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An Environmentally benign Synthesis and Characterization of Novel Sugar based Silver Nanocomposite Hydrogel for Antibacterial Applications

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Abstract : The incorporation of nanometals into hydrogels have been extensively studied and found to haveimmense potential for medical, therapeutic and diagnostic applications. In the present investigation, we report an environmentally benign synthesis of sugar based semi interpenetrating hydrogel (SIH) networks of cross-linked poly(acrylamide) utilizing carboxymethylcellulose (CMC) – starch (SR) biocomposites as a grafting backbone and N,N'–methylenebisacrylamide (MBA) as the cross-linker. Invariably sized silver nanoparticles were generated insitu in the swollen hydrogel by the reduction of silver nitrate ($AgNO_3$) using azadirachta indica (neem) leaf extract at room temperature. UV-Visible spectroscopy, FTIR spectroscopy, scanning electron microscopy (SEM) and thermogravimetric analysis (TGA) were used to characterize the formation of silver nanoparticles in the hydrogel. The antibacterial activity of the semi interpenetrating silver nanocomposite hydrogel was also investigated.
Keywords: Silver nanoparticles, environmentally benign synthesis, acrylamide, carboxymethyl cellulose, starch, hydrogel, nanocomposites.

Introduction

Hydrogels are generally synthesised from natural and synthetic polymers. They possess numerous advantages over inorganic materials such as lower material cost and easy fabrication, better biocompatibility and biodegradability and more versatile processability and functionalization[1]. Recently silver nanoparticles with unique optical, electronic and antibacterial properties have attracted much attention and have found many applications in the field of medicine and pharmaceutics. The most attractive features of silver nanocomposites is due to extraordinary enhancement in properties of hydrogels such as mechanical toughness, large deformability, high swelling/deswelling rates, excellent electrical conductivity, high transparency[2-4] and remarkably strong antibacterial activity in combination with a fairly low toxicity against human tissues[5]. Hydrogels with large free space among the cross-linked networks can not only act as a reservoir for massive nanoparticles loading, but also function as a nanoreactor template for the nucleation and growth of nanoparticles[6]. Free radical graft copolymerization of vinylic monomers onto sugar backbones followed by cross-linking of their chains is a well known method for the synthesis of sugar based hydrogels[7].The general methods used to incorporate nanoparticles into the hydrogels includes gelation of preformed nanoparticles in a solution of hydrogel forming monomers[8], embedding of nanoparticles in the hydrogel networks by swelling – shrinking process[9,10], repeated heating, centrifugation, redispersion[11] and entrapment of nanoparticles in hydrogel matrix followed by reduction

with common reducing agents. However drawbacks such as aggregation of nanoparticles in monomer solution before and during the gelation process, leaching of nanoparticles out of the network[12], forcing conditions for synthesis and the use of toxic reducing agents limit the potential of hydrogel nanocomposites for biomedical applications. This pave the way for biological methods, using plant extracts, which are cost effective and environmental friendly[13].

To the best of our knowledge, based on the wide literature survey on super absorbent hydrogels, it is concluded that there is no published report on the synthesis of hydrogels in which two sugars (CMC and SR) and a synthetic monomer (AM) are used. The above findings inspired us to synthesize poly(acrylamide) – carboxymethylcellulose – starch hydrogel with certain hydrophilic nature for embedding the silver ions and reducing it to silver nanoparticles insitu using neem plant extract[14] to obtain sugar based hydrogel – silver nanocomposite (HSN). The structure of HSN, effect of pH, monomer concentration on the swelling behavior of HSN and its antibacterial activity were studied.

Experimental

Materials

Acrylamide (AM), carboxymethylcellulose (CMC), starch (SR), ammonium persulphate (APS), silver nitrate ($AgNO_3$), N,N'-methylenebisacrylamide (MBA), N,N,N',N'-tetramethyl ethylenediamine (TEMED) of reagent grade were kindly supplied by S.D. Fine Chemicals (Mumbai, India) and used without further purification. Double distilled water (DDW) was used for the preparation of hydrogel and other solutions required in this study.

Synthesis of sugar based semi interpenetrating hydrogel

An improved single step method for the synthesis of SIH was conducted[15] as follows. CMC (0.05 g) and SR (0.05 g) were mixed in 5 ml of DDW to get a homogeneous mixture. AM (1.0 g) was dissolved in 6 ml of DDW followed by cross-linker MBA (0.01 g in 1 ml of DDW) and initiator APS (0.005 g in 1 ml of DDW). Both the solutions were mixed and TEMED (0.02 ml in 1 ml of DDW) was added which, together with APS, acts as redox-initiating pair and initiates free radical polymerization. The reaction mixture was stirred and heated at 60^o C for 5 minutes. The polymerization reaction results in the formation of SIH within 10 minutes of reaction time. The formed hydrogel was equilibrated with water for 3 days to remove unreacted monomers and reagents. The hydrogel was dried in hot air oven to constant weight.

Plant information

Scientific Name – Azadirachta Indica Common Name – Neem

Plant part used – Leaves Family Name – Maliaceae

Preparation of plant extract

Fresh leaves of Azadirachta indica (neem) were collected from the local territory. The leaves were cleaned by washing with double distilled water and dried using water absorbent paper. About 40 g of leaves were cut into small pieces using a sterilized scissor and grinded in mortar and pestle to obtain a paste. This paste was dispersed in 100 ml of double distilled water and heated for 15 minutes at 70 – 80^o C. The extract was filtered using Whatman's No.1 filter paper and the filtrate was collected in a presterilized conical flask and used on the same day.

Preparation of semi interpenetrating hydrogel – silver nanocomposite

Dried hydrogel (500 mg) was equilibrated with double distilled water for 3 days at room temperature. The swollen semi interpenetrating hydrogel was transferred to beaker containing 50 ml of 0.005 M silver nitrate solution and allowed to equilibrate for 24 hours. These silver salt loaded hydrogel was transferred into another beaker containing 50 ml of neem leaf extract and allowed to stand for 12 hours. During this period the silver ions were reduced to silver nanoparticles which were confessed by the development of brown color in the

hydrogel. The silver nanoparticles loaded semi interpenetrating hydrogel was dried at ambient temperature and often termed in the forthcoming sections as HSN.

Spectral methods

FTIR spectra of completely dried SIH and HSN were recorded with a Perkin Elmer FTIR spectrometer – Spectrum.RX1 (USA). UV – visible spectra of HSN (10 mg in 1 ml of methanol) was carried out on a Shimadzu 160A UV – visible spectrophotometer (Japan). For this, the HSNin methanol was grinded and stored for 10 days to leach out silver nanoparticles and then filtered using Whatman's No. 1 filter paper. This filtrate was used for recording the spectra.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopic (SEM) analysis was performed using Tescan Vega3 SBU variable pressure scanning electron microscope with 0.2 ml of finely grinded SIH and HSN dispersions on a copper grid dried at room temperature after removing excess solution using filter paper.

Thermogravimetric analysis

The thermal stability of HSN was evaluated using Mettler Toledo 851e thermal system (Switzerland) at a heating rate of 10°C per minute and a flow rate of 10 ml per minute under nitrogen atmosphere.

Swelling studies

The pre-weighed HSN of different CMC – SR concentrations were immersed in DDW at 37^{o} C. After specific time intervals, the gels were taken out and weighed after removing the surface adhered solution. Similarly, the swelling ability of HSN in various buffer solutions was studied. The swelling ratio (Q) of the gels was calculated from the equation

$Q = W_e/W_d$, where W_e is the weight of swollen hydrogel and W_d is the weight of dry hydrogel.

Antibacterial studies

The antibacterial activity of HSN was carried out on Mueller Hinton Agar (MHA) medium (Hi-Media Pvt. Ltd. Mumbai) using Kirby – Bauer disk diffusion method[16]. About 5 ml of the Mueller Hinton agar medium was poured into the sterile test plates and allowed to solidify. The plates were inoculated with test pathogens using sterile swabs. Sterile wells were dug inside the culture plates with the aid of a sterile cork borer at aseptic conditions. Samples (1 mg/ml) were then added to the wells at aseptic conditions. Stock solutions of the samples were prepared using DMSO. The test plates were incubated for 24hours. The zone of inhibition (in mm diameter) was measured and taken as the activity of the prepared HSN against the test organisms.

Results and discussion

In the present work, we have designed a novel pathway for the synthesis of HSN exploiting sugars (CMC and SR) which usually couple there biodegradability with stimuli sensitive response. In this method, the reduction potential/anchoring ability of the hydrogel is increased along with the stabilization of embedded nanoparticles[17-20]. In any conventional hydrogel networks, the functional groups present and its cross-linking density decides the stability of nanoparticles. Hence, we have synthesized nanometer sized homogeneous dispersion of silver nanoparticles in SIH networks employing poly(acrylamide) with CMC and SR matrices. A prominent feature of this methodology is that the nanoparticles were simply obtained at room temperature in presence of environmental friendly stabilizers. In general, the PAM – CMC – SR cross-linked networks acts as reservoir for metal ions uptake and anchoring it through carboxylic, amide and hydroxyl groups. The polymeric network also facilitates the reduction of silver ions into nanosilver and stabilizes it by preventing the aggregation of silver nanoparticles. It is quite interesting to indicate that the silver nanoparticles were formed solely inside the hydrogel networks and not in the surrounding medium. This confirms the binding of silver nanoparticles to the hydrogel networks as well as its storage without releasing into the media. The scheme of formation of silver nanoparticles inside the SIH networks is given below:

SIH + $AgNO_3$ $\longrightarrow$ SIH – Ag^+ $\xrightarrow{\text{Neem leaf extract}}$ SIH – Silver nanocomposite.

UV – Visible spectra

In this work, there is a development of opaque brown color after the addition of neem leaf extract to the swollen hydrogel. This observation can be explained based on the insitu formation of silver nanoparticles (Ag^o) by the reduction of silver ions (Ag^+) in the entire hydrogel. This also represents that the nanoparticles were entangled inside the swollen hydrogel networks through strong localization and stabilization established from the macromolecules of sugars. The perseverance of silver nanoparticles in the hydrogel networks was confirmed by UV – visible spectral analysis. Silver nanoparticles loaded hydrogel solution have shown a distinct peak around 400 – 414 nm in the UV – visible spectra[21] due to the surface plasmon resonance (SPR) effect caused by the quantum size of the silver nanoparticles[22].

FTIR spectra

IR spectra of the poly(acrylamide) based hydrogel (**Fig.1**) shows the presence of absorption bands characteristic to cross–linking bridges. Absorption band attribution was made in agreement with the values given in literature[23]. The absorption bands at 3450 and 1663 cm^{-1} corresponds to – NH stretching vibrations from the cross–linking bridges and – C=O extension vibrations of amide groups respectively[24]. Two bands at 3336 and 3194 cm^{-1} corresponds to – OH stretching as well as intra and intermolecular hydrogen bonding in the glycosidic ring. The symmetric valence vibration at2932 cm^{-1} is assigned to the – CH_2 groups between the macromolecular chains and cross–linking bridges. Another band at 2862 cm^{-1} is attributed to the – N – CH_2vibrations from the PAM – SR cross–linking bridges. The stretching vibration of ester groups of CMC is observed at 1610 cm^{-1}. Absorption bands assigned to the deformation vibrations of the – CN, – OH and – CH_2 groups in the 1400 to 1200 cm^{-1} range were also identified. Cluster of peaks from 800 to 1130 cm^{-1} range are assigned to stretching of C – O in C – O – C and C – O – H of glycosidic bonds. In the IR spectra of HSN, the peaks were shifted to lower wave numbers (3310, 3182, 2919, 1655, 1601 cm^{-1})due to the interaction of silver nanoparticles with hydrogel networks. Therefore, we can confirm the presence of silver nanoparticles in the hydrogel networks.

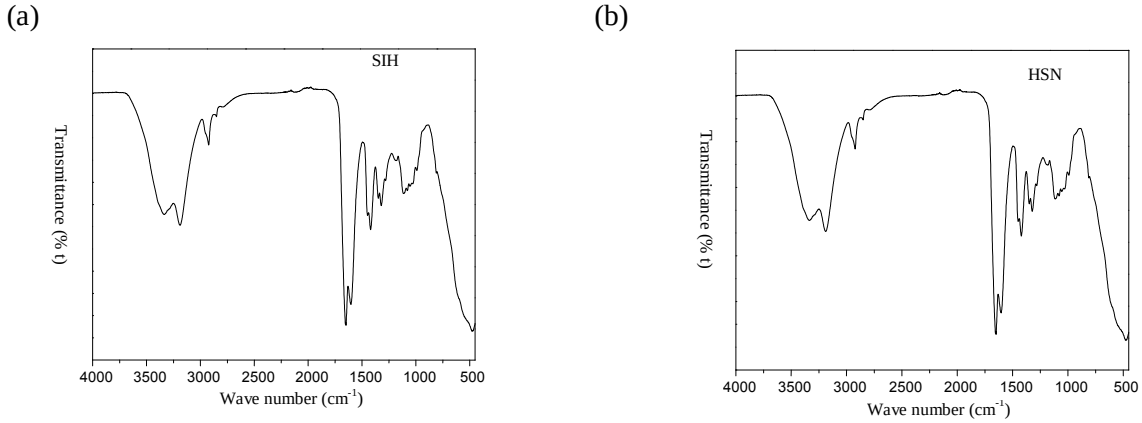

Figure 1. FTIR Spectra of (a) SIH and(b) HSN

Scanning Electron Microscopy (SEM)

The surface morphology of placebo SIH and the HSN were investigated with SEM. The placebo hydrogel showed a smooth surface feature (**Fig. 2a**), whereas HSN showed a shrunken surface throughout the gel (**Fig. 2b**). The particle size analysis confirms the presence of small, spherical and highly dispersed silver nanoparticles with an average size of about 50 – 100 nm. It is noticed that no individual silver particles were observed outside the HSN, suggesting a strong interaction between the polymer matrix and the silver particles.

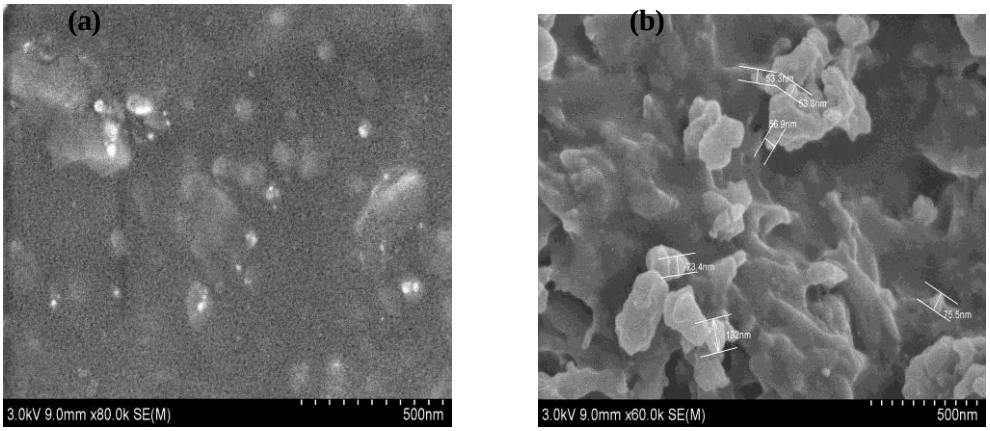

Figure 2. (a) SEM image of SIH and (b) SEM image of HSN

Thermal stability

The thermogram of HSN displayed distinctive thermal stability as shown in (**Fig.3**).The hydrogel has followed two decomposition steps and only 72.31 % degradation of the hydrogel occurred below 508^{o}C. The high thermal stability of the HSN can be attributed to the presence of large quantity of silver nanoparticles inside the hydrogel network.

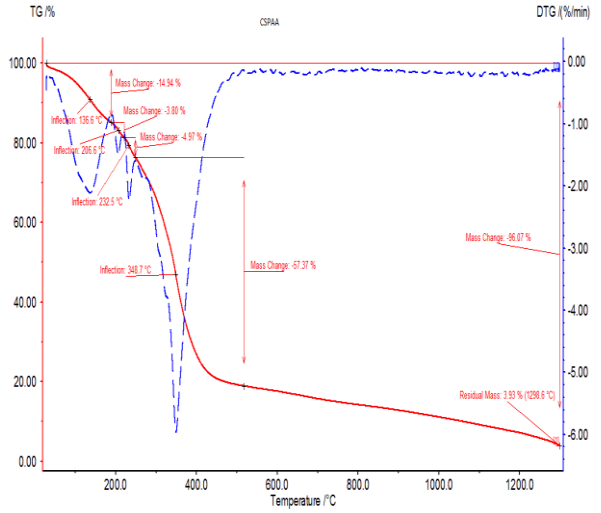

Figure 3. Thermogram of HSN.

Swelling studies

The swelling behavior of any polymer network depends upon the nature of the polymer, polymer – solvent compatibility and degree of cross-linking. Hence, we have chosen sugar polymers (CMC and SR) to improve the swelling capacity of poly(acrylamide) hydrogel. The capability of hydrogels to swell in water is due to the hydrophilic groups present in the polymeric chains and its mechanical resistance is due in part to the physical or chemical cross-linking[25-27]. It was known that polar head groups of polymeric chains such as hydroxyl, thiol, amine, and nitrile groups have high affinity for salts[28-30]. The loading of silver ions throughout the gel networks causes repulsion among the networks, which ultimately leads to an improved swelling behavior of hydrogel systems. Further increase in swelling capacity was observed after the addition of reducing agent (neem extract) to silver ions loaded SIH. This is due to the formation of silver nanoparticles throughout the gel networks which increases the overall porosity of system and makes entry for more number of water

molecules inside the gel. The other reason can be that the formed particles have different sizes and surface charges which cause absolute expansion of the networks. The order of swelling was**: HSN > Ag^+ loaded SIH >SIH**

The effect of CMC – SR concentration on water absorption of the HSN was investigated by varying the amount of CMC – SR from 0.1g [0.05g + 0.05g] to 1.0 g [0.5g + 0.50g] whereas the concentration of $AgNO_3$ was kept as 0.005M. As shown in (**Fig. 4a**), increasing CMC – SR amount up to 0.4g [0.2g + 0.2g] increases the swelling capacity. This was due to the introduction of more number of hydrophilic polymer chains inside the gel networks that assists in improving the swelling characteristics of gel systems. The decrease in swelling with further increase in CMC – SR amount [0.50g + 0.50g] can be attributed to the increase in viscosity of the medium which in turn hinders the movement of ions.

The swelling behavior of HSN in various pH was observed by placing 0.1 g of HSN in pH 2, 4, 6, 8 and 10 solutions respectively and examining them after an hour. It was noticed that the amount of swelling increases with increase in pH from 2 to 6, reaches maximum at pH 8 and then decreases as shown in (**Fig. 4b**). The repulsion between – COO^- groups in the hydrogel is the main reason for the maximum swelling at pH 8. Below pH 8, the H^+ ions in the external medium effectively suppress the ionisation of the carboxyl and hydroxyl groups of CMC – SR. This decreases the number of mobile ions inside the HSN which causes decrease in osmotic pressure and hence the swelling capacity of HSN. Above pH 8, the – OH groups can ionise to – OR which reacts with – COO^- to form esters. This increases the number of networks inside the hydrogel and also decreases the hydrophilicity which is responsible for the decrease in swelling.

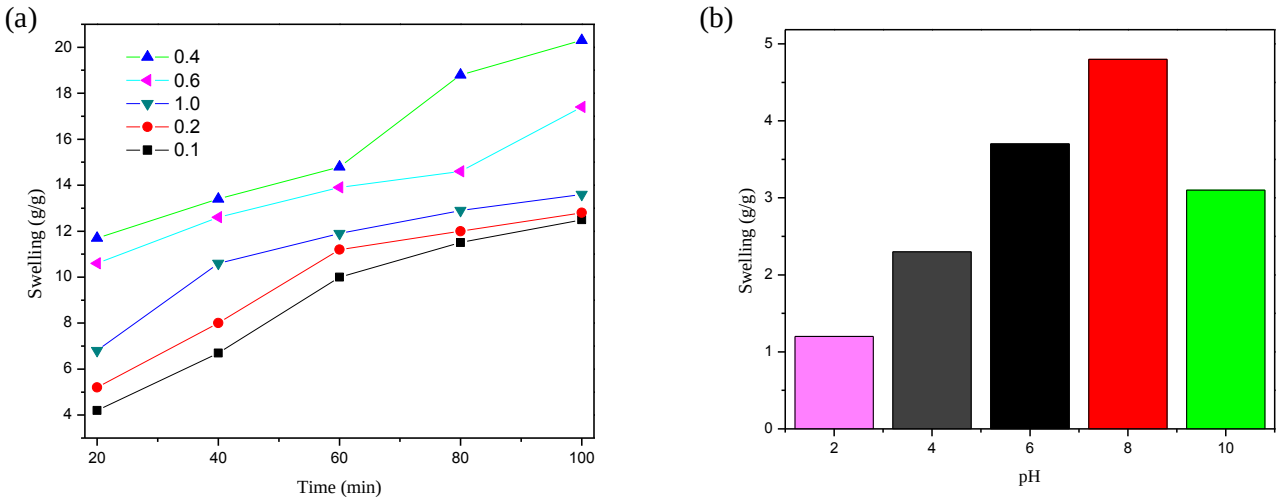

Figure 4. (a) Effect of concentration ofCMC-SR(g) on swelling capacityof HSN ($AgNO_3$ –0.005 M) and (b) effect of pH on swelling capacity of HSN.

Antibacterial analysis

The main course of this study is to unfold a new antibacterial material. The antibacterial activity of different molar weight ratios of HSN were examined against Staphylococcus aureus, E. coli and Aeromonas spp. All HSN proved effective against the tested microorganisms and the growth inhibitory effects showed variation form one another. **Fig. 5** exhibits the antibacterial property of HSN. The results indicate that the HSN exhibited greater reduction of bacterial growth which is due to the sustained release of silver nanoparticles form the hydrogel networks. The inhibition area of HSN for different organisms is given in **Table. 1**.

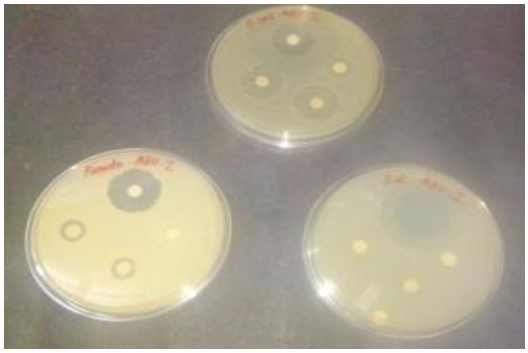

Figure 5. Antibacterial activity of HSN.

Table 1. Zone of Inhibition (mm)

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration(µg/ml)			
	1000	750	500	
Staphylococcus aureus	24	20	16	26
E. coli	21	17	14	23
Aeromonas spp.	16	13	8	17

Conclusion

CMC – SR / Poly(acrylamide) semi interpenetrating hydrogel – silver nanocomposite was successfully prepared via free radical polymerization followed by insitu reduction of silver ions to nanosilver using neem leaf extract as a clean and green reducing agent. The methodology developed for the synthesis is very simple and cost effective which makes it easy to implement in the industries. A number of HSN were formulated with high dispersion rates by varying the concentrations of CMC, SR and monomers. The formed HSN was characterized by different techniques. In the UV – visible spectra, nanosilver have shown good surface plasmon resonance behavior. In FTIR spectra, shifting of peaks to lower wave number occurred. SEM images confirm the presence of well defined silver nanoparticles and its stability was further confirmed by thermal analysis. The antimicrobial activities shown by silver nanoparticles make this method a potential route for metal nanoparticles preparation to be used for biomedical applications.

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