acidity, high thermal stability and most importantly ion exchange (Xu et al., 2009).

Zeolites though crystalline and microporous in nature are made up of alumina and silica tetrahedral frameworks with cations and water within their pores (Demirci et al., 2013). Due to the presence of these cations and pore spaces, nanomaterials (harmful to bacteria) can be incorporated into the zeolite structure through ion-exchange or covalent bonding and used to inhibit or kill bacteria. Several nanoparticles have

been put to these effects with considerably high success rate. This discovery has led to further studies into the bactericidal effect of other nanomaterials such as Carbon Nanotubes (CNTs).

CNTs as defined by Sumio Lijima in 1991 are nano-sized sheets of graphite rolled into cylinders. They have attracted attention in the biomedical fields as antimicrobial agents due to their unique structure and properties which include high stability, ability to be functionalized, high surface to volume ratio and larger inner volume. CNTs are of two major types; the single walled carbon nanotubes (SWCNTs) and multi walled carbon nanotubes (MWCNTs), both of which are toxic. Studies by Kang et al. (2008), and Yang et al. (2010) suggest that SWCNTs, due to their small diameter and long lengths which allows them penetrate the cells and form better cells-CNTs aggregates, are the most toxic to bacteria. Most studies on MWCNTs show no significant antimicrobial activity (Dizaj et al., 2015) but when functionalized with other antimicrobial agents show potency towards bacteria (Amiri et al., 2012).

This study, which is new, involves the incorporation of CNT into zeolite framework and investigating its antimicrobial activity. The bacteria to be studied include: *E. coli, S. typhi* and *P. Aaeruginosa*. The closest use of zeolites in antimicrobial activity study involved the incorporation of silver ions into zeolite framework by ion-exchange and Kwakye-Awuah (2008) and he reported significant success in his work. The zeolite A to be used in this work will be synthesized from Ahoko Kaolin using the hydrothermal synthesis method and the incorporation will be done via sol-gel method.

2. MATERIALS AND METHODS

2.1. Materials

Materials used in this study include: Raw Ahoko Kaolin from Kogi State, Sodium Hydroxide (NaOH) pellets from Panlac Pharmaceuticals, Minna, Nigeria, Distilled Water, Carbon Nanotubes and three gram-negative bacteria (*E. Coli, P. aeruginosa* and *S. typhi*) from Step B, FUT Minna, Bosso campus, Nigeria.

2.2. Methods

2.2.1. Pretreatment

Deionized water (28 ml) and crushed clay (5 g) were mixed to create a clay suspension which was allowed to settle in a measuring cylinder for 25 mins. After this, the mixture was decanted, and the supernatant was collected into a new measuring cylinder where it was allowed to settle for another 24 hours. A sample of clay (size, <2µm) was obtained after decanting the deionized water and it was dried to give refined Kaolin. This process was repeated to collect enough refined kaolin for subsequent experiment.

2.2.2. Metakaolinization

The metakaolinization was carried out by placing 5 g of the refined Kaolin in a crucible and calcined for 10 minutes in a furnace at a temperature of 800℃.

2.2.3. Synthesis of Zeolite A/CNT

The batch composition used for the synthesis is given by Kovo (2011) as: $3.75Na_2O:Al_2O_3:2.56SiO_2:243.7H_2O:xCNT$. To achieve this composition, 0.832 g of NaOH was dissolved in 12 ml of deionized water. Then, 0.693 g of metakaolin and 1.0 g (7 w⁄w%) of CNT were added to the mixture and stirred until a homogeneous solution was obtained. The solution was aged and stirred continuously at room temperature (27 ℃) for 6 hours. The aged gel was transferred into an autoclave and treated hydrothermally in an electric oven at 100℃ (crystallization temperature) and for 6 hours

(crystallization time). The synthesized sample was filtered using a filter paper and then washed using distilled water until the pH was 7 (neutral). The washed zeolite was then dried at 60 ℃ in an oven, then crushed into uniform powder, and then sieved.

2.2.4. Antibacterial susceptibility test

The zeolite A/CNT composites synthesized were investigated for antibacterial activity on the test organisms (*S. typhi, E. coli* and *P. aeruginosa*) using agar dilution method as described by Collins et al. (1995). 12000 µg/ml was affected by reconstituting 0.036g of the composite in 3cm3 of sterile distilled water for the polar solution while for the non - polar and mid polar solution the Zeo-A/CNT was first dissolved in 1.0 ml of dimethyl sulfoxide (DMSO) and then added to 2.0 ml of the distilled water. The mixtures were stirred and shaken vigorously for homogeneity. Under aseptic condition, a loopful of the standardized culture was plated into 19 cm3 of solidified nutrient agar containing 12000 µg/ml of the composite solution and was incubated for 24 hr at 37 0oC. The procedure was repeated for two more concentrations (16000 µg/ml and 20000 µg/ml) and control plates were prepared simultaneously. The efficacy of the composite was compared with Minimum inhibitory concentration (MIC) of chloramphenicol (250 µg/ml) for each organism. In the table of results positive and negative signs were used to describe activity and no activity, respectively.

2.2.5. Characterization

All the synthesized in the work were characterized with the aid of XRD, FTIR and SEM. The XRD pattern of the sample was recorded using Rigaku X-ray diffractometer operated at 30kV and mA at angular rate of 5^{o}/min. The Fourier Transform Infra-red spectroscopy (FT-IR) was performed in the range of 400-4000 cm^{-1} using kBr disk. The scanning electron Micro scope (SEM) image was obtained using environmental scanning electron microscope (ESEM) operated at an accelerating voltage of 3 kV.

3. RESULTS AND DISCUSSION

3.1. X-Ray Diffraction Analysis

The XRD result is as shown in Figure 1. The result showed that XRD pattern of the Zeolite A synthesized correspond to standard Zeolite A which is usually taken as fingerprint and definitive outcome of the synthesized composite via X-ray diffraction.

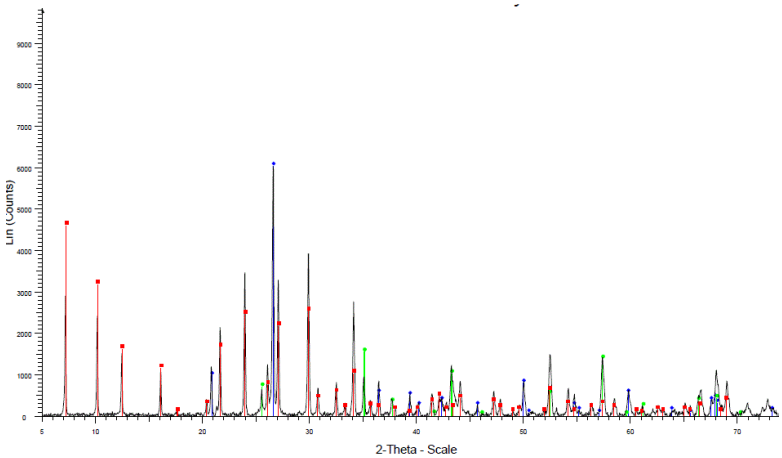

Figure 1: XRD result of synthesized zeolite A/CNT

The results confirm that the composite consist of Sodium Aluminum Silicate Hydrate which according to American Elements is a synonym for Zeolite type A. This was found as the major constituent phase in the specimen, whereas quartz and corundum (aluminum oxide) where found as minor phases. The peaks of the synthesized product matched the characteristic peaks of zeolite A at 2θ values of 7.2°, 10.3°, 12.6°, 16.2°, 21.8°, 24°, 26.2°, 30°, 30.9°, 32.6°, 33.4° and 34.3° that were reported by Treacy and Higgins (2001). Figure 1 also shows peaks attributed to the presence of an impurity, Aluminum oxide, which are not present even though both materials were synthesized from the same materials except that the result in Figure 1 is that of a sample containing CNT. This could serve as confirmation for the presence of CNT in the sample. The identified impurity (Al2O3) is a major part of a bimetallic catalyst used in the synthesis of CNT. The peaks of the presumed CNT first appear at 2θ value of 25.6° which correlates with that of Zhao and Ando (1998).

3.2. FT-IR Spectroscopy of Zeolite A/CNT

Figure 2 shows the FT-IR spectra of the synthesized zeolite A/CNT. It was observed that the major wave bands corresponding to the major functional groups of Zeolites are present as well as that of MWCNTs. This confirms the presence of the materials in the sample. The broad wave band at 3369 cm^{-1} represents the symmetric and asymmetric stretching vibrations of the OH functional group. This group can be found both in CNT (functionalized) and Zeolite. Since, the CNT is not functionalized, it can be assumed that this band is for the OH group on the Zeolite surface. Also, the vibrations generated by Si-O bond is a band located at 1684 cm^{-1} while the band located at 1237 cm^{-1} is due to the vibrations generated by Al-O bonds. Pristine CNTs usually have featureless FTIR spectra like the peaks at 1684 cm^{-1} which may represent C=O stretching and that at 3370 cm^{-1} which represents either COO-H or O-H stretching. This means that various carboxyl and hydroxyl functional groups were present on the surface of the synthesized composite. The peak at 2923 cm-1 represent vibrations generated by C-H¬x bond. This bond is common in the CNT (Misra et al., 2007). Kwakye-Awuah (2008) attributed the band located at 969 cm^{-1} to the overlap of asymmetric vibrations of Si-O- (non-bridging) and Si-O (bridging) bonds.

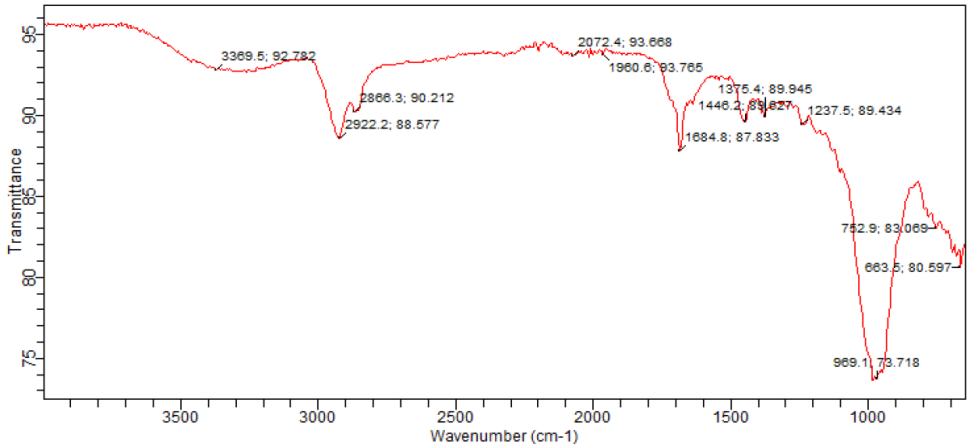

Figure 2: FTIR spectra of the synthesized Zeolite A/CNT

3.3. Morphology

SEM was used to study the morphology of the synthesized sample using different magnifications. The image of the synthesized Zeolite A/CNT composite is shown in Figure 3. The cubic nature of the crystals formed and the fibrous material within and surrounding them confirms the presence of the synthesized zeolite A along with CNT in the specimen. The Figure shows SEM image of well crystallized cubic

material which is consistent with that of zeolite A. Figure 3 shows CNT embedded within the zeolite crystals. The morphology as described here is supported by the FT-IR and XRD shown in Figures 1 and 2 respectively.

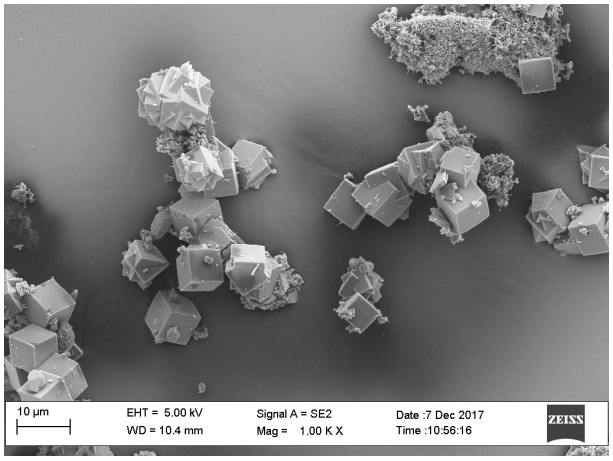

Figure 3: SEM image of the synthesized Zeolite A/CNT

3.4. Antimicrobial Activity

Table 1 shows the results of the susceptibility test and from there it can be deduced that no activity was recorded for any concentration of Zeolite A/CNT against any of the three bacteria. The two positive results are for the antibiotic, Chloramphenicol, at a concentration of 250 µg/cm^3 against *S. typhi* and *P. aeruginosa* (Plate Ic). Plate I(b) and Plate III (a, b and c) show that *E. coli* proved to be resistant even to the antibiotic probably because of its advanced eukaryotic cell walls (Ferreira et al., 2012). Kwakye-Awuah, (2008) stated that materials such as zeolites which do not dissolve upon solvation show no antimicrobial activity. This was the case with the Zeolite A/CNT composite. The composite failed to form a colloidal solution and diffuse through the nutrient agar which must have limited its ability to inhibit the bacteria. Plates II, III and IV contained *S. typhi, E. coli* and *P. aeruginosa*, respectively. Concentration of the composite was increased and each plate labelled (a) contained 12000 µg/cm^3, (b) contained 16000 µg/cm^3 and (c) contained 20000 µg/cm^3. Increasing the concentration of the composite against the bacteria did not cause any physical noticeable difference in their activity as shown in the results (Table 1).

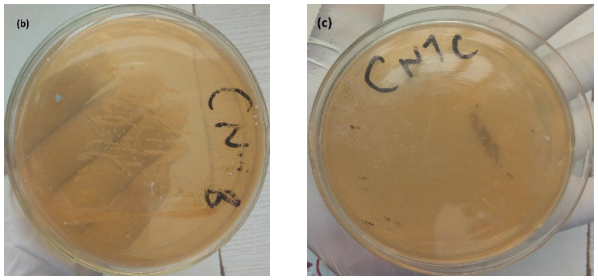

Plate 1: Image showing activity of Chloramphenicol against organisms: (b) *E. coli* and (c) *P. aeruginosa*

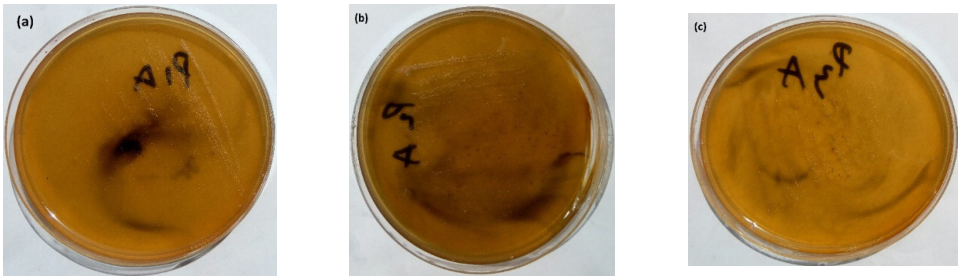

Plate II: Image showing activity of Zeolite A/CNT against *S. typhi*

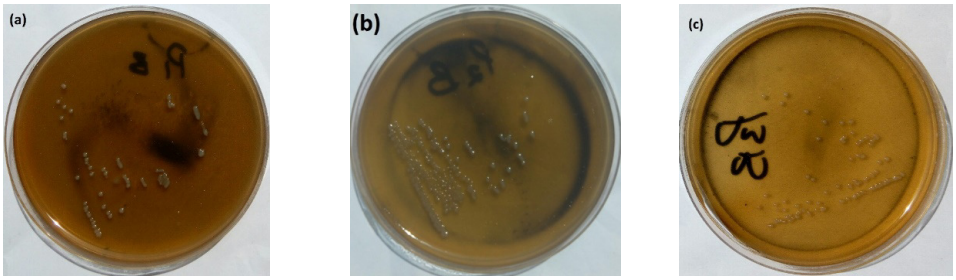

Plate III: Image showing activity of Zeolite A/CNT against *E. coli*

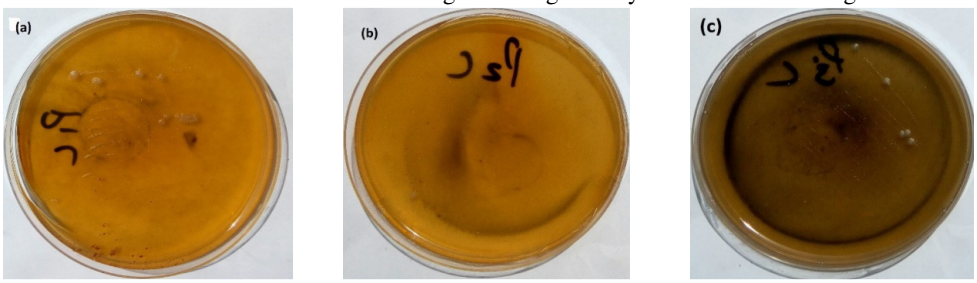

Plate IV: Image showing activity of Zeolite A/CNT against *P. aeruginosa*

Table 1: Antibacterial activity of Zeolite A/CNT and chloramphenicol against *S. typhi, E. coli* and *P. aeruginosa*

Organisms	Concentration ($\mu g/cm^3$)			
	Z/CNT 12,000	Z/CNT 16,000	Z/CNT 20,000	Chloramphenicol 250
Salmonella Typhi	-	-	-	+
Escherichia Coli	-	-	-	-
Pseudomonas Aeruginosa	-	-	-	+

Z/CNT=> Zeolite A/Carbon Nanotubes

To support these findings, Chen et al. (2013) stated that elevating the concentration of CNT may cause the CNTs to aggregate, thus, lessening their activity (toxicity). Bacteria in plate III and IV reproduced nearly as fast as they did in an uncontaminated nutrient agar while the bacteria in plate V experienced delayed reproduction. The findings could not be attributed to their cell structure as all the bacteria are gram negative bacteria, but as supported by Chen et al. (2013), it suggests that CNTs may have less antibacterial activity against rod-like bacteria as opposed to spherical ones.

Though results from the SEM and FTIR analysis confirmed the synthesis of the composite, Zeolite A/CNT, the antimicrobial activity test has proven to be a negative. Varying concentration and exposure time did not yield a different outcome.

4. CONCLUSION

CNT loaded zeolite A was synthesized using the sol gel method. Structural properties of the CNT loaded zeolite A were determined using FTIR, SEM and XRD. The results confirmed the presence of well-defined cubic crystals of zeolite A, and CNT with an average diameter of 33 nm in the synthesized specimen. The SEM and FT-IR results confirmed that Zeolite A/CNT composite was synthesized but no significant antimicrobial activity was recorded in the antimicrobial susceptibility test. This confirms that solubility and functionalization of CNT plays an important role in its antimicrobial activity. The antimicrobial activity of the composite was also found to be independent of concentration.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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