



**SILVER NANOPARTICLES EMBEDDED PAM/CMG HYDROGEL – IN-SITU GENERATION
BY GREEN SYNTHESIS AND ITS ANTIMICROBIAL ACTIVITY**

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ABSTRACT

Synthesis of silver nanoparticles using green chemistry process has become the prime focus of interest. This research article reports the in-situ generation of silver nanoparticles during copolymerization of polyacrylamide/carboxymethyl jargon crosslinked hydrogel through green route that is without using toxic reducing agents and solvents. The formation of silver nanoparticles was characterized by Ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, Energy dispersive analysis of X-ray, transmission electron microscopy and X-ray diffraction methods. All these methods confirmed the formation of Ag NPs during hydrogel synthesis. The silver nanoparticles produced in polyacrylamide/carboxymethyl guar gum hydrogel matrix were also examined for antimicrobial activity against *E. Coli*.

KEY WORDS: Silver nanoparticles, green synthesis, hydrogel, antimicrobial activity



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1. INTRODUCTION

The major thrust of nanoscience and nanotechnology research in recent years, has been the development and applications of nanomaterials and nanocomposites containing nanoparticles. The particles in the range of 1-100 nm are regarded as nanoparticles. They are the matter between molecules and bulk materials. These particles are characterized by high surface-to-volume ratio. Due to their extremely small size, they exhibit entirely different physical, chemical and mechanical properties than its macro counterpart^{1, 2, 3}. Among the nanoscale particles, silver nanoparticles (Ag NPs) has emerged as an important class of nanoparticles because of their biomedical, electronic, catalytic and optical applications. Ag NPs, in general, are synthesized by various physical and chemical methods. Some of the reported methods for Ag NPs synthesis are chemical reduction of AgBF₄ by NaBH₄⁴, electrochemical reduction of aqueous AgNO₃ solution⁵, thermal decomposition in organic solvent⁶, chemical reduction of aqueous AgNO₃ solution using ultrasonic waves (sono reduction)⁷, chemical and photo reduction in reverse micelles⁸, cryochemical synthesis of Ag NPs⁹, spark discharge¹⁰ etc. However, green synthesis route of producing Ag NPs has received much acceptance due to its less impact on environmental pollution and cost-effectiveness^{11,12}. Moreover, Ag NPs synthesized through green-route are biocompatible and non-toxic material as required for biomedical and pharmaceutical applications¹³. The green synthesis is the method of using a non-toxic solvent and reducing agents. Several researchers have reported the use of natural biomaterials for the green synthesis of Ag NPs such as *Murraya Koenigii* (green curry leaves)¹⁴, Pomegranate peel¹⁵, seaweed *Kappaphycus alvarezii*¹⁶, marine algae *Sargassum muticum*¹⁷, *Azadirachta indica* (Neem)^{2,18}, *Agaricus bisporus* (mushroom)¹⁹, Vitamin C²⁰, *Ocimum tenuiflorum* (Black Tulsi)²¹, Tryptophan²², *Fusarium semitectum* (Fungus)²³, Juniper Berry²⁴ etc. This method requires the use of stabilizer that controls the synthesis and dispersion stability of nanoparticles. Natural polymers have been widely used to control the particle growth, stabilize the metal nanoparticles and as the capping agent to limit the oxidation of particles. Hamedi et al. produced Ag NPs by reducing Ag⁺ ions by α - D-glucose and then starch was used as the stabilizer to prevent aggregation of nanoparticles¹³. Regiel A. et al. reported the use of chitosan for the in situ synthesis of embedded Ag NPs²⁵. Dawy et al. produced Ag NPs in a chitosan biopolymer by an in situ green chemical procedure²⁶. Hebeish A. et al. reported the synthesis of Ag NPs loaded Carboxymethyl cellulose based hydrogel²⁷. The nanoparticles are too small to be used for specific applications. Therefore, they are usually incorporated into the suitable matrix. The most preferred matrix is a polymer. The polymers or more precisely organic molecules aid in the stabilization of nanoparticles by binding themselves to the particles. The stabilization of nanoparticles may be due to the electronic interaction between the functional groups present in the polymers and the metal nanoparticles. The nucleophilic functional

groups are responsible for binding the metal particles by electron donation. Natural, as well as synthetic polymers, are used to effect the stabilization of nanoparticles²⁸. There are many methods to incorporate the nanoparticles into the polymer matrix. One of the earliest methods is to produce nanoparticles and then dispersing them in the polymer matrix. However, due to large surface areas of nanoparticles, they tend to aggregate and lose their inherent characteristics²⁹. The second method is layer-by-layer assembly technique in which oppositely charged thin films of polyelectrolyte and substrates are deposited alternatively resulting in electrostatic interaction between them. This method is not suitable to prepare bulk material³⁰. Alternatively, the nanoparticles can be stabilized by micro phase separation by an amphiphilic copolymer solution hence their growth is restricted³¹. An important method is the in-situ generation of nanoparticles in the polymer matrix. In this method, the solution of a polymer matrix and metal ions are exposed to counter ions in gas or solution^{32,33}. Ag NPs are extensively used as antimicrobial agents in the biomedical field. Ag NPs, due to their antimicrobial activity have received clinical approvals, but dermal toxicity was reported. Hence, Ag NPs in gel matrix can be a promising choice for wound dressing material^{19,27}. Before the discovery of Ag NPs, silver ions were the main ingredients in wound healing creams but the silver ions suffer from the disadvantage that they readily form complexes, and the effect of these ions was short lived. The above limitation of Ag⁺ ions was overcome by the use of neutral Ag NPs, which also possess an antimicrobial property by persuading the creation of reactive oxygen species^{31,34}. Moreover, Jha and Prasad reported that when AgNPs sol applied to the SiHa cancer cell line was found to reduce the growth by 70-80%³⁵. The present investigation aims for the in-situ synthesis of Ag NPs during copolymerization of polyacrylamide/carboxymethyl guar gum (PAM/CMG) hydrogel through green route that is without using toxic reducing agents and solvents. The Ag NPs produced in PAM/CMG hydrogel matrix has a potential application as a wound dressing material.

2 EXPERIMENTAL

MATERIALS AND METHODS

Silver nitrate solution (Qualigen, India) was used as precursor for the synthesis of Ag NPs. Acrylamide (AM) (S.D. Fine Chemicals Mumbai India), Carboxymethylated guar gum (CMG) viscosity 3000 CPS at 25°C (Courtesy Hindustan Gum Ltd. India), N,N'-methylene bisacrylamide (N,N'MBA) (Merck, Germany), Potassium persulphate (KPS) (CDH, New Delhi India) were used as received. The other reagents used were all of analytical grade, and all solutions were prepared with triple distilled water.

2.1 Green synthesis of silver nanoparticles embedded PAM/CMG hydrogel

The synthesis of Ag NPs embedded PAM/CMG hydrogel is discussed as below: (Figure 1) The Ag NPs embedded PAM/CMG hydrogel was synthesized by free radical copolymerization of acrylamide (AM) (7×10^{-2} mole) and 20 wt % CMG with respect to Am. The reaction was carried out by dispersing predetermined amount of CMG

and 25mM AgNO_3 solution in 50 mL Am solution. The reaction mixture was stirred for 2h in 250mL flat bottom flask. The solution turned yellowish brown during stirring. Then N,N'MBA (0.5 wt %) and 0.05g KPS were added to above solution while constant stirring. The stirring was continued further for 1h, till a homogenous, viscous but fluid system was obtained.

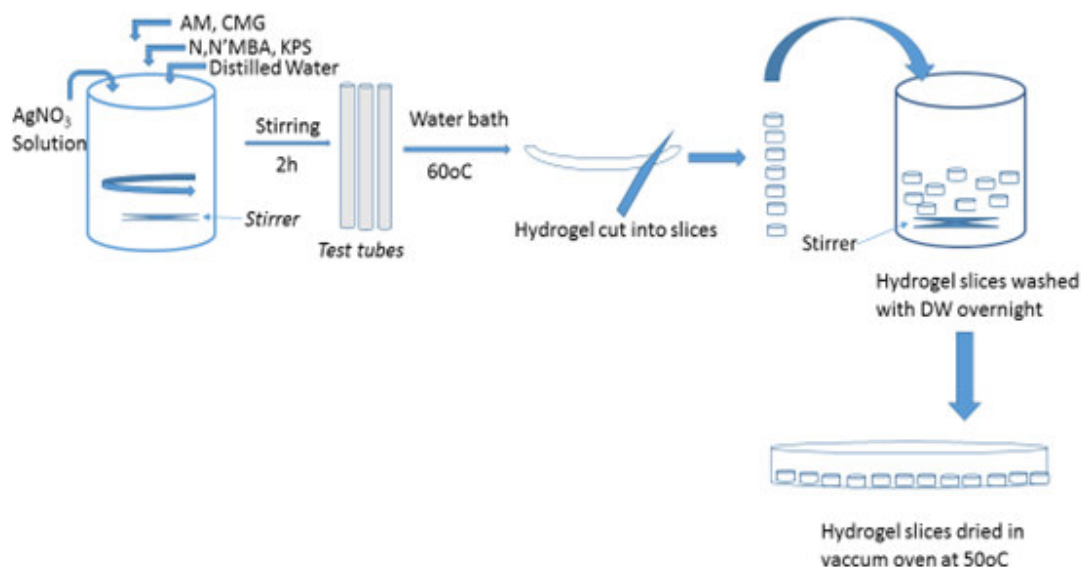


Figure1

Schematic flow diagram for Green synthesis of Ag NPs embedded PAM/CMG hydrogel

Subsequently the content of the flask was poured into test tubes and kept in a water bath maintained at $65 \pm 1^\circ\text{C}$ for 2h. The test tube was broken to release the Ag NPs embedded PAM/CMG hydrogel, which was then cut into small pieces of about 0.5cm thick. These slices were then swelled in distilled water for 24h with an intermittent stirring to remove any unreacted chemicals. Then the hydrogel pieces were first dried in the air and then in a vacuum oven at 50°C till constant weight.

2.2 Characterization

The synthesized Ag NPs embedded PAM/CMG hydrogel was characterized by following techniques to confirm the formation of Ag NPs:

2.2(I) Ultraviolet-Visible (UV-Vis) Spectroscopy

The synthesis of Ag NPs were monitored by measuring UV-Vis spectrum on UV-Vis Spectrophotometer (Perkin Elmer Lambda 750).

2.2(II) Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR spectra of Ag NPs embedded PAM/CMG and PAM/CMG hydrogels were obtained in KBr pellets on Thermo Scientific Nicolet 380 Spectrometer in transmittance mode to study the evidence of in-situ formation of Ag NPs.

2.2(III) Scanning Electron Micrography (SEM) and Energy dispersive analysis of X-ray (EDAX)

Surface morphology of Ag NPs embedded PAM/CMG and PAM/CMG hydrogels were investigated and compared using Scanning Electron Microscope with a Hitachi S3700N Microscope. The formation of Ag NPs in PAM/CMG hydrogel was confirmed by elemental analysis using energy dispersive X-ray spectrum (EDAX) equipped with SEM (HITACHI-S-3700N).

2.2(IV) Transmission Electron Microscope (TEM)

Microphotographs of Ag NPs embedded PAM/CMG hydrogels were obtained using transmission electron microscope (Tecnai, G2-200 KV HRTEM, SEI Company, Holland) to investigate the shape, size and microstructure of nanoparticles formed. The samples for TEM were prepared by dispersing small quantities of reaction mixture in distilled water by sonication (after the appearance of dark brown color during polymerization before hydrogel formation).

2.2(V) X-Ray Diffraction (XRD)

Ag NPs embedded hydrogel was characterized using X-Ray Diffraction Analyzer (Bruker D8-Advance).

2.2(VI) Antimicrobial activity

Antimicrobial activity of the Ag NPs embedded PAM/CMG hydrogel was examined using agar diffusion

test. The sterilized agar medium was taken in sterilized petri dishes and the gram negative bacteria *E Coli* strains were evenly spread on the medium. The Ag NPs embedded hydrogel slices were kept in the medium and incubated for 48 hours at 37°C.

3. RESULTS AND DISCUSSION

The in-situ generation of Ag NPs during the synthesis of PAM/CMG hydrogel was observed from the gradual colour change from pale yellow to brown. During the copolymerization reaction, Ag NPs were formed in-situ in the hydrogel that was evident from its dark brown colour (characteristic colour of Ag NPs), unlike PAM/CMG hydrogel that was transparent and colourless. The colour change indicated the formation of Ag NPs, which may be due to the excitation of surface plasmon vibrations in the Ag NPs³⁶. The CMG acted not only as reducing agent to reduce Ag⁺ ions to Ag but also as a capping agent. The –OH and –NH₂ groups present in acrylamide and CMG

are responsible for reduction of aqueous Ag⁺ ions to elemental Ag²⁶.

3.1 UV-Vis spectroscopy

The formation of Ag NPs was monitored by UV-Vis spectra. The UV-Vis spectra of the reaction mixture taken at different time interval must show surface plasmon resonance band (SPR) at around 400nm, a characteristic feature of Ag NPs^{11,37}. The slight left shift or right shift in the λ_{max} of the SPR peak could be due to the Ag NPs of different size, shape^{37,38}. The surface plasmon resonance is due to the collective excitation of conduction electrons in a metal on contact with the incident light²⁵. The figure 2 demonstrates that the Ag NPs showed the peak at around 425nm. The absorbance intensities were increased by the reaction time. The narrow width of SPR absorption bands signify the smaller size and more uniform size distribution of synthesized Ag NPs²⁷. The almost constant surface plasmon vibrations recorded as a function of reaction time is indicative of the stability of synthesized Ag NPs¹⁶.

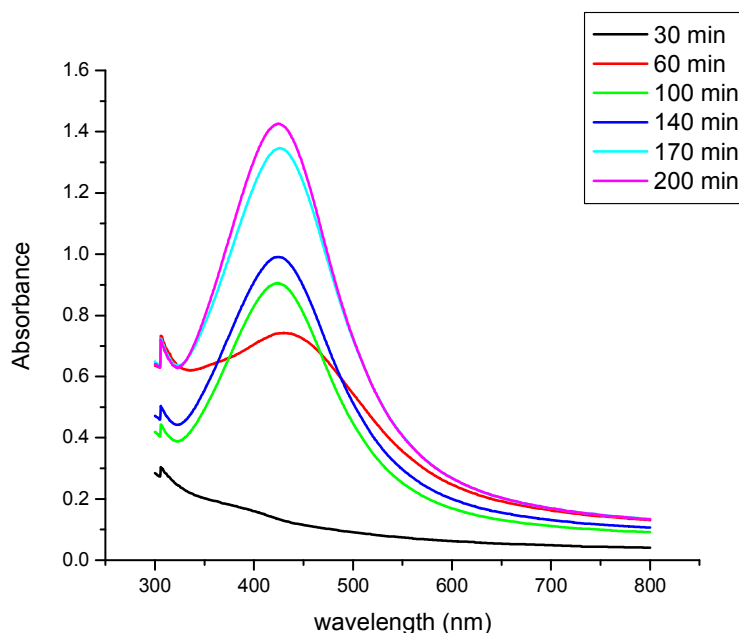


Figure 2

UV-Visible absorption spectra at different reaction time interval during Ag NPs synthesis

3.2 Fourier Transform Infra-Red Spectroscopy (FTIR)

The FTIR spectra of PAM/CMG hydrogel and Ag NPs embedded PAM/CMG hydrogel are shown in figure 3 (a) and (b). The stretching and deformation vibrations of Ag NPs embedded hydrogel (identical to the parent

PAM/CMG hydrogel) were observed at 3438.9 cm⁻¹ (O-H stretching), 2923.6 cm⁻¹ ((C-H stretching), 1651.1 cm⁻¹ (amide-I, C-O stretching and amide-II, N-H stretching), 1455.2 cm⁻¹ (N-H and C-N in-plane bending of amide).

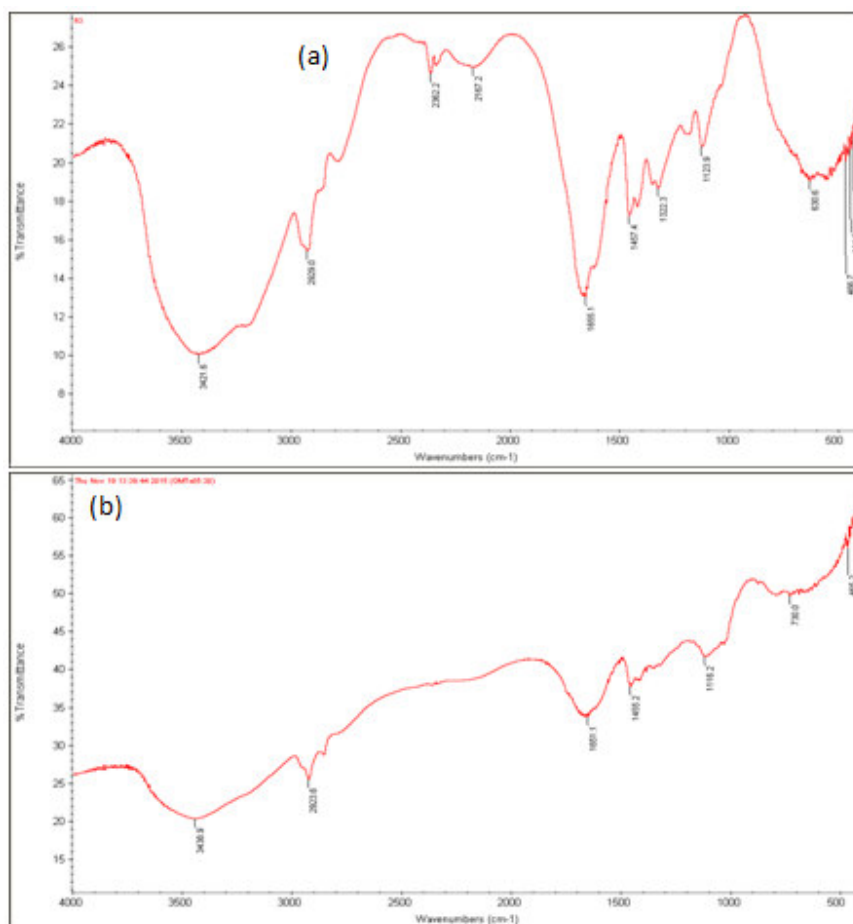


Figure 3
FTIR spectra of (a) PAM/CMG hydrogel and (b) Ag NPs embedded PAM/CMG hydrogel

3.3 SEM and EDAX analysis

The SEM images provided insight into the surface morphology of Ag NPs embedded PAM/CMG hydrogel. Figure 4(a) (b) and (c) shows the SEM images of PAM/CMG hydrogel and Ag NPs embedded hydrogel. The picture of PAM/CMG hydrogel manifests the presence of micropores through which water enters and cause the hydrogels to swell. On the contrary, figure 4(b) & (c) displays the presence of well distributed Ag NPs throughout the surface of the hydrogel without

aggregation. The chemical composition and stoichiometric proportions of the synthesized samples was confirmed by Energy dispersive analysis of X-ray (EDAX) study. It is well known that EDAX technique supplies the effective atomic concentration of different constituents on the top surface layers of the solid are investigated. The EDAX analysis confirmed the significant presence of elemental silver. Thus, the Ag NPs were synthesized in-situ in the PAM/CMG polymeric matrix as evident from figure 5 and Table 1.

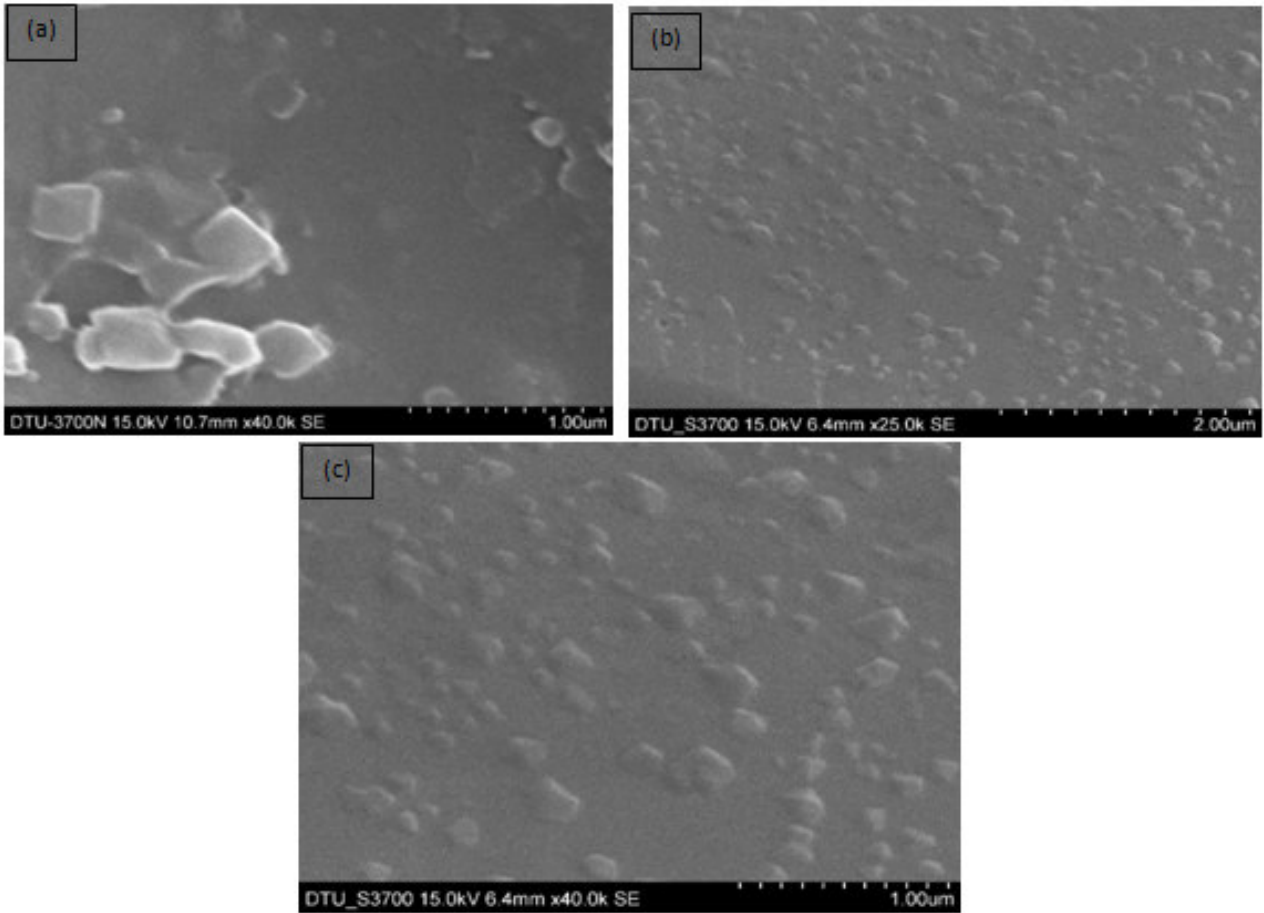


Figure 4

SEM image of (a) PAM/CMG hydrogel at 1 μm scale (X40K), (b) Ag NPs embedded PAM/CMG hydrogel at 2 μm scale (X25K) and (c) Ag NPs embedded PAM/CMG hydrogel at 1 μm scale (X40K)

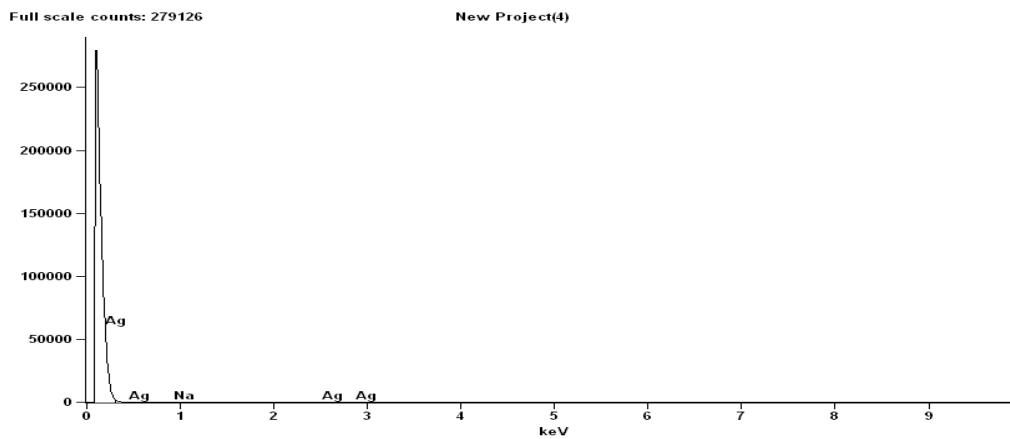


Figure 5

EDAX spectrum of Ag NPs embedded PAM/CMG hydrogel

Table 1
Elemental Analysis of Ag NPs embedded PAM/CMG hydrogel

Element Line	Net Counts	Weight %	Atom %	Formula
Na K	0	0.00	0.00	Na
Ag L	44	100.00	100.00	Ag
Ag M	435543	---	---	
Total		100.00	100.00	

3.4 TEM analysis

The transmission electron micrograph images of Ag NPs synthesized in PAM/CMG hydrogel matrix are shown in figure 6. The TEM images clearly show that the spherical Ag NPs ranging in size from 8 to 50nm were stabilized and encapsulated by the capping agent CMG.

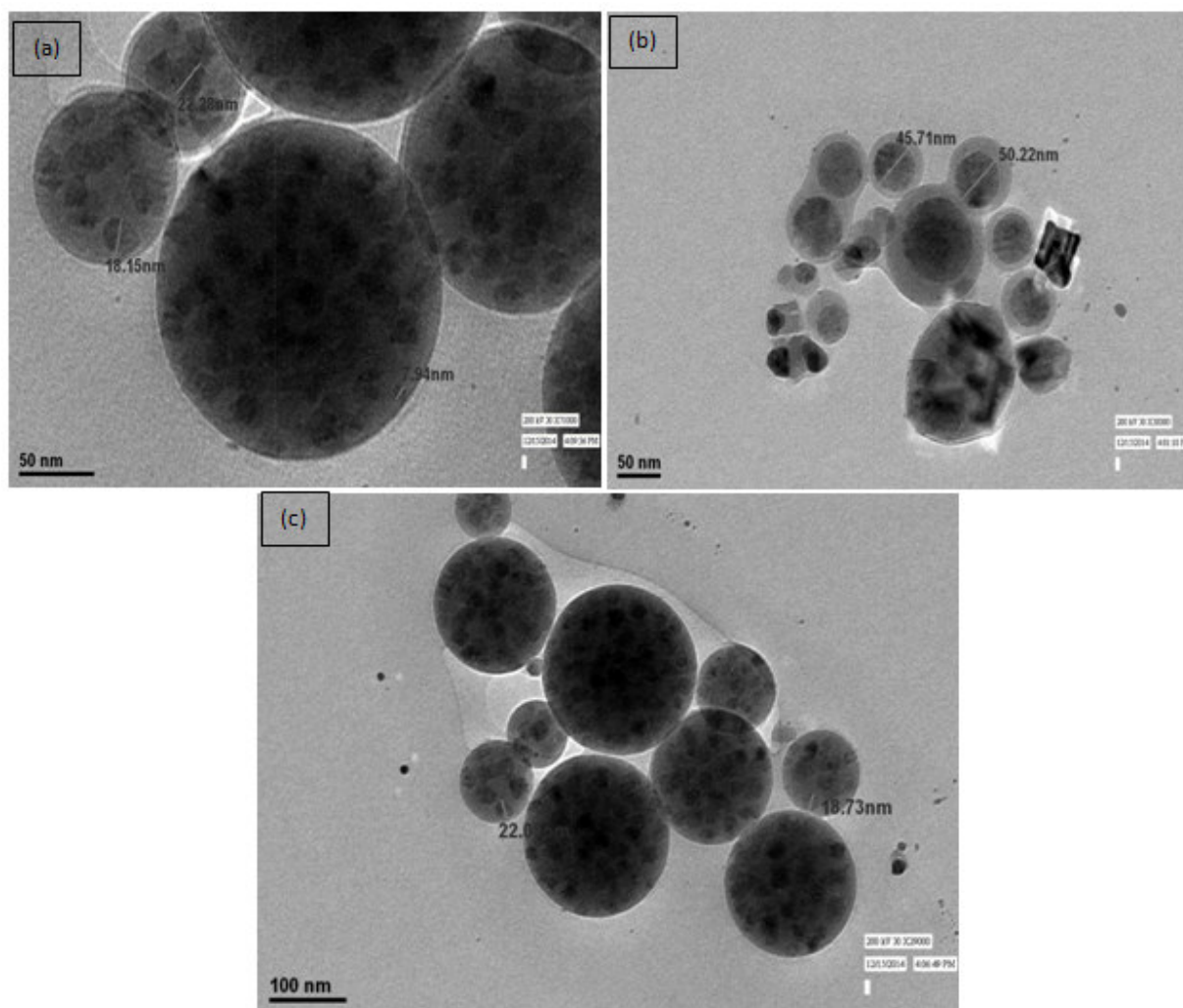


Figure 6

TEM image of (a) Ag NPs embedded PAM/CMG hydrogel at 50nm scale (X71K), (b) Ag NPs embedded PAM/CMG hydrogel at 50nm scale (X38K) and (c) Ag NPs embedded PAM/CMG hydrogel at 100nm scale (X29K)

3.5 X-ray Diffraction

The X-ray diffraction patterns obtained for Ag Nps embedded PAM/CMG hydrogel is shown in figure 7. The figure displays XRD peaks at 2θ of 39° , 44.5° , 64.5° and 77.5° can be due to the (111), (200), (220), and (311) crystalline planes of the face centered cubic (FCC) crystalline structure of metallic silver, respectively (JCPDS file No. 00-004-0783). The results thus indicated beyond doubt the presence of Ag NPs embedded in PAM/CMG hydrogel.¹¹

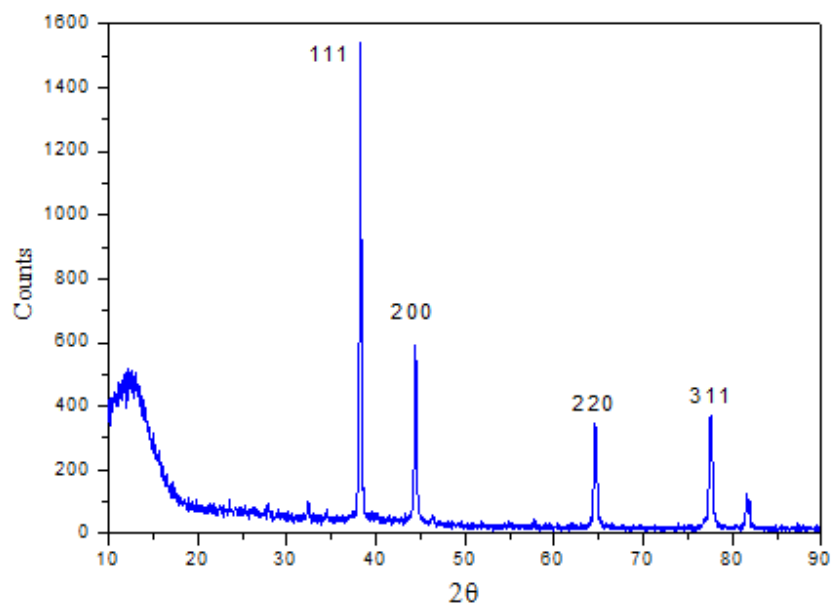


Figure 7
X-ray diffraction pattern for Ag NPs embedded PAM/CMG hydrogel

3.6 Antimicrobial activity

The antimicrobial activity of Ag NPs embedded hydrogel was examined against pathogen gram-negative bacteria *Escherichia Coli* by disc plate technique. The results shown in image (figure 8) evidently depicts that Ag NPs embedded PAM/CMG hydrogels has antibacterial properties as evidenced by inhibition zone after 48 hours of exposure (figure 8 a, b and c). On the contrary, PAM/CMG hydrogel without Ag NPs was attacked by bacteria (Figure 8 d). The results are consistent with the results reported by Hebeish A et al.²⁷

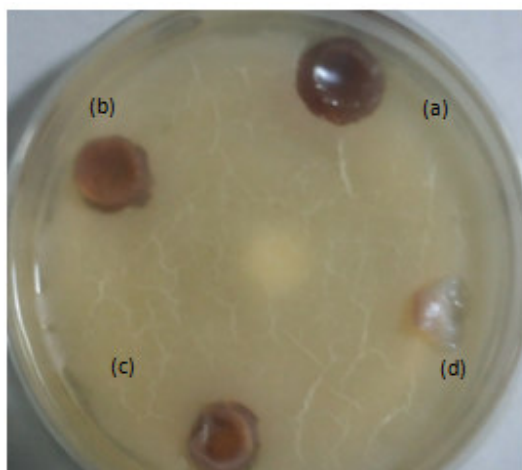


Figure 8
Comparison of Antimicrobial activity for (a, b & c) Ag NPs loaded PAM/CMG hydrogels and (d) unloaded PAM/CMG hydrogel

4 CONCLUSION

Silver nanoparticles were successfully synthesized in-situ by green route in PAM/CMG hydrogel polymeric matrix. Ag^+ ions in aqueous solution were reduced without using any toxic reducing agent. CMG acted as reducing, stabilizing and capping agent while forming Ag NPs embedded PAM/CMG hydrogel. It also aided in the

dispersity of these synthesized Ag NPs throughout the polymeric matrix. The synthesized Ag NPs embedded PAM/CMG hydrogel was also characterized by various techniques like FTIR, SEM & EDX, TEM, XRD and UV-Vis spectroscopy. All the techniques confirmed the formation of Ag NPs. UV-Vis spectra demonstrated the absorption maxima characteristic of surface plasmon resonance, characteristic of Ag NPs. Transmission

electron micrographs revealed the presence of spherical Ag NPs encapsulated by CMG and their sizes were in the range of 8-22nm. The prepared hydrogel was also examined for its antimicrobial activity against pathogen gram-negative bacteria, E Coli. It was observed that the Ag NPs embedded hydrogel demonstrated good resistant

to bacteria even after 48 hours of exposure. However, PAM/CMG hydrogel without Ag NPs suffered an attack from the bacteria. Therefore, the Ag NPs embedded PAM/CMG hydrogel has a potential application as a wound dressing material.

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