Chemical Methodologies 3(2019) 562-570

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Research article

Dithiocarbamate Modified SPION-Chitosan Nanobiocomposite, a Promising Adsorbent for Bovine Serum Albumin (BSA)

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ARTICLE INFORMATION

Received: 08 February 2019
Received in revised: 10 March 2019
Accepted: 14 April 2019
Available online: 01 September 2019

DOI: 10.33945/SAMI/CHEMM.2019.5.5

KEYWORDS

BSA
SPION
Chitosan
Protein extraction
Magnetic Chitosan

ABSTRACT

In the present paper, bovine serum albumin (BSA) was studied for extraction case from a buffer solution through a new version of modified magnetic chitosan nanocomposite. Post-modification of this magnetic chitosan led to conversion of amine groups to dithiocarbamate on the surface of chitosan which was wrapped to superparamagnetic iron oxide nanoparticles (SPION). Chitosan was converted to magnetic chitosan over co-precipitation of Fe^{2+} and Fe^{3+} under alkali conditions. Amines of chitosan were also converted to dithiocarbamate over the reaction of carbon disulfide. Study of the synthesized support in BSA extraction was achieved in further experiments.

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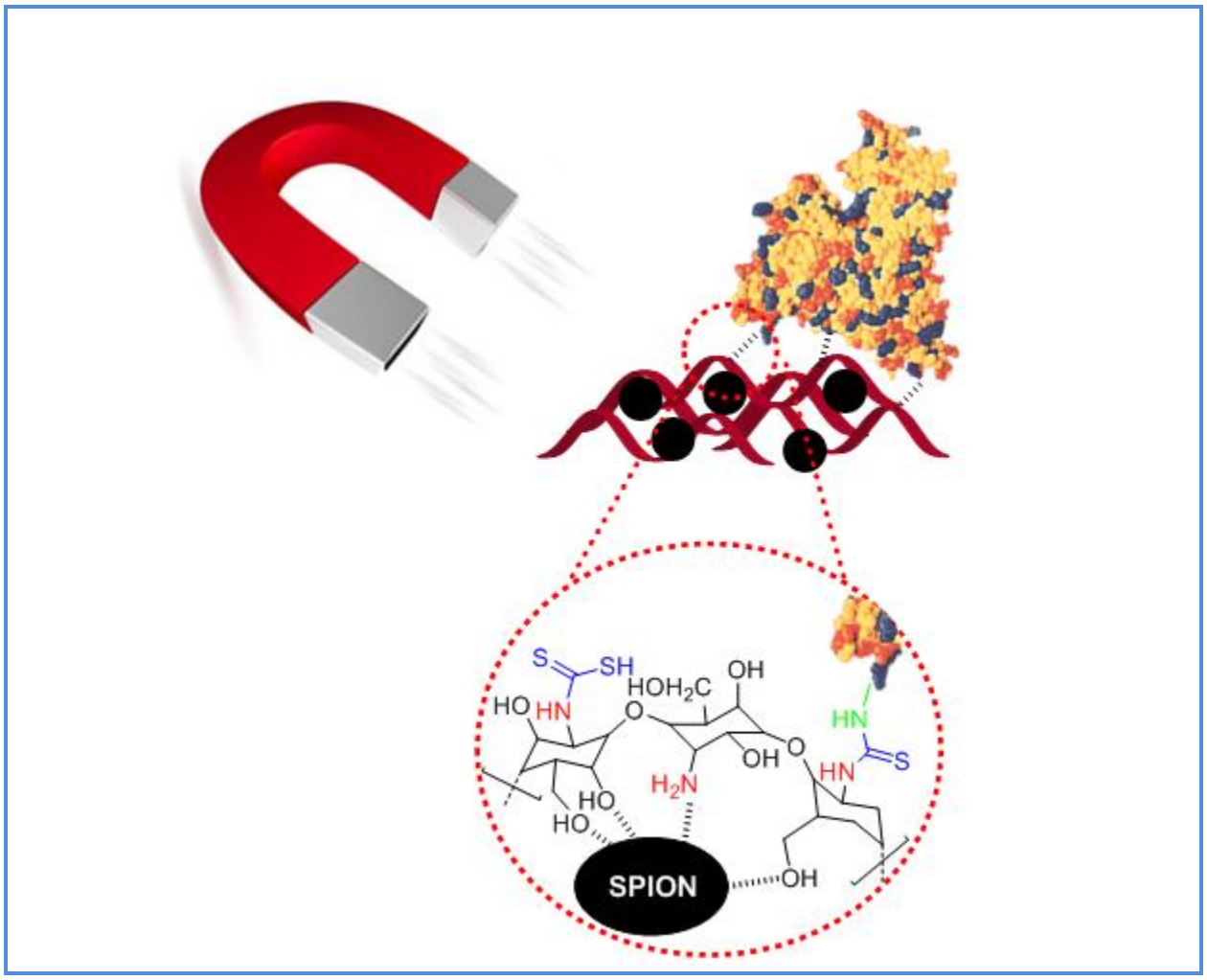
Graphical Abstract



Introduction

Protein extraction and purification methods are of important processes in the protein industry [1, 2]. These methods contribute to have proteins and pure and available enzymes for further studies or usages. However, selecting a suitable adsorbent to keep the extracted protein intact while having the process economical during the protein extraction from the viewpoint of adsorbent efficiency and preparation process expenses. Furthermore, finding a green adsorbent with higher surface area is another challenging aspect in the selection of an adsorbent [3]. Hence, Nano architecturing can play a significant role in reaching to the aforementioned parameters. Among them, the nanomaterials with high and accessible surface area for protein intercalation (which are assumed as supramolecules) can play a key-step role in efficient extraction of proteins [4].

Composites of superparamagnetic iron oxide nanoparticles (SPION) have been emerged as a milestone class of nanoadsorbents for many cases including water treatment, adsorption of heavy metals, dyes, and some proteins or enzymes [5-9]. These applications are owing to their capabilities in magnetic recovery and modification or encapsulation with other active species such as polymers, silica, metal oxides [10, 11]. Furthermore, non-toxicity and feasibility in fabrication steps can be other advantages of working with SPION in adsorption processes. These capabilities have caused the development of many types of successful designs for magnetic-based nanocomposites [12, 13]. Biopolymers with modifiable functions can play a major role in guiding SPIONs towards an efficient nanocomposite [14-17]. The easiest approach for obtaining a biopolymer-SPION nanocomposite is co-precipitation of Fe(II) and Fe(III) ions in the presence of the biopolymer under an alkaline solution which can be supplied by ammonia or NaOH addition [18]. Among biopolymers, tendency toward chitosan for biological uses is higher than the others. The reason may be related to the functional potency of chitosan owing to the amine functionality since amine can be easily post-modified by various types of ligands such as chloroacetate [19], aldehyde and ketone [20, 21], epichlorohydrin [19], salicylate [22], methionine [23]. On the other hand, having biocompatibility and hydrogel behavior are some other aspects that makes it more attractive towards chitosan's researches.

In the present paper, we synthesized SPION-chitosan nanocomposite and, then, we used CS_2 which led to the generation of dithiocarbamate on the surface of chitosan [17]. Thereafter, we used it as a biosorbent in the extraction of bovine serum albumin (BSA). Comparison of CS_2-modified and unmodified SPION-Fe_3O_4 in the extraction of BSA showed that the modification step plays a major key-factor in its capability.

Material and methods

Chitosan (low molecular weight) and BSA (MW = 66 kDa) were purchased from Sigma-Aldrich company and used without purification. Bradford indicator, CS_2, and iron salts were also purchased from Sigma-Aldrich. Scanning and transmission electron microscopy (SEM) was recorded on VEGA3 TESCAN and JOEL JEM 3010 instrument, respectively. FT-IR spectrums were recorded on Bruker Tensor 27 FT-IR spectrophotometer using KBr pellets, X-ray diffraction (XRD) spectra were recorded on Philips PW1800, and UV-visible spectra and analyses were measured and obtained by Shimadzu UV-1800 Spectrophotometer.

Synthesis of SPION-chitosan

Synthesis of SPION-Chitosan was achieved according to the literature [24] with a slight modification. Typically, 1 g of chitosan was dissolved in acetic acid solution (3 wt%, 100 mL) and vigorously stirred for 1 h to dissolve all chitosan in solution. Thereafter, $FeCl_3.6H_2O$ (5 g) and $FeCl_2.4H_2O$ (2 g) were added into the

chitosan solution and allowed for further stirring at 80 °C to obtain a homogeneous solution. At final step, an aqueous solution of ammonia (25 wt%, 15 mL) was added dropwise to solution under shaking mode. The solution was kept under shaking conditions for 30 min. The precipitate was then collected by an external magnet and washed with water and methanol for several times and finally dried at lower pressure for 6 h at 60 °C.

Synthesis of CS_2-modified SPION-chitosan

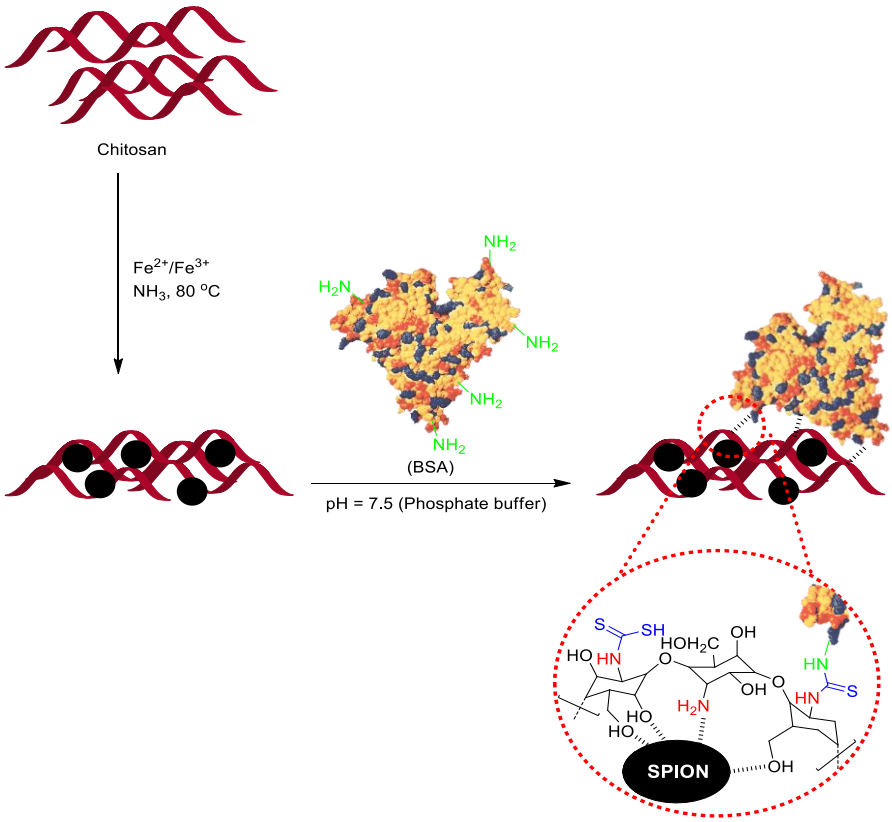
For the synthesis of CS_2-modified SPION-chitosan, 0.5 g of as prepared SPION-chitosan was dispersed in MeOH (20 mL) and, then, CS_2 (3 mL) was added to the dispersion and kept in the sealed pot. After vigorous stirring for 12 h, the reaction was stopped and the precipitate was collected by magnet and washed for several times with MeOH and dried in vacuum.

BSA extraction procedure

BSA solution was prepared in a phosphate buffer solution (pH=7.5) and for all tests, a constant concentration (M=3 mg/mL, 3 mL) was used. For homogeneous dispersion of adsorbent (10 mg) in all experiments, sonication (Ultrasonic Homogenizer-model APU500 Advanced Equipment Engineering Company-Adeeco, Iran) was used. The concentration of BSA was measured by adding Bradford indicator to the solution and measuring the intensity of absorption band at 595 nm though UV-visible spectrophotometer. After sonication and dispersion of adsorbent in buffer solution of BSA, magnetic stirring of the suspension was performed for the rest of treatment time. Treatment time started when the stirring was started. After 10 h, the reaction was stopped and the adsorbent was separated from mixture by an external magnet. The residual solution was kept for UV-vis spectrophotometry. For kinetic study, the amount of batch was 5 times more than other tests.

Results and discussion

Is this paper, we have synthesized SPION-chitosan nanocomposite through *in situ* co-precipitation of Fe(II), Fe(III) and chitosan. Instead of electrostatic interaction of biosorbent and protein, or using some toxic and unsuitable precursors to modify the surface and activate it for biosorption, we used another approach for the extraction of BSA. In this method which is recently developed by our group, amine groups undergo modification with carbon disulfide which eventually convert to an efficient biosorbent. This method is more rapid and facile than other methods like using glutaraldehyde. A schematic procedure for the synthesis of CS_2-modified SPION-Chitosan is depicted in Scheme 1.



Scheme 1. A glimpse at the preparation of CS_2 modified SPION-chitosan

X-ray diffraction pattern analysis of CS_2-modified SPION-chitosan can truly show us that SPIONs are embedded within the network of chitosan biopolymer. Furthermore, comparing the peaks before and after modification with carbon disulfide shows that there is an insignificant change in the crystalline structure of SPION. On the other hand, comparing the peak intensities of pure SPION and SPION-chitosan nanocomposite shows that the related peaks of SPION in SPION-chitosan are less intensive than SPION (Figure 1B). This is due to presence of biopolymer, a non-SPION and non-crystalline structure which decreases the intensities.

FTIR spectra of CS_2-modified and unmodified SPION-chitosan are depicted and compared in Figure 1A. According to the spectra, some new peaks such as 1541 and 1639 cm^{-1} have appeared after modification with carbon disulfide. The appearance of a peak at 1624 cm^{-1} can be due to the generation of C=S groups in CS_2-modified SPION-chitosan. Also, the presence of some other peaks in 2900-3000 cm^{-1} range, due to aliphatic chains, and 3000-3600 cm^{-1}, thanks to O-H and N-H stretching bonds, can be seen in both nanocomposites. This is due to the fact that the backbone of material structure stays intact during the modification with CS_2.

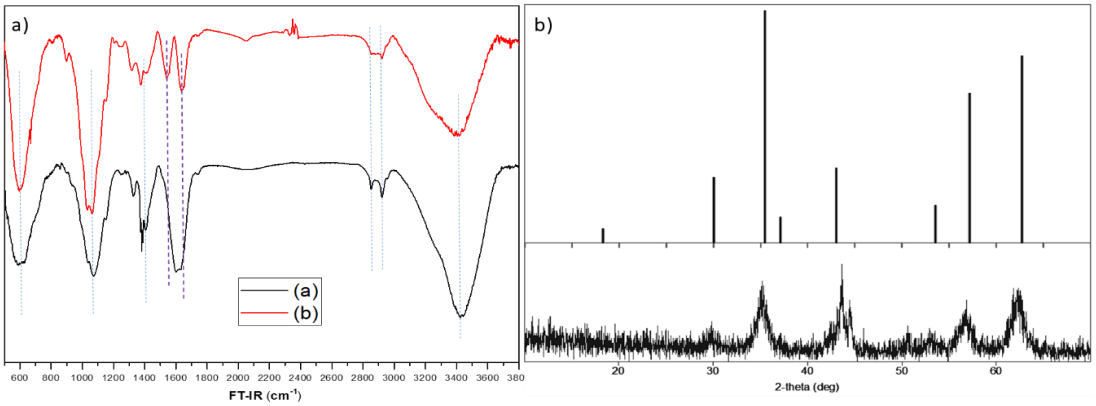


Figure 1. XRD pattern of SPION-chitosan and CS_2-modified SPION-chitosan. FTIR spectrums of SPION-chitosan and CS_2-modified SPION-chitosan

SEM images of CS_2-modified SPION-chitosan and unmodified SPION-chitosan as a nanocomposite morphology are shown in Figure 2C, D. Furthermore, comparing the two images shows that modification with carbon disulfide dos not have a destructive or deforming effect on the backbone of SPION-chitosan. TEM image of SPION-chitosan and CS_2-modified SPION-chitosan were obtained and showed in Figure 2A, B. Based on this image, there are some nanoparticles, which can be assigned to SPION. Distribution size of SPION was plotted in terms of a hysteresis diagram. Based on the diagram, the average size of SPION is less than 20 nm.

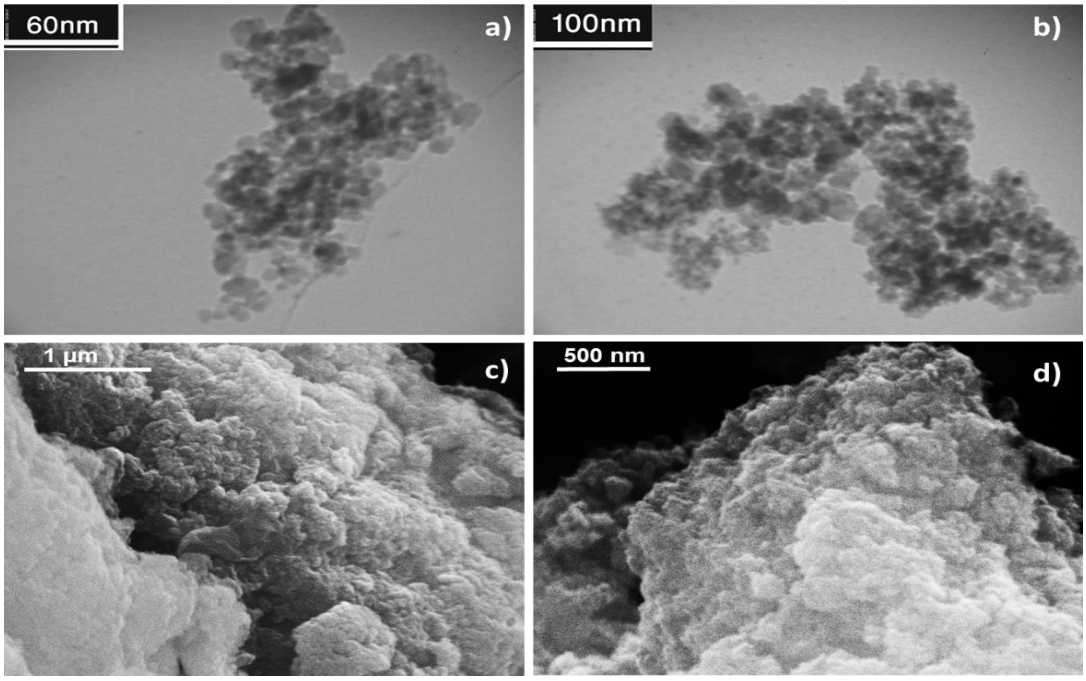


Figure 2. TEM images of (A) SPION-Chitosan and (B) CS_2-modified SPION-chitosan. (C) SEM images of Fe_3O_4-chit-CS_2H and (D) Fe_3O_4-chit-CS-lip

In our previous work, we equalized BSA with lipase to study the enzyme loading capacity of the modified magnetic-chitosan [9]. However, it was a glimpse on this case and there was no study in the extraction of BSA. Hence, in this paper, we wish to extend our study to various aspects of BSA extraction from solution using CS_2-modified SPION-chitosan. Therefore, we wish to extend our studies in this paper to uncover the effective parameters in the extraction of BSA.

For the study of temperature study on the amount of extraction of BSA through CS_2-modified SPION-chitosan, some different reaction setups were compared at various temperature under the similar conditions. Based on this investigation, there was lower amount of extracted BSA at room temperature. However, by enhancing the treatment time duration, the amount of BSA extraction improved for a small amount at room temperature (Figure 4). On the other hand, when the temperature was raised to 50 °C, there was a significant improvement in the extraction amount of BSA. However, when the temperature was raised to 80 °C and 100 °C, there was a very remarkable decrease in the biosorption activity of CS_2-modified SPION-chitosan toward BSA extraction (Figure 3B). In the justification of this observation, it can be pointed out that functional groups, dithiocarbamate, produced by reaction of amine and carbon disulfide is not stable at higher temperatures. Kinetics of BSA extraction from solution through CS_2-modified SPION-chitosan was analyzed at 50 and room temperature. In this study, the extraction amount had better efficiency at 50 °C. In case of 50 °C, the extraction rate was higher in the first 6 h.

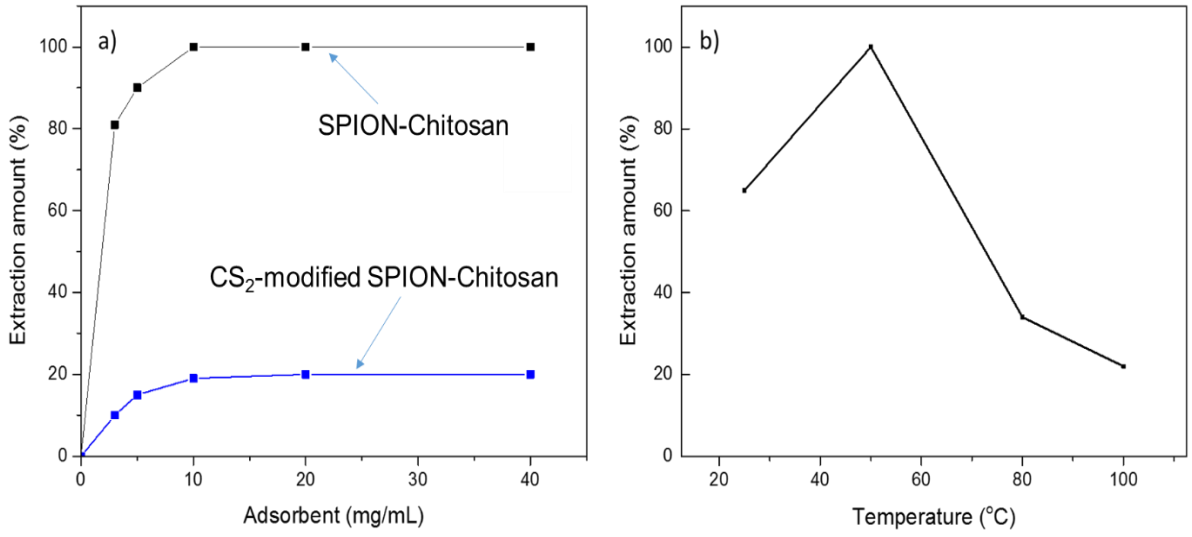


Figure 3. (A) Extraction of BSA in different amounts of SPION-chitosan and CS_2-modified SPION-chitosan; (B) Extraction of BSA using CS_2-modified SPION-chitosan at different temperatures

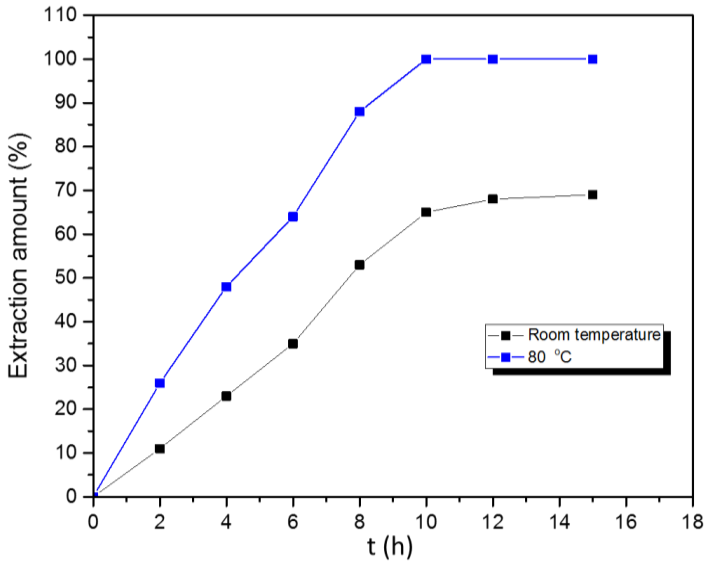


Figure 4. Kinetics of BSA extraction from solution at **5**0 °C and at room temperature

Conclusions

In summary, SPIONs were synthesized in the presence of chitosan biopolymer which led to the fabrication of SPION-chitosan nanocomposite. SPION sizes were less than 10 nm when generated within the chitosan network. By having amine functions, SPION-chitosan was successfully modified by CS_2 to generate new active functionality for the extraction of BSA. Comparison of SPION-chitosan and modified SPION-chitosan confirmed this claim. The use of the modified SPION-chitosan can be incorporated for other types of proteins which need extraction.

Acknowledgement

The first author is thankful from Payame Noor University for the financial supports.

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How to cite this manuscript: Esmail Doustkhah*, Mohammad Heydarizadeh, Hamed Mohtasham, Sadegh Rostamnia*, Morteza Hasani, Dithiocarbamate Modified SPION-Chitosan Nanobiocomposite, a Promising Adsorbent for Bovine Serum Albumin (BSA). Chemical Methodologies 3(5), 2019, 562-570. DOI: 10.33945/SAMI/CHEMM.2019.5.5.