# Green Synthesis of Silver Nano Particles using Plant Extracts in Semi-Hydrogel Networks of Poly(acrylamide) and Carbohydrates, Characterization and Biomedical Applications

M. Gulsonbi M.[1]*, Bharat Raj K.[2], and Jaisankar V.[2]
[1]Department of Chemistry, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai 600 018, India.
[2]PG and Research Department of Chemistry, Presidency College (Autonomous), Chennai – 600 005, India.
*Corresponding Author E-mail: mgzaa@yahoo.com

*Abstract*—**The preparation of silver nanoparticles on the nanometer scale by green synthesis have attracted considerable attention due to its widespread application in biomedical field. In the present investigation, we report the preparation of semi interpenetrating hydrogel networks (SIHNs) based on cross-linked poly (acrylamide) prepared through an optimized rapid redox-solution polymerization with N,N' - Methylenebisacrylamide (MBA) in presence of Carboxymethylcellulose (CMC). Silver nanoparticles have been obtained with hydrogel networks as nanoreactors via insitu reduction of silver nitrate ($AgNO_3$) using Azadirachta Indica (Neem) plant extract under atmospheric conditions. A systematic characterization of silver nanoparticles was performed using ultraviolet visible (UV–Vis) spectroscopy, FTIR, Scanning Electron Microscopy (SEM) and Thermo gravimetric analysis (TGA). The antibacterial activity was investigated for the synthesized hydrogel-silver nanocomposite.**

**Keywords-silver nanoparticles; green synthesis; carboxymethylcellulose; hydrogel; nanocomposites**

## I. INTRODUCTION

In recent years nanotechnology has become vital tool for manufacturing new materials with numerous applications in science and technology. Nanoparticles with sizes less than 100 nm possess unique properties such as high surface-volume ratio, high reactivities, etc., when compared to their bulk micron structure. Silver nanoparticles are studied extensively among noble metallic nanoparticles because they possess exceptional properties. For instance over the past decade, due to their uncommon size – dependent electronic, optical and magnetic properties made their use in the area of electronics, optics, catalysis and other areas of science and technology. Nowadays silver nanoparticles are used in treatment of water, textile engineering and in cosmetics. In addition, due to antimicrobial properties of silver nanoparticles, their inclusion in wide range of medical devices are being considered. The impregnations of surgical instruments with silver nanoparticles and also in wound dressing have also been reported [1-2].

Generally, metal nanoparticles agglomerate due to high active surface area. To stabilize and control the nanoparticle structures various polymers, biological templates, and biomacro-molecules were used [3-5]. Biological systems form sophisticated mesoscopic and macroscopic structures with tremendous control over the placement of nanoscopic building blocks within extended architectures [6]. The concept of green nanoparticles preparation was promoted [7] in which b-D-glucose act as reducing agent and starch played stabilizer role. In another study, silver nanoparticles were prepared with carbohydrate polymer, carboxymethyl cellulose sodium (CMCNa) that effectively works as both reducing and stabilizing reagent [8]. Among the recognized novel approaches, hydrogel or macroscopic gels have been used as promising templates or nanopots to prepare nanoparticles that brought a concept for newer composite/ hybrid materials [9]. The available free-network spaces between hydrogel networks reserve to grew and stabilize the nanoparticles. Moreover, these nanocomposite systems are highly suitable for bio-medical applications because of their good bio-compatibility over biological molecules, cells, tissues, etc. Another advantage of this method is that we can control overall size and morphology of the nanoparticles by changing its functionality and cross-linking points.

By considering the importance of hydrogel networks as effective carriers for nanosystems and natural polymers (carbohydrates) such as carboxymethylcellulose as prepolymer renewable materials with hydrophilic nature for anchoring/reduction of metal ions and stabilization, prompted us to draw a new idea in which semi IPN hydrogels prepared in presence of prepolymers that would be better choice to cook metal nanoparticle in their networks. For this purpose, the combination of poly(acrylamide) hydrogel and carbohydrates systems are selected because of their more relevance in pharmaceutical and biomedical applications.

## II. MATERIALS AND METHODS

### *A. Materials*

Acrylamide (AM), carboxymethylcellulose (CMC), ammonium persulphate (APS), Silver nitrate ($AgNO_3$), N,N'-methylenebisacrylamide (MBA), N,N,N',N' -tetramethylethylenediamine (TEMED) were supplied by S.D.

Fine Chemicals (Mumbai, India) and used without further purification. Double (DB) distilled water was used for the preparation of any solutions in this study.

*B. Preparation of semi IPN hydrogel*

Semi IPN hydrogel was prepared [10] by polymerizing 1 g of AM dissolved in 6 ml of distilled water with 0.10 g of CMC in presence of a cross-linker MBA (0.01g in 1ml of distilled water), initiating system APS(0.005g in 1 ml of distilled water)and TEMED (0.02 ml in 1ml of distilled water). Polymerization was performed in 100 ml beaker at room temperature. The polymerization reaction results in the formation of gel within 20 minutes of reaction time.

*C. Description of Plant*

Binomial Name - Azadirachta indica — Common Name – Neem
Plant part taken – Leaves — Family Name – Meliaceae

*D. Preparation of Plant Extract*

Neem Leaves were collected from the local region. They were washed and cleaned with double distilled water and dried with water absorbent paper. 40g of leaves were cut into small pieces with an ethanol sterilized knife and crushed with mortar and pestle, dispensed in 80 ml of sterile distilled water and heated for 5-10 minutes at 70-80°C. The extract was then filtered using Whatman's No.1 filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored.

*E. Preparation of semi IPN hydrogel–silver nanocomposite*

Semi IPN hydrogel–silver nanocomposite was prepared by accurately weighed dry semi IPN hydrogel was equilibrated with water for 3 days and these semi IPN hydrogel was transferred to another beaker containing 50 ml of 5mM $AgNO_3$ aqueous solution, allowed to equilibrate for 24 hours. Here most of the silver ions are exchanged from solution into hydrogel networks by anchoring through –COOH,–CONH2, –OH groups of hydrogel chains and rest of metal ions were occupied in free-network spaces of hydrogel. These silver salt loaded semi IPN hydrogel was finally transferred into a beaker containing 50 ml of neem plant extract and allowed for 2 hours to reduce the silver ions into silver nanoparticles. The colourless gel changed to brown shows the reduction of silver ions to silver nanoparticles as shown in Figure 1. The obtained silver nanoparticles in the semi IPN hydrogel is often termed in the forthcoming sections as semi IPN hydrogel–silver nanocomposite.

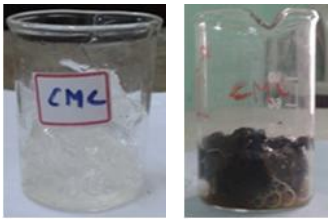


Figure 1. Semi IPN Hydrogel (Colourless) & Semi IPN Hydrogel-Silver Nanocomposite (Brown)

## III. CHARACTERISATION

*A. Spectral methods*

FTIR spectra of semi IPN hydrogel and semi IPN hydrogel silver-nanocomposite were recorded with a Perkin Elmer FTIR spectrometer-Spectrum.RX1 (USA). UV–visible spectra of semi IPN hydrogel silver-nanocomposite (10 mg in 1 ml of distilled water) was carried out on a Shimadzu 160A UV-VIS Spectrophotometer (Japan). For this, grinded sample (10 mg/ml) was stored for 10 days to leach out silver nanoparticles into water (medium) and then performed the measurements.

*B. Thermal Analysis*

The thermal stability of this nanocomposite was evaluated using Mettler Toledo 851e thermal system (Switzerland) at a heating rate of 10°C /min under nitrogen atmosphere.

*C. Scanning Electron Microscopy (SEM)*

Scanning Electron Microscopic (SEM) analysis was done using Tescan Vega 3 SBU Variable Pressure Scanning Electron Microscope with 0.2 ml of finely grinded semi IPN hydrogel silver nanocomposite dispersions on a copper grid dried at room temperature after removing excess solution using filter paper.

*D. Swelling studies*

The same weights of dried semi IPN hydrogels were equilibrated in distilled water at 25°C for 3 days. Swollen semi IPN hydrogels were treated first with $AgNO_3$ and then with plant extract as explained in the experimental section. The swelling ratio (Q) of the gels was calculated from equation: $Q = W_e/W_d$, where $W_e$ is the weight of swollen hydrogel and $W_d$ is the weight of dry hydrogel.

*E. Antibacterial activity*

The antibacterial activity of semi IPN hydrogel-silver nanocomposite was determined on Muller & Hinton Agar (Hi-Media Pvt. Ltd. Mumbai) using Kirby-Bauer disk diffusion method [11]. Test pathogens were spread on

the test plates- Muller Hinton agar (MHA) for bacteria using sterile swabs. Sterile wells were made with the help of a sterile cork borer at aseptic conditions. Samples (1 mg/ml) were added to the wells at aseptic conditions. Stock solutions of the samples were prepared using DMSO. The test plates were incubated for 24hrs. The zone of inhibition (in mm diameter) were read and taken as the activity of the extract against the test organisms.

## IV. RESULTS AND DISCUSSION

We have explored to design semi IPN hydrogel employing carboxymethylcellulose, to enhance the reduction potential/anchoring ability and stabilization of the formed nanoparticles[12-15]. In any conventional hydrogel networks the available functional groups and cross-link density decides the stability of nanoparticles. Therefore, in this study, we have developed smaller size and finer distribution of silver nanoparticles in semi IPN hydrogel networks composed of poly(acrylamide) with CMC carbohydrate polymeric chains. The advanced feature of this methodology is that the nanoparticles simply prepared at room temperature in presence of green stabilizers. In these experiments, the semi IPN hydrogel was allowed to swell in the $AgNO_3$ solution and reduced with neem plant extract throughout the gel networks. In detail, the PAM cross-linked networks act as reservoir for metal ions uptake and the ions are anchored through carboxylic, amide, and hydroxyl groups of carbohydrate polymer (CMC) and thereby holds large amounts of metal ions in their network and facilitate the reducing process as well as helps in stabilization. The carbohydrate polymer (CMC) in hydrogel networks arrest the agglomeration of silver nanoparticles. It is quite interesting to point out that silver nanoparticle are formed exclusively inside the hydrogel networks and no particles formation is observed in the surrounding medium, that strictly confirm that hydrogel networks are binding to the silver nanoparticles as well as storing the nanoparticles without releasing into the media. The scheme of silver nanoparticles synthesis in semi IPN hydrogel networks is given below:

$$\text{Semi IPN hydrogel} \xrightarrow{AgNO_3} Ag^{+}\ \text{Semi IPN hydrogel} \xrightarrow{\text{Neem plant extract}} \text{Semi IPN hydrogel –silver nanocomposite}$$

### *A. UV–Vis spectra*

The formation of silver nanostructures in the entire semi IPN hydrogel networks can be expected in our current strategy because the silver salts loaded in semi IPN hydrogel are readily reduced by neem plant extract which immediately turn into an opaque brown color. It represents that the particles were entrapped inside the networks through strong localization and stabilization established from the carbohydrate polymer (CMC). The existence of silver nanoparticles in the gel networks was tested by UV–vis spectral analysis. It was found that the absorption peak for semi IPN hydrogel–silver nanocomposite in the **380–500** nm wavelength range in UV–vis spectra that are assigned to silver nanoparticles which arose from the surface plasmon resonance(SPR).

### *B. FTIR spectra*

Poly(acrylamide) hydrogel exhibited broad bands at 3430 and 1664 $cm^{-1}$ due to amide groups stretching of poly(acrylamide) chains[16]. The band at 3432 $cm^{-1}$ are corresponding to hydroxyl and carboxyl groups of CMC [17, 9, 18]. The stretching vibration of ester groups of CMC is observed at 1603 $cm^{-1}$. As shown in Figure 2, after making semi IPN hydrogel using CMC the peak positions increased considerably from 3432, 1603 to 3477, 1640 $cm^{-1}$ for semi IPN hydrogel. Significant changes were observed for semi IPN hydrogel-silver nanocomposite due to silver nanoparticles interaction with hydrogel networks (peaks were shifted to lower wave numbers 3471, 1637 $cm^{-1}$). Therefore, we can confirm the presence of silver nanoparticles in the hydrogel networks.

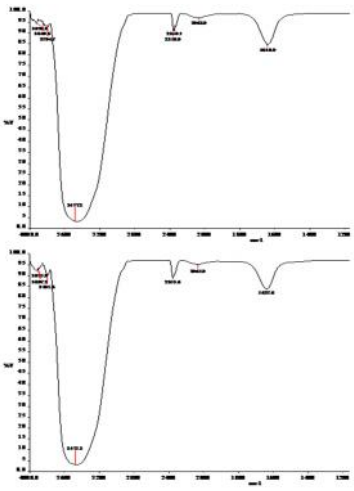

Figure 2. FTIR Spectra of Semi IPN Hydrogel & Semi IPN Hydrogel-Silver Nanocomposite

*C. Thermal stability*

The thermogram of semi IPN hydrogel–silver nanocomposite exhibited excellent thermal stability as shown in Figure 3. Three degradation steps were noticed as (i) 14.8% weight loss from 133°C to 225° C, (ii) 50.4% from 225°C to 512° C and (iii) 25% from 512°C to 800° C. The higher thermal stability was due to the presence of more amount of silver nanoparticles formation inside the semi IPN hydrogel-silver nanocomposite.

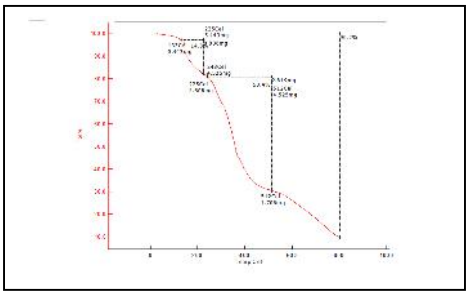

Figure 3. Thermogram of semi IPN hydrogel silver nanocomposite

*D. SEM Analysis*

The SEM micrographs of semi IPN hydrogel-silver nanocomposite showed that silver nanoparticles are spherical shaped, well distributed without aggregation in Semi IPN hydrogel with an average size of about 5-500 nm as Shown in Figure 4.

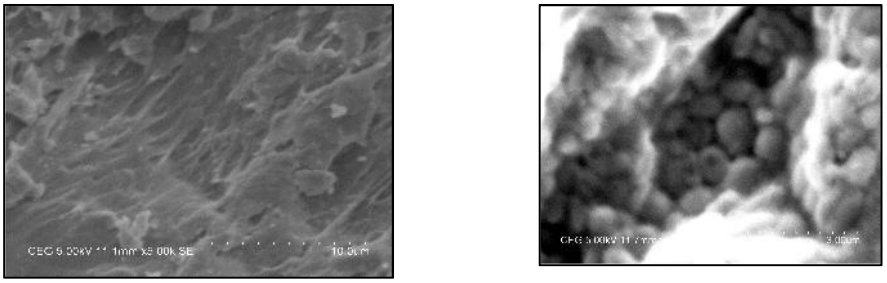

Figure 4. SEM image of Semi IPN Hydrogel & Semi IPN Hydrogel-Silver Nanocomposite

*E. Swelling capacity*

The basic feature of hydrogel is that it can absorb and hold huge amount of water/ solvent in its network structures and release over a period of time. This special property is very important not only to load the metal ions and formation of metal nanoparticles from reduction reaction, but also for its future studies including antibacterial activity or wound healing, and for any biomedical applications. Hence, we have chosen carbohydrate polymer (CMC) to improve swelling capacity of poly(acrylamide) hydrogel.

The swelling capacities of semi IPN hydrogel is because of hydrophilic polymeric chains were entrapped in the hydrogel networks which assist to improve swelling characteristic of gel systems. It was known that polar head groups of polymeric chains such as hydroxyl, thiol, amine, and nitrile groups have a high affinity for salts [19-21]. After treating the semi IPN hydrogel with silver salts, the silver ions loaded through the gel networks which are responsible to cause repulsion of networks, ultimately leads to an improved swelling behavior of hydrogel systems. It is quite uniform for all the semi IPN hydrogel systems as found in our previous studies [22]. Further increase in swelling capacity was recognized after addition of reducing agent (neem plant extract) to silver ions loaded semi IPN hydrogel. This pattern of swelling is reasonable because once the silver nanoparticles are formed throughout the gel networks overall porosity of system increased which allow for more number of water molecules inside the gel. The other reason can be that the formed particles have different sizes and different surface charges in the gel networks that cause absolute expansion of the networks. The order of swelling capacity was

semi IPN hydrogel–silver nanocomposite >$Ag^+$ loaded semi IPN hydrogel >semi IPN hydrogel.

*F. Antibacterial activity*

Nowadays nanotechnology has expanded its applications in biomedical field including fighting and preventing of diseases using atomic scale functional materials. Interestingly, silver nanoparticles have been considered to increase the resistant strains of bacteria to the most potent antibiotics.. Figure 5 exhibits the antibacterial property of semi IPN hydrogel–silver nanocomposite. The antibacterial activity was

TABLE I. ZONE OF INHIBITION (in mm diameter)

| No. | Organisms | Semi IPN hydrogel–silver nanocomposite |
|---|---|---|
| 1 | *Acinetobacterbaumannii* | R |
| 2 | *Pseudomonas aeruginosa* | 16 |
| 3 | *Staphylococcus aureus* | 14 |

R – Indicates resistance (Absence of activity)

mainly due to the release of silver nanoparticles from the semi IPN hydrogel-silver nanocomposite. The target strains used for screening antibacterial and antifungal activity were procured from MTCC, IMTECH Chandigarh. Antibacterial activities were performed for various test organisms. The inhibition area of semi IPN hyrogel-silver nanocomposite for different orgarnisms are given in Table I.

Figure 5. Antibacterial activity of Semi IPN Hydrogel-Silver Nanocomposite

## V. CONCLUSION

Controlled sized silver nanoparticles were synthesized using neem plant extract as reducing agent inside the semi IPN hydrogel templates that containg CMC polymeric networks. Under the UV-Visible wavelength, nano particles have shown quiet good surface plasmon resonance behaviour. Semi IPN hydrogel with Silver nitrate and reducing agent i.e. Neem plant extract has shown a remarkable color change. In FTIR spectra shifting of peaks to lower wave number confirmed the presence of silver nanoparticles in hydrogel networks. SEM images indicate that CMC contained semi IPN hydrogels produced well defined silver nanoparticles. The stability of semi IPN hydrogel-siver nanocomposite was further confirmed by thermal analysis. The antimicrobial activities shown by green synthesis of silver nanoparticles inside semi IPN hydrogel using neem plant extract indicates a potential route for metal nanoparticles synthesis applied in biomedical sector.

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