Asian Journal of Chemistry; Vol. 25, No. 10 (2013), 5658-5660

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.OH52

Preparation and Reaction of Fluorescently-Labeled Chitosan Nano Particles†

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AJC-13295

Chitosan nano particles were prepared firstly through the electrostatic interaction between chitosan polycation and sodium tripolyphosphate and then fluorescently-labeled chitosan nano particles was made by the reaction between the primary amino group (2-NH_2) of chitosan and isothiocyanate root (N=C=S) of fluorescein isothiocyanate. Finally the effects of reaction time and chitosan concentration on the fluorescence labeling efficiency of chitosan were discussed. The results showed that various reaction times and chitosan concentrations had great effects on the structure and fluorescence labeling efficiency of chitosan nano particles.

Key Words: Chitosan, Fluorescence labeling, Nano particles.

INTRODUCTION

Chitosan, β-(1-4)-2-amino-2-deoxy-D-glucose, is a derivative of chitin after deacetylation. In an acid solution, the amino group (-NH_2) of chitosan can combine with H^+ to form -NH_3^+, so that it dissolves in the acid solution. Due to the advantages of innocuity, easily being degraded, stable property and good biocompatibility, chitosan has been widely applied to food, medicine, environment, chemical industry and biotechnology[1]. In addition, because of having quantum size effect, surface effect and macroscopic quantum tunneling effect, nano particles show unique physical and chemical characteristics. Thus, chitosan nano particles have been extensively used as drugs' carrier and in gene therapy and have become a focus at present.

Fluorescence labeling means that fluorescent substances combine with a certain group of the studied object through covalent bonding or physical adsorption and the information of the studied object can be reflected by their fluorescent characteristics[2]. Fluorescein isothiocyanate, a common fluorescence labeling reagent, has a high quantum yield, good light stability and low temperature coefficient. Meanwhile, it also has good biocompatibility and less toxicity[3]. Through covalent bonding, the primary amino group of chitosan combines with isothiocyanate root (N=C=S) of fluorescein isothiocyanate to label the chitosan. The chitosan labeled by fluorescein isothiocyanate has a high fluorescence intensity, light stability and solid combination[4]. Additionally, it has fluorescence quenching effect under certain conditions, so it can be used in trace detection of heavy metal ions and pesticides.

Presently, there have been some reports on the application of fluorescently-labeled chitosan, that is, suitable fluorescein was used to label the substance detected, aiming at studying the substance detected through the detection of fluorescent derivatives' characteristics. However, there are few studies of fluorescence labeling reaction.

Here, chitosan nano particles were compounded by using ion induction firstly and then they were labeled by fluorescein isothiocyanate, finally the effects of reaction time and chitosan concentration on the fluorescence labeling efficiency of chitosan were discussed.

EXPERIMENTAL

Preparation of chitosan nano particles: Firstly, certain amounts of chitosan was added to 3 % acetic acid solution and was dispersed by ultrasonic wave for 10 min. Afterwards, 15 mL of 6 % hydrogen peroxide was added to a round-bottomed flask dropwise and the flask was kept in a water bath at 40 ºC to degrade chitosan for some hours[5]. After the degradation ended, chromatography was carried out by using 10 mol/L NaOH solution and then large quantities of white precipitate appeared rapidly. After the filtrated precipitate was dissolved by 50 mL of 3 % acetic acid solution, chromatography was carried out repeatedly by using 10 mol/L NaOH solution and the precipitate was washed by distilled water until it was

†Presented to the 6th China-Korea International Conference on Multi-functional Materials and Application, 22-24 November 2012, Daejeon, Korea

neutral. Hereafter, the final precipitate was dissolved in 3 % acetic acid and its pH was adjusted to 4-5 by using 10 mol/L NaOH solution and then the mixture was stirred by a magnetic stirrer at a speed of 1500 r/min. Meanwhile, a small amount of 1 % sodium tripolyphosphate was added to the mixture drop wise to form white flocculent precipitate.

After the reaction finished completely, the prepared chitosan nano particles were separated by using a high-speed centrifuge and they were washed three times by ethanol to remove residual organic monomers and other impurities. Finally, the prepared chitosan nano particles were dried in a vacuum oven until the weight was constant.

Preparation of fluorescently-labeled chitosan nano particles: Certain quantities of 1 % acetic acid solution was put in a flask to prepare a certain concentration of chitosan-acetic acid solution and then after 10 mL of methanol was added to the flask, certain quantities of fluorescein isothiocyanate-methanol solution were poured into the flask slowly. After several hours of reaction in a dark place, the labeled product was precipitated after 0.2 mol/L NaOH solution was added to the mixture. After the precipitate was centrifugated for 15 min at a speed of 10000 r/min, it was washed by methanol solution. The centrifugation and washing were repeated until there was no florescence found in the supernatant.

RESULTS AND DISCUSSION

Characterization of chitosan nano particles: As shown in Fig. 1, chitosan nano particles had an absorption peak of stretching vibration of N-H at 3431 cm^{-1}, revealing that the amino group of chitosan was not damaged. The result proved that the prepared chitosan nano particles were satisfactory, which laid a foundation for the preparation of fluorescently-labeled chitosan nano particles, because the highly active fluorescent group of fluorescein isothiocyanate combined with the primary amino group of chitosan to label the chitosan.

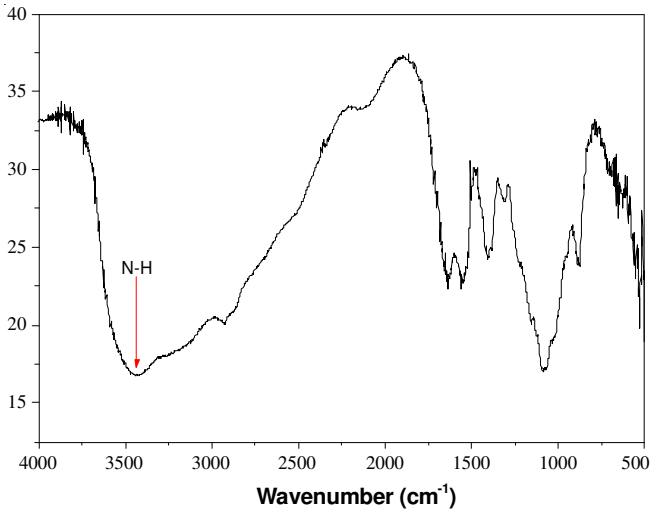

Fig. 1. Infrared spectroscopy of chitosan nano particles

From Fig. 2, we could find that chitosan nano particles could be prepared through ion induction under appropriate conditions, showing even spherical particles with a diameter of about 100 nm.

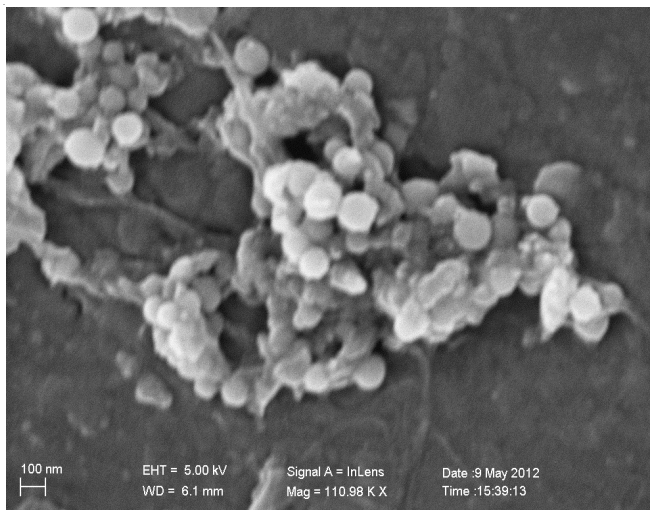

Fig. 2. SEM photographs of chitosan nano particles

Characterization of fluorescently-labeled chitosan nanoparticles: As shown in Fig. 3, fluorescently-labeled chitosan emitted extremely obvious green fluorescence observed under a UV lamp and it revealed that chitosan had been labeled by fluorescein isothiocyanate. In addition, the light emitted by fluorescently-labeled chitosan was detected by using a fluorescent visible spectrophotometer and strong green fluorescence could be found at 525-530 nm.

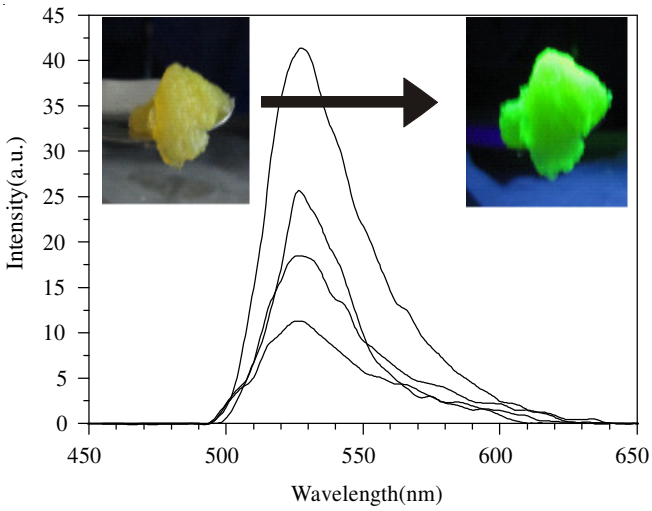

Fig. 3. Fluorescence spectra of FITC-CS

According to Fig. 4, there were no changes in the major adsorption peaks of chitosan before and after fluorescence labeling, showing that chitosan didn't react on other substances before and after fluorescence labeling, so the final substance must be fluorescently-labeled chitosan.

Effects of reaction time on the fluorescence labeling efficiency of chitosan: Fig. 5 showed that the fluorescence intensity of fluorescently-labeled chitosan went up gradually with the increase of reaction time, revealing that the fluorescence labeling efficiency of chitosan also enhanced gradually. It is because that as the increase of reaction time, chitosan and fluorescein isothiocyanate were in frequent contact with each other, promoting fluorescence labeling reaction.

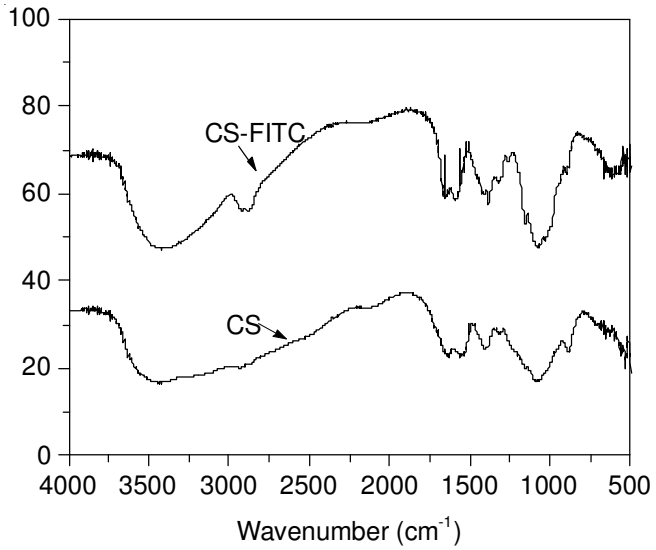

Fig. 4. Comparison between the infrared spectra of chitosan before and after fluorescence labeling

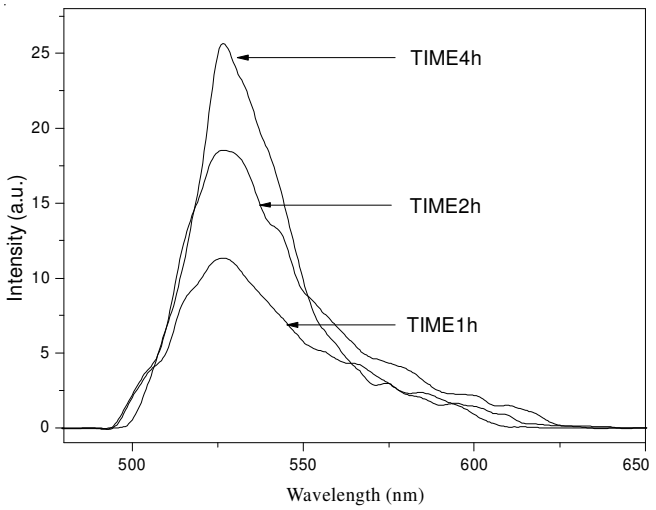

Fig. 5. Changes in the fluorescence spectra of fluorescently-labeled chitosan with reaction time

Effects of chitosan concentration on the fluorescence labeling efficiency of chitosan: When reaction time was 3 h and the concentration of chitosan solution varied from 0.005 to 0.015 g/mL, the fluorescence intensity of fluorescently-labeled chitosan was detected and shown in Fig. 6. The results revealed that the fluorescence intensity of fluorescently-labeled chitosan enhanced with the increase of chitosan concentration, indicating that the fluorescence labeling efficiency of chitosan also went up gradually. The reasons are that with the increase of chitosan concentration, more and more primary amino groups (2-NH_2) in chitosan solution could contact with fluorescein isothiocyanate, so that the fluorescence labeling efficiency of chitosan also increased. Therefore, the effects of various chitosan concentrations on fluorescence labeling efficiency resulted from the changes in the quantity of 2-NH_2 in chitosan solution.

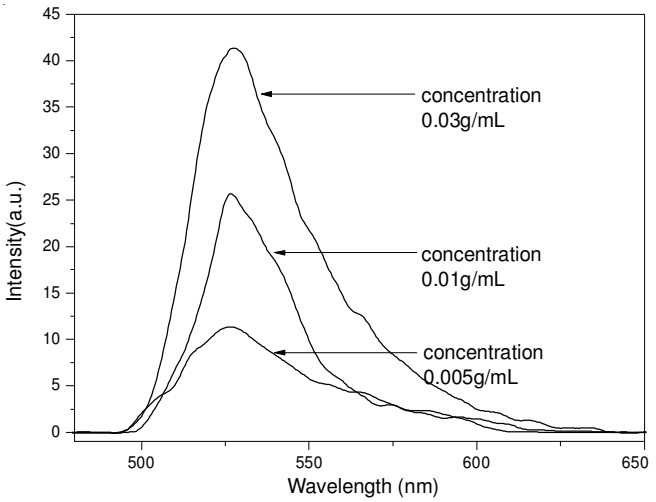

Fig. 6. Changes in the fluorescence spectra of fluorescently-labeled chitosan with chitosan concentration

Conclusion

By using ion induction, uniform chitosan nano particles in size were prepared and combined with fluorescein isothiocyanate to produce fluorescently-labeled chitosan through nucleophilic addition action, having good characteristics of both chitosan and fluorescein isothiocyanate.

Fluorescently-labeled chitosan observed under a UV lamp could emit obvious green fluorescence, which was proved by the analysis of fluorescence spectrum. Moreover, under the experimental conditions, the fluorescence labeling efficiency of chitosan went up gradually as the increase of reaction time and chitosan concentration.

ACKNOWLEDGEMENTS

This study was supported by the Hefei University Scientific Research Development Fund (Natural Science): 10KY05ZR.

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