ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Protein separation using chitosan immobilized magnetic nanocomposite

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ABSTRACT

Purification of proteins from fermentation broth containing various metabolites are complex and expensive. Selection of suitable methods determines the commercial viability of fermentation industry and also affects the cost of the final product. Use of novel biosorbent like nano-composite, which has increased affinity towards proteins and thereby easily recovering proteins, can make the bioseparation easier and less expensive. Magnetically immobilized chitosan nanocomposite was synthesized and utilized as a novel biosorbent for better separation of protein. The presence of chitosan on magnetic nanocomposite was confirmed by FTIR analysis. The microporous and spherical structure of magnetically immobilized chitosan nanocomposite were confirmed by Field Emission Scanning Electron Microscopy and X-Ray Diffraction analysis. The effect of varied concentration of immobilized chitosan nanocomposite as adsorbent on biosorption of protein was studied. The maximum protein recovery of 94.79% was obtained using magnetic chitosan nanocomposite. The magnetically immobilized chitosan nanocomposite can be used as a novel adsorbent for the efficient separation of protein.

KEY WORDS: Magnetic nanoparticle, Chitosan nanocomposite, Characterization, Protein Purification, Biosorption.

1. INTRODUCTION

The traditional methods of separating adsorbent from solution are filtration, sedimentation and centrifugation. These methods are time consuming and uneconomic. However, the magnetic separation technology is an efficient, rapid and inexpensive separation method. Magnetic chitosan can be prepared with the combination of chitosan and magnetic nanoparticles, and easily removed from the reaction medium using an external magnetic field (Kuang, 2013). Chitosan coated magnetic nanoparticles modified with α-ketoglutaric acid were used for the removal of copper ions from aqueous solution (Xu and Dong, 2008; Zhuo, 2009). These particles were used for removal the lead ions from aqueous solution. Chitosan coated magnetic nanoparticles were reported for the recovery of maximum number of metal ions which makes them attractive for removal of heavy metal ions from waste water (Liu, 2009; Lopez, 2013).

Purification of proteins from fermentation broth is a complex and expensive process. Suitable methods are selected for purification of proteins based on the commercial viability of the process and the cost of the final product. Partial cross linking of chitosan by di/polyfunctional reagents is generally carried out for protein adsorption in aqueous medium. The adsorption capacity depends on the extent of cross linking. Generally adsorption capacity decreases with increase in extent of cross linking. Further cross linking in homogenous condition leads to enhanced protein binding capacity as a result of increased hydrophilicity as compared to heterogeneous cross linking (Varma, 2004; Guibal, 2005; Liu, 2011).

Novel adsorbent such as nanocomposite can be used for separation of proteins. Nanocomposite with higher affinity towards proteins makes the bioseparation easier and less expensive. The binding of enzymes onto a nanosized magnetic particle provides better separation. Previous reports have suggested that the magnetic nanoparticle supports are less toxic and provide a higher surface area (Zhu, 2013). Magnetic nanocomposites find applications in medical, textile and waste recycling processes (Nagireddy, 2011; Ranjithkumar, 2014; Guibal, 2004). The use of magnetically immobilized adsorbent decreases the cost of operation by several folds due to the easy recovery and reusability (Kuang, 2013; Vold, 2003). Thus the chitosan immobilized magnetic nanocomposite was used as a novel adsorbent for better adsorption of protein in the present study. A novel magnetic chitosan nanocomposite (MCN) with better magnetic separation characteristics was successfully synthesized, characterized and utilized for the separation of protein.

2. MATERIALS AND METHODS

Materials used: Chemical such as Ferric chloride, ferrous sulphate and sodium hydroxide used for preparation of magnetic nanoparticle were obtained from Sd Fine Chemicals Limited, Mumbai, India in Analytical grade. The chitosan used for preparation of nanocomposite and Bovine Serum Albumin (BSA) for biosorption studies were obtained from SRL Pvt. Ltd., Mumbai, India in Analytical grade.

Preparation of magnetic chitosan nanocomposite: A 50 ml of 0.2M $FeCl_3$ and 50 ml of 0.1M $FeSO_4$ solutions were prepared and mixed together. Then 50 ml of 10% NaOH was added drop wise under constant stirring and black precipitate was obtained of magnetic nanoparticles were obtained. The resultant suspension was magnetically decanted and dried in oven at 80°C. The 100 ml of chitosan solution (1% w/v in 1% acetic acid) was mixed with the 3.5 gm of dried magnetic nanoparticle and stirred for 12 h at 30°C. The resultant homogeneous dark brown precipitate was separated using magnetic decantation. The precipitate was washed several times using distilled water to remove residual chitosan and dried (Shen, 2009; Dung, 2009; Mohsen, 2012).

Characterization of magnetic chitosan nanocomposite: The surface morphology and size of the synthesized MCN was characterized using Field Emission Scanning Electron Microscope (FE-SEM) (CARL ZEISS, GERMANY), the composition of the nanocomposite was analyzed using Energy Dispersive microscopy (EDS) (OXFORD Instruments, United Kingdom), functional groups present on the surface of the nanocomposite were studied using Fourier Transform Infra-Red (FTIR) spectroscopy (BRUKER) and the structural characteristics were studied using X-Ray Diffraction (XRD) (RIKAGU, JAPAN) analysis.

Biosorption of protein using magnetic chitosan nanocomposite: The prepared magnetic nano-composite of chitosan was utilized for biosorption of BSA. The effect of varied concentration of MCN from 1% to 5% (w/v) with an interval of 1% was studied on biosorption capacity and percentage adsorption. The protein uptake capacity and the percentage protein adsorption were studied at different MCN concentration.

3. RESULTS AND DISCUSSION

FE-SEM and EDS characterization of magnetic chitosan nanocomposite: Surface morphology and size of the prepared magnetic nanocomposite of chitosan was characterized using FE-SEM as shown in Fig. 1. The magnetic chitosan nanocomposite was found to be spherical in shape. The size of magnetic chitosan nanocomposite was in the range of 42.71 to 69.69 nm. The nanocomposites were found as very fine particles with an average size of 51.39 nm. The surface of the MCN was microporous in structure. Thus the magnetic chitosan nanocomposite was found to be an efficient sorbent for bioseparation of proteins (Liu, 2011). The composition of the nanocomposite was analyzed using EDS as shown in Fig. 2. The synthesized MCN was found to contain Fe, C and O. Thus, it infers that the magnetic particles and chitosan are present in the nanocomposite. Hence the prepared nanocomposite can be used as a novel adsorbent for protein purification.

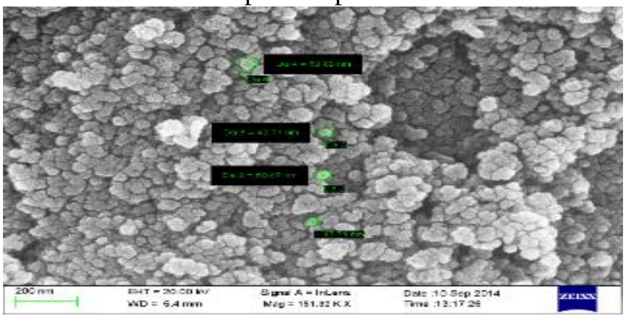

Fig.1. SEM image of synthesized magnetic chitosan nanocomposite

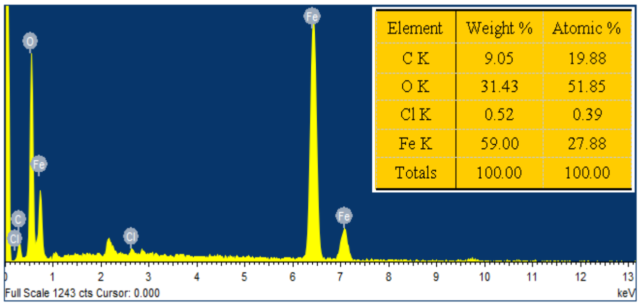

Fig. 2. EDS analysis of synthesized magnetic chitosan nanocomposite

FT-IR characterization of synthesized magnetic chitosan nanocomposite: The functional groups present on the surface of the synthesized MCN were observed using FTIR spectroscopic analysis as shown in Fig. 3. The peak at 2091.8 was found to have a weak appearance and it represents the presence of C-C group of terminal alkynes. The peak at 1623.8 was found to have a strong appearance and represents the presence of conjugated C-C group. The peak 1406.4 was found to have a weaker appearance to represent the presence of C-O group. The peak at 1105.4 was displayed a two strong and broad appearance of C-X group and a peak at 622.0 displayed the presence of one weak to medium C-X group (George, 2012; Peter, 2012). Thus the presence C-C and C-O and C-X group confirms the presence of chitosan on magnetic nanoparticle. The peaks at 3775.5 and 3422.0 confirmed the presence of N-H carrying secondary amines. The presence of N-H group on MCN confirms the adsorption of protein on to MCN.

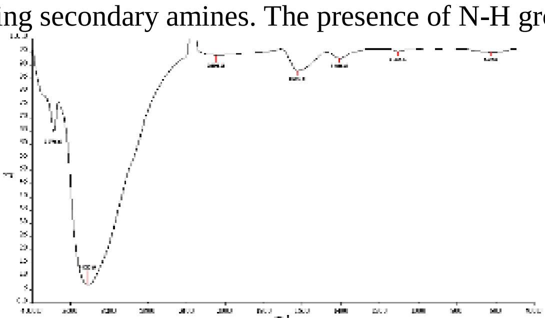

Fig.3. FTIR analysis of synthesized magnetic chitosan nanocomposite

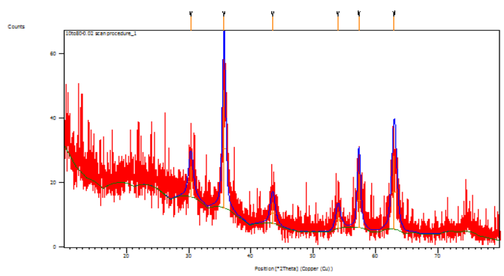

Fig.4. XRD analysis of synthesized magnetic chitosan nanocomposite

XRD analysis of synthesized magnetic chitosan nanocomposite: The structural characteristic of the synthesized MCN was observed using XRD as shown in Fig. 4.The 2θ values with maximum peak observed at 35.66 corresponds to diffraction plane (3 1 1), the peak at 57.31 corresponds to diffraction plane (5 1 1) and the at 62.98 corresponds to diffraction plane (4 4 0) plane. These diffraction planes confirmed the 90° inclined cubic structure of magnetic nanocomposite as per JCPDS 89–4924. A peak at 62.98 confirmed the presence of chitosan on nanocomposite. The peaks at 35.66 and 57.31 confirmed the formation of ferrite and ferromagnetic property of nanocomposite (Liu, 2009).

Biosorption of protein using magnetic chitosan nanocomposite: The effect of MCN concentration as adsorbent for biosorption of protein was studied by varying the concentration from 1% to 5% (w/v) with an interval of 1% under constant temperature, time, agitation and protein concentration as shown in Fig. 5. The protein uptake capacity was found to decrease with increase in MCN concentration from 1% to 5% (w/v). However the decrease in protein uptake capacity was found to be insignificant for increase in MCN concentration from 4% to 5% (w/v). The effect of MCN concentration on percentage of protein adsorption was observed as shown in Fig. 6. The percentage of protein separation was found to increase with increase in MCN concentration from 1% (w/v) to 4% (w/v). The increase in percentage of protein separation was found to be insignificant for further increase in MCN concentration from 4% (w/v) to 5% (w/v). Thus the 4% (w/v) MCN was found to be the optimum concentration for better separation of protein (94.79%).

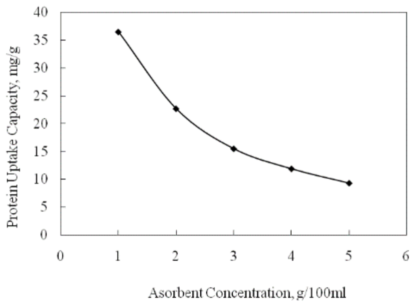

Fig. 5. Effect of adsorbent (MCN) concentration on protein uptake capacity

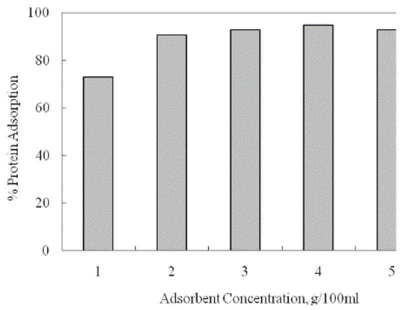

Fig. 6. Effect of adsorbent (MCN) concentration on percentage of protein adsorption

4. CONCLUSIONS

The magnetic chitosan nanocomposite was successfully synthesized and used as a novel adsorbent for the separation of protein from aqueous solution. The maximum of 94.79% of protein was separated using 4% (w/v) of magnetic chitosan. The presence of chitosan on magnetic nanocomposite was confirmed by FTIR analysis. The increased protein uptake of protein on to magnetic chitosan nanocomposite was might be due to its microporous and spherical structure. Thus the use of magnetically immobilised chitosan nanocomposite can be easily recovered and reused to reduce the cost of separation. The magnetic chitosan nanocomposite was found to be an efficient adsorbent for separation of protein.

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