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Comparative Studies of Fe(III) Chitosan Nanoparticle n-benzaldehyde Schiff base and Fe(III) Chitosan n-benzaldehyde Schiff base

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ABSTRACT
The present study was made by synthesizing Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base (Fe(III)CnSb) and Fe(III) chitosan n-benzaldehyde Schiff base (Fe(III)CSb). The two materials were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffractogram (XRD) and evaluated using the disc diffusion method against three gram-positive bacteria, two gram-negative bacteria and two fungi. The diffracted peaks of the Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base showed peaks at $2\theta=24^0$ and 42^0 relative to the peak of Fe(III) chitosan n-benzaldehyde Schiff base of $2\theta = 22.5^0$ and 34^0. The difference in peak shift was attributed to the ionic bonding of Fe(III) complexation with the benzaldehyde mixture of the chitosan-Tpp backbone structure. Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base has more antimicrobial activities than Fe(III) chitosan n-benzaldehyde Schiff base against the same bacteria and fungi tested

Keywords: Antibiotic Sensitivity, Antimicrobial agent, Chitosan nanoparticle, Complexation, Schiff base

INTRODUCTION

Chitosan is a natural linear cationic polyamino sugar obtained by N-deacetylation of shrimp shell (Ifeanyi *et al*., 2020; Ayodele, 2023; Jurca *et al*., 2017, Okunzuwa *et al*., 2024). Chitin is derived from the thick, hard shells of certain insects, molluscs, and especially crustaceans. Chitosan can be regarded as a copolymer of chitin and chitosan. The macromolecular backbone is composed of β-(1,4)-2-amino-2-deoxy-D-glucosamine and β-(1,4)-N-acetyl-D-glucosamine residues (Hernandez *et al.*,2002; Zhikuan *et al.*, 1999; Muzzerelli and Peter 1997; Wu *et al*., 2008). Chitosan can be easily obtained from chitin by N-deacetylation, and is easy-to-modify hydroxyl and amino groups. In recent years, various studies on the use of chitosan, especially in wastewater treatment, have attracted attention (Zhikuan *et al.*, 1999). Chitosan is essentially (1,4)-2-amino-2-deoxy-/3-D-glucan with enhanced chelating capacity, mainly due to the regular distribution of a large number of primary amino group (Muzzerelli and Peter 1997). Chitosan is a potentially useful material for cathode fabrication due to its low cost and abundance. Chitosan is used in many applications in the form of gels, films or fibers and is particularly useful as a hydrogel absorbent for environmental pollutants due to its low cost and small quantities. Efficient alkylation or arylation of chitosan amino groups is achieved by specific methods. Incorporation of hydrophobic segments into chitosan chains increases its antioxidant effect and its solubility in organic solvents, and modifies its thermal properties. Recently, nanoparticles have been considered as a viable alternative to antibiotics and appear to have an effective potential to address the problem of multidrug resistance in bacteria. Superparamagnetic iron oxide (Fe_3O_4) is most commonly used as drug delivery nanoparticles in biomedical applications due to its biocompatibility (Wu *et al*., 2008), biodegradability and ease of surface modification (Vita *et al.*, 2016). A promising technique for surface modification of nanoparticles is to coat the nanoparticles with polymers to prevent the oxidation of iron oxide. Polymers used to coat Fe_3O_4 nanoparticles include polyethylene glycol (PEG), dextran, polyethyleneimine (PEI), phospholipids, and chitosan. Natural polymers such as chitosan are highly recommended in drug delivery systems due to their biocompatibility and biodegradability (Vita *et al.*, 2016). Chitosan has the ability to interact with the negatively charged (hydroxyl groups) on the surface of Fe_3O_4 nanoparticles. Chitosan, Schiff bases, and metal complexes have been reported to have many antibacterial and antifungal properties. These results were obtained by (Nadia *et al.*,2013; Sashikala and Syed, 2014) and are also due to the increasing resistance of bacteria and fungi to available antimicrobials. This present work focuses on the comparison of the synthesis, characterization

and antimicrobial activities of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base and Fe(III) chitosan n-benzaldehyde Schiff base.

MATERIALS AND METHODS

Sodium Hydroxide, Hydrochloric Acid, Benzaldehyde, Sodium Hypochlorite, Ethanol, Acetic acid, Sodium tripolyphosphate, Distilled water, Ferric chloride; all were of analytical grade and used without further purification.

Preparation of chitosan nanoparticle:

For the preparation of chitosan nanoparticle through ionic gelation, the method of (Morteza *et al.,* 2011) was used. Chitosan 0.5% (w/v) was dissolved with acetic acid 1% (v/v) and then increased to pH 4.6 - 4.8 with 2 M NaOH. Chitosan nanoparticles were formed spontaneously upon addition of an aqueous tripolyphosphate solution (1mL) of 0.25% (w/v) to chitosan solution (3mL) under magnetic stirring of 1000 rpm for 1 hour at room temperature. The nanoparticles were purified by centrifugation at 16000 rpm for 30 mins. The supernatants were discarded and the chitosan nanoparticles were extensively rinsed with distilled water to remove any sodium hydroxide and then freeze-dried.

Preparation of chitosan n-benzaldehyde Schiff base

The method described by Jiao *et al.* (2011) and Omer *et al.* (2019) were employed, in which 1.0 g of Chitosan was dissolved in 50 mL of acetic acid 2% and stirred at room temperature for 6 hours. 10 mL of ethanol containing 0.0695g of benzaldehyde was added to the solution with stirring to form a homogenous solution. The mixture was stirred for 6 hours and heated at 50° C. A deep yellow gel indicating the formation of Chitosan Schiff base was obtained. The product was cooled and washed with distilled water and ethanol several times to remove unreacted benzaldehyde until colourless filtrate was obtained. The product was then dried at 50° C in vacuum for 24 hours.

The preparation of Chitosan nanoparticle n-benzaldehyde Schiff-base

The method described by Semahat *et al.* (2013) was followed, which involved 1.0 g of Chitosan was added to 100 mL of ethanol and the mixture was continuously stirred for 6 hours to produce a homogeneous solution. Afterwards, 0.0695g of benzaldehyde was added to the mixture. The obtained mixture was further stirred for 6 hours at 50° C. Chitosan nanoparticle Schiff base formed was separated by centrifugation at 16000 rpm and washed several times with ethanol. The product was dried in a vacuum oven at 50° C for 24 hours; a yellow powder of chitosan nanoparticle Schiff base was obtained.

Synthesis of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base (Fe(III)CnSb)

Synthesis of Fe(III)CnSb through complexation follows the method of (Jiao *et al.,* 2011). 1.0 g Chitosan nanoparticle n-benzaldehyde Schiff base was mixed with 1.0 g Fe (III) chloride in 50 mL ethanol solution, stirred and heated at 50° C for 12 hours in water bath. After cooling, the crude complex was washed with ethanol and subsequently washed to colourlessness by excess amounts of water, and then dried at 50°C in an oven for 24 hours.

Characterisation:

Materials were analysed with Fourier Transform Infrared (FTIR) Spectroscopy using KBR pellet method and recorded in the frequency range of 400-4000 cm^{-1} with model Cary 630 by Agilent technologies, USA and X-ray diffractograms of samples were obtained using an X-ray powder diffractometer (a Bruker AXS model, Germany) with Ni-filter and CuKα radiation source at an accelerating voltage/current of 50 KV/40 mA. The relative intensity was recorded in the scattering rage, varying from 5° to 90° at scanning rate $2°min^{-1}$.

Evaluation of antimicrobial activities:

The evaluation of antimicrobial activities of the synthesized materials through the agar disk diffusion method (Xiaohui *et al.,* 2005).

Preparation of materials / samples concentration

Fe(III) chitosan n-benzaldehyde Schiff base and Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base used in this study were dissolved each in diméthylsulfoxide (DMSO) to obtain varying concentrations (100%, 50% and 25%). Then, a concentration of 100% was achieved by dissolving 1g of the material in 1 mL of DMSO in a sterile universal bottle, 50% concentration was achieved by aseptically pipetting 0.5 mL of the 100% concentration and dissolving with 0.5 mL of DMSO and 25% concentration of each sample was achieved by dissolving 0.5 mL of the 50% concentration with 0.5 mL of DMSO (Saidu and Ogofure, 2018).

Antibiotic Sensitivity Test

The bacteria isolates were tested for resistance and sensitivity to different antibiotics using the standard disc diffusion method. The disc diffusion assay, bacteria were grown between 18 and 24 hours, while fungi were grown for 2-5 days on Mueller Hinton agar. The inoculated medium was maintained at 37° C. The results were recorded after 24 hours and 2-5 days respectively. Standard antibiotics (oxoid) were used to determine the resistance pattern of the isolates. The diameters of the zone of inhibition around each disc were

measured using a meter rule (Saidu and Ogofure, 2018).

Antibacteria and Antifungal Activity of the Materials

Screening of the materials Fe(III) chitosan n-benzaldehyde Schiff base and Fe (III) chitosan nanoparticle n-benzaldehyde Schiff base for antimicrobial activity was carried out by disc diffusion method which is normally used as a preliminary check and to select between efficient materials (Saidu and Ogofure, 2018). The tests were conducted with the authenticated pure culture of the tested pathogens to determine their respective susceptibility or resistance to the materials. Sterile agar plates were aseptically inoculated with a loop full of the test bacteria and fungi. Each inoculum was spread evenly over the surface of the agar plate. Under aseptic conditions empty sterile disc (Whatman filter paper no. 5, 6 mm diameter) were impregnated with 1000 µL of different concentrations (100%, 50% and 25%) of each of the materials and placed on the agar surface. Paper disc moistened with DMSO, was placed on the seeded plates as a negative control. The plates were left for 30 mins at room temperature to allow the diffusion of the materials and then they were incubated at 37° C for 24hours at a pressure of 15 psi for bacteria and 2-5 days for fungi. The plates were observed for the presence of the inhibition zone around the disc. The extent of inhibition was determined by measuring the diameter of the inhibition zone using the transparent meter rule. Measurements were made across the discs.

RESULTS AND DISCUSSION:

Degree of deacetylation of chitosan:

The Degree of deacetylation is used to establish that chitosan has been successfully prepared before further analysis. The degree of deacetylation of the prepared Chitosan was 90.1%.

Ionic interaction between Chitosan and Tripolyphosphate (TPP):

The reaction of Chitosan with tripolyphosphate (TPP) leads to the formation of intermolecular and/or intramolecular network structure by ionic interaction between NH_4^+ protonated groups and negatively charged ions OH^- and $P_3O_{10}^{5-}$ of tripolyphosphate. Due to hydrolysis, the small molecules of polyelectrolyte, sodium tripolyphosphate, dissociates in water and released out OH^- ions.

Complexation of chitosan n-benzaldehyde Schiff base with metal ion:

The prepared chitosan Schiff base was complexed with Fe^{3+} to form chitosan Schiff base Fe^{3+} complex which was brown in colour.

Data Analysis:

It is mandatory to determine the structure and properties of the synthesized materials in order to know the specific functional groups, chemical bonds that exist in a material, surface and structural morphology, including particle size and crystalline nature.

Fourier Transform Infrared (FTIR) Spectroscopy:

The important FT-IR spectroscopy results confirm the formation of the prepared materials for Fe(III) chitosan n-benzaldehyde Schiff base and Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base.

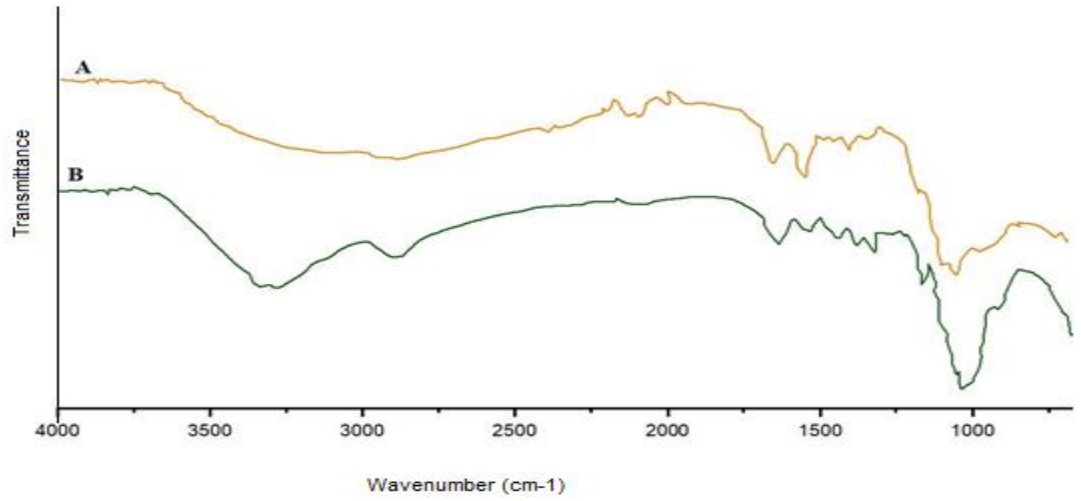

Figure 1: FTIR spectra of Fe(III) Chitosan n-benzaldehyde Schiff base (A) and Fe(III) Chitosan nanoparticle n-benzaldehyde Schiff base (B).

Table 1: FTIR spectroscopy of Fe(III) Chitosan n-benzaldehyde Schiff base

Absorption frequency (ν cm^{-1})	Responsible functional groups of Fe(III) chitosan n-benzaldehyde Schiff base
3753.4 - 3045.2	OH, H- bonding, N-H in NH_2
2873.8	C-H axial stretching band
1625.1	C=N (Imine)
1528.2	Aromatic C=C Stretching
1423.8 – 1379.1	C-H bend
1082.3	C-O stretching
1021.3	C-O-C stretching vibration
678.4	Cl^-

Table 2: FTIR Spectroscopy of Fe(III) chitosan n-benzaldehyde Schiff base against FTIR Spectroscopy of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base

Fe(III)CSb (cm^{-1})	Fe(III)CnSb	Functional groups
1625.1	1616.3	ν(C=N)
678.4	682.1	Cl^-

From Figure 1 (A) and (B), it was observed that the spectrum of Fe(III) Chitosan nanoparticle n-benzaldehyde Schiff base was different from that of Fe(III) Chitosan n-benzaldehyde Schiff base. The absorption peaks of Fe(III) Chitosan Schiff base at 1625.0cm^{-1} and 678.4cm^{-1} of C=N (imine) vibration and Cl^- was shifted to a lower wavenumber at 1616.3 cm^{-1} and 682.1cm^{-1}in Fe(III) Chitosan nanoparticle n-benzaldehyde Schiff base. These clearly indicate the interaction between chitosan-Tpp nanoparticle n-benzaldehyde Schiff base and Fe^{3+}in the formation of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base.

X-ray diffractogram (XRD):

The XRD diffractogram was used to determine the structural morphology and phase identification of the materials produced.

Table 3: The XRD patterns results for Fe(III) chitosan n-benzaldehyde Schiff base (Fe(III)CSb) and Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base (Fe(III)CnSb)

Materials	Diffracted peaks at 2θ (degree)
Fe(III)CSb	11.5,18.5, 22.5, 27, 34
Fe(III)CnSb	12, 18.5, 24, 27, 42

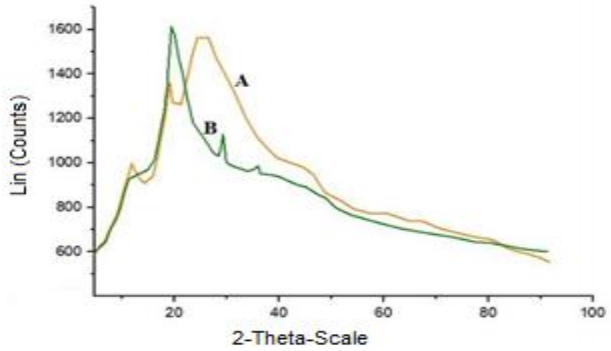

Figure 2: XRD Pattern of Fe(III) Chitosan n-benzaldehyde Schiff base (A) and Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base (B)

From Table 3 and Figure 2, it was observed that there were few differences between the X-ray diffractogram of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base and Fe(III) chitosan n-benzaldehyde Schiff base. However, the differences in the diffracted peaks of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base are new peaks at 2θ - 24° and 42°when compare to Fe(III) chitosan n-benzaldehyde Schiff base at 2θ - 22.5° and 34°. The difference in peak change may be attributed to the ionic bonding of the complexation of Fe(III) with chitosan-TPP n-benzaldehyde Schiff base backbone structure leading to amorphous nature of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base.

The experimental results of antimicrobial activity of all the materials using the inhibition zone method:

The antimicrobial activities of Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base and Fe(III) chitosan n-benzaldehyde Schiff base with varying concentrations of 100%, 50%, 25% on three Gram positive bacteria: *Bacillus Subtilis*, *Bacillus cereus* and *Staphylococcus aureus*, two Gram-negative bacteria: *Samolena* and *Pseudomonas aeruginosa* and two fungi: *Candida albicans* and *Aspergillus niger*. In a typical experiment, the inhibition zone results were recorded after 24 hours of incubation at 37° C for bacteria and 3 days of incubation at 37° C for fungi. The obtained experimental data are clearly shown in Tables 4 and 5.

Table 4: The antibacterial activity of the materials (mm) against different bacteria

		Fe(III)CSb			Fe(III)CnSb		
Entry	**bacteria**	**100%**	**50%**	**25%**	**100%**	**50%**	**25%**
1	*Bacillus substilis* (Gram + bacteria)	16	12	00	18	16	10
2	*Bacillus cereus* (Gram + bacteria)	24	22	10	26	24	20
3	*Staphylococcus aureus* (Gram + bacteria)	20	18	16	24	20	18
4	Samolena (Gram - bacteria)	16	12	10	18	14	12
5	*Pseudomonas aeruginosa* (Gram - bacteria)	10	00	00	12	10	00

Table 5: The antifungal activity of the materials (mm) against *Asperigillus niger* and *Candida albicans*

		Fe(III)CSb			Fe(III)CnSb		
Entry	**Fungi**	**100%**	**50%**	**25%**	**100%**	**50%**	**25%**
1	*Asperigillus niger*	10	08	00	12	10	08
2	*Candida albicans*	10	08	00	16	14	08

Results from Tables 4 and 5, shows that the tested materials exhibited excellent antimicrobial activities against all selected strains and these could be related to the following factors: The structural properties and functional groups of all the materials $–NH_2$, OH, P=O, P-O, C=N and Fe^{3+}, Particle size, Type of organism and Concentration of the inoculates (Okunzuwa *et al.*, 2024, Kinga *et al.*, 2013 and Xiaohui *et al.*, 2005)

The antimicrobial activities of the Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base have better activity than the Fe(III) chitosan n-benzaldehyde Schiff base. This is because nanoparticles have small quantum size effect, large surface area to volume ratio, enhanced solubility, increased rates of dissolution of molecules into microbial cell and also it has the ability to target specific sites of the microbial cells easily than microparticles.

Comparison of the antimicrobial activities of the nanoparticle materials against microparticle materials:

The antimicrobial activities of the nanoparticle material and microparticle material were compared as presented in the Tables 4 and 5. Obviously, the antimicrobial activities of the nanoparticle materials have better activity than the microparticle materials. This is because nanoparticles have small quantum size effect, large surface area to volume ratio, enhanced solubility, increased rates of dissolution of molecules into microbial cell and also it has the ability to target specific sites of the microbial cells easily than microparticles.

CONCLUSION

Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base and Fe(III) chitosan n-benzaldehyde Schiff base have been synthesized through the reaction of Chitosan with Tpp, Benzaldehyde and Fe^{3+} and characterized by conventional methods: degree of deacetylation, FTIR and XRD. All results showed Fe(III) chitosan n-benzaldehyde Schiff base and Fe(III) chitosan nanoparticle n-benzaldehyde Schiff base are good antimicrobial agents.

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Disclosure of conflict of interest

We declare that we have no conflict of interest.

REFERENCES

Ayodele TO. (2023) Ease to Challenges in Achieving Successful Synthesized Schiff base, Chirality, and Application as Antibacterial Agent. *BioMed Research International*. Volume 2023, Article ID 1626488, 1-22.

Ifeanyi E, Otuokere J. C, Anyanwu and Igwe KK (2020). Ni(II) Complex of a Novel Schiff Base Derived from Benzaldehyde and Sulphathiazole: Synthesis, Characterization and Antibacterial Studies. Communication in physical sciences. 145-155.

Jiao TF, Juan Z, Jingxin Z, Lihua G, Yuanyuan and Xuhui L (2011). Synthesis and Characterization of Chitosan-based Schiff Base Compounds with Aromatic Substituent Groups. *Iranian Polymer Journal*. **20**(2): 123-136.

Jurca, T., Marian, E., Vicaş, L. G., Mureşan, M. E., & Fritea, L. (2017). Metal Complexes of Pharmaceutical Substances. Spectroscopic Analyses - Developments and Applications, 7: 123-142. http://dx.doi.org/10.5772/65390.

Kinga, S., Andrzej M. and Tadeusz, L. (2013). Nitrogen, Oxygen or Sulfur containing heterocyclic compounds as analgesic drugs used as modulators of the nitroxidative stress. *Mini-reviews in medicinal chemistry*. 13: 335-352

Hernandez M, Angel M, Michael KK, Timothy S, Keizer BC, Yearwood and Atwood DA (2002). Six- coordinatinate Aluminium cations: characterization, catalysis and theory. *Journal of the Chem. Soc.*, Dalton Trans (3) 410-414.

Morteza HK, Mohammad K, Mobina K and Sahar K (2011). Reinforcement of Chitosan nanoparticles obtained by an Ionic Cross-linking process. *Iranian Polymer Journal*. **20** (5):455-456.

Muzzerelli RAA., and Peter MG (1997). Some modified chitosans and their niche applications, Chitin handbook. *European Chitin Society, Italy*: 47-52.

Nadia AM, Magdy WS, Ahmed HH, El-ghandour MM, Abel-aziz O and Abdel-gawad F (2013). Preparation, characterization and antimicrobial activity of carboxymethyl chitosan Schiff base with different benzaldehyde derivatives. *Journal of American Science*.**9**(3):247-264.

Okunzuwa IG, Omamoke OE, Okunzuwa IS (2024). Synthesis and characterization of Fe(III) Chitosan nanoparticles n-benzaldehyde Schiff base for biomedical application. Chemical papers, Springer. https://doi.org/10.1007/s11696-024-03309-5

Omer A.M., Y.A. Ammar, Ahmed Mohamed, Y. M. Abdelbaky, T. M. (2019). Preparation of Isatin/chitosan Schiff base as Novel Antibacterial Biomaterials. Egypt. J. Chem. Vol. 62, Special Issue (Part 1), pp. 123- 131.

Saidu JZ, Ogofure AG (2018). Cannamon (cinnamomumzeylanicum) and Rosemary (rosemarinusofficialis). Essential oils possess antibacterial activity against bacterial strains of public health importance. Nigerian Journal of Applied Scie nce, 36 : 170-178

Sashikala S and Syed SS (2014). Synthesis and characterisation of chitosan Schiff base derivatives. *Scholars Research Library*.**6** (2): 90-97.

Semahat K, Zehra OE, Jiri BMS and Nuriye K (2013). A Novel Chitosan Nanoparticle-Schiff Base Modified Carbon Paste Electrode as a Sensor for the Determination of Pb(II) in Waste Water. *Int. J. Electrochem. Sci.* **8**: 2164-2181.

Vita TM, Ika OW, Rizky AS, Dionysius JDHS, Akhmad S (2016). In-situ synthesis and characterization of chitosan-fe3o4 nanoparticles using tripolyphosphate/citrate as cross-linker. *Scientific Study & Research.* 17 (3), pp. 249-260.

Wu W, He Q and Jiang C (2008). Magnetic Iron Oxide Nanoparticles: Synthesis and Surface Functionalization Strategies. *Nanoscale Research Letter*, **3** (11), 397-415.

Xiaohui W, Yumin D, Lihong F, Hui L and Ying H (2005). Chitosan- metal complex as antimicrobial agent: Synthesis, Characterization and Structure–activity study. *Polymer Bulletin*. **55**: 105-113.

Zhikuan Y, Yuting W and Yurong T (1999). Preparation and Adsorption Properties of Metal Ions of Crosslinked Chitosan Azacrown Ethers. *Journal of Applied Polymer Science*, Vol. **74**, 305-3058.