Terpolymeric Nanocomposites of Silver for Wound Healing Applications

Seema Tiwari, Nidhi Jain, Aniket Aggarwal

***Abstract:** In the present work, silver nanoparticle, sodium alginate, chitosan and gelatin are used to form nanocomposite films and films have been prepared for wound healing application. These films have been characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM) and an antibacterial study of the sample. The dynamic release data were interpreted with various kinetic models. These film has shown remarkable antibacterial property against E.Coli, thus offering their candidature for the application in the near future.*

Keywords **:** ***silver nanoparticle; nanocomposites; wound healing.***

I. INTRODUCTION

Today the nanoscience can without much of a stretch be assumed as the key component of current world innovation. In this manner, because of the arranged field of usages, it is assuming an urgent job in the material science industry. Its applications can financially grow the properties and estimations of material getting ready and things. The nanomaterials are arranged either by consolidating into the centre frame of the material or through cleaning over the outside of planned materials. The broad utilization of nanomaterials ranges from catalysis to gadgets and optics just as in magnetics close by the wellbeing and condition applications. However, the fate of nanotechnology in material applications lies in domains where the new measures will be joined into solid, multifunctional material systems without dealing with the inherent material properties.

Silver nanoparticles are tyrannical among the most significant and hypnotizing nanomaterials among a couple of metallic nanoparticles that are locked in with the biomedical applications. They display astounding antibacterial, antifungal, mitigating, and antiviral properties liberally or either in the wake of responding with explicit components to grant such utilitarian properties. To a degree, the silver nanoparticles can be used against an expansive assortment of contaminations.

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The utilization of silver nanoparticles isn't limited to the restorative field just; they have been likewise utilized as self-cleaning, UV insurance, improving strength and optoelectronics.Silver is steady in unadulterated water and air situations yet the encompassing of ozone, hydrogen sulfide or sulfur if present in air or water may achieve silver discolouring due to the arrangement of silver sulfide. Nano-silver has been commonly used as a result of its antibacterial microbial development for the headway of things containing silver fuse sustenance contact materials, (for instance, compartments, bowls and cutting sheets), fragrance safe materials, devices, and nuclear family mechanical assemblies, decorating operators and individual consideration things, remedial contraptions, water disinfectants, room sprinkles, youngsters' toys, infant kid things and wellbeing supplement.

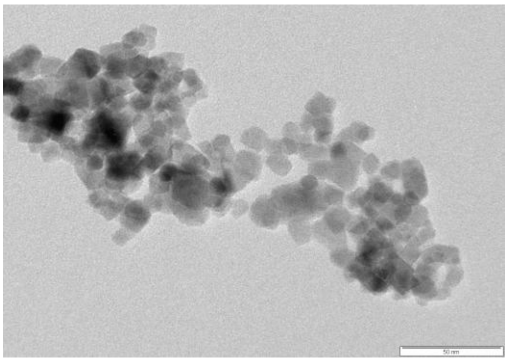

Fig.1- TEM of nanosilver

Chitosan is a generally cheap financially accessible material. It is gotten from chitin, an insoluble straight polymer of N-acetylglucosamine found in the hard shells of shellfish e.g., shrimp, lobster, crab, which are blessed to receive expel unessential material.

Chitosan is gotten from chitin by the expulsion of an extent of the N-acetyl bunches which renders it dissolvable in numerous acids, including certain weaken natural acids, for example, formic, acidic and propionic acids. Both chitin, what's more, chitosan has been utilized for an assortment of purposes, ordinarily as powders, in arrangement or films in the type of viscose practically equivalent to cellulose gluey.

It has been discovered that a gel or gel-like layer, which meets the above prerequisites for use on wounds can be effectively produced using chitosan broken down in a corrosive water-glycerol arrangement which is then killed with a base. The resultant unbiased arrangement out of the blue transforms into a gel after standing. In event that a slight layer of the corrosive water-glycerol-chitosan arrangement is utilized, upon the balance, a gel-like layer is formed.

The chitosan beginning material is ideally utilized in finely powdered structure with the goal that it will break down less demanding in the dissolvable arrangement.

The centralization of chitosan can be around 1-4%, with 1% being favoured.

The corrosive to be utilized in the dissolvable arrangement can be any pharmaceutically adequate corrosive in which chitosan is dissolvable, with the favoured acids being acidic, formic also, propionic corrosive, and acidic corrosive the most favoured.

The glycerol (1,2,3-propanetriol) which is utilized can be available in a wide scope of extent of 10-90% of the corrosive water-glycerol arrangement, yet to get great gel-like consistency necessitates that a grouping of at least half of glycerol is utilized. Along these lines, the lesser extents of glycerol are helpful when a gel-as is film wanted, however in event that at any rate half glycerol is utilized either a gel or on the other hand a gel-like layer can be gotten.

chitosan has been used as an antimicrobial material against varied objectives of living beings like green growth, microscopic organisms, yeasts and parasites in investigations including the in-vivo and in-vitro examinations of chitosan in various structural arrangements and composites.

The chitosan-containing gels or gel-like films of the present innovation are fantastic bearers for various medicaments, for example, antibacterial specialists e.g., silver sulfadiazine, quaternary ammonium specialists, iodophors, vasodilators, for example, epinephrine compounds which advance injury mending, analgesics, hostile to provocative specialists and the numerous different medicaments which are generally connected to wounds in such way. The gels obviously remain generally steady what's more, hold their three-dimensional structures for long timeframes over a wide variety in temperature e.g. from 4 to 40 C., and are reasonable for pharmaceutical use.

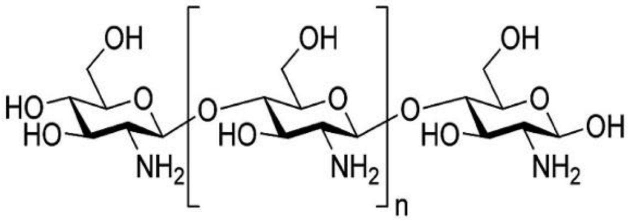

Fig.2- Structure of Chitosan

Sodium Alginate is a polysaccharide which is dissolvable in cold and high temp water with strong unsettling influence and gets thick after cooling. In cooking, sodium alginate is generally used with calcium salts to form a caviar-like colossal cavity with liquid inside that burst in the mouth. Sodium alginate is used to adjust emulsions or froths and to casing films. furthermore, it is used to fabricate thick liquid-like structure and as an emulsifier. It is used in heartburn tablets and it has no detectable flavour in the mouth. The ordinary limit of alginate is to offer versatility to the sea development, so more often than not to find the higher substance of alginate in species found in lamented waters. Sodium alginate was first packed in 1881 by English logical master ECC Stanford. Sodium alginate is used to adjust emulsions or froths and to casing films.

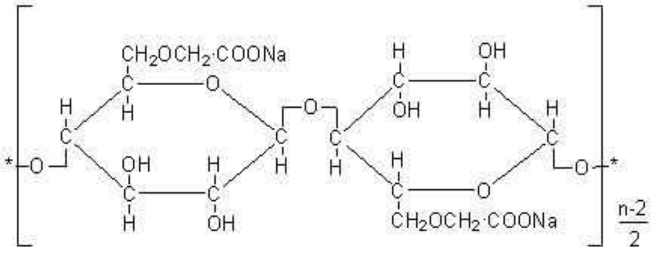

Fig.3-Structure of Sodium Alginate

Gelatin mixture of peptides and proteins obtained from the skin, bones, and connective tissues of creatures, for example, cows, chickens, pig, and a variety of fishes. During the process of hydrolysis, common atomic bonds between individual atoms are separated into a structure that revamps more effectively. Its synthetic arrangement is similar to its parent collagen in terms of Photographic and pharmaceutical evaluations of gelatin.by and large it is sourced from cows bones and pigskin. Gelatin structure shows complex chains of proline, hydroxyproline and glycine in its polypeptide structural chain. The nearness of proline limits the adaptation. This is significant for the gelation properties of gelatin.

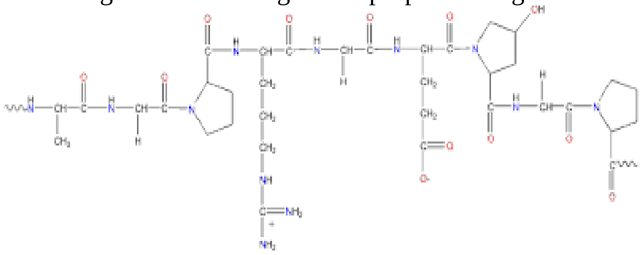

Fig.4- Structure of Gelatin

II. EXPERIMENTS

A. Materials

Chitosan (Ch; molecular weight 340 k Da) obtained from Research Lab, Mumbai, India. Gelatin (molecular weight 25K Da) obtained from Hi-Media Chemicals, Mumbai, India and were of analytical grade. Glacial acetic acid and other chemicals were purchased from Merck, Mumbai, India. The double-distilled water used throughout the process of synthesis of the polymeric sheet.

B. Methods for preparation of nanocomposite

1. **Preparation of silver nanoparticle solution:**For the preparation of silver nanoparticle, firstly we take 50 ml of water in a conical flask. Then we measured 50ml of 0.2M Aqueous Sodium Citrate in the water in the flask. Blend it well to prepare a homogeneous solution. Also, we have to warm 50ml of water in a different measuring flask to prepare the other part of the nanoparticle solution. Then we Included 50ml of 0.002M Aqueous Silver Nitrate in the water warmed in the previous steps. Blend it well at 80°C for 15 minutes to obtain its homogenous solution. Blended both arrangement`s solutions in a single flask and Heated it till the shade of overall solution change to Yellow in colour. This shows the development of Silver Nanoparticles in the solution.

2. **Preparation of nanocomposite of silver nanoparticle with chitosan and gelation:** In the secondary phase of polymer making, we broke down 0.3g of Chitosan in 5ml of half weakened Glacial Acetic Acid and prepare its solution. We included 5ml of Ag Nanoparticle and 0.3g gelatin in this solution and stir it well. The above arrangement is warmed till 50°C and mixed by Magnetic Stirrer for 30min. After 30min Polymeric Material was moved to Petri dish and dried at room temperature for 12 hours to get the required polymeric sheet.

C. Characterization: Terpolymeric composites and terpolymeric nanocomposites were characterized by FTIR, SEM and XRD. The Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu 8400, Japan, and spectrophotometer using KBr pellets of samples from CIF, SPPU, Pune. The X-ray diffraction (XRD) method was used to measure the amorphous, semi-crystalline and crystalline nature of composites and nanocomposites of nanosilver. All samples were dried in vacuum and coated with platinum before SEM. Surface morphology studies were investigated at different resolutions.

D. The antibacterial study by the 'zone inhibition method': The antibacterial study was done with ut and with silver nanoparticle nanocomposites by 'zone of inhibition' method. The nutrient agar medium was prepared by mixing nutrient agar and agar powder in 100 mL of distilled water and was autoclaved in a conical flask for 45 min. The agar medium was transferred into sterilized Petri dishes. After some time, E. coli culture was added on the surface of the media. The Petri dish was incubated for 2 days at 37 °C in an incubator.

III. RESULT AND DISCUSSION

A. Preparation of silver nanoparticle polymeric film :

A1. Preparation of silver nanoparticle :

1. For the preparation of silver nanoparticle firstly we take 50 ml of water in a conical flask.
2. Then we Included 50ml of 0.2M Aqueous Sodium Citrate in the water in the flask. Blend it well to prepare a homogeneous solution.
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5. Blended both arrangement`s solutions in a single flask and Heated it till the shade of overall solution changes to Yellowish shade.
6. This shows the development of Silver Nanoparticles in the solution.

A2. Preparation of nanocomposite of silver nanoparticle with chitosan and gelation

1. In the secondary phase of polymer making, we broke down 0.3g of Chitosan in 5ml of half weakened Glacial Acetic Acid and prepare its solution.
2. We included 5ml of Ag Nanoparticle and 0.3g Gelatin in this solution and stirred it well.
3. The above arrangement i.e the blend was warmed till 50°C and mixed by Magnetic Stirrer for 30min.
4. After 30min Polymeric Material was moved to petri-dish and dried at room temperature for 12 hours to get the required polymeric sheet.

B. Characterisation of terpolymeric composite and nanocomposite

B1 .FTIR SPECTRUM:

The FTIR spectra of silver nanoparticle loaded nanocomposite is recorded on FTIR- spectrophotometer (Shimadzu, 8400S) at CIF, SPPU, Pune.FTIR spectrum of terpolymeric composite broadband at 3400 cm-1 is observed because of overlapping of O-H and N-H stretching vibrations of polysaccharides. A peak at 2900 cm-1 is because of stretching vibrations of the aliphatic C-H bond. A characteristic band between 1653 to 1706 cm-1 shows the presence of C-O group. The transition of peaks between 3649 to 3325 shows the chelation of silver with amino and hydroxyl groups of chitosan.

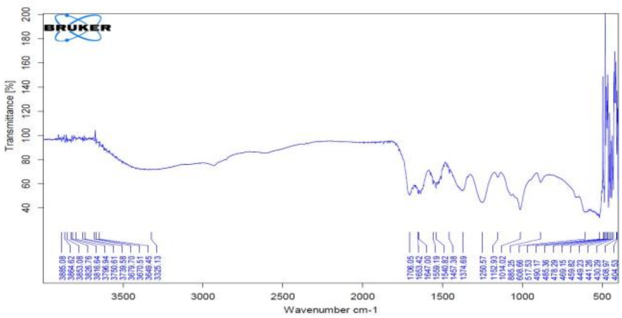

Fig. 5: FTIR of terpolymeric nanocomposite of silver

B2. SEM analysis

In order to know the surface details of the silver nanoparticle loaded in a polymeric nanocomposite, SEM images were taken at a magnification of 10,000 X. The SEM images of the terpolymeric nanocomposite are shown in Figure 6. The SEM image of silver nanoparticle loaded terpolymeric nanocomposite is shown in `Figure 7

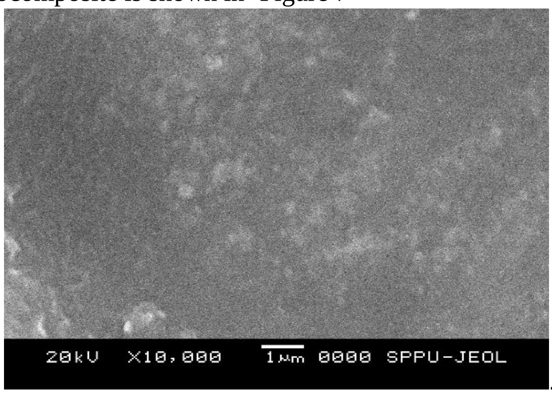

Fig.6 SEM image of terpolymeric composite (without silver nanoparticle)

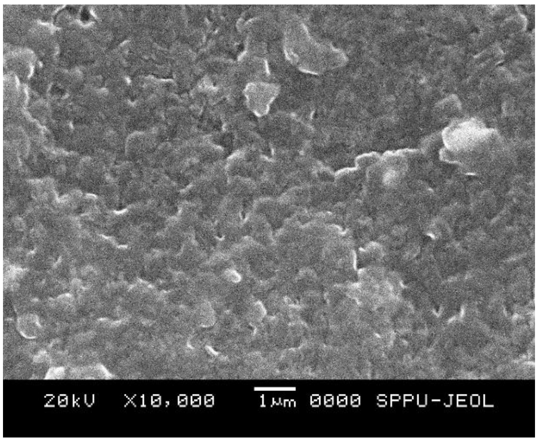

Fig.7 SEM image of terpolymeric composite with silver nanoparticle

B3. XRD analysis

The XRD pattern of terpolymeric nanocomposite of without silver nano particles and with silver nanoparticles are shown in Fig.8 & Fig.9 respectively. Figure 8 shows the amorphous nature of polymer and peaks were observed in Figure 9 indicates the crystalline nature of silver nanocomposite. The XRD pattern of silver nanoparticle loaded composite also shows peaks in fig 9 indicates less amorphous or semi-crystalline nature of the material

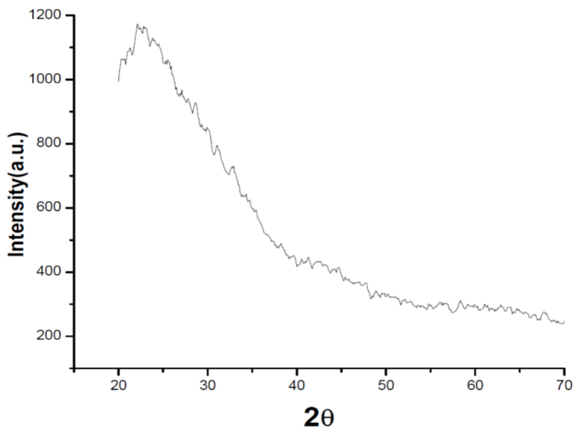

Fig.8: XRD of terpolymeric composite(without silver nanoparticles)

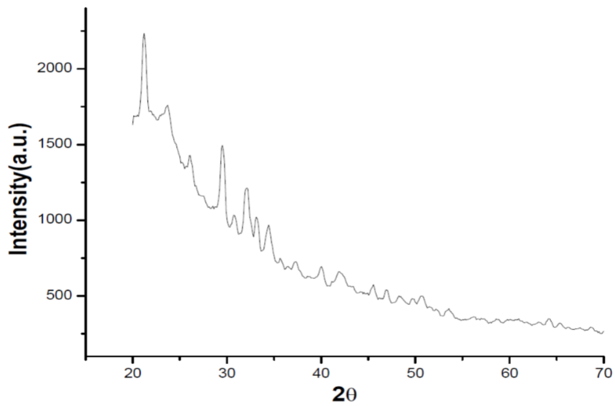

Fig.9: XRD of terpolymeric nanocomposite(with silver nanoparticles)

B4. Antibacterial study*:* The results of the antibacterial study of *E.Coli* are shown in Figure 10 (a) and (b). Here it is visible that Petri dish, with the plain film, shows small 'zone of inhibition' whereas the Petri dish with silver nanoparticle loaded nanocomposite shows a large 'zone of inhibition' with an approximate diameter of 3.8 cm. This shows that nanocomposite film processes fair antibacterial property. Biocidal action of the plain film is attributable to chitosan as one of the components of the film. Antimicrobial nanosilver loaded film shows stronger antibacterial activity as indicated by the area of 'zone of inhibition' is a fair indication of antimicrobial property of nanocomposite containing nanosilver.

Fig.10. Photograph showing 'Zone of Inhibition' in (a) without silver nanoparticles, (b) with Silver nanoparticle loaded nanocomposite

IV. CONCLUSION

From the study, we can conclude that silver nanoparticle loaded nanocomposite prepared from chitosan and gelatin has the potential to be used due to their antimicrobial property and can be used for the wound healing applications. This film is free from toxicity so may be recommended for effective wound healing application in the near future.

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