



Preparation and Characterization of Silver Nanoparticles Incorporated Chitosan Hydrogel Sheet for Wound Healing

J. Sindhu*, J. Sundaraseelan

1. Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor, Andhra Pradesh, India.

ABSTRACT

The present work aimed to develop a novel chitosan-PVA-based hydrogel sheet which forms an immobilizing matrix for silver nanoparticles (AgNPs). Silver nanoparticles were prepared by chemical reduction method. Silver nitrate was taken as the metal precursor and tri-sodium citrate as a reducing agent. These AgNPs were incorporated in to chitosan-PVA hydrogels in different concentrations (1%, 2%, 4% and 6%) to form F1, F2, F3 and F4 formulations respectively. The prepared chitosan and silver nanoparticle composite hydrogel sheets were characterized using U.V, FTIR, water absorption studies, SEM and Anti-microbial studies. The data shows the average diameter of AgNPs is in the range of 60-70 nm and is roughly spherical in shape. The AgNPs incorporated hydrogel sheets were bactericidal against *Escherichia Coli* (gram positive) and *staphylococcus aureus* (gram negative) and it showed good antibacterial effect by disc diffusion method. These results suggested that AgNPs incorporated chitosan hydrogel sheet could be a promising candidate for wound dressing applications.

Keywords: Chitosan, Hydrogel, Silver Nanoparticles, Chemical reduction.

*Corresponding Author Email: sindhujpharma308@gmail.com

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INTRODUCTION

Hydrogels are three dimensional materials (3D) with the ability to absorb large amount of water while maintaining their dimensional stability. The 3D integrity of the hydrogel in their swollen state is maintained either by physical or chemical cross-linking^{1,2}. In the present study hydrogels acts as moist wound dressing materials and has the ability to absorb and retain the exudates within its network structure. These exudates are important in wound healing process to keep the wound moist as wound healing process gets faster in a moist environment. Exudate provides nutrition for the injured tissue and enhances the migration of epithelial cells. Exudate supplies the wound with leucocytes, which control bacterial growth and reduce the incidence of infection³. Within this hydrogel network we are incorporating the silver as a nanoparticle to treat the wound infections⁴. Silver was the most important antimicrobial agent available before the introduction of antibiotics. Silver is a non-toxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of disease causing microorganisms⁵. Silver nanoparticles (AgNps) have the ability to anchor to the bacterial cell wall and subsequently penetrate it thereby causing structural changes in the cell wall and leads to the death of microbial cell⁶. So AgNps which is a broad spectrum antibiotic is incorporated in to the hydrogel so that it is effective against gm positive and gm negative bacteria⁷ and decreases the duration of wound healing.

MATERIALS AND METHOD

Chitosan was purchased from OTTO's Chemicals. PVA was procured from SD Fine Ltd, Mumbai, and was used without purification. Silver nitrate was obtained from Merck, India Pvt Ltd. All other chemicals used were of analytical grade.

Formulation of Chitosan and Poly Vinyl Alcohol (Ch/PVA) Hydrogel Sheet

A predetermined amount of PVA as shown in (Table No.1) was dissolved in 80⁰ c distilled water with stirring for a period until a clear solution was obtained. A weighed quantity of chitosan was dissolved separately in 100 ml of 1% acetic acid solution. To this solution add required quantity of glutaraldehyde and stirred it for 10 minutes. The resulting two mixtures were mixed with addition of glycerol until a homogenous mixture was obtained. This resultant mixture was poured in to OHP boats and kept for drying at room temperature for a period of 40 to 48 hours.

Table 1: Formulation plan of Ch/PVA Chitosan hydrogel sheet

S. No	Formulation	Chitosan (gm)	Acetic acid (%)	Poly vinyl alcohol(gm)	Glycerol (ml)	Glutaraldehyde (ml)
1	F1	1	1	0.5	0.5	0.5
2	F2	1	1	1	1	1
3	F3	1	1	1.5	1.5	1.5
4	F4	1	1	2	2	2

Preparation of Silver Nanoparticles

There are many synthetic routes that have been developed to synthesize silver nanoparticles (Ag-NPs) due to its tremendous applications. Chemical reduction method is widely used to synthesize Ag-NPs because of its readiness to generate Ag-NPs on a large scale. 100 ml of 0.001M AgNO₃ was heated to boiling point. To the heated solution 10mM sodium citrate was added drop by drop with vigorous stirring until a bright yellowish colour is obtained. The appearance of colour indicates the formation of silver nanoparticles. The resulting solution was then removed from the heating element and stirred until cooled to room temperature.

Preparation of Silver Nanoparticles Incorporated Hydrogel Sheet

For the preparation of silver nanoparticles incorporated Ch/PVA hydrogel sheets, a predetermined amount of PVA was dissolved in 80⁰ c distilled water with stirring until a clear solution was obtained. 1% chitosan solution prepared in acetic acid was added to the above PVA solution. The mixture was stirred with addition of glycerol. The silver nanoparticles solution was added in a required quantity and stirred for 15 minutes (Table 2). The resulting mixture was poured in to OHP boats and kept for drying at room temperature for a period of two days.

Table 2: Formulation plan for CH/PVA/Ag hydrogel

S. No	Formulation	Chitosan (gm)	PVA (gm)	Glycerol (ml)	Silver Nanoparticle solution (%)
1	F1	1	0.5	0.5	1
2	F2	1	1	1	2
3	F3	1	1.5	2	4
4	F4	1	2	3	6

Characterization of Chitosan-Pva Hydrogel Sheet

Generally hydrogels are characterized for their morphology, swelling property and elasticity. Morphology is indicative of their porous structure. Swelling determines the release mechanism of the drug from the swollen polymeric mass while elasticity affects the mechanical strength of the network and determines the stability of these drug carriers. Some of the important features for characterization of hydrogels are as follows:

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a useful technique for identifying chemical structure of a substance. Here FT-IR spectra of pure chitosan, pure PVA and CH/PVA hydrogel sheet were taken on analytical technologies model 2202 spectrometer. Powdered samples were prepared into pellets with KBr.

Swelling and Absorption Capacity

The water absorption of CH/PVA hydrogel sheet was examined by water uptake capacity. Hydrogel sheet was cut into $1 \times 1 \text{ cm}^2$ and note down their dry weight (W_1). The swelling behaviour of the hydrogel was measured by immersing them in distilled water at room temperature in petri dish. At different time intervals, remove hydrogel and the excess water on the surface of it was removed by blotting the surface with tissue paper and the weight was then recorded (W_2). This process was repeated until the swelling equilibrium was achieved. The degree of swelling was calculated using the following equation

$$\text{Degree of swelling (\%)} = [(W_2 - W_1) / W_1] \times 100$$

Where, W_1 is the weight of completely dried sample and

W_2 is the weight of swelled sample.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy is an excellent tool for physical observation of morphological features of hydrogel sheet. This is a powerful technique widely used to capture the characteristic 'network' structure in hydrogels. Here, the hydrogel sheets were treated with liquid nitrogen to make them rigid and focused with the 'ZEISS' equipment to get the surface morphology of hydrogels.

Characterization Studies of Prepared Silver Nanoparticles

Visual Inspection

For the preliminary determination of silver nanoparticles, the formation of yellow colour is necessary. This yellow colour is due to the reduction of silver nitrate to silver ions in the presence of sodium citrate as a reducing agent.

UV-Visible Spectroscopy

Ag nanoparticles synthesized from chemical reduction method were characterized in a 'SHIMADZU' UV-VIS spectrophotometer. The scanning range for the samples was 200-800 nm. The spectrophotometer was equipped with "UV Probe" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference.

Characterization of AgNps Incorporated Chitosan-Pva Hydrogel Sheet

Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR spectra of chitosan-PVA-AgNPs hydrogel sheet were taken on a analytical technologies model 2202 spectrometer. Powdered samples were prepared using KBr pellet method.

Scanning Electron Microscopy (SEM)

Here, the hydrogel sheets were treated with liquid nitrogen to make them rigid and focused with the 'ZEISS' equipment to get the surface morphology and the particle size range of the silver nanoparticles.

Antimicrobial Studies

The antimicrobial activity of silver nanoparticles is tested on two types of human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* which were cultured on nutrient agar plates supplemented with different concentration of silver nanoparticles (Table 3).

Table 3: Composition of Nutrient Agar medium (100 ml)

S. No	Ingredients	Amount to be taken (gm)
1	Beef extract	0.15
2	Yeast extract	0.2
3	Peptone	0.6
4	Glucose	0.1
5	Agar	1.5
6	Distilled water	100 ml

This prepared nutrient agar medium was autoclaved for 15 minutes. Then the medium was transferred to the sterilized petri plates and allowed to solidify. After solidification, under the laminar air flow chamber inoculate the bacteria present in the nutrient broth (100µl) in to the nutrient agar medium with the help of 'L' shaped spreader. After that Silver nanoparticles incorporated hydrogel sheets were placed over the plates in the form of disc shape and were incubated for 24 hr at 37 °C.

RESULTS AND DISCUSSION

Compatibility Studies by FT-IR

The Fourier transform infrared (FTIR) spectra of pure chitosan, PVA Chitosan/PVA hydrogel sheet and Ag incorporated Chitosan/PVA hydrogel sheet were recorded with an FTIR-Spectrophotometer using KBr pellet method. There is no significant change in the peak of chitosan after the addition of PVA and Ag nanoparticles to it and this reveals that they are compatible with each other.

Swelling and Absorption Capacity of Hydrogel Sheet

With the increase in time the water absorption capacity of the hydrogel increases and the formulation F4 shows maximum water uptake of 142.86% (Table 4). This reveals that as the PVA concentration increases the absorption capacity increases.

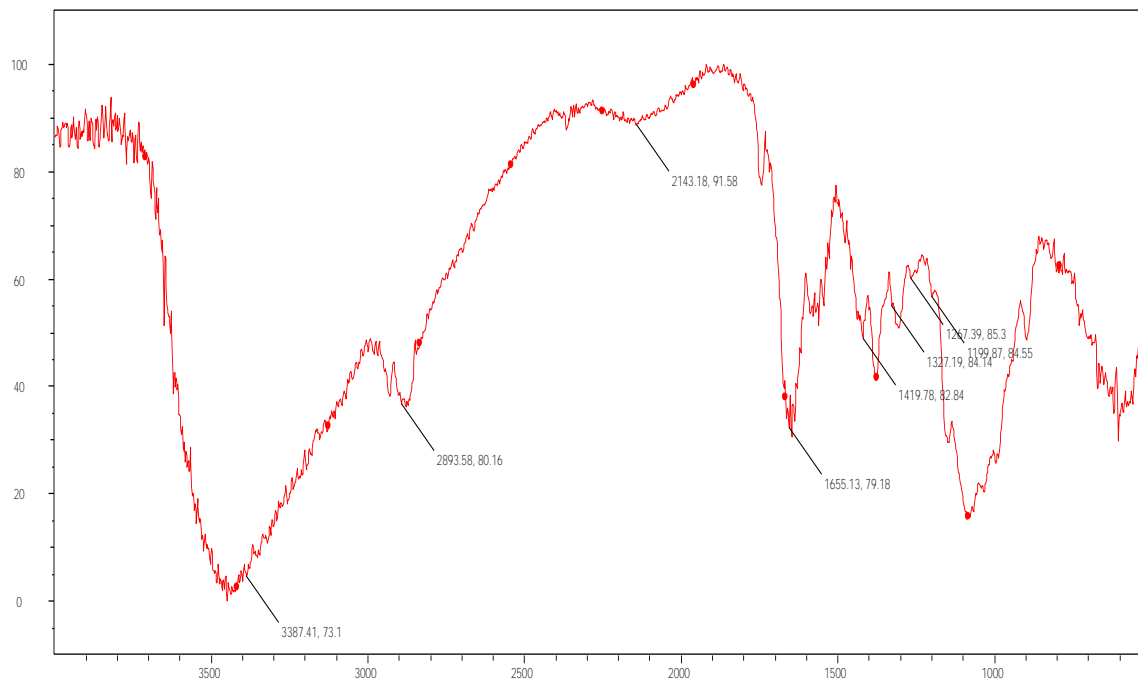


Figure 1: FTIR spectra of pure chitosan

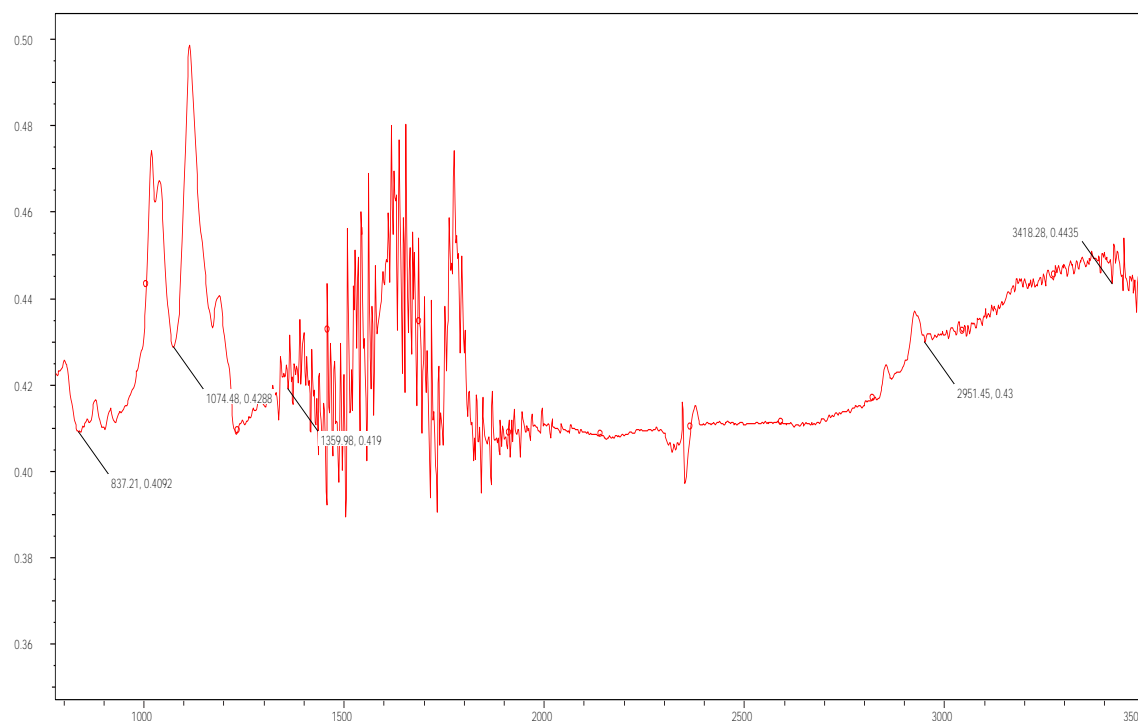


Figure 2: FTIR spectra of Pure PVA

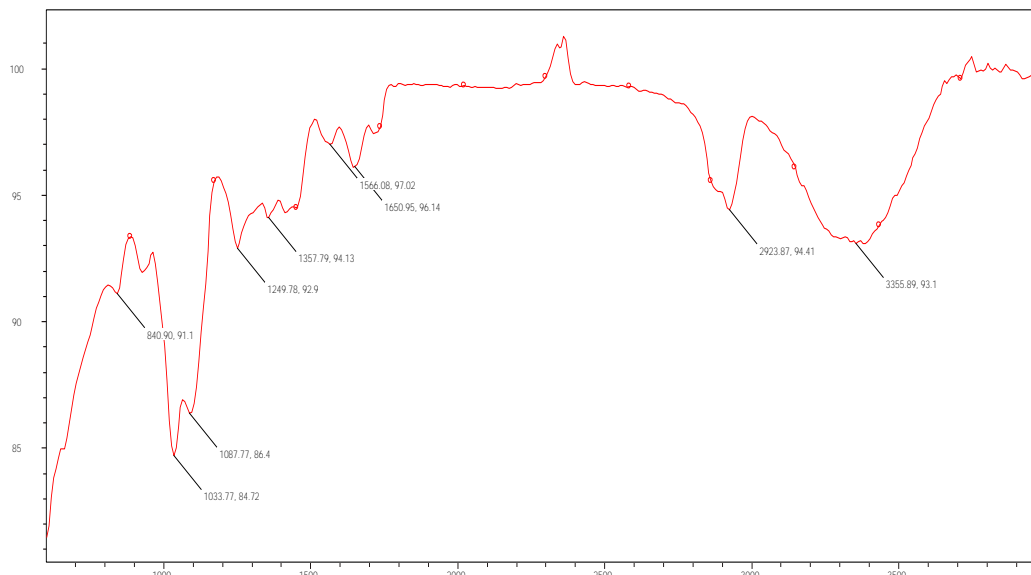


Figure 3: FTIR spectra of Chitosan and PVA hydrogel base

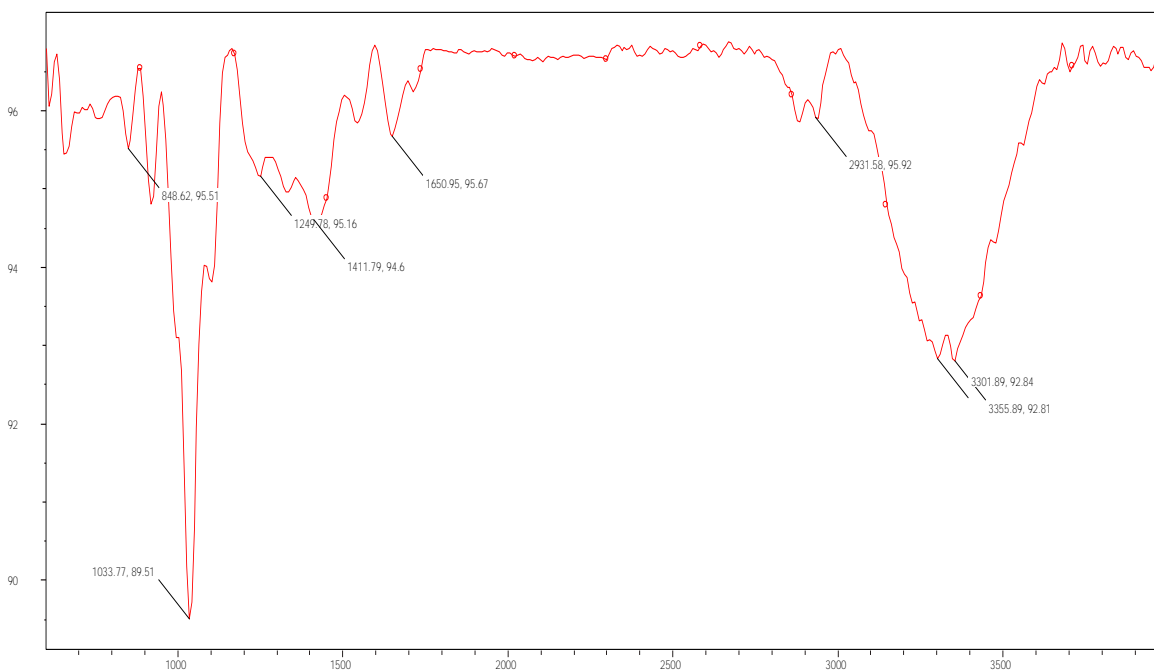


Figure 4: FTIR spectra of Chitosan – PVA-Silver nanoparticles hydrogel sheets

Table 4: water absorption capacity of F1, F2, F3 and F4 formulations

S. No	Time(min)	Formulations(Weight)				% of water absorption			
		F1	F2	F3	F4	F1	F2	F3	F4
1	0	0.046	0.052	0.019	0.035	0	0	0	0
2	5	0.052	0.059	0.023	0.047	17.39	18	21.05	34.28
3	10	0.058	0.072	0.028	0.053	30.43	44	57.89	51.43
4	15	0.063	0.084	0.032	0.061	41.30	68	78.95	74.28
5	20	0.074	0.098	0.038	0.075	60.87	96	100	14.28
6	25	0.082	0.106	0.043	0.085	78.26	112	126.31	142.86

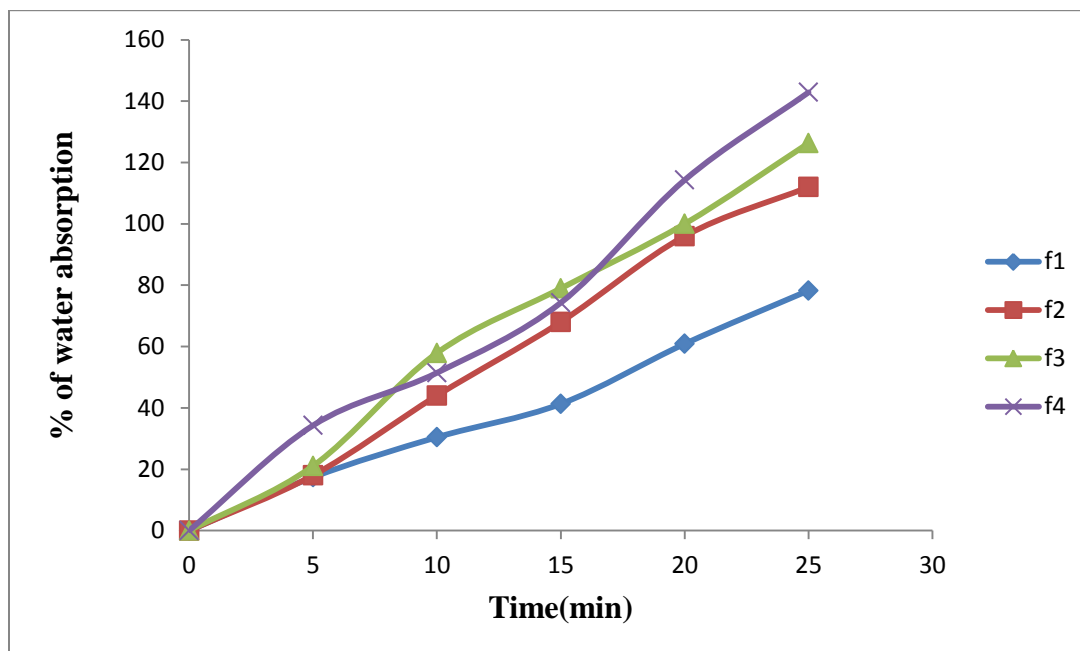


Figure 5: Swelling capacity of F1, F2, F3 and F4 formulations

Scanning Electron Microscopy (SEM)

The morphology of hydrogel sheet were evaluated through SEM images (Figure 6). Here, the hydrogel sheets were treated with liquid nitrogen to make them rigid and focused with the 'ZEISS' equipment to get the surface morphology of hydrogel at different magnifications.

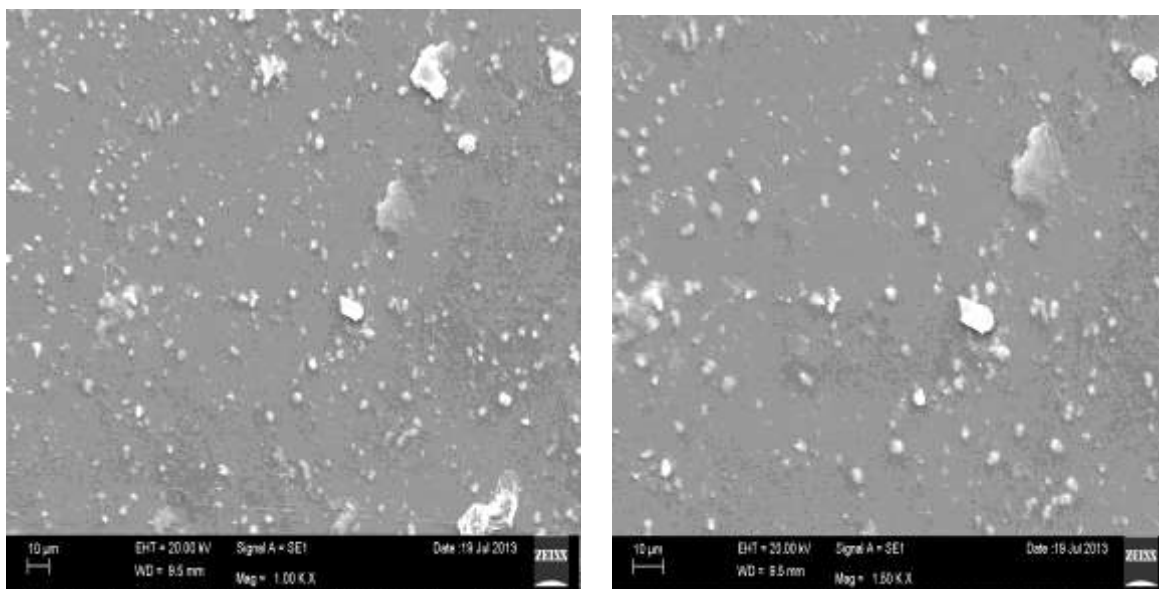


Figure 6: SEM image of chitosan hydrogel sheet A) at 1.00KX magnification B) at 1.50KX magnification

CHARACTERIZATION STUDIES OF PREPARED SILVER NANOPARTICLES

Visual Inspection



Figure 7: A) Image of Silver Nitrate solution B) Silver Nanoparticles produced using sodium citrate as reducing agent

Ultraviolet-Visible Spectroscopy

The prepared silver nanoparticles showed an absorption bands at 420 nm and 397 nm as shown in Figure 9, which is a typical absorption bands of spherical Ag nanoparticles. The absorption band in visible light region (350 nm – 550 nm, plasmon peak at 420 nm) is typical for silver nanoparticles.

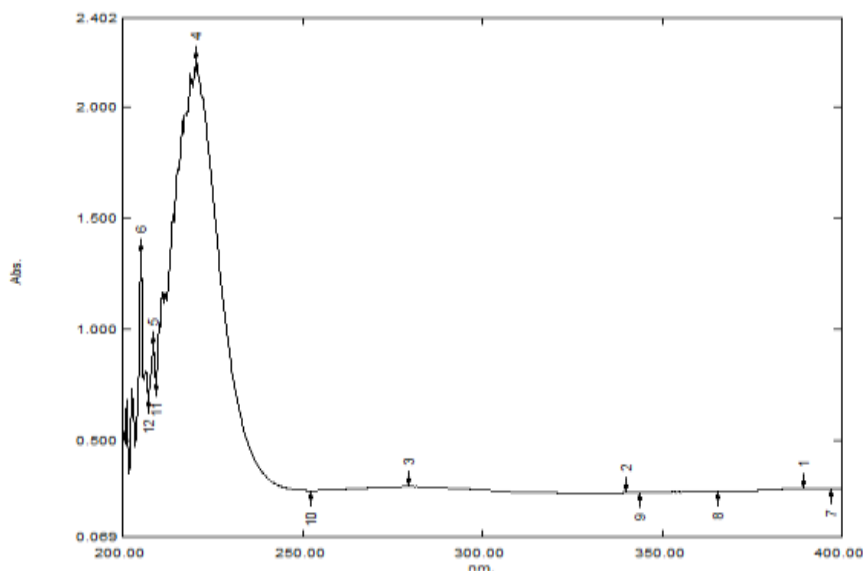


Figure 8: UV-VIS spectrum of silver nitrate solution before chemical reduction

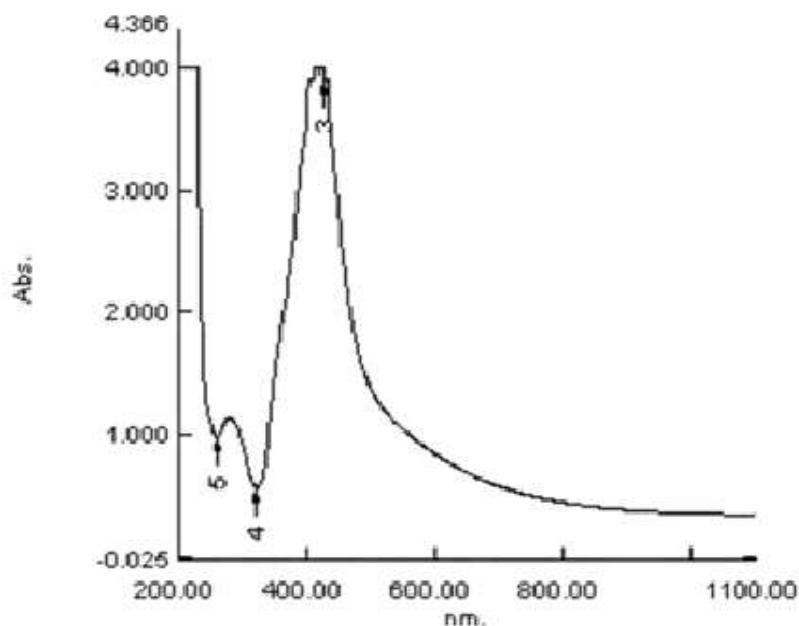


Figure 9: UV-VIS spectrum of silver nanoparticles formed after chemical reduction
Characterization Studies of AgNPs Incorporated Chitosan-PVA Hydrogel Sheet Scanning
Electron Microscopy (SEM)

The SEM image of Silver nanoparticles synthesized by chemical reduction method by using 1mM AgNO₃ concentration and 10mM of sodium citrate was shown in Figure 10. It gave a clear image of Silver nanoparticles. The SEM image showing silver nanoparticles confirmed the development of silver nanostructures.

Antimicrobial Study

The antimicrobial activity of silver nanoparticles is tested on two types of human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* which were cultured on nutrient agar plates supplemented with different concentration of silver nanoparticles and it was shown in Figure 11.

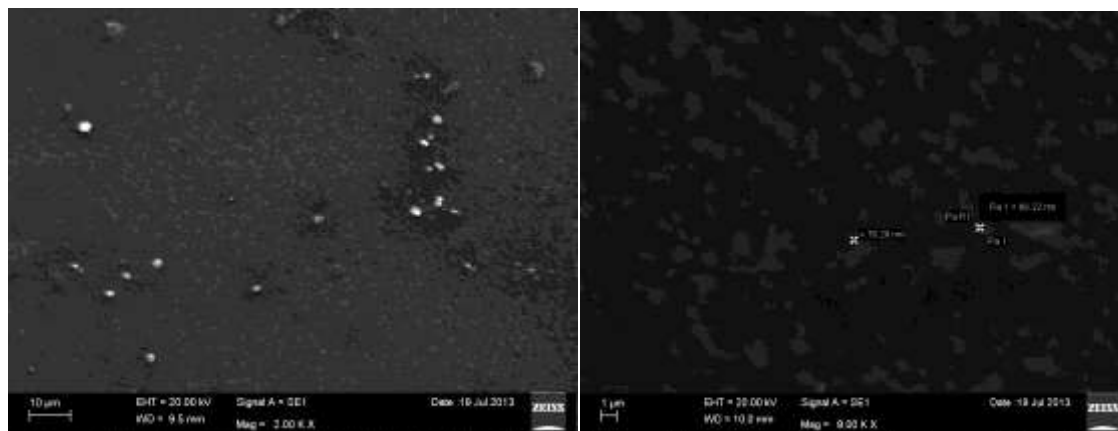


Figure 10: SEM image of Ag-NP's incorporated chitosan hydrogel sheet at A) 2.00KX magnification B) 3.00KX magnification

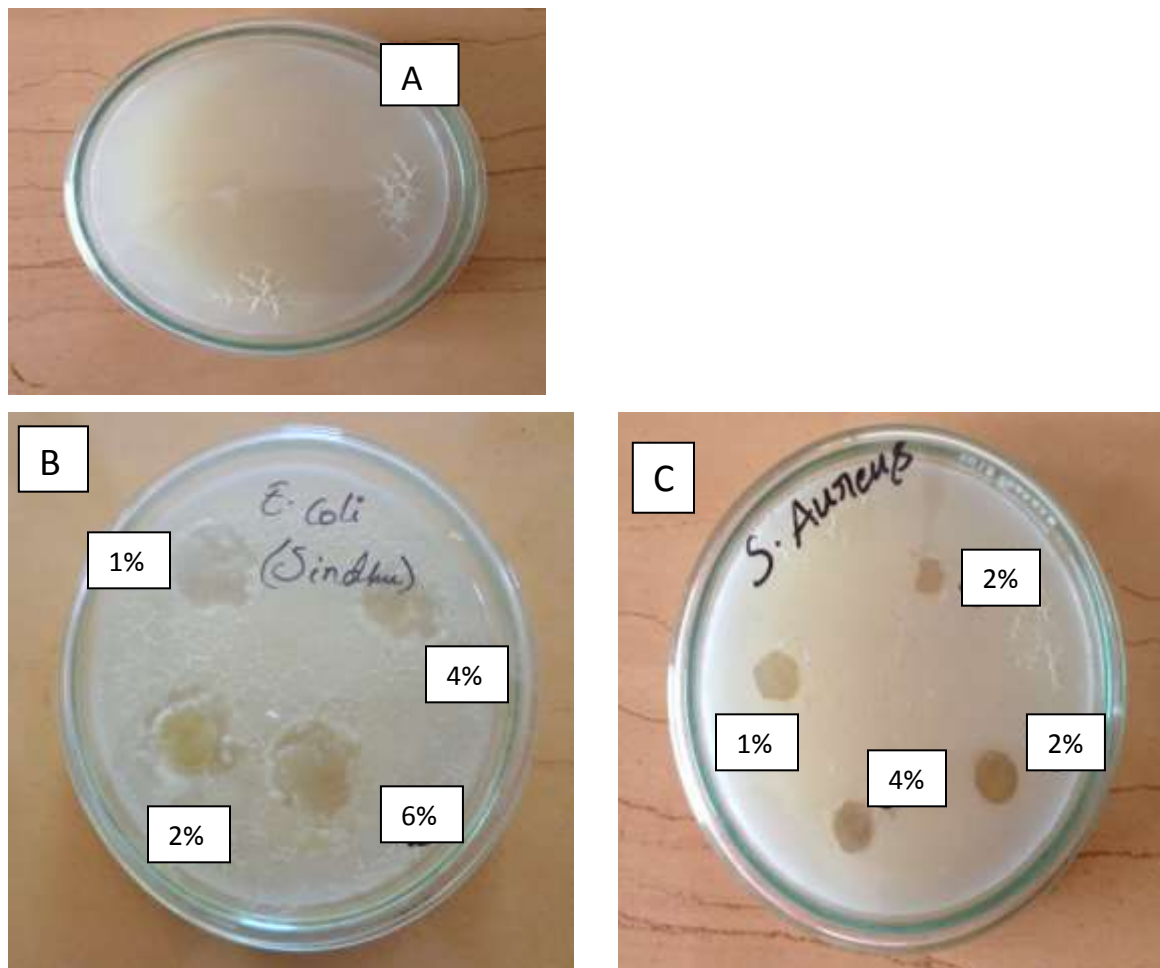


Figure 11: Images of antibacterial activities of discs of different concentration of Ag nanoparticles (1%, 2%, 4% and 6%) on B) *Escherichia coli* (gram negative) bacteria C) *Staphylococcus aureus* (gram positive) bacteria

Table 5: Zone of inhibition of Antibacterial test of AgNps

Bioactive agent	Concentration of AgNp (%)	Zone of inhibition(diameter, cm)	
		<i>Staphylococcus aureus</i> (gram positive)	<i>Escherichia coli</i> (gram negative)
Silver nanoparticles (0.5cm diameter)	1	1.2	2.3
	2	1.3	2.4
	4	1.3	2.4
	6	1.1	2.5

CONCLUSION

Silver nanoparticles with mean diameter of 60-70 nm were synthesized using silver nitrate as a metal precursor and citrate of sodium as a reducing agent. The nanoparticles were characterized by UV/Vis and SEM. UV/Vis spectra show the characteristic plasmon absorption peak for the silver nanoparticles ranging from 380 to 420 nm. Additionally, the antibacterial activity of the nanoparticles was measured by disc-diffusion method. The results of this study clearly demonstrated that the silver nanoparticles inhibited the growth and multiplication of the tested bacteria, including highly multi resistant bacteria such as *Staphylococcus aureus* (gram negative) and *Escherichia coli* (gram positive). Such high antibacterial activity was observed at very low total concentrations of silver. A successful attempt for the preparation of chitosan hydrogel was formulated and characterized with silver nanoparticles. It has proved near optimal requirements for a hydrogel. The prepared hydrogel are promising to give better healing of wound compared to conventional dressing materials. The major advantage being to provide moist environment for the wound to heal fast. These hydrogel can be utilized for incorporation of wound healing growth factors to fasten the wound healing process and minimize the duration of wound healing.

REFERENCES

1. Yaszemski, M.; Trntolo, D.; Lewandrowski, K-U.; Hasirci, V.; Altobelli, D. & Wise, D. (Eds.), Tissue Engineering and Novel Delivery Systems, CRC Press, (2004).
2. Slaughter, B.; Khurshid, S. Fisher, O.; Khademhosseini, A.& Peppas, N. Hydrogels in regenerative medicine. *Advanced Materials*. 2009; 21: 3307-3329.
3. Benskin L. Activation of a Stalled Traumatic Finger Wound With Silver Polymeric Membrane Dressings. Poster presentation the 3rd Congress of the World Union of Wound Healing Societies. Toronto, Ontario, Canada. 2008:4–8.
4. Kubo, M.; Matsuura, T.; Morimoto, H.; Uno, T. & Itoh, T. Preparation and polymerization of a water-soluble, nonbonding crosslinking agent for a mechanically cross-linked hydrogel. *Journal of Polymer Science Part A-Polymer Chemistry*. 2005; 43(21): 5032-5040, ()
5. Castellano JJ.; Shafii SM; Ko, F; Donate G; Wright TE; Mannari RJ; Payne WG; Smith DJ; Robson MC. Comparative evaluation of silver-containing antimicrobial dressings and drugs. *Wound healing*, 2007; 4: 114–122.
6. Sondi, I, Salopek-Sondi, B: Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 2004; 275: 177–182.

7. Kirsner, R, Orsted, H, Wright, B: Matrix metalloproteinases in normal and impaired wound healing: a potential role of nanocrystalline silver, Wounds 2001; 13:5–10.



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