6.1 Introduction

Polysaccharides are capable of coordinating several metal ions, resulting new biological properties on the basis of their activities [1-2]. Several copper complexes were studied and tested for use in different applications. Copper is one of the most largely analysed elements. Among the eight biologically essential metals (Ca, Mg, Mn, Fe, Cu, Zn, Co and Mo), it can indeed be considered as the most versatile one [3-5].

Recent research has been aimed at exploiting metallic and bi-metallic nanoparticles in polymeric network. Products prepared by these approaches are leading to the development of new hybrid or composite systems in chemistry and engineering science [5].

Recently, Reddy et al.[6] have reported a hydrogel Au-Ag bimetallic nanocomposites of acrylamide using a crosslinker. In their studies the nanocomposites are nanoparticles within the hydrogel network which exhibited superior antibacterial properties. Dongwei Wei et al [7] reported chitosan based silver nanoparticles that were synthesized by reducing silver nitrate salt with chitosan. Chitosan has attracted much attention not only due to its broad spectrum antibacterial activity but also most abundant natural, renewable, biocompatible, and biodegradable polymeric nature with enormous metal complexation capacity [8]. Chitosan, a polysaccharide biopolymer derived from naturally occurring chitin, displays unique polycationic, chelating, and film-forming properties due to the presence of active amino and hydroxyl functional groups. The choice of the chitosan as stabilizer of the Cu-NPs is because of its ability to chelate metals, This makes a perfect applicant for metal NP synthesis[9]. Generally, the use of bio-polymers as
stabilizers for the synthesis of Cu-NPs is gaining momentum because of their availability, biocompatibility and low toxicity. Chitosan exhibits a number of interesting biological activities, including antimicrobial activity [10,11]. It has been recorded that chitosan itself has antimicrobial activity due to its cationic properties that cause a membrane-disrupting effect [7]. Rhim et al [12] suggested that the increased antimicrobial activity of silver incorporated chitosan materials arise from the high infiltration of the silver component and further investigation has only rarely reported.

The development of nanocomposites and nanostructural materials have opened a new era for constructing well designed nanostructures that have been considered as a novel class of materials for catalytic, optical,[13,14] electronic,[14,15] and biomedical applications [16–18]. Vulnerability of hydrogels against thermal circumstances may be substantially eliminated via incorporating nanoclay to prepare nanocomposite hydrogels. Chitosan–interrelated montmorillonite (chitoMMT) was used as bionanoclay to yield novel composite hydrogel based on 2-acrylamido-2-methyl-1-propanesulfonic acid with superior thermal properties and non toxic material suitable for biomedical applications[19]. It is widely accepted that nano-sized metal particles such as silver, gold and copper are highly toxic to microorganisms exhibiting strong biocide effects on bacteria. 2-Acrylamido-2-methyl-1-propanesulfonic acid is one of the strong acid[8,20] and it can copolymerize with other monomers to synthesize water-soluble and nonwater-soluble copolymers due to its good reactivity [20].
Hence poly(acrylamide) hydrogel and acrylamide-2-acrylamido-2-methyl-1-propanesulfonic acid hydrogel systems are selected because of their relevance for pharmaceutical and biomedical applications[21]. The present work is to improve the swelling properties as well as improved wound dressing properties of hydrogels generating bi-metallic nanoparticles using poly (acrylamide –AMPS) hydrogel in the presence of chitosan, copper and silver salts.

6.2 Experimental

6.2.1 Materials

Chitosan (CS) (high MW, >75% deacetylated) Sigma Chemical, St.Louis, USA, Acrylamide (AM) Merck. N,N\textsubscript{1}-methylenebisacrylamide (MBA) as crosslinking agent, ammoniumpersulphate(APS), acrylamide-2-acrylamido-2-Methyl-1-propanesulfonic acid (AMPS) N,N,N\textsubscript{1},N\textsubscript{1}-tetraethylenediamine (TMEDA), silver nitrate(AgNO\textsubscript{3}), copper sulphate(CuSO\textsubscript{4}) and sodium borohydride(NaBH\textsubscript{4}). Double distilled water was used throughout the investigation for the preparation of all solutions.

6.3 Preparation of Hydrogels

Poly(acrylamide) combination of Chitosan hydrogels were prepared by aqueous redox free radical polymerization using AM, a crosslinker N,N-methylene bisacrylamide, MBA and an APS initiator the system. In a typical polymerization reaction, chitosan (10gm) dissolved in 2% acetic acid (75 ml), and acrylamide (1gm) 14.08mM dissolved in 2mL of distilled water were mixed in a 250mL beaker. To this mixture 0.2412 of mM AMPS was added as a co-monomer. Then 0.0648 mM of MBA as a hydrophilic crosslinker and also 2.191 mM of APS/0.172mM of TMEDA as an
initiating pair system were added. The temperature of the system was raised to 50°C for 30 min. After the reaction has been completed, the hydrogel formed was immersed in distilled water at room temperature for 24 h to remove the unreacted materials present in the hydrogel network. Finally, the hydrogel was dried at room temperature for 2 days. Similarly, other hydrogels were prepared by the above procedure.

### 6.3.1 Formulation of Cu-Ag Bimetallic Nanocomposites

The dried hydrogels (2 gm) were immersed for 24 hours in a large amount of distilled water for 2 days to swollen to full extent. Then the hydrogels were transferred to a beaker containing 50 mL of 5 mM AgNO₃ and Copper sulphate 50 mL of 5 mM (CuSO₄) aqueous solutions to permit equilibrating for 1 day. The gel was removed and transferred to NaBH₄ solution for reducing the metal ions for 4 hrs to yield a final Cu-Ag bimetallic nanocomposite hydrogel. The gel was dried at ambient temperature for 48 hours. It was stored in a desiccator at room temperature for further experiments.

### 6.3.2 Characterization

The UV-vis spectra are recorded on an ELICO SL 210 Model UV-vis spectrophotometer (The Elico co, Hyderabad, India). The morphological variations are observed (coated with a thin layer of palladium gold alloy) by using a JOEL JSM 840A (Tokyo, Japan) scanning electron microscope (SEM). FT-IR spectral measurements were performed with a Bomem (MB-3000, Canada) spectrophotometer. Polymeric MNPs were finely ground with KBr to prepare pellets under a hydraulic pressure of 400 kg for MNPs and spectra were scanned between 4000 and 600 cm⁻¹.

### 6.3.3 Antibacterial Activity
The antibacterial activity of the composite hydrogels under study was investigated by disc method, using the standard procedure[6]. Nutrient agar medium was prepared by mixing peptone (5.0g), beef extract (3.0g), and sodium chloride (NaCl) (5.0 g) in 1000mL of distilled water and the pH was adjusted to 7.0. Finally, agar (15.0g) was added to the solution. The agar medium was sterilized in a conical flask at a pressure of 15 lbs for 30 min. This mixture was transferred into sterilized petri dishes in a laminar airflow chamber. After solidification of the media, bacillus culture (50 μL) was spread on the solid surface of the media. To the inoculated Petri dish, one drop of nanoparticles solution (20mg/10mL distilled water with alkali treatment) was added using 50 μL tip and incubated for 2 days at 37°C in the incubation chamber.

6.4 Result and Discussion

6.4.1 Characterization

6.4.2 Swelling studies

The equilibrium swelling ratio of hydrogels was measured by gravimetric method. The swelling characteristic of the hydrogels provides the information about the hydrogel network integrity after loading gold and silver salts and formation of bimetallic nanocomposites inside the hydrogel networks. To study this phenomenon, the same weights of dried hydrogels were equilibrated in distilled water at ambient temperature for 2 days. The swollen hydrogels were treated first with AgNO₃ + CuSO₄ and then with NaBH₄ solutions. The swelling ratio (Q) of the gels was calculated from the following equation

\[
\text{Swelling ratio (Q)} = \frac{\text{The weight of the swollen hydrogel}}{\text{The weight of the dry hydrogel}}
\]
Figure 1 (A) Swelling behavior of pure hydrogel, ions loaded hydrogel, and nanohydrogels. (B) Influence of AMPS content on swelling behavior of hydrogels (pure hydrogels, Cu-Ag ions-hydrogel, and bimetsals-nano hydrogel).
6.4.3 UV-vis spectra of Cu-Ag nanocomposite

![UV-vis spectra of Cu-Ag nanocomposite](image)

Figure 2: UV-vis spectra of Cu, Ag and Cu-Ag bimetallic nanocomposites

The formation of Cu-Ag nanoparticles in the hydrogel network can be analyzed by comparing the UV-spectra of metallic and bimetallic nanocomposite solutions (Fig. 2). Hydrogels containing silver nanoparticles show a characteristic peak at 417.55 nm which indicates the formation of silver nanoparticles. Similarly, UV abortion peak of Cu nanoparticles was obtained at 589 nm, which indicates formation of Cu-NPs. However, the mixture of pure Cu and pure Ag nanoparticles (spectrum) shows a red shift at 573–600 nm range which indicates the formation of both silver as well as copper nanoparticles in the gel network.
6.4.4 FTIR spectra of Cu-Ag nanocomposite

For identification of the hydrogel, infrared spectroscopy was used. In the spectrum (blank hydrogel), two bands at 3206 and 1660 cm\(^{-1}\) correspond to the primary amides and amide –NH stretching vibrations, respectively. Band at 1418 cm\(^{-1}\) and 1013 cm\(^{-1}\), belongs to C–H bending and –C–O skeletal stretch ing. Where as in Cu-Ag nanocomposite hydrogel, bands corresponding to the primary amides and amide –NH stretching vibrations were observed at 3202 and 1672 cm\(^{-1}\) and the bands corresponding to C–H bending, –C–O skeletal stretching were observed at 1422 cm\(^{-1}\).
and 1028 cm\(^{-1}\) respectively. The shifting in frequencies confirms the presence of Cu-Ag in the hydrogel nanocomposites.

### 6.4.5 Scanning electron microscopy (SEM)

The morphology of P(AM-AMPS) chitosan hydrogel and P(AM-AMPS) chitosan bimetallic nanocomposite hydrogels were examined by SEM images and presented in the Fig.4 (A-D). It was observed that P(AM-AMPS) chitosan hydrogel has smooth channel network structure. But nanocomposites hydrogels show particles distributed throughout the hydrogel network which are clearly appearing on the surface of the hydrogel network.
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Fig 4: Scanning electron microscopy of (A) pure hydrogel (B) Ag (Ag NCH), (C) Cu (A-AS3 CuNCH), and (D) Ag and Cu nanocomposite hydrogels (Cu&Ag NCH).

6.4.6 Evaluation of antibacterial activity

The main aim of this study was to develop a new antimicrobial/wound dressing agent. By addition of bimetallic compounds the bacterial inhibition was increased. The antimicrobial activities of the nanocomposite (Blank, Cu, Ag and Cu + Ag nanocomposite) hydrogels were tested with nutrient agar media. Figure (D) shows the higher inhibition capacity than others. The hydrogel containing bi-metallic nanoparticles, which are highly inhibited the *bacillus* growth compared to Blank<Cu<Ag hydrogels as shown in Fig.5 (A-D).
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Fig. 5: Antibacterial activity of (A) blank hydrogel (B) Ag nanocomposite hydrogel (Ag NCH), (C) Cu nanocomposite hydrogel and (D) Cu-Ag bimetallic nanocomposite hydrogel.

6.5 CONCLUSIONS

Cu-Ag bimetallic nanocomposites were synthesized by varying the feed composition of hydrogels. Chitosan is not toxic and biocompatible in nature. It is clearly illustrated that the Cu-Ag nanoparticles are being formed not only on the surface of hydrogels but also throughout the networks. The developed Cu-Ag bimetallic nanocomposites have exhibited superior antibacterial properties than the chitosan, chitosan-copper and chitosan-silver nanocomposites.
6.6 References


The Synthesis of Chitosan based Polymer Bimetallic Cu-Ag Nanocomposites and their Antibacterial Activity


