Characterization of squid pens extracted beta-chitosan coated magnetite nanoparticles

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Abstract

Aim: Squid pens, a feather shaped structure in squid species, are waste material which are successfully utilized for the production of industrially important chitosan. The present study deals with characterization of squid pens extracted beta-chitosan coated magnetite nanoparticles

Methodology: Beta-chitosan was extracted from squid pens. The extracted beta-chitosan was used as a surface amino coating polymer onto solvothermally prepared magnetite nanoparticles (MNs). Various characterization analysis was performed to understand the properties of MNs, chitosan and beta-chitosan coated MNs (β_c -MNs).

Results: Peak at 448 nm in UV-Spectrum corresponded the magnetite and required absorption peaks in IR spectrum showed perfect magnetite and chitosan formation. Also, electron micrographs revealed the flaky structure of chitosan, spherical structure of MNs and less aggregated structures of beta-chitosan coated MNs (β_c -MNs). Diffraction pattern from X-ray diffraction (XRD) suggested the formation of MNs and β_c -MNs and crystallite size (13.9 nm) was determined using Debye-Scherrer equation. Superparamagnetic behaviors of MNs were observed from the magnetization curve of vibrating sample magnetometer (VSM) plot.

Interpretation: The characteristic studies revealed the extraction of beta-chitosan from squid pens and formation of MNs and β_c -MNs.

Key words: Beta-Chitosan, Magnetite, Nanoparticles, Squid pens

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Introduction

In general, various food industries produce processing waste, out of which seafood industries release products like chitin (Meanwell and Shama, 2008; Tsai *et al.*, 2011) that are potentially rich source of commercially important chitosan. Chitin waste from these processing industries can be utilized effectively. Chitosan is the most ubiquitous natural polymer (Je, 2012; Kumirska *et al.*, 2010) and is produced by deacetylation of chitin (Rinaudo, 2006); wherein the amide group is hydrolyzed to a primary amine group altering its reactivity. Rapid degradability and lack of toxicity makes chitosan suitable for different environmental uses (Uthairatanakij *et al.*, 2007).

Commercially available chitosan is extracted mostly from crustacean wastes like shrimp, crab, krill (Kumar *et al.*, 2018) while those obtained from squid pens yield beta structured chitosan (Huang *et al.*, 2018; Raigond. P. *et al.*, 2017) with higher reactivity due to weaker inter molecular hydrogen bonds and crystalline stability and better anti-microbial activities (Chaussard and Domard, 2004; Virk. P., *et al.*, 2017). *Illex argentinus* and *Todarode spacificus* predominate the Squid species for chitosan production (Vazqueza *et al.*, 2016; Parthiba *et al.*, 2016). Currently, alpha-chitosan are used as functionalizing agents (Doan *et al.*, 2018) providing natural amino groups on the surface of nanocarriers in addition to other industrial applications, but this study involves coating with beta-chitosan (Tolaimate *et al.*, 2000). The free amino groups attached are enabled for numerous reactions for further cross-linking and attachments extending applications.

Magnetite is the most widely used nanoparticles because of its versatile physical and chemical properties that can be easily controlled using magnetic field (Seenuvasan *et al.*, 2014; Keyhanian *et al.*, 2016; Nagarajan *et al.*, 2016.). Among various methods, solvothermal synthesis results in uniform sized monodisperse (Yanez-vilar *et al.*, 2009; Sahoo *et al.*, 2012; Zhang *et al.*, 2013; Wang *et al.*, 2013; Dar. *et al.*, 2015) nanoparticles as organic solution is used and gains benefits of both sol-gel (Gao *et al.*, 1994) and hydrothermal process (Cheng *et al.*, 1996; Chaisri *et al.*, 2015; Shalini *et al.*, 2016.).

In the present study, MNs were prepared using solvothermal process, coated with beta-chitosan extracted from the squid pens. Properties of MNs and resultant product β c-MNs were further studied by various characterization analyses.

Materials and Methods

Cleaned squid pens were procured from Victoria Fresh Sea Food Pvt. Ltd., Chennai, India. Initially, desalination reaction was carried out by adding 4% HCl to clean dried squid pens at room temperature for about 7 hrs followed by deproteinization at 90 °C for 3 hrs by adding 1% NaOH to the neutralized material. The obtained beta-chitin was deacetylated by treating with 4%

alkali solution at 95 °C for 6 hrs to yield beta-chitosan that was neutralized by continuous aqueous wash and further drying at 105 °C (He *et al.*, 2015). Depolymerization was performed with chitosan solution (in 2 % acetic acid) at 30 °C for 3 hrs in the presence of 3% sodium nitrite. The neutralized mixture was then concentrated by rotary evaporator and dried beta-chitosan was stored at 4 °C for further use.

Solvothermal technique (Li *et al.*, 2016) was carried out for preparing magnetite nanoparticles (MNs) with the homogenous mixture of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (18.5 mM) and sodium acetate (3 mM) with ethylene glycol and ethylene diamine. The reaction was allowed to occur at 200 °C for 8 hrs in a Teflon lined autoclave (200 ml) and the resultant black precipitates of MNs were magnetically decanted and vacuum dried at 60 °C for 8 hrs.

Chitosan (in 1 % acetic acid) and TPP solution was added simultaneously onto MNs and complete dispersion along with uniform coating was ensured by ultrasonication for half an hour (Pan *et al.*, 2009). β c-MNs were recovered by magnetic decantation, washed, freeze-dried and stored at 4 °C.

Maximum absorption wave length (λ_{max}) was found using UV-Vis spectroscopic analysis (JASCO V 650) in 400-600 nm range and the crystallite size and structure were estimated using diffraction patterns from X-ray diffractometer (XRD, X'Pert3 Powder X-ray Diffractometer) with Cu K α radiation at 30 kV and 30 mA. Functional groups were studied using FT-IR (Perkin Elmer FTIRC 100566) spectrum at 4000-400 cm^{-1} and the morphological features and magnetic properties were understood through FESEM (FEI quanta FEG 200, Operating voltage 30 kV) and VSM (Lakeshore 7407, United States) analyses respectively.

Results and Discussion

UV-Vis spectrum of solvothermally synthesized MNs showed maximum optical absorption at 430 nm corresponding to that of magnetite. The FTIR spectrum of MNs, β c-MNs and beta-chitosan is shown in Fig. 1, which exhibited the presence of required characteristic peaks. NH stretching and bending vibrations were seen at 3335 cm^{-1} and 1644 cm^{-1} . The peaks corresponding Fe-O stretching vibrations were seen at 552 cm^{-1} (Seenuvasan *et al.*, 2013), CH stretch and COC stretch were observed at 2675 cm^{-1} and 1044 cm^{-1} , respectively and also the presence of COC peak in β c-MNs may be due to coating of beta-chitosan onto MNs.

Fig. 2 depicts the diffraction pattern of MNs and β c-MNs that revealed the characteristic extreme peaks at 30.2°, 35.4°, 43°, 57.2° and 63.1° with indices (2 2 0), (3 1 1), (4 0 0), (5 1 1), and (4 4 1) attributing to the inverse spinel structure without any phase change. Sharp and broadened peaks indicated the crystalline nature and smaller size of nano particles and the weaker peak observed at 20° for β c-MNs illustrated the perfect coating of beta-chitosan onto MNs. With the help of Debye-

Fig. 1 : FT-IR Spectrum of (a) MNs (b) β c-MNs and (c) Beta-Chitosan.

Fig. 2 : XRD pattern of (a) MNs and (b) β c-MNs.

Scherrer equation ($d = 0.9 \lambda / \beta \cos \theta$; β is the full width at half maximum) on the most extreme peak (Malar *et al.*, 2018), the size was 13.92 nm and 13.95 nm for MNs and β c-MNs respectively.

Fig. 3 shows asymmetric membranous nature of beta-chitosan. Spherical particles with few agglomerations due to dipole-dipole interactions can be noticed from the image of MNs

Fig. 3 : SEM micrographs of (a) Beta-Chitosan (b) MNs and (c) β c-MNs.

Fig. 4 : VSM plot of (a) MNs and (b) β c-MNs.

and the coating of non-magnetic beta-chitosan reduced aggregation among β c-MNs. As seen from the image, uniformity in distribution and higher dispersion revealed the finest coating of beta-chitosan on the surface of MNs.

The magnetization measurements performed at room temperature are expressed as hysteresis loops in magnetization curve (M-H curve) of Fig. 4. Loss of long range ferromagnetism and surface spin disorder by particle size reduction are exhibited by lower saturation magnetization than bulk iron oxide (Phan *et al.*, 2016). It can also be seen that both coercivity and retentivity reduced to zero indicating their super paramagnetic behavior (Malar *et al.*, 2018).

Beta-chitosan extracted from squid pens were coated onto the solvothermally prepared MNs and their various properties were studied using characterization analyses. Presence of all stretches in FTIR spectrum revealed the successful formation of MNs and β c-MNs. Also, crystalline structure and non-significant increase in particle size by coating of beta-chitosan were clearly visible from the diffraction patterns. Spherical and less aggregated nanoparticles were observed from the electron micrographs and superparamagnetic behaviors were exhibited from the magnetization curves. Hence, this study presents an insight view on unaltered properties of MNs through introduction of amino groups on the surface and suggests that β c-MNs can be used effectively for extended future applications.

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