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Green synthesis and characterization of hydroxyethyl cellulose based silver nanoparticles and its antibacterial activity

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Abstract: In this study we report an environmentally friendly synthesis of silver nanoparticles (Ag-NPs) using hydroxyethyl cellulose (HEC), which acts as a reducing and a stabilising agent in an aqueous medium. The synthesis of Ag-Np was achieved by reduction of silver nitrate ($AgNO_3$) using HEC dissolved in water. The change of colour from colourless to pale yellow indicated the formation of silver nanoparticles. The optimum reaction conditions were obtained after studying various parameters such as the concentration of $AgNO_3$, concentration of HEC, reaction time and temperature. The optimum conditions to form the Ag-NPs are to use 800 µl of 0.05 molar (M) of silver nitrate, 3 ml of 10 wt. % of HEC at 100 ˚C for 30 minutes. The synthesized nanoparticles were characterised by UV visible spectroscopy, X-ray diffraction (XRD), Energy dispersive X-ray diffraction (EDX) and Transmission electron microscopy (TEM). The size of the nanoparticles was in the range of 100-200 nm and the UV-visible absorption peak was at 410-430 nm. The Ag-NPs showed good antibacterial activity against various pathogens. The antibacterial property of Ag-NPs against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were evaluated by agar well diffusion method. This HEC-Ag-NP is an excellent candidate for wound dressing material.

Key words: Hydroxyethyl cellulose; environmentally friendly synthesis; aqueous medium; silver nanoparticles; antibacterial activity

1. Introduction

Materials containing silver have attracted immense research because of their applications in various fields like catalysis, sensors, surface-enhanced Raman Scattering, food industries, medical fields and so on (Li et al., 2006; Chen et al., 2005; Setua et al., 2007). The antibacterial property of silver and its compounds has been known for centuries. Due to the antimicrobial activity silver containing materials have been widely used in food industries in packaging and plastics as well as food additives (Panacek et al., 2006; Wei et al, 2009). In medical field silver has been used in catheters, dental materials, medical devices, implant and burn dressings (Sambhy et al., 2006). Compound of bulk silver, silver nanoparticles have shown improved activity because of the reduction of particle size and high surface to volume ratio. Moreover the residual silver ions are highly toxic and affect the human health severely. Recently, intense research in being carried out in various fields using silver nanoparticles.

Several methods have been developed to synthesize silver nanoparticles with different size and shape, including chemical reduction, electrochemical techniques and photochemical reduction. The most common chemical synthesis includes the reduction of silver salts with sodium borohydride, sodium citrate and ascorbic acid (Chen et al., 2006; Lou et al., 2006; Kuo & Chen, 2003).

The solvents used in these chemical reduction methods cause toxicity and biological risk to the environment. In order to save the environment, research is being focused on the development of silver nanoparticles in a green chemistry approach using plant extracts (Gnanadesigan et al., 2011; Vijayakumar et al., 2013), microorganism like bacteria and fungi(El-Rafie et al., 2010; Fayaz et al., 2010; Sastry et al., 2003), chitosan (Venkatesham et al., 2012), enzymes, seaweeds or algae (El-Rafie et al., 2013), natural gums (Kora et al., 2010) and sodium hydroxide (El-Sheikh et al. 2013).

The mechanism of the antibacterial activity of silver nanoparticles involves the attachment of silver nanoparticles to bacterial cell wall, thus disturbing the cell-wall permeability and eventually the cellular respiration. In addition to this the silver nanoparticles also penetrate inside the cell and denature the DNA (Deoxy Ribo Nucleic Acid) and protein of the bacteria by interactions with the phosphorus and sulphur (Sambhy et al., 2006; Sondi & Salopek-Sondi, 2004; Kumar et al., 2004; Cho et al., 2005; Ruparelia et al., 2008). The bactericidal effect

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of silver nanoparticles depends on size and shape of the particles, the effect decreases with increase in the particle size.

Although many routes have been developed for synthesizing silver nanoparticles using polysaccharides, natural biopolymers, have attracted extensive attention due to its rich source, biocompatibility and biodegradability. The biopolymers such as starch, chitosan, glucose and heparin have been successfully used as reducing agents for silver nanoparticles synthesis.

Hydroxyethyl cellulose (HEC), a polysaccharide is a cellulose derivative with excellent biocompatibility, biodegradability, film forming prospect and water solubility. HEC is widely used in several fields including food and cosmetics industries which functions as a thickeners and viscosity increasing agents, biotechnological and biophysical industries (Gorgieva & Kokol, 2011). Due to the viscosity properties, it is used as viscosifier and filtrate-loss controllers for drilling fluids, gelling agents for fracturing fluids, water plugging/profile modifying polymers and flooding agents for enhanced oil recovery (Zhang, 1999).

In this research, we report the synthesis of silver nanoparticles using HEC as the reducing and stabilizing agent with water as the only solvent and also report the antibacterial efficacy of the silver nanoparticles towards pathogenic bacteria. HEC a natural polymer will play the role of particle stabilizer to prevent agglomeration and control the silver nanoparticles structure. In our research, we have produced silver nanoparticles with high stability using water as the solvent. The plenty of O-H groups in HEC reduce Ag^{+} to Ag^{0} and being a polymer it prevents agglomeration and thus HEC acts as a stabilizing as well as a reducing agent.

2. Experimental methods

2.1. Materials and bacterial strains

2-Hydroxyethyl cellulose (HEC) (average molecular weight Mw ~250,000) and silver nitrate ($AgNO_3$) 99.99% were purchased from Sigma-Aldrich. All chemicals were of highest purity and used without further purification. Prior to experiments, all glass wares were thoroughly washed with detergent and copiously rinsed with millipore water. Nutrient agar and nutrient broth were purchased from Merck. Bacterial strain such as *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 10536), *Enterococcus faecalis (*ATCC 14506), *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus (*ATCC BAA1026) were purchased from Fisher. The control antibiotic used was Penicillin G Sodium from Duchefa Biochemie.

2.2. Preparation of silver nanoparticles

3 ml of HEC with different wt. % (3, 5, 7 and 10) were prepared by dissolving HEC in millipore water and keeping it under continuous stirring overnight to get a homogenous solution. A stock solution 0.05M of $AgNO_3$ solution was prepared using millipore water for the synthesis of Ag-NPs. In a typical synthesis, different volume of $AgNO_3$ (200 µl, 400 µl, 600 µl and 800 µl) was added into 3 ml of 2-hydroxyethyl cellulose and 2 ml of deionized water under constant stirring. For optimisation the reaction was carried out at different temperature 70 °C, 80 °C, 90 ˚C and 100 °C and at different time intervals of 10, 15, 20, 25 and 30 minutes. The sample bottles were covered with aluminium foil and the experiment was run in a dark room to avoid photo reduction as silver nitrate is photo sensitive. The optimum reaction condition was used to study antibacterial activity.

2.3. Characterization of synthesised silver nanoparticles

The UV-visible absorption spectrum was recorded using a Shimadzu 3101 PC spectrophotometer using a 1 cm quartz cell. The absorption wavelength was set up in the range of 300 nm to 700 nm. A solution containing HEC alone was dissolved in deionised water and used as a blank. . X-ray diffraction measurements were carried out using a D5005 Siemens X-ray diffractometer with Cu-Kα (λ = 1.54 Å) radiation at 40 kV and 40 mA. All samples were scanned over a 2θ range of 30 – 80° at a step size of 0.02°. The energy dispersive x-ray analyser (EDX) was used to perform the elemental analysis. For transmission electron microscopy , a drop of sample solution was dispensed directly onto a carbon coated copper grids and blotting out the excess solvent using a filter paper and left the material to dry at room temperature. The copper grid was then placed in a JOEL JEM 3010 (TEM) operating at the voltage of 120 kV to obtain the TEM images.

2.4. Antibacterial assay

2.4.1. Agar well diffusion method

The agar well diffusion method was used to study the antibacterial property of the HEC stabilized silver nanoparticles. The glass wares and all the reagents were sterilized in an autoclave at 121˚C for 20 min. *Escherichia coli* (ATCC 10536), *Staphylococcus aureus (*ATCC BAA1026), *Pseudomonas aeruginosa* (ATCC 15442), *Enterococcus faecalis (*ATCC 14506) and *Bacillus subtilis* (ATCC 11774) were used as model test strains. Bacterial suspension was cultured in Mueller Hinton Broth (MHB) at 37 ˚C overnight and the turbidity was adjusted to 0.5 McFarland standards [14]. Then, 100 µl of this bacterial suspension was used to inoculate the Mueller Hinton Agar (MHA) plate and 800µl of silver nanoparticles was added to the centre well with a diameter of 8 mm. A standard antibiotic penicillin g sodium or known as

benzylpenicillin sodium at 1 mg/ml was used as a positive control. These agar plates were incubated at 37 ˚C for 24 h in a bacteriological incubator and the inhibition zone around the well was measured using a ruler up to 1 mm resolution. This experiment was done in triplicates for all the bacteria.

3. Results and discussion

3.1. UV-vis spectroscopy

The formation of Ag-NPs could be followed by visual colour changes of the reaction solution. These colour forming phenomenon were attributed to the excitation of surface plasmon resonance (SPR) in metal NPs. UV-visible absorption is used to detect the presence of Ag-NPs at the visible region of 400-500 nm. At this region, a strong SPR transition appeared and confirmed the production of Ag-NPs (Kanmani & Lim, 2013). Fig. 1 is the photograph of the Ag-NPs suspensions at various concentrations. The colour of Ag-NPs solutions changed from white to yellowish brown within 30 min. It is well known that the absorption band of Ag-NPs in UV-Vis shows the successful preparation of the nanoparticles due to the stimulation of surface plasmon vibrations in the metal nanoparticles (Shankar et al., 2004).

Fig. 1: Photograph of Ag-NP suspension at various concentrations of 0.05M $AgNO_3$. From left 200, 400, 600 and 800 µl

The UV-Visible spectra in Fig. 2(a) shows the effect of $AgNO_3$ volume at the same concentration (0.05 M) on Ag-NPs (200, 400, 600 and 800) µL at 100 ˚C, with 10 wt. % of HEC in 30 min. It reveals that the absorption intensity increases as the volume of $AgNO_3$ increased. At 800 µL of $AgNO_3$, the higher absorbance values are reached with the formation of bell shaped bands. This is because more conversion of Ag-NPs occurs at high volume of $AgNO_3$. High amount of $AgNO_3$ will increase the size of nanoparticles (Xiong et al., 2013). Thus, 800 µL was kept as an optimum volume of $AgNO_3$ to produce Ag-NPs.

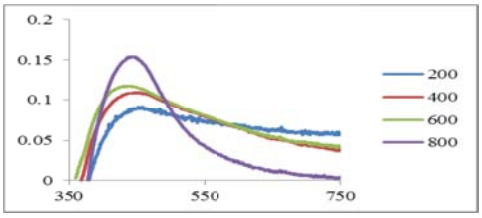

Fig. 2(a): The UV-vis absorption spectra of silver nanoparticles synthesized at varying volume (µl) of $AgNO_3$ (0.05 M)

A sharp absorbance peak was formed for the high concentration of HEC as shown in the Fig. 2(b). Thus, it shows that the optimum HEC concentration to produce silver nanoparticles was 10 wt. %. The absorbance value is high this indicates the better conversion of Ag^+ to Ag^0. The increase in concentration of HEC contributes to the increase in hydroxyl groups which reduces silver ion to metallic silver in nanosize (Mochochoko et al., 2013; Park et al., 2011).

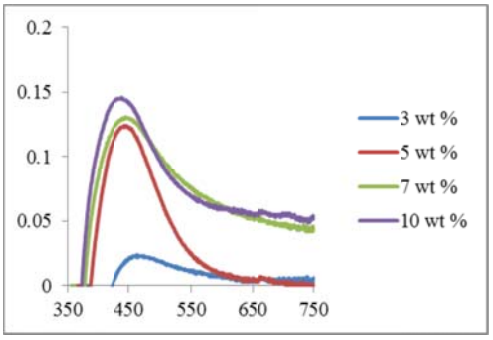

Fig. 2(b): The UV-vis absorption spectra of silver nanoparticles synthesized at varying wt. % of HEC.

The UV-Vis spectra in Fig. 2(c) show the effect of temperature in the synthesis of Ag-NPs. Both temperature and time played an important role, to increase the synthesis rate and final conversion to Ag-NPs (Song & Kim, 2009). The maximum temperature used was at 100 ˚C due to the boiling point of water which used as a solvent in this experiment. It was noticed that the low temperature at 70 ˚C takes a long time to synthesize Ag-NPs. However, for the reaction at 100 ˚C, the rate of reduction and reaction time increased respectively. Moreover, at 100 ˚C, the absorption band increased and solution turns from colourless to brownish colour due to increase in number of nanoparticles formed. The colour of the reaction solution changed fast at the high temperature because it speeds up the synthesis rate.

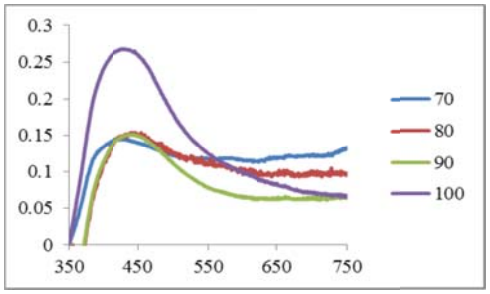

Fig. 2(c): The UV-vis absorption spectra of silver nanoparticles synthesized at varying temperature (degree Celsius)

Fig. 2 (d) shows the effect of heating time on silver nanoparticle synthesis. It is observed that a levelling of is reached at the time of 35 minutes, results in the appearance of sharp bell shaped band. This shows that the reaction time increases which ends up in the conversion of silver nanoparticles.

Similar results were reported with sharp band at increased reaction time (Venkatesham et al., 2012).

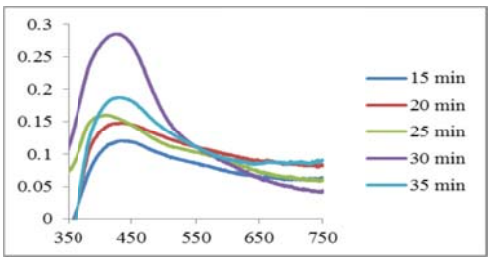

Fig. 2(d): The UV-vis absorption spectra of silver nanoparticles synthesized at different reaction time (minutes)

3.2. X-ray diffraction (XRD) analysis

X-ray diffraction pattern (XRD) was studied to determine the crystal structure of the Ag-NPs. Fig. 3 shows the XRD pattern of HEC synthesised Ag-NPs. Four main peaks were observed at 2θ = 38.18° correspond to (111), is stronger as compared to the peaks found at 2θ = 44.6°, 64.2° and 77.1° corresponding to (200), (220) and (311) lattice planes of face-centred cubic (fcc). The XRD pattern in the Fig. 3 clearly illustrates that the Ag-NPs synthesized using HEC are crystalline in nature.

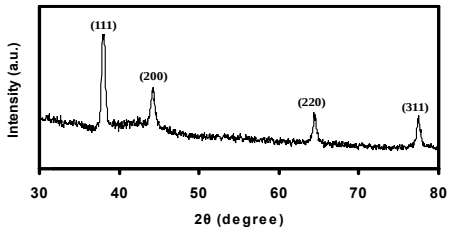

Fig. 3: X-ray diffraction diagram of HEC stabilized silver nanoparticles.

3.3. Energy dispersive X-ray spectroscopy (EDS) analysis

EDS of the sample was analysed, to check the presence of other metal ions. The strong signal in the silver region at 3 KeV confirmed the formation of silver nanoparticles (Fig. 4). The metallic silver nanoparticles show a typical optical absorption peak approximately at 3 keV due to surface plasmon resonance (Magudapatty et al., 2001). Another peak at 8 keV belongs to the copper (Cu) which is from the Copper grid used as the substrate for the samples (Xiong et al., 2013).

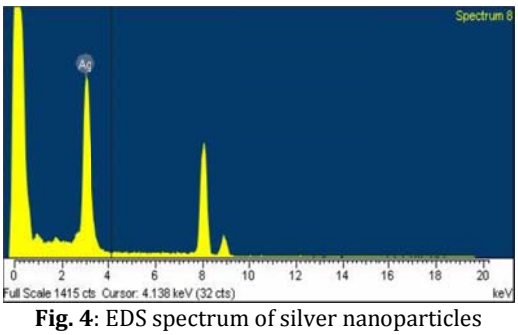

Fig. 4: EDS spectrum of silver nanoparticles

3.4. Transmission electron microscopy (TEM)

The formation of silver nanoparticles is further confirmed by TEM analysis. The TEM micrograph in the Fig. 5 shows the surface morphology of the well-dispersed Ag-NPs synthesized by HEC which acts as a good reductant and a good stabilizer. The average particle size was in the range of 100-200 nm and the particles are spherical in shape (Bankura et al., 2012). The synthesized Ag-NPs are well capped with hydroxyl group of HEC biopolymer and thus it stabilized the particles by preventing the agglomeration (Xu et al., 2014).

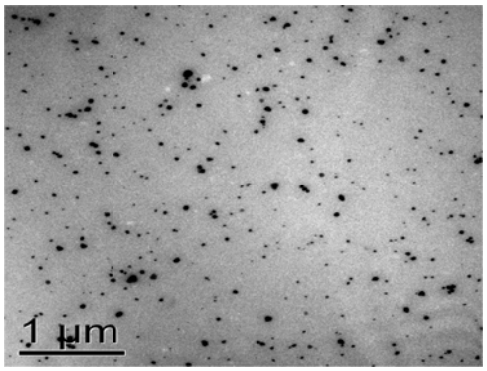

Fig. 5: TEM images of HEC synthesized silver nanoparticles.

3.5. Mechanism of silver nanoparticles formation

As reported earlier for the starch by Vigneshwaran et al, (2006) the silver ion from the $AgNO_3$ interacts with the O-H functional groups present in the cellulose backbone. The natural polymer of HEC has a large number of O-H groups which acts as an effective reducing agent for silver ions. The process involves complexation of silver ions with O-H groups in HEC and reduction of the metal precursor $AgNO_3$ by HEC. The HEC caps and stabilizes these Ag-NPs, since the structure of this polymer is very complex, it is believed that more than one mechanism might involve in the reduction of silver ion.

3.6. Antibacterial activity

3.6.1. Agar well diffusion method

The optimised conditions to form silver nanoparticles were used (800 µl of 0.05 M of $AgNO_3$, 3 ml of 10 wt. % of HEC at 100 ˚C for 30 min) for evaluating antibacterial activity. Thus, the synthesized Ag-NPs were investigated by using the agar well diffusion method against the test bacterial strains. Clear zone of inhibition around the sample on agar well plates after 24 h of incubation at 37 ˚C was observed (Fig. 6). The pure HEC did not show any growth inhibition, whereas Ag-NPs presented a good antibacterial activity with a diameter range of 12 to 18mm. The diameter of inhibition zone shows magnitude of susceptibility of the microorganism towards antimicrobial agent.

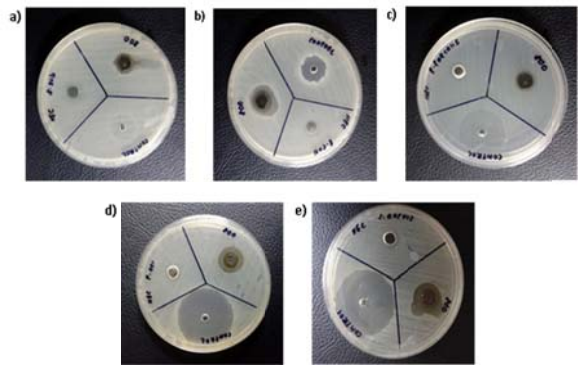

Fig. 6: Plates showing zone of inhibition for different microbial strains. (a) *B. subtilis* (b) *E. coli* (c) *E. faecalis* (d) *P. aeruginosa* and (e) *S. aureus.*

The diameter of inhibition zone around the well is tabulated in Table 1. Each bacterium shows the different inhibition zone probably due to the difference in cell walls between Gram-positive and Gram-negative bacteria. It was observed that the test bacterium showed a wide range of sensitivity towards silver nanoparticles. Among all the strains, *Staphylococcus aureus* was more sensitive to Ag-NPs followed by *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Enterococcus faecalis* respectively (Fig. 7). It is evident from this study, that HEC synthesised nanoparticles can acts as an antimicrobial agent to different microbial strains.

Table 1: Antibacterial activity of HEC synthesized silver nanoparticles.

S. No	Test bacterium	Diameter of inhibition zone for Ag-NPs (mm)	Diameter of inhibition zone for control antibiotic (mm)
1.	B. subtilis	12	14
2.	E. coli	15	18
3.	E. faecalis	12	23
4.	P. aeruginosa	16	34
5.	S. aureus	18	35

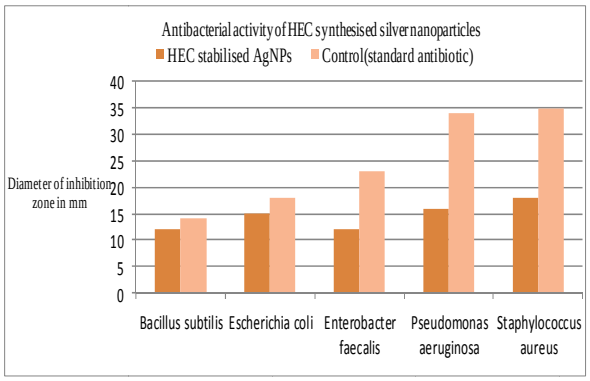

Fig. 7: Graph of the antibacterial activity of HEC synthesized silver nanoparticles

4. Conclusion

In the present investigation, we have reported a simple and facile process for the preparation of Ag-NPs by using non-toxic and biodegradable HEC as a reducing and stabilizing agent. The synthesized Ag-NPs were characterized using UV-Visible, TEM, XRD, and EDS to support the stability of biosynthesized nanoparticles. The average particle size was in the range of 100-200 nm. This study shows that the silver nanoparticles obtained have antibacterial activity towards pathogenic bacteria. This experiment suggests that the HEC stabilized Ag-NPs can be used for biomedical applications as it shows a good antibacterial activity.

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