Vol III Issue VII August 2013
Impact Factor : 0.2105 ISSN No : 2230-7850

Monthly Multidisciplinary
Research Journal

Indian Streams Research Journal

IMPACT FACTOR : 0.2105

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Indian Streams Research Journal
ISSN 2230-7850
Volume-3, Issue-7, August-2013

Preparation And Characteristics Of Magnetic Nanoparticles With Chitosan For Food Bacterial Detection

V. Manonmani And Vimala Juliet
Research Scholar, Sathyabama University, Chennai, India
Professor and Head, Department of ICE, SRM University, Chennai, India

Abstract:A world wide foodborne illness due to viruses remains a major concern, despite improving hygienic conditions. Magnetic nanoparticles have attracted recent researchers for their intensive applications as biomaterials and magnetic storage materials. The goal of this study is to synthesize Mg- NP's by using ultrasonic-assisted chemical coprecipitation method under different conditions. The Synthesized Mg- NP's was coated with chitosan for capturing the bacterial concentration from contaminated food sample. Chitosan from amine group specifically binds with bacteria on their surface of most food pathogenic bacteria (Staphylococcus aureus). The binding characteristic of chitosan coated Mg- NP's with bacterial cell wall was obtained by using UV-Visible spectroscopy and Transmission Electron Microscopy. UV-Visible Spectroscopy gives the reading of absorbance when the binding of receptor proteins occured with the sample. By using TEM, the Mg NP's shape and size was investigated. The product consisted of ferrous, ferrite (Fe3O4) nanosized cubic particles with a high level of crystallinity. This kind of Chitosan-magnetite nanocomposite has the potential to be used in biosensors to identify and to detect foodborne pathogens.

Keyword: Mg- NP's (Magnetic Nanoparticles), TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy),OD(Optical Density)

INTRODUCTION:

The molecular biology and medicine incorporated into nanotechnology has resulted in active developments of new trends in research area, nanomedicine which offers excellent opportunities for discovering new materials, processes, and phenomena. Nanoparticles play major role as imaging contrast agents in various bio-imaging and pathogenic detection modalities including fluorescence microscopy, Confocal laser scanning microscopy, positron emission tomography, single photon emission computed tomography ultrasound imaging, magnetic resonance imaging, plasmon resonance scattering, optical coherence tomography and magnetomotive[2]. Bacteria can lead to serious diseases and environmental contamination, and bring a huge public health burden. Staphylococcus aureus is a spherical gram-positive food borne bacterium; infections by this pathogen are often found in the skin, soft-tissue, bone, joint, and endovascular disorders.

MATERIALS AND SYNTHESIS METHODS:

Chitosan polymer 150 kDa. Aqueous acetic acid solution was used as a solvent for the chitosan polymers and glutaraldehyde was used as the cross-linker. All chemicals were of analytical grade and no further purification was required.

PREPARATION OF MAGNETIC NANOPARTICLES

Fe3O4 nanoparticles were prepared by coprecipitaion method with a ferrous complex in presence of NaOH. Firstly, FeCl2.4H2O and FeCl3.6H2O [Fe2+: Fe3+= 1:2] were dissolved in about 50 ml Millipore water, and stirring this solution under magnetic stirrer while heating solution to 70°C. Next, this iron solution source was added drop-wise into NaOH under magnetic stirrer agitation for 30 minutes, and bubbling N2 gas [6].

The chemical reaction of Fe3O4 precipitation is expected as follows:

$$FeCl_2.4H_2O + 2FeCl_3.6H_2O + 8NaOH \rightarrow Fe_3O4 + 8NaCl + 20H_2O$$

Figure 1: Preparation of Mg-Np's using Magnetic stirrer

Black Fe3O4 particles were decanted by permanent magnet and cleaned by Millipore water several times [1].

ISSN 2230-7850
Volume-3, Issue-7, August-2013

PREPARATION OF MAGNETIC CHITOSAN NANOPARTICLES

The suspension cross-linking technique was used for the preparation of magnetic chitosan nanoparticles. In this specific procedure, a 5% chitosan solution was prepared using a 2% aqueous acetic acid solution containing 0.2 g Fe3O4 dry magnetic nanoparticles. And then, this solution was poured, drop-wise, into the dispersion medium, which was composed of 30 ml paraffin and 0.5 ml span-80. During this process, the dispersion medium was stirred with a magnetic stirrer at room temperature. Next, an additional 3 ml 25% glutaraldehyde solution was added to the dispersion medium and then solution was stirred for further 5 h. At the end of this period, the chitosan-magnetite nanocomposite particles were recovered from the reaction mixture by using a permanent magnet; the products were washed with ethanol and dried with acetone overnight [5].

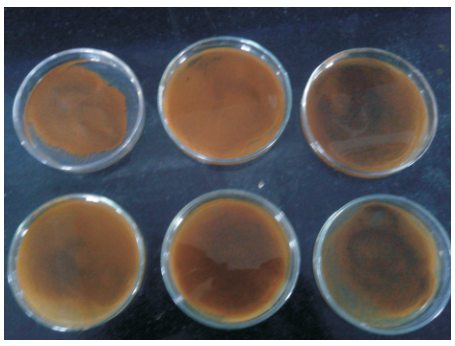

Figure 2: Chitosan-magnetite nanocomposite particles for drying

PREPARATION OF SAMPLE (PATHOGENIC STRAIN)

Stock cultures of Staphylococcus aureus, were kind gifts from Bharathidasan University, Trichy. Cultures were grown at 37 °C on a rotary shaker at 150 rpm overnight. The bacterial cells were centrifuged at 5000 rpm for5 min. For safety considerations, all of the bacterial samples were placed in an autoclave at 121 °C for 20 min to kill bacteria before disposal and all glassware in contact with bacteria was sterilized before and after use.

BACTERIAL CAPTURE EFFICIENCY OF MG-NP'S

The bacterial concentration was adjusted to a desired level, a certain amount of chitosan coated magnetic nanoparticles suspended in PBS (10 mg mL-1) was then added into the bacterial solution, and the solution volume was fixed to2 mL. The solution was incubated by a rotary shaker at 250 rpm for a specific period (15min), and then an external magnet was employed for magnetic separation. The supernatant was then carefully pipetted into a cell to measures its OD570 using UV-visible spectroscopy (Table 1). The relative efficiencies of the magnetic capture of bacteria by chitosan coated magnetic nanoparticles were calculated from the decrease of turbidity relative to a reference before magnetic capture [3].

Figure 3: Chitosan coated Mg-Np's with bacterial solution for magnetic seperation

RESULTS AND DISCUSSION

From the TEM analysis the shape and size of magnetic nanoparticles was determined. The shape was found to be cubical and the average size was measured to be in the range of 25-40nm. From Figure 4(b), it was observed that the binding of Mg-Np's coated chitosan with the bacterial surface or cell wall. From figure 4(b), (c) & (d), there was no significant agglomeration and there was no structural difference found between the chitosan coated and uncoated Mg-Np's. The size of the Mg-Np's before and after coating also found to be in nanoscale level.

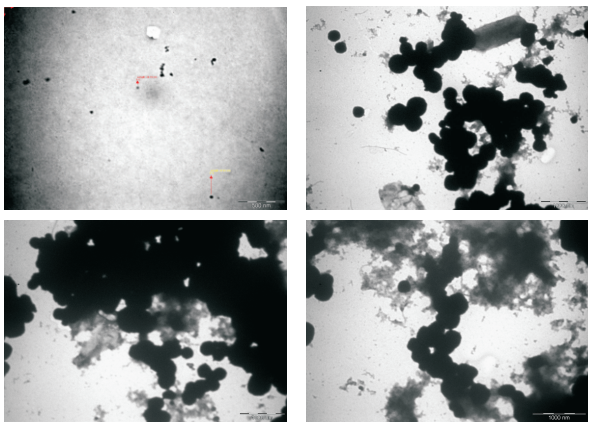

Figure 4(a) shows TEM Image of synthesized magnetic nanoparticles, (b) TEM image - Binding event of magnetic nanoparticles coated with chitosan & bacteria (Staphylococcus aureus), (c) & (d) TEM image of Binding event of magnetic nanoparticles alone with bacteria (Staphylococcus aureus)

BINDING OF MAGNETIC NANOPARTICLES WITH BACTERIAL SURFACES BY UV VISIBLE SPECTROSCOPY

From the reading of below table it was observed that after magnetic stirrer of magnetic nanoparticles with chitosan along with bacteria (Staphylococcus aureus), due to magnetic separation, about 90% of bacterias were settled down in the pellet and hence the optical density (OD) was found to be maximum at 1.942.The supernatant measures the

ISSN 2230-7850
Volume-3, Issue-7, August-2013

OD of 0.301 at 570nm (Table 1). This confirms the binding occurence of Magnetic nanoparticles with chitosan and bacterial surfaces.

SEGMENT	OD @570nm
SUPERNATENT	0.301
MIDDLE SUSPENSION	0.339
PELLET	1.942

Table 1: OD reading using UV- Visible Spectroscopy

CONCLUSION

Food safety is the world's major health goal. This new concept of detection of food borne pathogens using chitosan nano composite binding with bacterial surfaces is an integrated application of Nanomaterials and Molecular biology [4]. From present work, it was proved by UV-Spectroscopy and TEM measurements that the prepared Mg-Np's with chitosan could bind well to the bacterial cell wall/surface and thus used to capture the presence of food borne bacteria. TEM results confirmed the nanosized and cubic shape of Mg-Mp's. Further, this work will be extended to isolate and detect concentration of bacteria when mixed with dye/marker like rhodamine to emit fluorescence on the captured bacteria with input light.

REFERENCES

1.Doan Thi Kim Tung, Tran Hoang Hai, “Preparation and characterization of Magnetic nanoparticles with chitosan coating”, Journal of Physics: Conference series 187(2009) 012036.IOP Publishing, doi: 10.1088/1742-6596/1871/012036.

2.T.K.Indira, P.K.Lakshmi, “Magnetic Nanoparticles – A Review”, International journal of Pharmaceutical Sciences and Technology,Issue 3, Vol 3,October - December 2010.

3.Shen Wu, Aizhi Sun, “Fe3O4 Magnetic nanoparticles synthesis from tailings by ultrasonic chemical co-precipitation”, Materials Letters 65(2011) 1882-1885,www.elsevier.com/locate/matlet.

4.Jilan Xia,Peng fei Liao, Zhen Yuan Nie, “Preparation and characterization of magnetic carboxylated chitosan-Fe3O4 nano composites for isolation of genomic DNA” http://www.edu.cn

5.X.X.Xu and Y.F.Zheng, “Synthesis and Characterization of Magnetic nanoparticles and their reinforcement in polyurethane film”. Key Engineering Materials Vols: 324-325(2006), pp.659-662, copy at (2006) Trans Tech Publications,Switzerland.

6.S.F.Hasany,I.Ahmed,Rajan,J.A.Rehman, “Systematic Review of the preparation Techniques of Iron oxide Magnetic Nanoparticles”, Journal of Nanoscience and Nanotechnology, 2012,2(6); 148-158,doi: 10.5923/j.nn 201202016.01.