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Effect of Treatment time on Synthesis of silver Nanoparticles using Chitosan and its stability during Freezing

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Abstract

Chitosan, a bioactive polysaccharide extracted from shell fish waste can act as both reducing and stabilising agent for the synthesis of metal nanoparticles. In the present study, silver nanoparticles (AgNPs) were synthesised using chitosan with thermal treatment. Two treatment times studied were 15 and 30 min. The formation of chitosan silver nanocomposites resulted in change of visible colour from colourless to yellow in both samples. The characterisation was done using UV-visible spectroscopy. Both samples exhibited the surface plasmonic peak at ~420 nm. The absorbance and wavelength of peak maxima increased with increase in treatment time. The full width half wavelength of the samples reduced with increased treatment time. Hence the polydispersity of silver nanoparticles can be modified using the time of thermal treatment. The samples were studied for the changes under frozen conditions and both samples were found to be stable under storage at -18±2°C.

Keywords: Chitosan, silver, nanoparticle, time, freezing

Introduction

Silver nanoparticles are among the extensively used nanomaterials for different biomedical applications. The unique physiochemical properties like antibacterial, anti-inflammatory, antiviral and anti-angiogenesis activities imparted a significant role in medical field (Kalaivani et al., 2018) along with their use in medical imaging. Another speciality of these metal ions is with their surface plasmon resonance (SPR) properties and related colour formations. AgNPs also have applications in optics, catalysis (Malassis et al., 2016) and photoelectrochemicals (Chandrasekharan & Kamat, 2000). The distinguishable SPR properties in silver and gold nanoparticles depends on their shape, size and stability which are affected by the method of preparation. Metal nanoparticles can be synthesised through two approaches. The physical approach includes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach (Abou El Nour et al., 2010). Because of the deleterious effects of chemicals, the use of biocompatible compounds for the preparation of these nanoparticles are studied enormously (Jabbar et al., 2020; Hebeish et al., 2010).

The β-(1–4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-*d*-glycopyranose functions as structural polysaccharides in chitosan (Tyagi et al., 1996). The shell fish processing industry generates a huge amount of waste and the composition of shell waste depends on season and species. The specific applications of chitosan are affected by properties like molecular weight and degree of deacetylation which in turn depends on the processing conditions. Chitosan is gaining importance widely because of the presence of amino groups in its chain. The glucosamine units which exist in open chain forms at the end of chitosan undergoes oxidation and helps in nanoparticle formation.

The synthesis of silver nanoparticles using chitosan has been reported using ascorbic acid and NaOH reduction by Badawy et al. (2019), using laser ablation method by Kalaivani et al. (2018), using autoclave by Venkatesham et al. (2014) and using NaOH and thermal treatment by Akmaz et al. (2013). There is no data on the effect of time of thermal treatment on the one pot synthesis of silver

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nanoparticles using chitosan. The AgNPs have found to undergo aggregation on freezing. Hence this study was undertaken to standardise the one pot synthesis of AgNPs using chitosan at two different treatment time and to assess their stability up on frozen storage.

Material and Methods

The chitosan sample was purchased from SRL chemicals, Mumbai, India. The silver nitrate and acetic acid were obtained from Merck Life Science Ltd., India. Two AgNP samples were prepared for studying the effect of treatment time. The chitosan was dissolved in 1% acetic acid (w/v). Both samples were prepared at 0.5% chitosan concentration. The samples were heated iso-thermically at 90°C for 45 min with continuous stirring of 500 rpm for dissolving chitosan completely. Ten millilitre of silver nitrate (1 M) was added to the dissoluted chitosan of 100 ml volume. Heating was continued and visible colour change was monitored. Time of heating studied were 30 min and 15 min based on the preliminary studies conducted (data not given). The samples were cooled to room temperature and stored in a refrigerator at 4°C for storage stability studies.

The AgNPs synthesised using different treatment times were assessed for their efficiency to withstand the frozen conditions. A known volume of synthesised AuNPs were taken in disposable cuvettes and top layer was sealed. From each batch, sufficient numbers of cuvettes were exposed to frozen temperature (-18±2°C) for 7 days and change in visible colour and UV-Visible spectra were monitored to assess the effect of frozen storage.

The change in surface plasmon resonance, before and after frozen storage of AgNPs, was monitored by UV-Visible spectroscopy measurements (Jasco Dual Beam Spectrophotometer, Model V-570, Jasco International Pvt Ltd., Japan). UV-Visible spectra of nanoparticle solutions were taken over the wavelength range of 300 – 700 nm (Anandalakshmi et al., 2016). The full width half maximum (FWHM) was calculated from the spectral data according to Keijok et al. (2019).

Results and Discussion

Generally silver nanoparticles exhibit yellow to brown colour depending on the size, shape and aggregation of colloidal nanoparticles. The samples treated for 15 and 30 min were observed for the visible colour changes (Fig. 1). The chitosan solution was almost colourless on dissolution in acetic acid. The dissolution of chitosan in acidic solution is due to the protonation of amine groups and chitosan can exist with an extended configuration because of the ionic repulsion between the protonated amine groups present (Sreelakshmi et al., 2021). On addition of silver nitrate, slight yellowish colour appeared which increased with treatment time. Both samples have turned pure yellow colour at end of treatment time. This indicates the formation of silver nanoparticles. The formation of silver nanoparticles from silver precursor involves three steps as reported by Ahmed et al. (2003) and Iravani et al. (2014). First the reduction of Ag+ occurs which results in the formation of metallic silver. These undergo agglomeration and form oligomeric clusters. These clusters will eventually form colloidal AgNPs.

Fig. 1. Colour change of chitosan solution on formation of silver nanocomposites

The synthesised chitosan silver nanocomposites were characterized by UV–visible spectroscopy. Surface plasmonic resonance occurs due to energy equivalence between energy of conductor electrons in metal and energy of electromagnetic waves applied (Somee et al., 2018). The SPR band of AgNPs depends on morphology and distribution of silver nanoparticles (Wei & Qian, 2008). The characteristic UV-Vis spectra of silver nanoparticle occurs at ~420 nm. In the present study there was no peak in the UV-Vis spectra of chitosan solution and silver precursor at ~420 nm (Fig. 2). Both the chitosan silver nanocomposites have depicted the typical SPR peak due to the synthesised silver nanoparticles (Fig. 3 and 4). When the time of treatment increased, the wave length of maximum absorption (l_{max}) showed a red shift (Table 1). The absorbance of the band (A_{max}) also increased as the concentration of nanoparticles formed increases with increase in time of treatment. The full width half maximum is used as an index for the polydispersity of nanoparticles. The FWHM reduced with increase in treatment time of nanoparticles. This shows that higher treatment

time can aid in the formation of uniform sized nanoparticles.

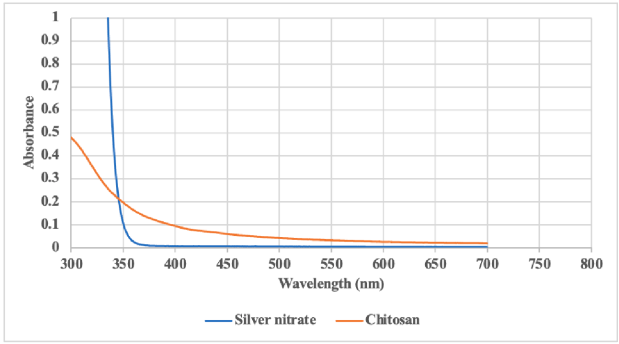

Fig. 2. UV-Vis spectra of silver precursor and chitosan

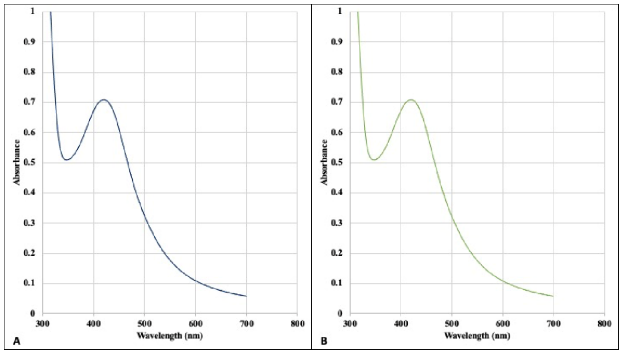

Fig. 3. UV-Vis spectra of silver nanocomposite with 15 min treatment time. A is the fresh sample and B is the frozen-thawed sample

The freeze stability of silver nanoparticles helps to increase scope of its applications. Hence the chitosan AgNP nanocomposites prepared were studied for their stability on frozen storage at -18±2°C. The samples were observed for visual colour changes (Fig. 5) and UV-Vis spectra (Fig. 3 and 4) after thawing at room temperature for 2 h. Both the samples retained the initial yellow colour after freezing and thawing. The UV spectra had no change in the sample synthesised with lower treatment time. The sample synthesised with higher treatment time showed a reduction in the spectral

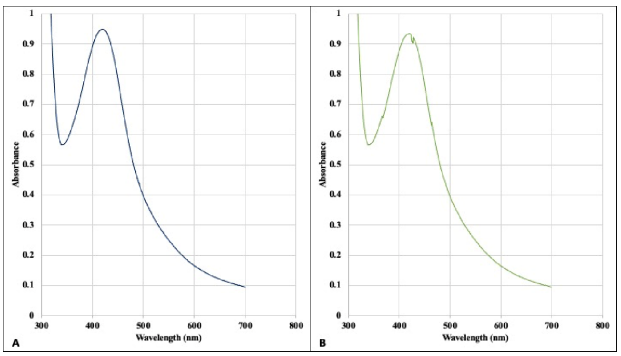

Fig. 4. UV-Vis spectra of silver nanocomposite with 30 min treatment time. A is the fresh sample and B is the frozen-thawed sample

absorbance (Table 1) without any change in l_{max}. Both samples had no variation in FWHM after freezing. The lack of fluctuation from the original characteristics shows the absence of aggregation

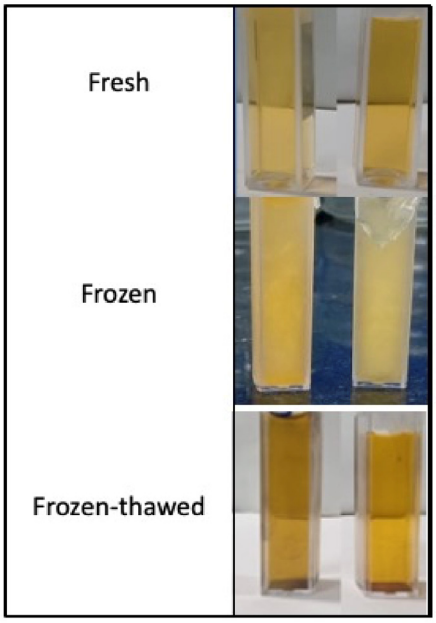

Fig. 5. Colour change of silver nanocomposites before freezing, immediately after freezing and after thawing

Table 1. UV-Vis characteristics of silver nanocomposites

Sample treatment time	λ_{max} (nm)	Fresh A_{max}	FWHM	λ_{max} (nm)	Frozen A_{max}	FWHM
15 min	419.5	0.71	80	419.5	0.71	80
30 Min	421	0.949	60	421	0.934	60

and/or the changes in morphology of the nanoparticles. Hence the samples synthesised were stable on freezing and can be frozen for transportation and storage purposes.

Polydispersity of the nanoparticles influences its applications and the present study showed that the polydispersity of silver nanoparticles synthesised using chitosan can be modified suitably using thermal treatment time. Further, the chitosan silver nanocomposites synthesised were stable under frozen conditions which enhances its utility in biomedical applications like drug delivery, antimicrobial and tissue regenerative formulations and in electrochemical applications.

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References

Abou El Nour, K. M., Eftaiha, A. A., Al-Warthan, A. and Ammar, R. A. (2010) Synthesis and applications of silver nanoparticles. Arab. J. Chem. 3(3): 135-140

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R. and Sastry, M. (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf. B. 28(4): 313-318

Akmaz, S., Dilaver Adýgüzel, E., Yasar, M. and Erguven, O. (2013) The effect of Ag content of the chitosan-silver nanoparticle composite material on the structure and antibacterial activity. Adv. Mater. Sci. Eng. 2013: 1-6

Anandalakshmi, K., Venugopal, J. and Ramasamy, V. (2016) Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Appl. Nanosci. 6(3): 399-408

Badawy, M. E., Lotfy, T. M. and Shawir, S. M. (2019) Preparation and antibacterial activity of chitosan-silver nanoparticles for application in preservation of minced meat. Bull. Natl. Res. Cent. 43(1): 1-14

Chandrasekharan, N. and Kamat, P. V. (2000) Improving the photoelectrochemical performance of nanostructured TiO2 films by adsorption of gold nanoparticles. The J. Phys. Chem. B. 104(46): 10851-10857

Hebeish, A. A., El-Rafie, M. H., Abdel-Mohdy, F. A., Abdel-Halim, E. S. and Emam, H. E. (2010) Carboxymethyl cellulose for green synthesis and stabilization of silver nanoparticles. Carbohydr. Polym. 82(3): 933-941

Iravani, S., Korbekandi, H., Mirmohammadi, S. V. and Zolfaghari, B. (2014) Synthesis of silver nanoparticles: chemical, physical and biological methods. Res. Pharm. Sci. 9(6): 385

Jabbar, A. H., Al-janabi, H. S. O., Hamzah, M. Q., Mezan, S. O., Tumah, A. N. and Ameruddin, A. S. B. (2020) Green Synthesis and Characterization of Silver Nanoparticle (AgNPs) using Pandanus Atrocarpus Extract''. Int. J. Adv. Sci. Technol. 29(3): 4913-4922

Kalaivani, R., Maruthupandy, M., Muneeswaran, T., Beevi, A. H., Anand, M., Ramakritinan, C. M. and Kumaraguru, A. K. (2018) Synthesis of chitosan mediated silver nanoparticles (Ag NPs) for potential antimicrobial applications. Frontiers in Laboratory Medicine. 2(1): 30-35

Keijok, W. J., Pereira, R. H. A., Alvarez, L. A. C., Prado, A. R., Silva, A. R. D., Ribeiro, J., Oliveira, J. P. D. and Guimarães, M. C. C. (2019) Controlled biosynthesis of gold nanoparticles with *Coffea arabica* using factorial design. Sci. Rep. 9(1): 1-10

Malassis, L., Dreyfus, R., Murphy, R. J., Hough, L. A., Donnio, B. and Murray, C. B. (2016) One-step green synthesis of gold and silver nanoparticles with ascorbic acid and their versatile surface post-functionalization. RSC adv. 6(39): 33092-33100

Somee, L. R., Ghadam P., Abdi-Ali, A., Fallah, S. and Panahi, G. (2018) Bio- synthesised AgCl NPs using Bacillus sp. 1/11 and evaluation of their cytotoxic activity and antibacterial and anti-biofilm effects on multi-drug resistant bacteria. IET Nanobiotechnol. 12(6): 764-72

Sreelakshmi, K. R., Mohan, C. O., Remya, S., Raj, R. and Ravishankar, C. N. (2021) Time dependent synthesis of gold nanoparticles using chitosan as reducing agent: A spectroscopic approach. Indian J. Fish. 68(2): 112-117

Tyagi, R., Kumar, A., Sardar, M., Kumar, S. and Gupta, M. N. (1996) Chitosan as an affinity macroligand for precipitation of N-acetyl glucosamine binding proteins/enzymes. Isolation and Purification. 2: 217-226

Venkatesham, M., Ayodhya, D., Madhusudhan, A., Babu, N. V. and Veerabhadram, G. (2014) A novel green one-step synthesis of silver nanoparticles using chitosan: catalytic activity and antimicrobial studies. Appl. Nanosci. 4(1): 113-119

Wei, D. and Qian, W. (2008) Facile synthesis of Ag and Au nanoparticles utilizing chitosan as a mediator agent. Colloids Surf. B. 62(1): 136-142