Effects of Halloysite Nanotubes (HNT) Structures on Antimicrobial Activity on TPSS Film

Nader Abuhamed, Zuraida Ahmad*, Maisarah Tajuddin

Department of Manufacturing and Materials, Kulliyyah of Engineering, International Islamic University Malaysia, Gombak, 53100 Kuala Lumpur, Malaysia

Abstract

Halloysite nanotubes (HNT) build good interest in biological technologies attributable to their excellent loading capacity and hollow tubular structures. HNT is also naturally available, low toxicity, and inexpensive. This paper investigates the HNT structures' effect in encapsulating the antibiotics for antimicrobial activities in thermoplastic sago starch (TPSS) wound dressing film. The control TPSS film was produced using sago starch, glycerol, and water. In the nanocomposite films, the HNT was diluted in 0.25 and 0.5 mL, without and with immersion in 2 ml antibiotics, with a TPSS solution with the following names: TPSS/HNT/0.25, TPSS/HNT/0.5, TPSS/HNT/0.25A, and TPSS/HNT/0.5A, respectively. All samples were characterized by Fourier transform infrared, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) for the morphological analysis and tested for antimicrobial activity using a disc diffusion test. Micrographs from SEM and TEM demonstrated that pristine HNT had an average length of 400 nm with an average outer and inner diameter of 90 nm and 30 nm, correspondingly. The HNT with empty lumen tubes shows high absorption and loading capability of the antibiotic. It is believed that the antibiotic was released over a prolonged time or responded to when the bacteria approached. The inhibition zone sizes designated the antimicrobial activity on nano-composites films were 67.3 ± 0.5 and 100 ± 0.3 mm^2 for TPSS/HNT0.25 and TPSS/HNT0.5A, respectively, and zero inhibition zone for other samples.

Keywords: halloysite, nanotube, tubular, encapsulation, antibiotics

Received 23rd December 2019
Accepted 29th December 2020
Published 1st December 2020
*Corresponding author: Zuraida Ahmad; email: zuraidaa@iium.edu.my

ISSN: 1823-7010, eISSN: 2600-7444

Introduction

Nowadays, there is a growing interest in biodegradable polymers as wound healing materials to reduce environmental pollution caused by synthetic dressing materials. The development of new biopolymers has been motivated by the need to minimize polymer products' carbon footprint, the ecological and fossil resource shortages. Interestingly, starch is a promising and widely available candidate on the market. Starch has economic advantages, high availability, and total compost ability without toxic residues [1]. Native starch alone, however, is brittle, and its mechanical properties are low [2-3]. Therefore, starch needs to be transformed into a thermoplastic to manufacture thermoplastic starch (TPS) via the introduction of plasticizers, including glycerol, to improve its plasticity and reduce its fragility. Nanoclays, on the other hand, are added to TPS for reinforcement or fillers, which lead to the formation of composites and improve their mechanical and physical properties. A good example is halloysite nanotube, as it is cytocompatible and biocompatible [4-6].

Halloysite is a natural, kaolinite-like aluminosilicate nanotube from a clay mineral. It has a two-layer aluminosilicate structure, in which layer rolling forms the hollow tubular morphology [7]. HNT exhibits a high aspect ratio with the length ranging typically from about 500 nanometers to over 1.2 microns, and diameters typically smaller than 100 nanometers. In another study, common HNT has a length of 300nm ~1,500nm, while the inner and outer diameters are 15nm ~100nm and 40nm ~120nm, respectively [8]. The high length-diameter aspect ratio allows HNT to be an excellent nano-reinforcement for polymer nanocomposites.

Halloysite has a similar stoichiometrically composition to kaolinite, except for its water content. According to Duarte and colleagues [9], the hydrated form (with an interlayer spacing of 7 Å), the formula $Al_2Si_2O_5(OH)_4.2H_2O$ and the anhydrous (in interlayer spacing of 10 Å) and the kaolinite composition $Al_2Si_2O_5(OH)_4$ transpire mainly in the polymorphous layer.

Besides, halloysite aluminosilicate comprises two hydroxyl groups: internal layer-to-external groups located on the surface, as shown in **Figure 1** [10]. The density of the halloysite surface hydroxyl groups is much smaller than other silicates, such as kaolinite and montmorillonite. Therefore, with the combination of a high aspect ratio and low-density surface hydroxyl group, HNTs are very promising as reinforcing fillers for polymer materials.

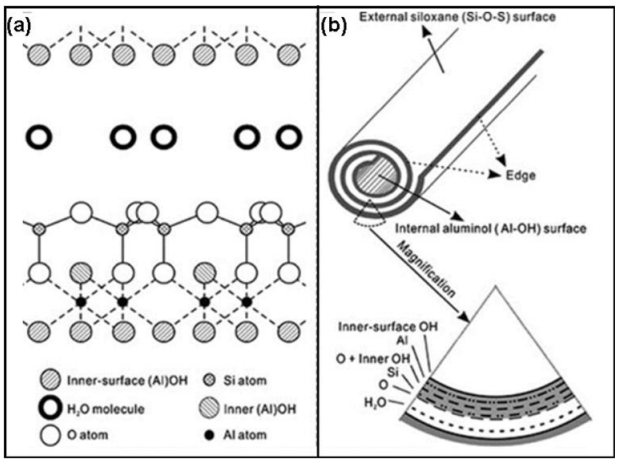

Figure 1: Schematic diagrams of (a) the crystalline structure halloysite and (b) the structure of a halloysite nanotube [10]

HNTs are also sufficiently able to bind with many synthetic and biological components because of the larger surface area, positively charged inner surface (Al–OH groups), and a negatively charged outer surface (Si-OH and Si-O-Si groups). The functional groups present on the surface of HNT aid in the loading of negatively charged bio-macromolecules into the positive lumen of the nanotube, such as DNA and antimicrobial agents' encapsulation. Combined with improved biocompatibility and lower cytotoxicity, this make-up plays a vital role in advancing recent applications, such as in biomedical sciences, namely the development of novel drug and gene delivery agents, such as tissue engineering scaffolds, wound dressings, tumour cell isolation, and enhanced adhesion of human cells [9-10].

Henceforth, this paper reports from earlier research [11-12], using sago (*metroxylene rottb*) to produce TPSS film, and using HNTs as a filler. In this study, the advantage of the tubular structure and the positive and negative charges on the inner and outer surface of HNT were investigated to optimize TPSS's potential as an antibiotic carrier in the treatment of antimicrobial activities.

Materials and Methods

Characterization of the as-received HNTs includes particle distribution and morphological studies. Thermoplastic sago starch (TPSS) acted as control samples made from the composition of 6.5% sago starch, a 3.5% glycerol, and 90% distilled water [12]. Initially, sago starch and glycerol were added to distilled water. Next, the solution was heated and magnetically agitated until gelatinized at 75 ℃. The mixture was then poured on a smooth flat surface as film samples and placed in the oven at 40 ℃ for 20 hours.

The fabrication of TPSS wound healing film nanocomposites was carried out by dispersing 0.35 grams of HNT in 15.0 ml of distilled water and magnetically stirring at room temperature for 60 minutes. Later, 2.0 ml of antibiotics (chloramphenicol) was added to the HNT solution and continue mixing for 10 minutes.

The mixture was then poured onto a smooth flat surface as film samples and dried for 20 hours inside the oven at a temperature of 40 ℃ [12]. These film samples were peeled and ready for further testing. The prepared samples and its composition are listed in **Table 1**.

Table 1: Composition of nano-composites film samples of TPSS /HNT and seaweed with TPSS composition of 6.5: 3.5: 90

Sample's Name	HNT solution (ml)	Antibiotic (ml)
TPSS	0	0
TPSS/HNT/0.25	0.25	0
TPSS/HNT/0.25A	0.25	2.0
TPSS/HNT/0.5	0.5	0
TPS/HNT/0.5A	0.5	2.0

The Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to classify HNT structures and film samples at different majors. The samples have been gold-coated and placed on the rod to create high-quality images before characterization.

In the meantime, in this analysis, the antimicrobial test was the disc diffusion test called Kirby-Bauer through the inhibition zone calculation. The previously prepared film samples were dipped into distilled water to turn into a hydrogel. They were then placed in a well in diameter with grams positive, cultivated agar, and left in the incubator for 24 hours at 37 ℃.

The FTIR (Spectrum 2000 Perkin Elmer) was used with 20 scans and an 8 cm^{-1} resolution in the range of 500 to 4000 cm^{-1} to scan and analyze the chemical group functionality of TPSS nano-composites TPSS/HNT sample.

Results and Discussion

Characteristics of HNTs

Figure 2 (a) shows the micrograph of SEM for the as-received HNT with visible tubular shapes under magnification of 30,000X. Some HNT particles were naturally agglomerated due to their high surface to volume ratio, as the HNT had a specific gravity of 2.5 g/cm^3 [8]. The pore size distribution for HNTs in this characterization is shown in Figure 2(b). This distribution, however, is contradicted the finding by Liu et al. [13], who claimed that the pore size distribution for HNTs is broadly bimodal. This contradiction can be explained by the different dispersion medium used to disperse these HNTs before it was characterized by particle distribution. At the same time, it might be the inaccuracy of particle analyser results due to the significant difference between diameter (<100nm) and length of HNTs (>100nm), and detection was only for larger particles.

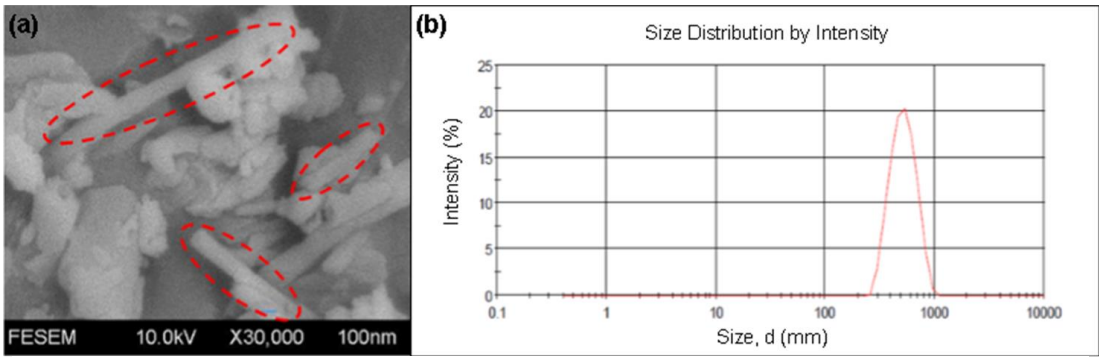

Figure 2: (a) SEM image of HNT particles (b) Particle size distribution of HNT

Figure 3(a) and **(b)** illustrate the TEM micrographs of the detailed appearance of pristine HNT with an empty lumen with hollow microtubes. These tubular lumens have inserts inside provide gains to uptake substances with the uncovered open-ended lumen. The small molecules' substances to large complex molecules could fill inside the lumens through various mechanisms, such as adsorption, intercalation, and tubular entrapment [8].

Moreover, it was observed that HNT varies in their length, inner and outer lumen diameter with the calculated average of 400, 13.2, and 22.0, respectively, as illustrated in Figure 3(b). These values were similar to the finding of earlier researches [5, 8]. Geometrically, the tube-like morphologies with a proper aspect ratio generate few opportunities for large area contact between tubes. The regular tubular morphology, bulk structure, rich mesopores, small dimension, and high strength suggest that HNTs have potential uses in high-performance bio-polymeric nanocomposites.

According to Ferraril et al. [14], HNTs have a small permanent negative charge independent of pH due to the tubular form of halloysite. The Si-tetrahedral sheet on the external surface with the Al-octahedral sheet on the inside. Alumina has a positive charge of pH 8.5, while silica is a negative charge above pH 1.5 (physiologically relevant pH range). The dispersion behaviour of HNTs is, therefore, more strongly influenced by the external Si-tetrahedra, with a shallow point of zero charges (pH <3.0). This allows for the selective loading of negatively charged molecules inside the HNTs lumen, thus suits to be carrier by encapsulating antibiotics inside it.

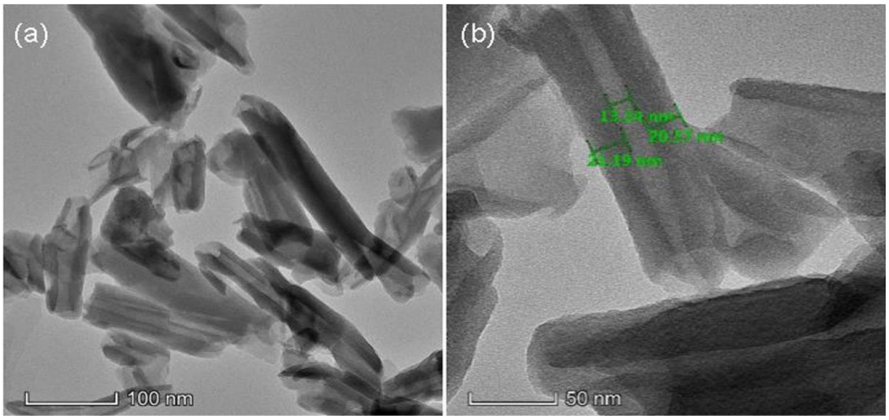

Figure 3: HNT structures under TEM at magnification of (a) 30,000X and (b) 50,000X

Characterizations of nano-composites TPSS/HNT

Figure 4(a) shows the amorphous starch granules have successfully fused with glycerol and water to form a homogenous phase, resulting in a clear and smooth surface film. The nanocomposite film's SEM image incorporated with 0.25 wt.% HNT, as in Figure 4(b) and 4(c), shows that HNT is uniformly dispersed in the TPSS matrix. This can be indicated by HNTs with few hydroxyl and siloxane groups on the HNT surface, which offer a relatively low interaction of two HNTs and, therefore, a consistently scattered morphology obtained by this nanocomposite film. No significant difference was observed for the film with its HNTs without and with the HNTs immersed in the antibiotic (chloramphenicol)(Figure 4(d)). The surface becomes slightly rougher due to a random arrangement of HNT on the surface of the film.

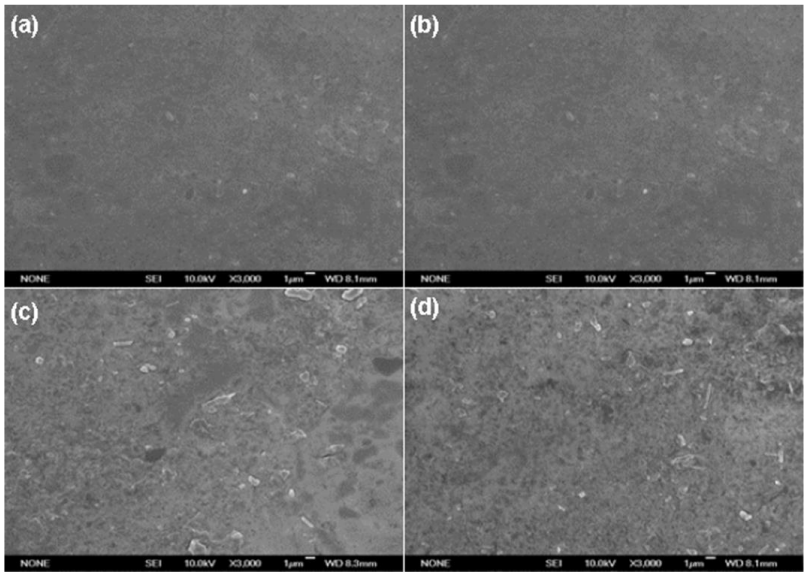

Figure 4: FESEM micrographs of (a), (b) and (c) of theTPSS and nanocomposite TPSS/HNT films at 0.25, 0.5 of HNT contents respectively and (d) with the antibiotic, (TPSS/HNT0.5A) at the magnification of 3,000X

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra observed the interfacial interaction between the TPSS and the HNTs without and with antibiotics. As shown in Figure 5, the absorption band for all the samples in the functional group region was almost identical between the TPSS and nano-composites TPSS/HNTs film. No new peaks appeared in the nano-composites film, revealing that the hydrogen bond interactions occur between the TPSS and HNTs. Still, no reaction occurs suggested that the structure of the HNTs is retained in the composites film. A trivial shift of the nanocomposites' peaks to the lower wavenumber was observed for the peak of 3730 cm^{-1} indicated that the bond length is increasing or stretching vibrations exist. This may be attributed to the hydroxyl (O-H) groups that are placed on the inner-surface of Al-O octahedron and deformation vibration of Si-O tetrahedron interfaced layer. Stretching happens due to a change in the surrounding atom's electronegativity and increases with an increase in HNT loading.

The antibiotic in this study was loaded into the tubular lumen through an adsorption process through stirring to ensure thermodynamic equilibrium has been attained between the loaded antibiotics and solution involves. Antibiotic was expected to encapsulate inside the hollow structure of tubular HNT and did not disrupt any changes or affect the structures of the film samples. To prove that the antibiotic was encapsulated in HNTs, the fingerprint region at the wavenumber of 1450cm-1- 500cm-1 in FTIR was studied to detect any new peak for identifying any new compound evidence for the existence of the antibiotic in the HNTs. In the complexity of the peaks in this region, there is no new peak observed indicated that antibiotic is not detected here through FTIR spectroscopy.

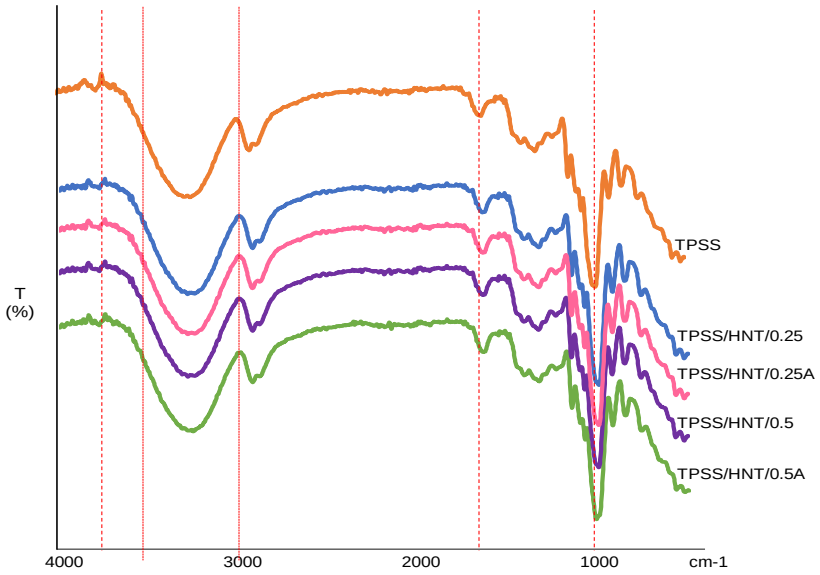

Figure 5: FTIR spectra of TPSS samples with additional HNT and antibiotics

Antimicrobial activities

Figure 6 shows the results obtained for the disc diffusion test. It was found that the TPSS and TPSS/HNT nanocomposites were zero inhibited without an antibiotic. This behavior elucidates sago starch like a polysaccharide that attracts and attacks bacteria, as well as the antibiotic-free nano-composites film. Nanocomposites, on the other hand, display a 67.3 ± 0.5 ± 100 ± 0.3 mm2 inhibition zone (TPSS/HNT/0.25A and TPSS/HNT0.5A), suggesting the presence of any antimicrobial activities. The inhibition zone's size shows that antibiotics are successful. The wider inhibition region means that antibiotics are susceptible to bacteria more sensitive.

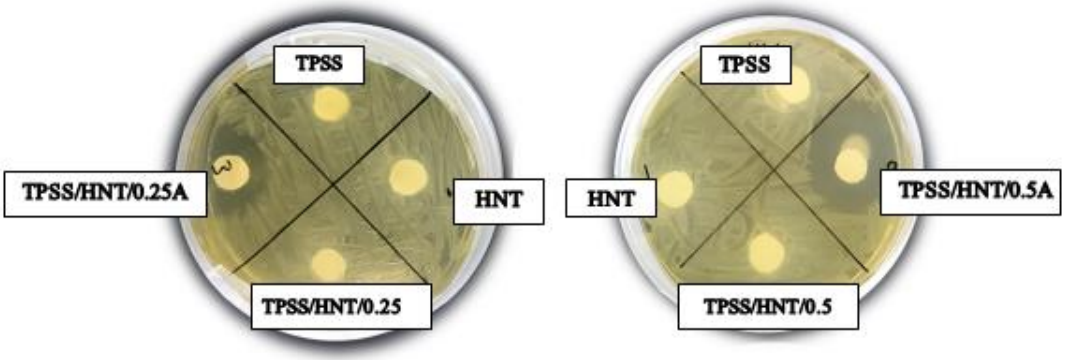

Figure 5: Disk diffusion test on the film samples contain HNT

The high loading capacities of HNT are expecting to work well as entrants for the sustained and controlled release of drugs, including antibiotics [13]. Moreover, these properties confine detrimental side effects such as non-swelling nature, good regeneration ability, and high efficiencies; and protects the encapsulated antibiotics within the lumen from the external environment while prolonged and waiting to release their contents in response to a chemical trigger [8, 13-14].

Conclusions

The structures of Halloysite Nanotubes (HNTs) revealed from SEM, and TEM micrographs have the advantages of encapsulating and releasing antibiotics in TPSS/HNT film even though it is not detected in FTIR spectroscopy. The results have been confirmed by the disc diffusion test showing a broad zone of inhibition with antibiotics used. HNT, therefore, reacts as good antimicrobial agents that are advantageous and relevant in the medical and technical sectors and that have tunable functionality such as wound dressing.

Acknowledgments

This work was financially supported by the IIUM Research Initiative Grant Scheme (RIGS17-146-0721) and International Islamic University Malaysia (IIUM).

Author Contributions

Zuraida Ahmad gave idea and helped supervised the project. Nader Abuhamed and Maisarah Tajuddin fabricated the TPSS/HNT films and carried out the experiment. All the authors contributed to analysis and writing of the manuscript.

Disclosure of Conflict of Interest

The authors declare that there is no conflict of interest.

Compliance with Ethical Standards

This manuscript does not contain any studies with human or animal subjects.

References

[1] Xie, F., Pollet, E., Halley, P.J. Avérous, L. (2013) Starch-based nano-biocomposites. *Progress in Polym. Sc.* 38(10) 1590-1628.
[2] Stavitskaya, A., Batasheva, S., Vinokurov, V., Fakhrullina, G., Sangarov, V., Lvov, Y., Fakhrullin, R. (2019) Antimicrobial Applications of Clay Nanotube-Based Composites. *Nanomaterials* 9(5) 708.
[3] Garcia, N.L., Fama, L., Dufresne, A., Aranguren, M., Goyanes, S. (2009) A comparison between the physio-chemical properties of tuber and cereal starches. *Food Res. Int.* 42 976–982.
[4] Abdullayev, E., Lvov, Y. (2013) Halloysite clay nanotubes as a ceramic "skeleton" for functional biopolymer composites with sustained drug release. *J. Mater Chem. B.* 1(23) 2894-2903.
[5] Lvov, Y.M., Abdullayev, E. (2013) Functional polymer–clay nanotube composites with sustained release of chemical agents. *Prog. in Polym. Sci.* 38(10) 1690–1719.
[6] Yendluri, R., Lvov, Y., de Villiers, M.M., Vinokurov, V., Naumenko, E., Tarasova, E., Fakhrullin, R. (2017) Paclitaxel encapsulated in halloysite clay nanotubes for intestinal and intracellular delivery. *J. Pharmaceut. Sci*. 106 (10) 3131-3139.
[7] Xie, Y.F., Chang, P.R., Wang, S.J., Yu, J.G., Ma, X.F.(2011). Preparation and properties of halloysite nanotubes/plasticized Dioscorea opposite Thunb. Starch composites. *J. of Carb. Poly.* 83(1)186-191.
[8] Kamble, R., Ghag, M., Gaikawad, S., Panda, B.K. (2012) Halloysite nanotubes and application: A review. *J. of Adv Sc. Res.* 3(2) 25-29.
[9] Duarte, H.A., Louren, M.P., Heine, T., Guimaraes, L. (2012). Clay mineral nanotubes: Stability, structure, and properties. *In-Tech* pp. 1-24
[10] Bac, B.H., Dung, N.T. (2016). Finding nanotubular halloysite at Lang Dong kaolin deposit, Phu Tho province. *Vietnam J. of Earth Sc.* 37 299-306.
[11] Zuraida, A., Hermain, H.Y., Abdul Razak, N.H. (2015) Mechanical and Physical Properties of Sago Starch/Halloysite Nanocomposite Film. *Adv. Mat. Res.* 1115 394-397.
[12] Zuraida, A., Ahmad Ulwi, A.K., Maisarah, T., Maisarah, A. (2015) Characterization and Preparation of Sago Starch (SS) Based Reinforced with Silver Nanoparticle (SNP). *Adv. Mat. Res.* 1115 369-372.
[13] Liu, M., Jia, Z., Jia, D., Zhou, C. (2014) Recent Advanced in Research of Halloyiste Nanotubes-Polymer Nanocomposites. *Prog. Polym. Sci.* 39 1498-1525.
[14] Ferrari1, P.C., Araujo1, F.F., Pianaro, S.A. (2017) Halloysite nanotubes-polymeric nanocomposites: Characteristics, modifications, and controlled drug delivery approaches. *Cerâmica*, 63 423-431.