Chapter-5 Other applications of starch nanoparticles

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5.1 Biological applications of starch nanoparticles

5.1.1 Introduction

Starch-based polymers have recently been proposed as having great potential for several applications in the biomedical field such as bone replacement implants [1], bone cements [2], drug delivery systems [3] and tissue engineering scaffolds [4]. The development of new processing technique [5] and the reinforcement with various fillers results in materials with mechanical properties matching those of bone [6]. However, other conditions should be met for a material to be considered suitable for any biomedical use. The evaluation of the in vitro cytotoxicity of a biomaterial is the initial step on a biocompatibility study, and is usually performed using immortalised cell lines [7,8].

Chitin and chitosan are biocompatible, biodegradable and nontoxic polymers. Because of these properties, they have many applications such as biomaterials for tissue engineering, in wound healing, as excipients for drug delivery [9] and also in gene delivery [10]. Chitosan nanoparticles used for the delivery of polypeptides such as insulin, tetanus toxoid, and diphtheria toxoids are widely explored [11,12,13]. Chitosan is soluble only under acidic conditions, which limits some of its applications. The limited solubility of chitosan in water can be overcome by chemical modification. Thus chemical modifications of chitin/chitosan are generally preferred to improve the polymer processability as well as to modify some of its properties such as solubility, antimicrobial activity and the ability to interact with other substances.

Cytotoxic drugs continue to play a major role in cancer therapy but often produce side effects, especially through the destruction of lymphoid and bone marrow cells [2]. Therefore, strategic improvements in cancer therapy are needed to improve efficacy while decreasing side effects. Over the past decades, nanoparticles (NPs) have been of great interest in applications for biological fields such as drug delivery systems and anticancer applications. The antitumor activities of natural biopolymer chitosan and its NPs are well reported. Another abundant polysaccharide starch, is relatively, more competent than chitosan due to its low cost, easy availability and better solubility but suffers from drawback of hydrophilic nature.
In recent years, many researches [14,15] have been focused on interaction of small molecules with DNA. DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death [16]. Small molecule can interact with DNA through the following three non-covalent modes: intercalation, groove binding and external static electronic effects. Among these interactions, intercalation is one of the most important DNA-binding modes, which is related to the antitumor activity of the compound. Recently, there is a great interest on the binding of nanoparticles with DNA, owing to their possible applications as new cancer therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation [17,18,19].

As seen in earlier chapters nanosized derivatives of starch showed excellent reinforcing abilities. In order to assess their potentiality as nanocarriers for drugs and also their anticancer properties we investigated the cytotoxic potential of nanosized acyl derivatives of starch with A549 human lung carcinoma cells as well as mouse embryonic fibroblast (3T3L1) cells. DNA binding studies were also carried out.

5.1.2 Experimental

5.1.2.1 Measurement of cell viability by MTT assay
A549 cells (5.0 × 10^3 cells/well) were maintained in 96 well cell culture plates (Tarson India Pvt Ltd.) for 24 hour in absence or presence of acetylated StNPs (1000-10,000 µg/ml). At the end of incubation period 10 µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT; 5 mg/ml) was added to the wells and plates were incubate at 37 °C for 4 hours. Later, culture media was discarded and wells were washed with Phosphate Buffer Saline (HiMedia, India Pvt. Ltd.), followed by addition of 150 µl DMSO and subsequent incubation for 30 min and absorbance was read at 540 nm in ELX800 Universal Microplate Reader [20].
5.1.2.2 Statistical analysis
Data was analyzed for statistical significance using one way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test and results were expressed as mean±S.E.M. using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, USA.

5.1.2.3 DNA solution preparation
CT-DNA was dissolved in Tris buffer (50 mM Tris-HCL pH 7.2) overnight at 4°C. DNA concentration was adjusted with the buffer to 3 mg/mL. This stock solution was stored at 4°C and was stable for several days. A solution of CT-DNA in water gave a ratio of UV absorbance at 260 and 280 nm, A260/A280 of 1.89–2.01, indicating that DNA was sufficiently free of protein. The concentration of DNA was determined from the UV absorbance at 260 nm using the extinction coefficient ε260=6600 M⁻¹ cm⁻¹. ⁵

5.1.2.4 UV titration
The absorbance titrations were performed at a fixed concentration of the compounds (0.1mg/mL) and varying the concentration of double stranded CT-DNA within the range 200–400 nm. For an individual experiment, 2 mL of a stock solution of compound in DMSO was added to DNA solution of varying concentrations.

5.1.2.5 Fluorescence titration
Fluorescence experiments were conducted by adding different concentrations of the solution of compound to a mixture containing 40 μM EB and 50 μL DNA. All the samples were excited at 340 nm and emission was recorded at 650–700 nm.

5.1.3 Results and discussion

5.1.3.1 Cytotoxicity study
The MTT assay and the MTS assay are colorimetric assays for measuring the activity of enzymes that reduce MTT or close dyes (XTT, MTS, WSTs) to formazan dyes, giving a purple color. A main application allows to access the viability (cell counting) and the
proliferation of cells (cell culture assays). It can also be used to determine cytotoxicity of potential medicinal agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), is reduced to purple formazan in living cells [21]. A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employ.

These reductions take place only when reductase enzymes are active, and therefore conversion is often used as a measure of viable (living) cells. However, it is important to keep in mind that other viability tests (such as the CASY cell counting technology) sometimes give completely different results, as many different conditions can increase or decrease metabolic activity. Changes in metabolic activity can give large changes in MTT results while the number of viable cells is constant. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death, or changing metabolism of cells, can be deduced through the production of a dose-response curve.
The in vitro cytotoxicity was evaluated at various doses by exposure of A549 cells as well as 3T3L1 cells to acetylated StNPs. A dose range of 1000-10,000 µg/mL recorded moderate cytotoxicity in 3T3L1 cells (Figure. 5.1.2) while StNPs induced practically no cell death. At this dose, in tumor cells interesting dose dependent cytotoxicity was observed within 24 h (Figure. 5.1.2). Among all the NPs tested herein, StcinNPs showed highest cytotoxicity (table-1) and its highest dose (10,000µg/mL) recorded nearly 80% cytotoxicity (Figure. 5.1.2). On the other hand, StpalNPs recorded lowest cytotoxic potential.

In biological activities, cell must interact with the extracellular environment which is generally through chemical, electrical or mechanical signaling. In the present studies, DS of acetylated starch NPs are ≥ 2 which indicates the hydrophobic nature of acetylated starch NPs. Cytotoxicity is reported to increase with increasing hydrophobicity [22]. Based on this it can be concluded that there is hydrophobic interaction between tumor cells and starch derivatives which is responsible for cytotoxicity. The order of anticancer activity was StcinNPs > StbenNPs > StphNPs > StpalNPs respectively (table 5.1.1) shows that the cytotoxicity of derivatives of StNPs containing aromatic groups was found to be more compared to that containing aliphatic group. There may be a structure activity relationship which requires further investigation.

The cytotoxicity of chitosan NPs and derivatives has been attributed to positive surface charge. However zeta potential measurements of acetylated StNPs (table 5.1.1) showed that they have negative surface charge. This is because, neither size nor zeta potential alone determine the optimal cellular response induced by NPs [23]. It has also been proposed that, in regions where the columbic repulsion of similar charges is not too pronounced, the presence of a high electric field may cause local electroporation. So, high electrical fields of the NPs of about 50 nm, which may succeed even with negative zeta potential, may eventually lead to cytotoxicity. This phenomenon is known to facilitate permeation of various nanoscale objects through biological membranes. It is reported in literature, that the proteins from the growth media adsorb to the surfaces of both cationic and anionic NPs, increase their hydrodynamic radius, and flips their charge immediately to similar negative value of the serum proteins in the original media [24].
Thus size, aggregation state, surface charge and surface chemistry would be significantly acetylated via electrostatic screening which in turn could influence their ability to interact with or enter cells [25,26]. Therefore, NPs that had a positive effective surface charge upon preparation are no longer cationic in the cellular media. This is important when considering the molecular effect of charge on toxicity and cellular uptake, and argues against the simple picture, still propagated in the literature, that cationic nanoparticles disrupt the negatively charged cellular membrane by electrostatic interactions. Protein adsorption to the NPs surface can mediate the uptake of the nanomaterial via receptor-mediated endocytosis [27,28,29]. This is believed to be the reason for the interaction of the nanosized derivatives with biological systems.

### Table 5.1.1 Degrees of substitution, thermal analysis, IC$_{50}$ values, thermal degradation and zeta potentials of acetylated starch nanoparticles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ value (µg/mL)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>StPhNPs</td>
<td>2010±120</td>
<td>-24.52</td>
</tr>
<tr>
<td>StCinNPs</td>
<td>1110±110</td>
<td>-45.14</td>
</tr>
<tr>
<td>StBenNPs</td>
<td>1410±140</td>
<td>-23.41</td>
</tr>
<tr>
<td>StPaNPs</td>
<td>4450±200</td>
<td>-15.01</td>
</tr>
<tr>
<td>StNPs</td>
<td>-</td>
<td>-26.70</td>
</tr>
</tbody>
</table>

Previous studies have reported polysaccharide NP induced cytotoxicity against various tumor cell resulting due to oxidative damage to the cell membrane and mitochondrial dysfunction [30,31]. Similar alterations observed in our study are in accordance with these reports and thus establish the doses and the resultant cellular damage caused by acetylated StNPs.
Figure 5.1.2 Effect of acetylated starch nanoparticles exposure on cell viability in 3T3L1 and A549 cells. Results are expressed as mean±S.E.M. for n = 3 (replicates). Where NS = non-significant, *p < 0.05, **p < 0.01 and ***p < 0.001 compared to untreated cells.
5.1.3.2 DNA binding studies

UV-visible spectroscopy
The binding interaction of many organic carcinogens such as polycyclic aromatic hydrocarbons with DNA is the key step in their genotoxic effect. Titration with UV absorption spectroscopy is an effective method to examine the binding mode of DNA with the molecules [32]. In general, the spectra of the compounds show UV absorption bands that are usually symmetrical with no obvious splitting. In the UV region, compounds exhibited bands between 240-320 nm, which are assigned to the $\pi\rightarrow\pi^*$ transitions, due to long living triplet excited state. Hypochromism results from the contraction of DNA helix axes as well as the conformational changes on molecule of DNA, while hyperchromism results from the secondary damage of DNA double helix structure [33,34,]. Upon increasing concentration of CT DNA, the UV region exhibited an increase in absorption intensity ‘hyperchromic’ effect with a blue shift of 2-10 nm in $\pi\rightarrow\pi^*$ region (Figure.5.1.3). The strong hyperchromic effect with a blue shift is suggestive of higher binding propensity to CT DNA due to stabilization of the nanoparticle-DNA adduct. These changes are typical of compounds bound to double stranded DNA through non-covalent interaction [35]. In the present case, the complete intercalation of the compounds between a set of adjacent base pairs seems sterically impossible, but some partial intercalation can be envisioned [36].

Photoluminescence spectroscopy
Enhancement of the fluorescence emission when binding with the biomacromolecules (such as DNA and proteins), is a very useful fluorescent probe in genomics and proteomics [37]. In present study we found that luminescence was not observed for compounds either in DMSO or in presence of DNA. Hence, competitive binding studies using ethidium bromide (EB) bound DNA was carried out. EB is a conjugate planar molecule. Its fluorescence intensity is very weak but it is greatly enhanced when EB is specifically intercalated into the adjacent base pairs of double stranded DNA. The enhanced fluorescence can be quenched by the addition of a second molecule [38]. The
addition of compounds to DNA-EB system displayed an increase in emission intensity of the DNA-EB system (Figure. 5.1.4).

Increase in fluorescent intensity indicates that the StNPs derivatives have not completely intercalated into the DNA helix, as complete intercalation would decrease the emission intensity due to the replacement of the intercalated EB from DNA. The observed results suggest that the nanoparticles can make a contraction in the helix axis of DNA [39]. The binding affinity seems to follow the order StpalNPs ≥ StphNPs > SteinNPs > StbenNPs.

Figure. 5.1.3 Absorption spectra of (a) StpalNPs, (b)StphNPs, (c) SteinNPs and (d) StbenNPs (0.1 mg/mL) without and with CT-DNA at different concentrations (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55 and 0.60 mL of stock solution). The arrow shows the intensity changes on increasing the acylated StNPs concentration
Figure. 5.1.4 Emission spectra of EB bound to DNA in the absence and presence of (a) StpalNPs, (b) StbenNPs, (c) StcinNPs and (d) StphNPs [EB] = 40 µM, [DNA] = 50 mL, [St- benzoate NPs] = 2, 4, 6, 8, 10, 12 and 14 µL, respectively; $\lambda_{\text{max}} = 340$ nm. The arrow shows the intensity changes on increasing the acylated StNPs concentration.
5.1.4 Conclusions

The present study reports cytotoxic potential of the acetylated starch nanoparticles along with its biocompatible nature and warrants further evaluation at preclinical and clinical levels. The non-cytotoxicity to noncancerous cells suggest promising drug delivery applications of these materials at lower concentrations while higher doses would be useful as anticancer agents. The cytotoxicity of derivatives with aromatic groups and high degree of substitution was higher relative to those containing aliphatic group. The details of the mechanism of action, especially to clarify the mode of interaction with tumor cells, effect of degree of substitution and particle size are still to be investigated. Despite negative zeta potential the nanoparticles exhibited reasonable binding propensity with CT-DNA, although complete intercalation was not observed.
5.2 Starch nanoparticles as crosslinkers for polyurethane

5.2.1 Introduction

Polyurethanes (PUs) are unique polymer materials with a wide range of physical and chemical properties [40]. With well-designed combinations of monomeric materials, PUs can be tailored to meet diversified demands of various applications such as coatings, adhesives, fibers, thermoplastic elastomers, and foams. Extensive work has been focused to develop chemical and physical methods for their surface modification and treatments. PU materials and especially PU membranes and coatings need to bear functionalities to improve their intrinsic properties such as wettability, adhesion, biocompatibility, conductivity, cross-linking density and many others [41]. There are some interesting areas such as agricultural and medicinal applications, wherein it is desirable to introduce biodegradability by using natural materials for the synthesis of PUs [42,43]. Their non-biodegradability is restricting their utility in commodity applications. Hence, the possibility of converting them into partially biodegradable products is investigated. Carbohydrates are multihydroxyl compounds and hence can be employed as crosslinkers for PU. Among carbohydrates, the use of starch in the synthesis of PUs has been reported in only a few publications [44,45,46,47,48]. Dosmann and Steel [34] added starch to urethane systems to yield shock-absorbing foams. Lu et al. [35] have studied the miscibility and physical properties of plasticized starch acetylated with PUs. They observed that the occurrence of hydrogen bonding interaction between starch and PUs plays a key role in the improvement of the performance of the material. A study of rheological properties of PU incorporated with starch granules was carried out by Ha and Broecker [49]. Biodegradable PUs containing starch have been synthesized and characterized in our laboratory [50]. Further, synthesis of biodegradable PUs using a series of carbohydrate like glucose (monosaccharide), sucrose (disaccharide), and starch (polysaccharide) as crosslinkers by varying the NCO : OH and diol : triol ratios were also carried out [51].
5.2.2 Experimental

5.2.2.1 Materials
The polypropylene glycol, molecular weight 2000 g / mole 1,4- hexamethylene diisocyanate (HMDI) Dibutyl tin dilaureate (DBTDL) and Tetrahydrofuran (THF) were purchased from Fluka AG, Switzerland.

5.2.2.2 PPG-HMDI modified StNPs PU nanocomposite film
PPG and 1, 4-hexamethylene diisocyanate were reacted at room temperature in the presence of nitrogen to form a urethane linkage followed by crosslinking with StNPs. PPG modification involves a two-step process. The first step requires the reaction of PPG with one isocyanate functionality of HMDI. During the second step, the unreacted isocyanate of HMDI is then reacted with the surface hydroxyl groups of the starch nanoparticles.

PPG (1 mole) was vacuum dried at 80 °C. Dry THF was added followed by dropwise addition of 1.9 moles of HMDI with stirring in nitrogen atmosphere. The prepolymer formation was continued for 1 hour. Previously dried starch nanoparticles (1 mole) were added as dispersion in THF. Stirring was continued till homogeneous mixture was achieved and then catalytic amount of DBTDL was added. When the solution attained certain viscosity, degassing was carried out and polyurethane film was obtained by solution casting.

Previous studies showed that the properties of waterborne PU could be significantly improved when a small amount of nanoparticles were well mixed in the polymer matrix [52]. Introduction of nano Ag could not only improve the physical properties and biocompatibility of PU, but also inhibit the growth of bacteria even when nano Ag were embedded in the PU matrix in low concentrations. Metal nanoparticles have been doped in polymer matrix by a variety of chemical and physical methods in which the formation of metal nanoparticles was performed first. However, it is extremely difficult to disperse metal nanoparticles homogeneously into polymer matrix by these methods because of easy agglomeration of metal nanoparticles and high viscosity of polymer solution [46].
Hence, for practical applications it is important to get nanoparticles uniformly dispersed into polymer matrices.

The swelling and electrical properties of polyurethanes containing starch as crosslinker have been previously investigated [53,54]. With a view to enhance the conducting properties we attempted the incorporation of metal nanoparticles into the crosslinked PU membranes by novel sorption method in a manner similar to hydrogels [55]. The sorption ability of polyurethane could be very well exploited to facilitate transport of metal ions into the membranes. Subsequent reduction of the ions within the membrane resulted into the formation of stable nanoparticles.

5.2.2.3 Synthesis of polyurethanes immobilization with metal nanoparticles for electrical applications

From the kinetic study of sorption of alcohol in the PU membrane the optimum time was observed to be about 5 h. Circular shaped sample was immersed in 10 ml of 0.1 M alcoholic solution of metal salt for 5 h. During this equilibrium stage the metal ions were exchanged from solution to PU network through their free space between the cross-links or anchored to the –NHCOO- groups of PU chains (Scheme 5.2.1). The metal salt loaded membranes were wiped with tissue paper and immersed in 10 ml of 50 % alcoholic hydrazine hydrate solution. The swelling was continued till the color of the membrane changed to the characteristic wine reddish in case of copper and yellowish brown in case of and silver respectively. This indicated the formation of metallic nanoparticles within the PU network. The membranes were dried under vacuum at 40 ºC. At the end of the process there was no change in shape and size of PU membrane.

Alternatively PU membrane was immersed in alcoholic solution containing mixture of cupric acetate and 20 % ascorbic acid for 5 h. The solution containing the membrane was subjected to microwave irradiation for 2 min whereby formation of copper nanoparticles (CuNPs) occurred.

5.2.2.4 Characterization

Characteristic optical properties of the nanoparticle solutions were recorded using PerkinElmer Lambda 35 UV–vis spectrophotometer. Emission spectrum was recorded by
photoluminescence (PL) spectroscopy using spectrofluorometer from JASCO. Size and shape of the metal immobilized nanoparticles was determined by using TEM on a Philips, Holland Tecnai 20 model operating at 200 kV. AFM measurements were performed using an AFM Explorer microscope (ThermoMicroscopes, USA) in air and at room temperature, in a non-contact mode with Si cantilevers of a 1650-00 type (ThermoMicroscopes) with a nominal tip radius of 10 nm and resonant frequencies of about 220 kHz. XRD was determined by using PANalytical ‘X’PERT-PRO XRPD of Cu Kα radiation (λ=0.15406 nm) with scanning rate of 2º/min and 2θ ranging from 10º to 80º. Thermal Gravimetric Analysis (TGA) was recorded on TG-DTA 6300 INCARP EXSTAR 6000. The glass transition temperature (Tg) was measured using DSC200 F3MAIA, NETZSCH. Both TGA and diffential scanning calorimetry (DSC) were carried out under nitrogen atmosphere at heating and cooling rate of 10 ºC/min respectively. Conductivity spectra of PU membranes have been recorded by Solartron Impedance analyzer in the frequency range 100Hz-1MHz at temperature from 298 to 383K.

Figure. 5.2.1 Schematic representation of formation of PU-Cu and PU-Ag.

5.2.3 Results and discussion

The white translucent PU membrane gained a blue color upon sorption of the copper acetate solution, (Figure. 5.2.1 (a)) changing to reddish brown after reduction (Figure. 5.2.1 (b)), whereas in case of silver the white membrane changed to yellowish brown (Fig. 5.2.1 (c)). The process of immobilization has been represented in scheme 5.2.1. The metal nanoparticle immobilized membrane was removed from the reducing agent and wiped. It was dried at room temperature for an hour followed by vacuum drying.
Subsequently it was immersed in fresh alcohol for 72 h. There was no leaching out of the nanoparticles which was confirmed by UV-vis spectrophotometer. The color of the membrane remained unchanged which ensured immobilization of the nanoparticles in the membrane. Similar results were obtained with dichloromethane and acetone.

![Digital photographs of PU membranes](image)

**Figure. 5.2.1** Digital photographs of PU membranes to demonstrate (a) swelling in alcoholic solution of 0.1 M cupric acetate reduction of (b) Cu ions and (c) Ag ions to obtain immobilized Cu and Ag nanoparticles PU membranes respectively.

### 5.2.3.1 Optical properties

Formation and stability of metal nanoparticles in aqueous solution was confirmed using UV-vis spectral analysis of the CuNPs solution. The extinction spectra of CuNPs at different time intervals are shown in Figure. 5.2.2 (a). The characteristic absorption peak at 569 nm is due to the surface plasmon resonance (SPR) of CuNPs, which can be predicted by the well-known Mie resonance condition [56]. The surface plasmons are collective electronic excitations at the interface between metal and dielectrics. The optical properties of Ag and Cu nanoparticles change with size, shape, and dielectrics of the medium. The intensity of the peak increases with time. Figure. 5.2.2 (b) represents
photoluminescence spectrum of the CuNPs which shows emission at 660 nm on excitation at 569 nm due to the electronic transitions from excited states to d orbitals [57].

Figure. 5.2.2 (a) UV–vis absorption spectra (b) photoluminescence spectra of CuNPs

5.2.3.2 Transmission electron microscopy
As per our earlier observations the solubility parameter of the starch crosslinked PUs matched with that of acetic acid [53]. Hence when the silver nanoparticles immobilized
PUs membrane (PU-Ag) was immersed in acetic acid extensive swelling of the membrane took place. As equilibrium approached the membrane disintegrated. The TEM image of this membrane clearly reveals that the particles are somewhat spherical with a diameter ranging from 10 - 20 nm dispersed in the PU matrix (Figure. 5.2.3 (a)). However the particles are not very monodisperse. The surface morphology of PU-Ag was recorded using AFM. The two- and three-dimensional topography of the nanoparticles is shown in Figure. 5.2.3 b and c. Direct observation of the image revealed that the size of Ag nanoparticles was of the order 20–30 nm. The particles appeared to be almost spherical in shape. The size distribution of the nanoparticles along with the line profiles drawn at 9.87 μm horizontally and vertically indicates that the majority of the Ag nanoparticles were falling in the range of 20–30 nm. The data obtained from the AFM matched well with the TEM results.

5.2.3.3 X-ray diffraction

The XRD pattern of metal immobilized membranes showed the crystalline phases of the metals embedded in the amorphous matrix of crosslinked PU. In case of both PU-Cu and PU-Ag, the pattern matches well with the cubic phase of Cu and Ag (JCPDS 04-0836). In case of PU-Ag all Bragg's reflections representing b111N, b200N b220N and b311N planes of fcc crystal structures due to metallic silver are observed at 38.3 º, 44.7 º, 65.1 º and 77.7 º respectively [58]. While in case of PU-Cu all Bragg's reflections due to metallic copper are observed at 43.29, 50.42 and 74.11 representing b111N, b200N and b220N planes of fcc crystal structures of bulk copper [58] (Figure. 5.2.4). The average sizes of the NPs within the PU matrix were determined from the width of the reflection according to the Debye–Scherrer equation (eq 1). It was 27±2 for Cu and 33±2 nm for Ag respectively [58].

\[
D(\text{nm}) = \frac{K\lambda}{\beta\cos\theta} \quad \ldots \ldots \ldots \ldots \ldots (1)
\]

where K is a constant equal to 0.89, \( \lambda \) is the X-ray wavelength (1.54 Å), \( \beta \) is the full-width at half-maximum (fwhm) of the major peak expressed in radians, and \( \theta \) is the Bragg angle (deg) corresponding to that peak.
5.2.3.4 Thermal properties

TGA curves of PU membranes are shown in Figure 5.2.5 (a). From the decomposition temperatures in the Table 2 it can be seen that the metal nanoparticles considerably improve the thermal stability of PU. This indicates effective entrapment and a high degree of adhesion between the NPs and the PU matrix. The DSC curves shows that the Tg of PU containing immobilized NPs is higher as compared to PU (Figure 5.2.5 (b)). The increase is nominal and is greater in case of PU-Cu, compared to PU-Ag. Inorganic nanoparticles can usually hinder the motion of polymer chains, leading to an increase in α-transition peak temperature of the PUs [59]. The variation in Tg was also observed due to effect of hydrogen bonding between the polymer matrix and metal nanoparticles and improved dispersion as also seen in TGA. Thus incorporation of CuNPs improves the thermal stability as well as increases the glass transition temperature even more than nanosilver. This indicates a greater compatibility between copper and the PU matrix which can be explained as follows. Due to greater valency, initially the cuprous ions bind more strongly with the urethane groups through the nitrogen atom compared to silver. This facilitates the anchoring and alignment of the nanoparticles onto the PU network on subsequent reduction so that continuity of metal nanoparticles is better.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Ea KJ/mol</th>
<th>Glass Transition Temperature (Tg) °C</th>
<th>Degradation temperature (°C) for % Wt. loss</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PU</td>
<td>227</td>
<td>-55.2</td>
<td>101</td>
</tr>
<tr>
<td>PU-Ag</td>
<td>107</td>
<td>-54.0</td>
<td>72</td>
</tr>
<tr>
<td>PU-Cu</td>
<td>81</td>
<td>-53.0</td>
<td>82</td>
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Figure. 5.2.3 (a) TEM and (b) 2 and 3-D AFM images of PU-Ag membranes
5.2.3.5 Electrical properties

The PU membranes were examined for the electrical properties to see how the metallic nanoparticles improve the ionic conductivity of the polymer chain. The electrical properties of metal immobilized nanoparticles are strongly influenced by the metal filling factor and nanoparticle sizes [60,61]. The important mechanisms of electric conductivity in polymer metal nanoparticles dispersed in polymer matrix are

1. Ion conductivity, due to the ions remaining dispersed in the polymer matrix;
2. Electron conductivity in the metal nanoparticles network,
3. Tunnelling conductivity.

Generally, at high volume fractions of metal nanoparticles a 3D conducting network can develop in the matrix, leading to a sudden increase in the electric conductivity known as percolation. Our sample is well below the percolation threshold.

The results showed that ionic conductivity increases with temperature, with activation energy of 0.15 eV which indicates a thermally activated conduction mechanism in metal immobilized polymer membrane (Figure. 5.2.6 (B)). This behavior is attributed to increase of charge carrier energy with increase in temperature. The frequency dependent conductivity of the metal immobilized nanoparticles at different temperatures exhibits a typical frequency independent plateau at lower frequencies and a crossover or a
dispersive region at higher frequencies (Figure. 5.2.6 (A)). At higher frequencies, the mobility of charge carriers is high due to which the conductivity increases with frequency. As frequency decreases, more and more charge accumulation occurs at the electrodes, which leads to a decrease in the number of mobile ions and eventually to a drop in conductivity at low frequency. The conductivity increases in the order PU-Cu > PU-Ag > PU which again may be due to better compatibility of Cu nanoparticles with polymer matrix. This assumption is well supported by thermal analysis. Thus, the electrical conductivity along with better thermal stability of these materials can lead to the development of novel sensors for biomedical applications.

Figure. 5.2.5 (a) Thermogravimetric and (b) Differential Scanning Calorimetry curves of PU membranes.
Figure 5.2.6 (A) shows frequency dependent conductivity at different temperatures of PU membranes and (B) shows variation of ionic conductivity with temperature (a) PU (b) PU-Ag (c) PU-Cu
5.2.4 Conclusions

A novel sorption method of immobilizing metallic nanoparticles on starch crosslinked PU membranes was developed. The immobilization of the metal NPs was ensured by microscopy. The resulting membranes exhibited high thermal stability and higher Tg. The metallic nanoparticles improved the ionic conductivity of PU which exhibited Arrhenius relation. AC conductivity is found to increase with frequency as well as temperature. Copper nanoparticles exhibited a pronounced effect compared to silver. The temperature sensing ability, thermal stability and permeability of these novel membranes can lead to development of sensors for biomedical applications or conducting wires for connection of minitype devices.
5.3 References