STUDY OF POLYLYSINE AND CHITOSAN NANOPARTICLES SYNTHESIZED USING VARIOUS CROSS-LINKERS AND THEIR APPLICATIONS FOR HEAVY METAL ION RECOVERY

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ABSTRACT OF THE THESIS

Study of Polylysine and Chitosan nanoparticles synthesized using various cross-linkers and their applications for heavy metal ion recovery

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Nanotechnology offers the possibility of an effective and efficient removal of pollutants from waste water. This removal of pollutants and heavy metal ions is being accomplished using nanoparticles, nanomembranes and nano powders of various materials. Chitosan, a polysaccharide, and polylysine, a polypeptide, have been receiving increasing attention for their metal scavenging and sequestering properties. One part of this study focused on synthesizing nanoparticles of polylysine, chitosan and mixed chitosan-polylysine nanoparticles using various cross-linkers involving ionic and covalent interactions. The effect of pH on the type of bonding between polymers and cross-linkers as well as the properties of nanoparticle suspensions were studied. Nanoparticles synthesized were characterized based on their size and zeta potential using dynamic light scattering techniques. Second part of the study focused on using these nanoparticles for adsorbing copper from aqueous solutions and analyzing the amount of adsorption using NMR spectroscopy technique.

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Chapter 1

Introduction

1.1 Problem Statement and Objective

Clean water is essential for the survival, sustenance and propagation of life on the planet. We are facing huge challenges today in providing humanity with clean water and fresh water resources are depleting at a very fast rate because of various factors such as extended droughts and famines, population growth and industrial uses. Industries like manufacturing, defense and pharmaceuticals use chemicals to improve the standard of living, where as these same chemical's invasion into the environment reverses the standard of living that they were intended to foster [3]

As two million tons of industrial waste and sewage are being dumped into worlds water bodies each day (UN WWAP 2003), clean water is becoming one of the leading environmental concerns. Common waterborne pollutants include microorganisms such as bacteria, fungi, viruses and algae apart from organic solvents and heavy metals, which are usually found in industrial effluent streams. The uncontrolled dumping of non-biodegradable wastes, mainly heavy metals, into water bodies,has already reached alarming rates. [4]

These heavy metal ions form complexes with natural organic matter present in soil and water resources and removing them poses a significant challenge [5–7] . The presence of heavy metals such as Copper (Cu), Lead (Pb), Nickel (Ni), Arsenic (As), Cadmium (Cd) and Chromium (Cr) in water streams is a matter of serious concern for the public health as well as the environment. Copper is one of the most widely used heavy metals and is mostly used for electrical wiring, plumbing and industrial machinery. An essential element when present in minute quantities in human body, it plays a vital role in metabolic activities but acute doses can be extremely dangerous and even fatal at

times. Copper, when bound to an organic ligand, as in food, is a beneficial nutrient needed for good health. On the other hand, in its inorganic form, such as the one found in industrial streams, cooking utensils, plumbing pipes, it is a neurotoxic heavy metal on par with mercury and lead [8].

Traditional methods of water purification include filtration, precipitation, electro-chemical treatment, ion exchange, adsorption and chelation. In recent years, adsorption has been the major focus of research and various adsorbents are being tested to increase their adsorptive capacity, and in particular by incorporating nanoparticles. Advancements in the fields of nano science and technology are showing promising signs in tackling the problem of water purification using nanoscale catalysts, sorbents, biomaterials, polymers and membranes. Most of the purification techniques that are being used today, while effective, are often very costly and time consuming. The ultimate goal is to remove these toxic compounds effectively and efficiently at affordable costs [9]. Using nanoparticles as adsorbents addresses two important properties which make them very effective sorbents.

1) On a mass basis, they have very high surface area to mass ratio compared to bulk particles

2) They can be functionalized with different reactive groups using chemical techniques to increase their affinity in adsorbing the desired components from the waste water

Naturally available polymers such as Chitosan and Polylysine are receiving increasing attention due to their excellent affinity towards heavy metal ions. Both of them are abundantly available and environmentally friendly, which makes them suitable adsorbents for removing toxic compounds from waste water. The low cost as well as very good selectivity for metal ions make these biopolymers practical and sustainable for removal of these compounds [1, 10].

For this study, the polymer that was selected for the adsorption experiment was the α-poly-L-lysine Br. This polymer is prepared artificially, whereas the other form of this polymer, ϵ-PLL occurs readily in nature. The reason for selecting α-poly-L-lysine is its high molecular weight which makes it viable for metal recovery . Even though

research has been conducted using ϵ-poly-L-lysine [11] for removal of copper not much research has been done in using α-PLL as a possible absorbent for divalent copper after Shima.et.al, especially using the nanoparticles of poly-L-lysine. To our knowledge, even the synthesis of α-PLL nanoparticles using TPP and genipin that were achieved by this study are novel.

1.2 Main objectives of this study

The major purpose of this study was to synthesize nanoparticles of polylysine, chitosan as well as chitosan-polylysine mixed particles using different crosslinkers, namely tripolyphosphate (TPP) and genipin, and characterize the resulting particles based on their size and zeta potential. The study contains an innovative comparison of the cross-linking behavior of a carbohydrate polymer and a polypeptide polymer, under the action of an ionic cross-linker or a covalent cross-linker. Moreover, we aimed to study the adsorption capacity of polylysine nanoparticles cross-linked with TPP with divalent copper ions and to test if polylysine in nanoparticle form it is a very good adsorbent for the removal of copper. The specific objectives are as follows:

1. Synthesize chitosan, polylysine and chitosan-polylysine nanoparticles using different cross-linkers such as TPP and genipin and characterize them according to size and zeta potential. Also to determine the effect of pH on the size and zeta potential of the nanoparticles

2. Perform the adsorption experiment using polylysine nanoparticles crosslinked with TPP and determine the metal uptake capacity in mg/g using NMR spectroscopy

1.3 Heavy Metal ion Contamination

Heavy metals are classified as those elements whose atomic weights range from 63.5 to 200.6 and have a specific gravity greater than 5.0 [12]. Industries such as metal plating, pesticides, fertilizers, tanneries, batteries, mining, paper, etc., use heavy metals and

these metals are discharged either directly or indirectly into the water bodies in the form of effluents. These heavy metal ions, in trace amounts are essential for normal functioning of the human body, but elevated amounts cause significant threat to the health. Intake of metals such as Cadmium, Zinc, Copper, Arsenic, Mercury, and Lead can lead to severe chronic disorders and might also result in death.

1.4 Copper Contamination and effects

Copper, one of the widely distributed elements in nature, is one such heavy metal that is used in various applications such as electrical wiring, architecture, automotive, plumbing and industrial machinery. Copper is a transition metal, available in its pure form in nature and also has the oxidation states of Cu (+I) and (+II). It is a soft and ductile material and has excellent electrical and thermal conductivities resulting in a variety of applications.

Large scale usage of this metal results in the metal getting exposed to the environment which is a severe threat to the ecological system. The provisional WHO guideline for copper in drinking water is 2 mg/L [13]. Experimental studies have shown that drinking water with more than 3mg Cu/L will produce gastrointestinal symptoms including nausea, vomiting, and diarrhea. The highest exposure to copper occurs by consuming food cooked in uncoated cookware, or from drinking water with excess copper levels or from other environmental sources. Excessive exposure to copper results in Wilsons disease which is characterized by accumulation of copper in the liver, brain, kidneys and cornea [14].

Traditional methods of copper remediation involve methods such as chemical and electrochemical precipitation, adsorption, ion exchange and chelation. Some of these methods such as ion exchange are very effective in removing copper when the initial concentration is high but for the cases with lower concentration they are ineffective. Hence cheaper and efficient alternatives are being researched for the removal of low concentrations of toxic metals [15].

1.5 Biopolymers as Adsorbents

In recent years chitosan, alginate and polylysine are being extensively researched for removal of metals because of their affinity towards them. Chitosan, used in this study for synthesizing the nanoparticles with crosslinkers, is obtained from chitin, which is a homopolymer of α (1-4) N- acetyl -D-glucosamine residues and is found in the shell of crabs and other crustaceans [1]. Polylysine, used in this study for synthesizing nanoparticles using crosslinkers as well as for adsorption of copper ions, is a cationic polymer at neutral pH, is very well used for its biological applications such as a cell adhesion agent for cell culturing experiments (Krikorian, et al., 2002). Apart from synthesizing various nanoparticles, one aspect of this study focused on using α-PLL for adsorption of copper ions from aqueous solutions.

1.6 Equilibrium adsorption isotherms

Analysis of adsorption data is essential to determine the efficiency of the adsorbent and for doing this, we need to correlate the metal uptake with the amount of adsorbent used. Two adsorption isotherms that are widely in use are the Langmuir and the Freundlich isotherms. Experimental data obtained is generally fit to these isotherms, which give the relation between Q (mg/g), the amount of metal adsorbed per unit mass of the adsorbent, to Ce(mg/L), which is the equilibrium metal ion concentration in the solution after adsorption [1].

For the adsorption part of the project, we used the Langmuir isotherm for evaluating the data and Langmuir isotherm is based on the following set of assumptions:

1. The surface of the adsorbent is in contact with a solution containing an adsorbate which is strongly attracted to the surface.

2. The surface has specific number of sites where the solute molecules get adsorbed and all the sites are equivalent.

3. The adsorption involves the attachment of only one layer of molecules to the surface i.e., monolayer adsorption.

4. The adsorbed species do not interact with one another but interact only with the

adsorbent.

The general form of the Langmuir adsorption model is given by

$$Q = \frac{Q_{max} K_s C_e}{1 + K_s C_e} \tag{1.1}$$

Where Q is the metal uptake concentration (mg/g adsorbent), Q_{max} is the asymptotic maximum metal uptake (mg/g adsorbent), K_s is the Langmuir equilibrium constant (L/mg) and C_e is the bulk liquid phase metal concentration (mg/L solution).

The linear form of the Langmuir equation is used to estimate the parameters Q_{max} and K_s and it is given by the following equation:

$$\frac{C_e}{Q} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_s} \tag{1.2}$$

The values of Q_{max} and K_s can be obtained from the slope and intercept of the straight line graph. [1, 16]

Chapter 2

Materials and Experimental methods involved in the synthesis of nanoparticles

2.1 Materials used for the synthesis of nanoparticles

2.1.1 Chitosan

Among many naturally available biosorbents, chitosan has been receiving a lot of attention due to its effective metal scavenging properties [17]. Chitosan is derived from chitin, which is the second most abundant natural biopolymer after cellulose and is found in the exoskeletons of shellfish and crustaceans. Chitosan, obtained from chitin comprises of poly (b-1-4)-2-amino-2-deoxy-D-glucopyranose and the chemical structure is shown in Fig. 1. In neutral conditions, the amine and hydroxyl groups crystallize forming hydrogen bonds and chitosan remains stable [18]. But in acidic conditions it becomes protonated because of the amine groups. Research has shown that chitosan can be used to effectively remove metals such as chromium [17, 19–21], copper [17, 19–22], mercury [23, 24] and lead [25, 26] from aqueous solutions. Apart from heavy metal recovery applications, chitosan is also being used in the fields of drug delivery [27], flocculation [28] and also for antimicrobial treatment [29, 30].

Fig 2.1 [1] shows Chitosan which contains several reactive groups that are involved in the removal of the metal ions and the amount that can be removed depends on various conditions such as the pH of the solution, metal ion involved and the solution composition [31–33].

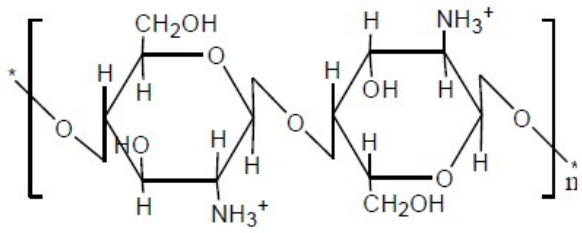

Figure 2.1: Structure of Chitosan in an acidic solution with protonated amine groups [1]

Amine groups as well as hydroxyl groups present in the chitosan are responsible for the majority of the adsorption [34]. The adsorption mechanism follows ion exchange, chelation or formation of complexes and in the case of chitosan, it is believed that chelation is the main mode of adsorption. Nitrogen present in the amine groups forms coordinate covalent bonds with the copper ions where as some hydroxyl groups also might take part in coordination by release of protons. Chitosan binds with metals in the form of two models, the bridge model and the pendant model as shown in the following figure 2.2. The metal ion is bound to two amine groups from the same chain or different chains in the case of bridge model whereas in the pendant model it is bound to only one amine group and hydroxyl groups and pairs of oxygen atoms in water molecules in a pendant fashion [34–42].

Figure 2.2 [27] shows the formation of Chitosan chelates with Copper ion in the form of bridge model and pendant model.

Chitosan has been modified chemically using different crosslinkers to enhance the performance of the polymer in adsorbing the metal ions. Even though adding the crosslinkers decreases the available number of sites for metal adsorption, it increases the stability of the polymer in the acidic solution [17].

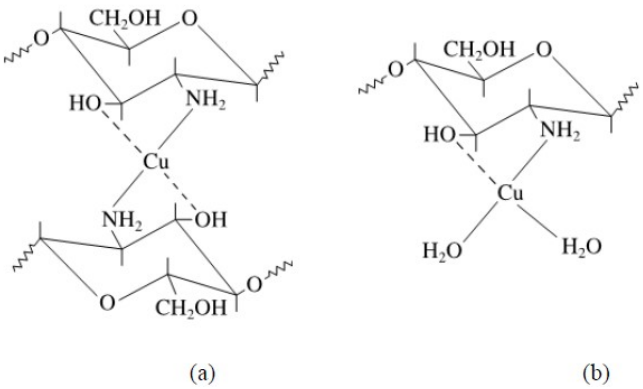

Figure 2.2: Chitosan copper interaction (a) Bridge model (b) Pendant model

In this study we synthesized nanoparticles of chitosan using two cross-linkers, TPP and genipin. Chitosan cross-linked with TPP has been used for metal ion removal applications where-as chitosan cross-linked with genipin has been used in films.The specific type of chitosan used was the "low molecular weight" chitosan (Sigma-Aldrich) with a low molecular weight of range of 50-190 kDa. We synthesized nanoparticles of chitosan with genipin at different pH conditions and measured the size and zetapotential of the nanoparticles to determine the stability of the colloidal solution. The way that chitosan binds with TPP and genipin is discussed in detail in the results section.

2.1.2 Poly-L-lysine

Poly amino acids refer to a specific set of polyamides that consist of only one type of amino acid linked by amide bonds [43]. These polymers are being used for a variety of applications such as drug delivery, cell culture, pharmacetical applications and also for metal ion recovery. Lysine monomer is an amino acid with an amine group providing its charge. Poly-L-Lysine is a polymer formed by condensation and contains peptide bonds between each two monomers [44]. The conformational states of polylysine in aqueous solutions have been extensively studied and are shown to be dependent on a wide range of solution conditions such as pH, temperature and salt concentration. At neutral pH, polylysine exists as random coil in solution due to the high charge of

lysine side chains. But at a pH above 10.6, it has been observed that PLL transforms into α-helix conformation because the charge on PLL is reduced at a pH above the pK_a (10.5) of the lysine side chains. Upon heating to 51 degrees Celsius followed by cooling to room temperature at pH above 10.6, the α-helix structure transforms into β sheet conformation [45]. Polylysine, one of the the several type of lysine homopolymers, has two different structures, one that is naturally occuring and the other one which is chemically synthesized. ϵ-PLL is a naturally occuring cationic polymer and it is made of L-lysine residues connected between ϵ-amino and α-carboxyl groups [43]. α-PLL is manufactured synthetically from ϵ-PLL and we used α-PLL for metal ion recovery applications in this study. Compared to ϵ-PLL, α-PLL has a higher molecular weight and hence is mainly preferred for metal recovery applications. On the other hand ϵ-PLL shows a variety of anti-microbial properties and hence is used mainly as a food preservative [46]. Fig 2-3 [46] shows the two structures of Poly-L-Lysine

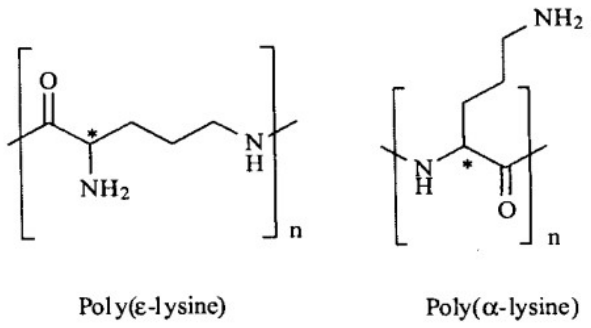

Figure 2.3: Structures of ϵ and α polylysine

In this study, we used α-PLL that was synthetically obtained from Sigma Aldrich for the adsorption experiment. ϵ-PLL has 36 to 40 Lysine molecule residues and the molecular weight of one lysine residue is 146.19 Da where as a polymer with a high molecular weight will have more number of sites available for adsorption. Out of the different molecular weights of α-PLL that are available (15000-30000 Da, 30,000-70,000 Da, 70,000-150,000 Da, 150,000-300,000 Da) we used the 30,000-70,000 Da as it is less viscous in solution and also costs less compared to the other molecular weights. Even though higher molecular weight α-PLL (greater than 300,000 Da) might provide

more adsorption sites, we used the 30,000-70,000 Da molecular weight α-PLL as it produced the most stable nanoparticles when cross-linked with TPP, and the molecular weight range was comparable to that of the lower molecular weight chitosan. The lower molecular weight α-PLL has around 450 lysine molecule residues and is a white powder that is water soluble. α-PLL .HBr is a positively charged amino acid polymer with approximately one HBr molecule per lysine residue. The hydrobromide allows the poly-L-lysine to stay in crystalline form and also soluble in water.

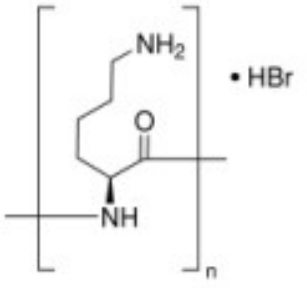

Figure 2.4: Structure of α-PLL hydrobromide showing the HBr molecule

We synthesized the nanoparticles of α-PLL using two different cross-linkers, TPP and genipin, and characterized them using zeta potential and sizing.Apart from synthesizing plain nanoparticles of chitosan and polylysine, we also focussed on synthesizing mixed nanoparticles of Chitosan and polylysine (mass ratio 10:1) using the same cross-linkers.

Even though polylysine has been used for metal recovery applications, it was mainly used in the form of molecules in solution, for recovering the metal ions. Our study focused on using the nanoparticles of polylysine,mainly cross-linked with TPP for metal ion recovery.

2.1.3 Cross-linkers

Cross-linkers are chemical compounds that contain specific functional groups and are used to link one polymer to another with covalent or ionic bonds. Cross-links are formed by chemical reactions that can be triggered by heat, pressure, changes in pH or even by using radiation. In this study, the cross-linkers that were used are TPP and genipin.

Tripolyphosphate (TPP) is a non-toxic polyanion which interacts with chitosan via electrostatic forces to form ionic cross-linked networks. The protonated amine groups in chitosan as well as polylysine interact with the negatively charged ion in TPP, through an ionic network and thus helping the polymers remain stable in acidic conditions [1]. Apart from TPP, we used genipin, which is obtained from geniposide,and is much less toxic compared to gluteraldehyde. Genipin binds with polymers in a variety of ways depending on the pH of the medium and forms cross-linked products. The following figure depicts the chemical structure of genipin molecule [18]

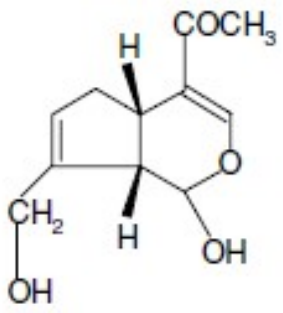

Figure 2.5: Chemical structure of genipin

The various binding mechanisms of genipin with polymers are discussed in the later sections.

2.2 Characterization techniques: Zeta Sizing

Zeta potential is the potential difference existing between the surface of a solid particle immersed in a conducting liquid and it is a key indicator in estimating the stability of colloidal suspensions. Zeta potential in aqueous solution is dependent on charge at the shear plane and free salt concentration. The square of potential is inversely proportional to the force of electrostatic repulsion between charged particles thereby indicating the stability of a colloidal suspension. When the Zeta potential approaches zero, vander-waal's forces dominate over electrostatic forces thereby resulting in aggregation(From Brookhaven Instruments Corporation) .

In this study, we measured the zeta potential of the nanoparticle suspensions to determine the stability of the colloidal suspensions. Different types of nanoparticles were synthesized and the nanoparticle pellets were suspended in water and were sonicated using a Misonix 3000 sonicator (Qsonica, Newtown, CT). Malvern Zetasizer was used to perform dynamic light scattering to determine the size of the nanoparticles as well as the zeta potential. Colloidal suspensions are considered stable when they have very high values of zeta potential

2.3 MRI Scanning : Adsorption measurement technique

Even though adsorption is most commonly measured by using ICPMS (Inductively coupled plasma mass spectroscopy), in this study, we tried using MRI scanning to determine the amount of adsoprtion by measuring the difference in intensities of the standard and supernatant samples. When a sample is put in a magnetic field, the protons in the system align with the magnetic field either parallel or antiparallel. Then they precess or wobble at Larmor frequency. This frequency can be calculated by using the following equation.

$$\omega_0 = \gamma B_0 \tag{2.1}$$

Where

ω_0 = Precessional or Larmor Frequency (MHz)

γ = gyro magnetic ratio (MHz/T)

B_0= Magnetic field strength (T)

Before the system starts acquiring the data, the center frequency is determined and an RF (radio frequency) pulse is generated which matches with the center frequency of the system. By sending an RF pulse with the center frequency, with a certain strength for a certain period of time, it is possible to rotate the net magnetization to the perpendicular axis depending on the alignment, which results in excitation.

Due to the rotation of the net magnetization, all the protons reach an excited state

and will try to reach the lower energy state which results in relaxation. They do so by releasing the absorbed energy in the form of RF waves,which produce the observed free induction decay (FID) signal. Relaxation occurs by T1 and T2, which are two independent processes. T1 relaxation describes what happens in the Z direction(i.e.,the rate of relaxation back to the ground state) where as T2 tells about the X-Y plane (i.e.,the additional rate of decay of the signal while in the excited state). Some of the the MRI parameters are as follows :

a) TR (Repetition time) : It is the time between two excitation pulses

b) TE (Echo time) : Time between excitation and echo pulse

c) Flip angle (FA) : Estimates the net magnetization directed towards XY plane

d) Inversion time (TI) : The inversion time is the time between an 1800 excitation pulse and the 900 excitation pulse

e) Slice thickness (ST) : The slice thickness influences the amount of signal as well as the sharpness of the image.

f) Slice gap (SG) : The SG parameter describes the amount of space (in percentage of slice thickness) between slices [47]

For the current study, we used GRE (gradient Echo) scan, which is a manipulation of the FID signal and the values of different parameters for the run are as follows:

Number of Slices = 32

Slice thickness (ST) = 2 mm

TR (repetition time) = 15.4 ms

TE (Echo time) = 3.8 ms

Number of excitations = 3 [47]

All the above details regarding the MRI scanning have been taken from mrisource.net

2.4 Experimental methods

2.4.1 Chitosan Nanoparticle preparation

2.4.1.1 Materials

Chitosan (low molecular weight) (CAS no.9012-76-4) was obtained from Sigma-Aldrich. Sodium tripolyphosphate and genipin were obtained from Sigma Aldrich too. Water was obtained from Milli Q water system in the lab for all the experiments.

2.4.1.2 Particle Preparation

Chitosan nanoparticles were prepared in this work using two different cross-linkers. One type of nanoparticles was produced by using Sodium tripolyphosphate, which simply followed the protocol from previous work of the group [1]. They are named CSNPs with TPP". The second type of nanoparticles were made by using genipin as the cross-linking agent. We named them CSNPs with genipin.

2.4.1.3 Chitosan Nanoparticles cross-linked with TPP

Plain chitosan nanoparticles were prepared using the regular chitosan nanoparticle preparation protocol, involving ionic interactions between chitosan and TPP. 2 mg/ml of Chitosan solution was prepared by dissolving 0.8 grams of Chitosan in 400 ml of DI water and acetic acid, 1.75 times the mass of chitosan, was added to adjust the pH of the solution to 3.5. This solution was left overnight under magnetic stirring. TPP solution was prepared by adding 0.2 grams of Sodium tripolyphosphate to 100 grams of DI water and was left under magnetic stirring overnight. 0.7 ml of TPP solution (2 mg/ml) and 0.3 ml of deionized water were added dropwise to 5 ml chitosan solution (2 mg/ml) while stirring in a 20 ml vial. The mixture was kept under magnetic stirring for another 30 minutes and was then left overnight for it to reach equilibrium. On the next day, the mixture was centrifuged for 30 minutes at 20,000 rpm (48,400 g) (Beckmann Coulter). The wet pellet was then washed using DI water, dried and then weighed.

Then it was redispersed in 3 ml of DI water by probe sonication (Misonix Sonicator) for approximately 30 s. The wet mass of the pellet produced per 4 vials ranged from 0.09 to 0.13 g [1].

2.4.1.4 Chitosan nanoparticles cross-linked with Genipin

The aqueous solutions of genipin turn brown in color and viscous when the solution is made highly basic. Genipin solutions prepared at pH 2.0, pH 5.0, pH 7.0 and pH 10.0 remained transparent, while the ones prepared at a pH of 12.0 and above became dark brownish and viscous [2]. 2 mg/ml of this genipin solution was prepared by dissolving 0.2 grams of genipin in 100 ml of DI water and the pH of this solution was adjusted to 12.7 by adding concentrated KOH (potassium hydroxide) to it. The mixture was left overnight under magnetic stirring. Volume of the cross-linker genipin, used in making chitosan nanoparticles was varied ranging from 0.02 ml to 1.5 ml. For the regular particles, 0.7 ml of genipin and 0.3 ml of DI water were added dropwise to 5 ml of chitosan solution (2 mg/ml) while stirring in a 20 ml vial. The mixture was left under magnetic stirring for another 30 minutes and was then left overnight for it to reach equilibrium. The mixture was centrifuged next day for 30 minutes at 20,000 rpm (48,400 g) (Avanti J-E, Beckmann Coulter, Brea, CA). Wet pellet that was obtained was washed using DI water and was then redispersed in 3 ml of DI water by probe sonication (Misonix Sonicator) for approximately 30 s. The wet mass of the pellet produced per 4 vials was around 0.09 to 0.13 g.

2.5 Polylysine nanoparticle preparation

2.5.1 Materials

Poly-L-Lysine hydrobromide (low, medium and high molecular weights) (CAS 25988-63-0), Sodium Tripolyphosphate and genipin were obtained from Sigma Aldrich too.

2.5.2 PLNPs using TPP as cross-linker

Polylysine nanoparticles were prepared by cross linking polylysine with TPP which involves ionic interactions between polylysine and TPP. Polylysine solution (2 mg/ml) was prepared by dissolving 0.8 grams of polylysine in 400 ml of DI water and the solution was left overnight under magnetic stirring. TPP solution was prepared by dissolving TPP in water and adjusting the concentration to 2 mg/ml. 5 ml of Polylysine solution (2 mg/ml) was taken into a 20 ml vial and 0.7 ml of TPP (2 mg/ml) and 0.3 ml of DI water were added dropwise to the solution under magnetic stirring. The mixture was left under the stirring for an additional 30 min and then left at rest overnight for it to reach equilibrium. On the following day, the mixture was centrifuged for 30 min at 48,400 g (20,000 rpm) in the Beckman Coulter centrifuge and the wet pellet that was obtained was weighed. The wet pellet obtained was redispersed in DI water by probe sonication for approximately 30 s. The wet mass of the pellet produced per 4 vials was around 0.1 to 0.15 g. Different molecular weights of Polylysine (3000-15000 Da, 30,000-70,000 Da, 70,000-150,000, 150,000-300,000 Da) were used in the preparation of nanoparticles and all the preparation methods followed the protocol mentioned above.

2.5.3 PLNPs using genipin as cross-linker

2 mg/ml concentration of genipin solution was prepared by dissolving 0.2 g of genipin in 100 ml of DI water and the pH of solution was adjusted to 12.7 by adding concentrated KOH (potassium hydroxide) to it. This mixture was left overnight under magnetic stirring. The amount of genipin used to make the nanoparticles was varied from 0.02 ml to 1.5 ml to understand the effects of cross-linking on the size and the zeta potential of the particles. 5 ml of polylysine was taken in a 20 ml vial and 0.7 ml of genipin and 0.3 ml of DI water were added to it dropwise under magnetic stirring. The mixture was then left under stirring for an additional 30 min and was left at rest overnight for it to reach equilibrium. The mixture was then centrifuged at 48,400 g (20,000 rpm) for 30 min the next day. The wet pellet that was obtained was weighed and then redispersed in DI water by probe sonication for around 30 s. The wet mass of the pellet that was

obtained per 4 vials was around 0.07-0.13 g.

2.6 Chitosan-Polylysine mix nanoparticle preparation

2.6.1 Chitosan-Polylysine nanoparticle preparation (CPNPs) with TPP

Chitosan-polylysine nanoparticles were prepared in a slightly different method compared to the plain chitosan and polylysine nanoparticles. This type of nanoparticles was prepared by incorporating polylysine inside while forming the chitosan nanoparticles. Chitosan (2 mg/ml) and polylysine (2 mg/ml) solutions were mixed with each other in order to achieve a mass ratio of 10:1 of chitosan and polylysine. The pH of the solution was adjusted to 3.5 by adding concentrated acetic acid. This chitosan and polylysine mixed solution was used to prepare the nanoparticles instead of pure chitosan solution. 0.7 ml of TPP (2 mg/ml) and 0.3 ml of DI water were added dropwise into 5 ml of the mixed solution (2 mg/ml total polymer concentration) while stirring. The mixture was left under magnetic stirring for an additional 30 minutes and was left at rest overnight to reach equilibrium. On the next day, the mixture was centrifuged for 30 min at 48,400 g (20,000 rpm). The wet pellet obtained was washed several times by DI water and weighed. Then it was redispersed in DI water by probe sonication for approximately 30 s. The wet mass of the pellet produced from 4 vials was around 0.1 to 0.14 g.

2.6.2 Chitosan-Polylysine nanoparticle preparation (CPNPs) using genipin

This type of nanoparticles also were prepared by incorporating polylysine inside chitosan while forming nanoparticles. Chitosan (2 mg/ml) and polylysine (2 mg/ml) solutions were mixed with each other in order to achieve a mass ratio of 10:1 and the pH of the solution was adjusted to 3.5-4 by adding concentrated acetic acid. This chitosan polylysine mixed solution was used to synthesize the nanoparticles instead of chitosan solution. 5 ml of this mixed solution was transferred into a 20 ml vial and 0.7 ml of polymerized genipin and 0.3 ml of DI water were added under magnetic stirring. The

mixture was left under stirring for another 30 min at 48,400 g (20,000 rpm) and sat overnight to reach equilibrium. The wet pellet obtained was washed several times by DI water, dried and weighed. Then it was redispersed in DI water by probe sonication for approximately 30 s. The wet mass of the pellet produced per 4 vials was around 0.08 g - 0.14 g.

Chapter 3

Characterization of Polymer nanoparticles using Zetasizing

3.1 Particle characterization

In this study we synthesized nanoparticles of chitosan, polylysine and chitosan-polylysine nanoparticles using two different crosslinkers namely

1) TPP (Tripolyphosphate), an inorganic salt that crosslinks with the polymers using ionic interactions.

2) Genipin, an organic compound, obtained from its parent compound, which is isolated from the fruits of Gardenia jasminoides ELLIS and bonds covalently with the polymers under different pH conditions

and characterized them using Zeta sizing and potential techniques.

This chapter deals with characterization of different types on nanoparticles synthesized using different cross-linkers on the basis of size and potential. The various nanoparticles synthesized are as follows:

1. Chitosan nanoparticles cross-linked using TPP and genipin

2.Polylysine nanoparticles cros-linked using TPP and genipin

3. Chitosan-polylysine mix nanoparticles cross-linked using TPP and genipin

Apart from synthesis, a section of the chapter deals with the adsorption capacities of polylysine nanoparticles cross-linked with TPP in removing divalent copper ion from aqueous solutions.

3.2 Characterization of chitosan cross-linked with TPP

3.2.1 Size

The size of chitosan nanoparticles that were cross-linked with TPP were characterized using zetasizer and the diameter of the particles were measured by dynamic light scattering as shown in Fig 3-1.

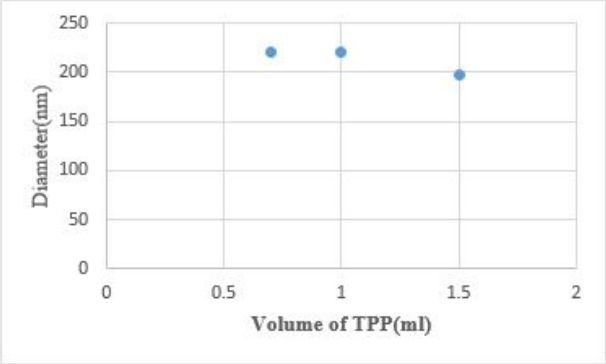

Figure 3.1: CSNP's crosslinked with TPP- size

The concentration of the chitosan solution and TPP that were used for making the nanoparticles were 2mg/ml and the volume of TPP was varied. We observed that the size decreased as the amount of TPP was increased.

3.2.2 Zeta Potential

Fig 3-2 reveals the zeta potential of chitosan nanoparticles produced using varying amounts of TPP. Zeta potential is an estimate of the surface charge density and high magnitude of zeta potential indicates the stability of the colloidal suspension. As the magnitude of zeta potential decreases, the electrostatic repulsion forces between the particles dominate over the Van der waals forces resulting in aggregation of the particles and therefore leading to the instability of the colloidal suspension.The pH of the solution was maintained at acidic conditions (pH = 3.5) by adding acetic acid to the chitosan solution to ensure that most of the amine groups remain protonated.

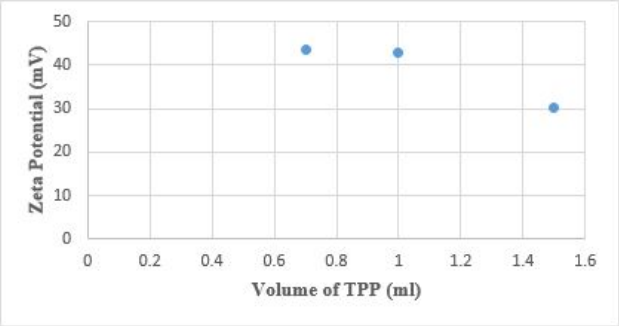

Figure 3.2: CSNP's crosslinked with TPP- zeta

TPP is known to form five links with the amine groups of chitosan and the number of links might change with the concentration of the synthesized solution. The zeta potential of the solution decreased with increasing amount of TPP, which shows that increasing the salt concentration decreases the zeta potential of the solution. Therefore, higher the zeta potential, lower the fraction of TPP present in the particle. If the salt amount is further increased, at high amounts of salt, the double layer collapses to the extent that ever present Van der waals forces overcome the repulsive forces between the particles resulting in destabilization of the colloidal solution, thereby forming aggregates. We prepared nanoparticles of chitosan linked with TPP by varying the concentrations of both the chitosan and TPP solutions and measured the size and potential of the suspensions which are presented in the table 3.1.1.

Chitosan conc(mg/mL)	TPP conc(mg/mL)	Avg Diameter(nm)	Zeta(mV)
1	1	248.2	34
2	2	220.8	44.7
4	2	334	18.5
4	4	298.3	27.6

Table 3.1: Chitosan nanoparticles synthesized with TPP by varying concentrations

3.2.3 Interaction between chitosan and TPP

TPP interacts with chitosan via ionic interactions where the protonated amine groups of chitosan interact with the negatively charged phosphate ion of the salt forming ionic cross linked networks [48]. The extent of crosslinking depends on the concentration of the solutions as well as the reaction rate between them. Fig 3-3 illustrates the mechanism of the binding between TPP and Chitosan [48]. Even though the crosslinking reduces the number of adsorption sites, it has been observed that it helps the polymer remain stable in acidic conditions which are preferred for metal removal applications. Chitosan films as well as nanoparticles cross-linked with TPP have been extensively studied for removing heavy metal ions such as cadmium, copper, lead and manganese.

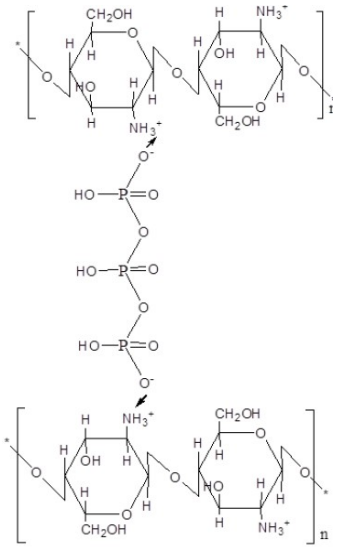

Figure 3.3: Chitosan cross-linking mechanism with TPP

3.3 Chitosan cross-linked with genipin

3.3.1 Ring opening polymerization of genipin

Genipin, a naturally occurring compound, has been widely used antiphlogistics and cholagogues in herbal medicine [2]. One of the advantages of genipin is that it is less toxic compared to other cross-linkers. It is about 5000-10000 times less cytotoxic compared to glutaraldehyde and has been used as a cross-linking agent for the fixation

of biological tissues as bio-prostheses [49, 50]. Genipin has been used as cross-linking agent with chitosan and they are being used as hydrophilic gels for the purpose of biomedical applications [2]. Not much research has been done in the field of using chitosan nanoparticles cross linked with genipin for metal recovery applications. In this study we synthesized nanoparticles of chitosan cross-linked with genipin at different pH conditions and characterized them using size and potential. In the preparation of aqueous genipin solution, it has been observed that the solution turns brownish and viscous under highly basic conditions [2]. There was no interaction between genipin and chitosan when the aqueous solution of genipin (neutral conditions) was used and we couldnt synthesize any particles. Earlier research conducted by Fwu-Long et.al has shown that genipin undergoes a polymerization reaction under highly basic conditions to form macromers via aldol condensation. The polymerized genipin were found to have a molecular weight of 1600-20000 and had approximately 7-88 monomer units [2]. To make the aqueous genipin solution highly basic, we added 1 M KOH (potassium hydroxide) solution and adjusted the pH of the solution to 12.7. At highly basic conditions, the nucleophile OH- in the aqueous solution attacks the genipin molecules resulting in the ring opening reaction to form aldehyde groups and the mechanism is illustrated in the Fig 3-4. It has been observed that without the ring opening reaction of genipin, where it polymerizes with itself via aldol condensation, no cross-linking with chitosan is possible [2]. We prepared the polymerized genipin by adjusting the pH of the solution using KOH and used it in synthesizing the chitosan nanoparticles at different pH conditions.

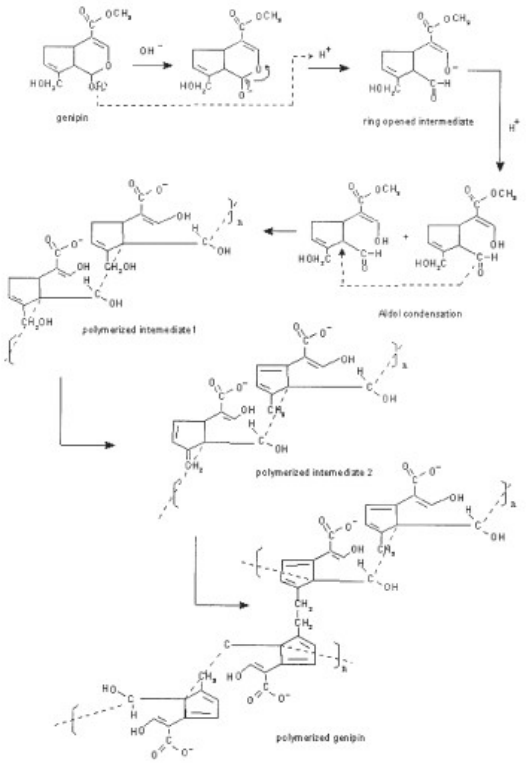

Figure 3.4: prepolymerization of genipin under basic conditions

3.3.2 Chitosan-Genipin nanoparticles synthesized at acidic conditions

3.3.2.1 Size

5 ml of 2 mg/ml chitosan solution was mixed with a certain quantity of polymerized genipin and the pH of the solution was adjusted to 3.5 by adding concentrated acetic acid. We used different volumes of genipin ranging from 0.02 ml to 1 ml for cross-linking and observed that there was cross-linking even when very low volume of genipin was used. We synthesized the particles and determined the size using dynamic light scattering using Malvern Zetasizer. The size of the nanoparticles increased gradually as the amount of genipin was increased but later on decreased when the amount of genipin kept on increasing. We observed the formation of particles indicating cross-linking even when the volume of genipin used was as low as 0.02 ml indicating the strong associating properties of genipin.

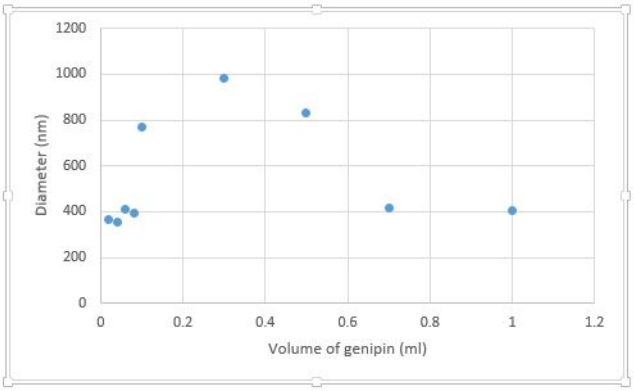

Figure 3.5: Chitosan-genipin nanoparticles-size

Research conducted by Fwu-Long et.al showed that genipin binds with chitosan in different ways, depending on the pH of the solution. Figures 3-6 and 3-7 illustrate the mechanism of genipin cross-linking with chitosan under highly acidic conditions. At acidic and neutral conditions, a nucleophile attack by the amino groups of chitosan on the olefinic carbon atom at C-3 occurs and is followed by opening the dihydropyran ring to form a heterocyclic amine. The intermediate compounds could further associate to form to cross linked networks with short chains of cross-linking bridges [2].

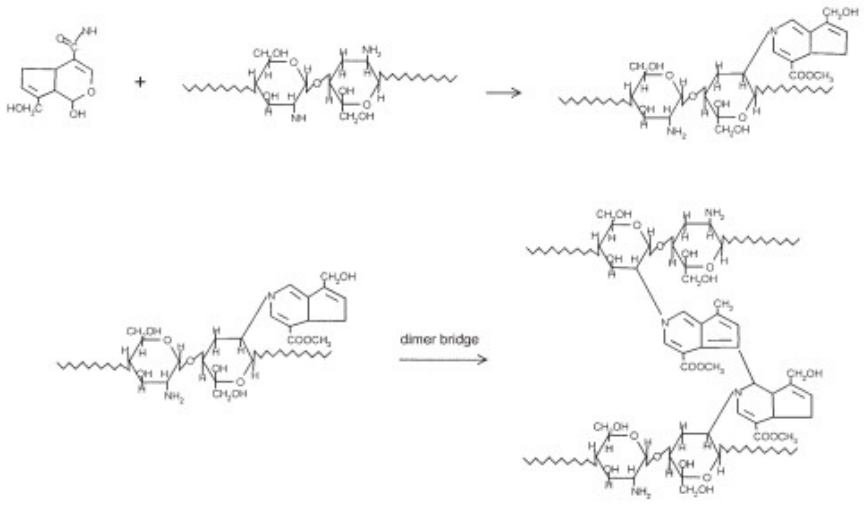

Figure 3.6: Presumed mechanism of Chitosan-genipin cross-linking under acidic conditions- step 1

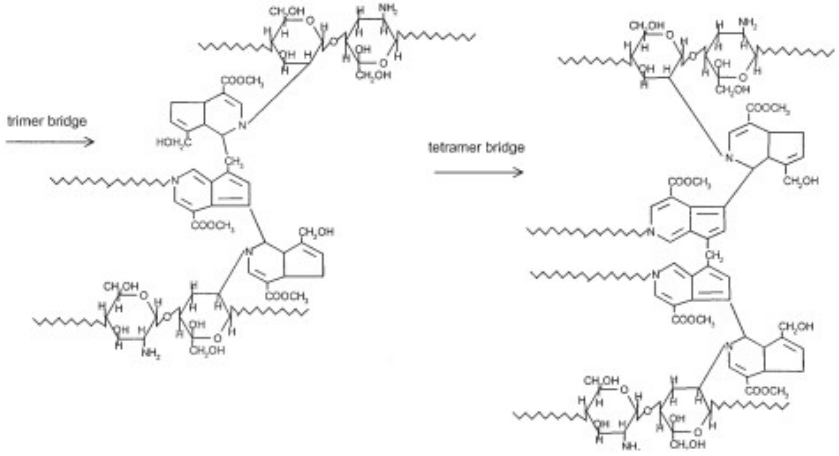

Figure 3.7: presumed mechanism of Chitosan-genipin cross-linking under acidic conditions- step 2

3.3.2.2 Zeta Potential

Fig 3-8 reveals the zeta potential of chitosan nanoparticles cross-linked with genipin. Genipin crosslinks with chitosan forming covalent bonds and the zeta potential followed similar trends to that of TPP. For lower volumes of genipin, the zeta potential was almost the same but as the volume of genipin was increased, the zeta potential increased gradually and later on decreased for much higher concentrations of genipin.

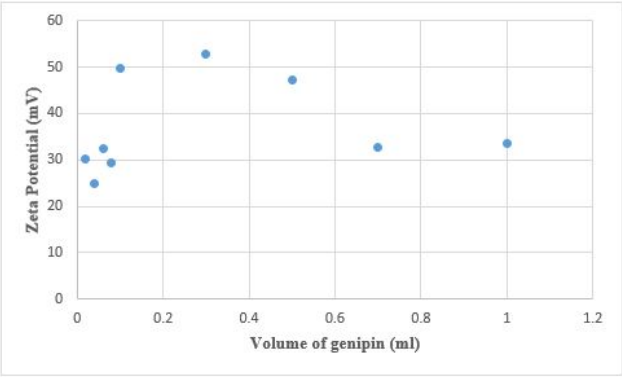

Figure 3.8: Zeta potential of Chitosan-genipin nanoparticles

Zeta potential of chitosan bonded with genipin followed similar trend to that of

chitosan bonded with TPP. Values of zeta potential increased gradually and after a certain point they started decreasing indicating the salting out effect.

Volume of genipin (ml)	Zeta Potential (mV)
0.02	30.1
0.04	24.8
0.06	32.4
0.08	29.3
0.1	49.7
0.3	52.9
0.5	47.1
0.7	32.8
1	33.6

Table 3.2: Zeta Potential of CSNP's with genipin

We also varied the concentrations of the chitosan and genipin solutions and synthesized the nanoparticles under acidic conditions and measured the potential and size of the particles.

chitosan conc(mg/ml)	Genipin(mg/ml)	Volume of genipin(ml)	Diameter(nm)	Zeta(mV)
4	2	0.3	566.8	31.8
4	2	0.7	434.5	31.1
4	4	0.3	415.6	30.6
4	4	0.7	382.5	28.1

Table 3.3: Zeta Potential of CSNP's with genipin using different concentrations at acidic pH

3.3.3 Chitosan-genipin cross linking under basic conditions

Under basic conditions, we managed to synthesize a film but there was no formation of nanoparticles. 0.7 ml of genipin was added to 5 ml of chitosan solution in a 20

ml vial and the pH was adjusted to 12.0 by adding 1 M KOH and was left under stirring. Centrifugation followed by sonication didnt yield any nanoparticles but there was formation of a film in the test tube.

Under basic conditions, the terminal aldehyde groups on the polymeric genipin could undergo a Schiff base reaction with the amino groups on chitosan to form cross linked networks.

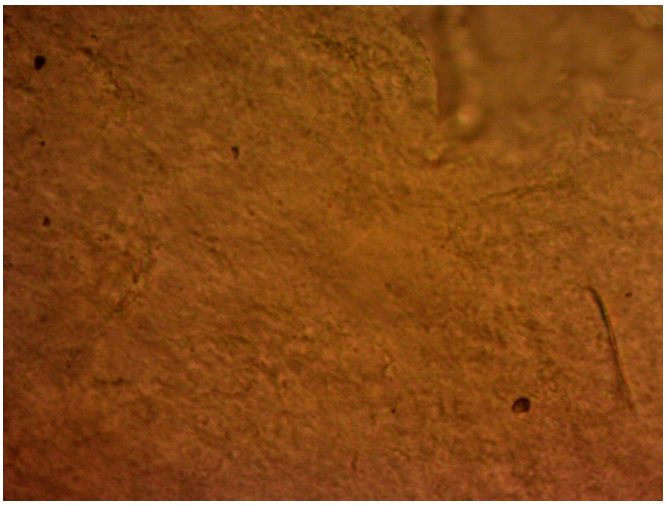

Figure 3.9: Chitosan-genipin film under highly basic conditions (pH = 12)

Therefore the genipin crosslinked chitosan networks prepared in strong basic conditions consist of primary polymer chains of chitosan and long cross link bridges of polymerized genipin as shown in Fig 3.9 and 3.10. It can be understood that pH plays a very important role in determining the cross-linking reactions between genipin and chitosan. As proved by Fwu-Long et.al, under acidic conditions, a nucleophilic attack of genipin at C-3 carbon by chitosan is inhibited by protonation of amine groups.

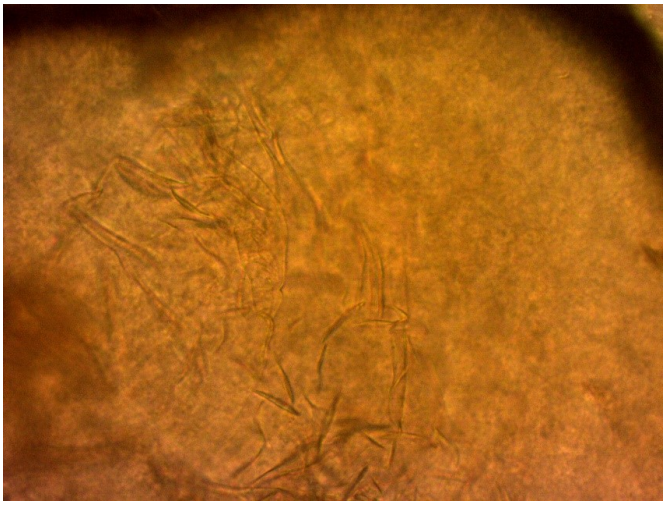

Figure 3.10: Chitosan-genipin film under basic condition (2)

In contrast the presence of acid improves the nucleophilic substitution of the ester group on genipin, enabling the formation of secondary amide with genipin [2].

Therefore chitosan cross-linked with genipin under acidic conditions contains short chains of cross-linked bridges which enabled us to synthesize the nanoparticles.

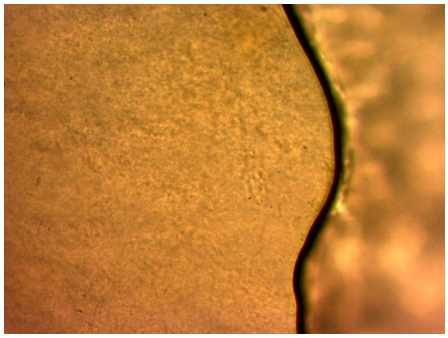

Figure 3.11: Boundary of chitosan-genipin crosslinked film under basic conditions (pH=12)

In contrast, under basic conditions, genipin undergoes self-polymerization reaction prior to chitosan cross-linking resulting in long chains of cross-linked bridges with itself and then with chitosan.

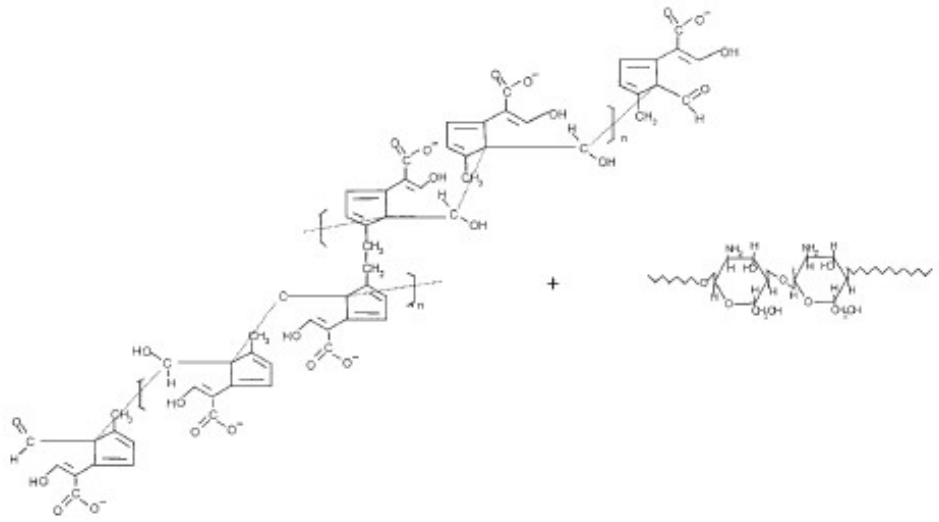

Figure 3.12: Presumed mechanism of genipin chitosan crosslinking under basic conditions (pH=12) step 1 [2]

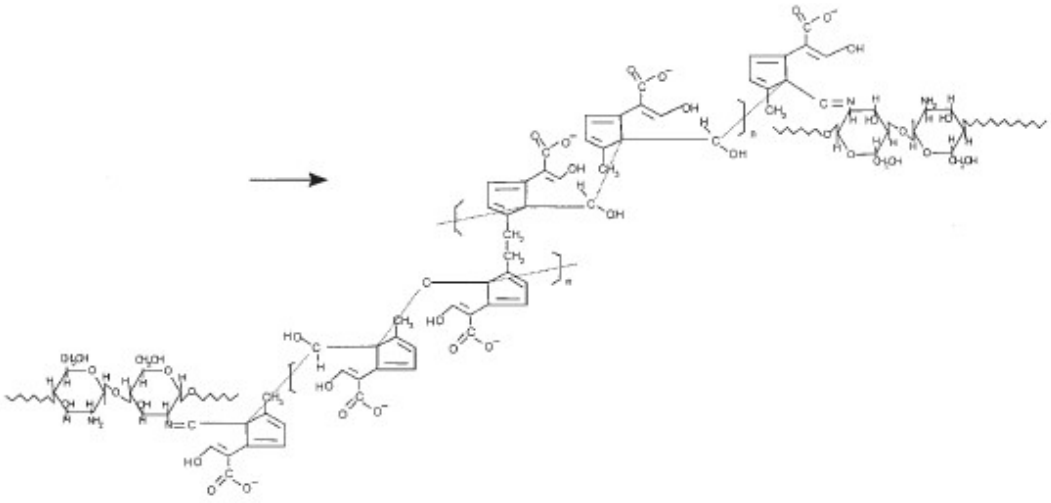

Figure 3.13: Presumed mechanism of genipin chitosan crosslinking under basic conditions (pH = 12) step 2 [2]

We believe this might be the reason for non-formation of particles under highly basic conditions. These films are used a lot in the field of biomedical applications and drug delivery [2].

3.4 Characterization of Polylysine nanoparticles crosslinked with TPP

3.4.0.1 Size

Our study focused on synthesizing the most stable PLNPs crosslinked with TPP for the adsorption experiment. α-PLL has two amine groups, one which participates in the elongation of the chain where as the other which is bonded to the alkyl part of the chain.

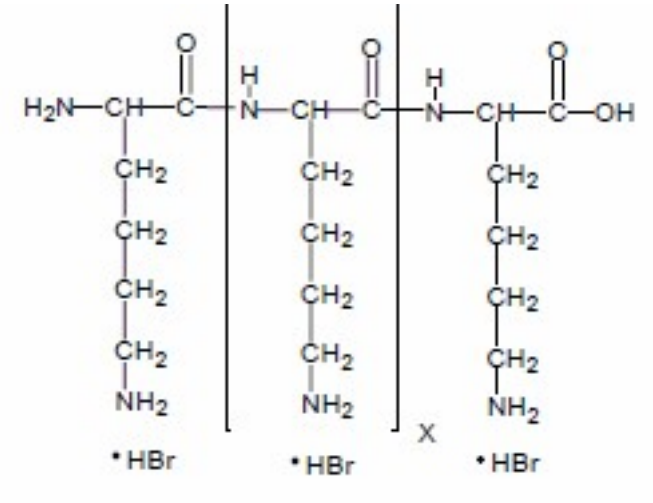

Figure 3.14: α PLL Hydrobromide polymer structure [Sigma Catalogue]

Under acidic and neutral conditions the amine group attached to the alkyl part of the chain is protonated and it forms cross-links with the negatively charged counterion of TPP resulting in a crosslinked network. We synthesized the nanoparticles by adding 0.7 ml of TPP and 0.3 ml of DI water to 5 ml of 2 mg/ml -PLL solution in a vial and left them under stirring followed by centrifugation and sonication. Concentration of Polylysine as well as TPP solutions used were 2 mg/ml. As the amount of the TPP used was increased in small amounts, there was a gradual increase in the size of the nanoparticles and the particles prepared using 1.5 ml of TPP had the highest size.

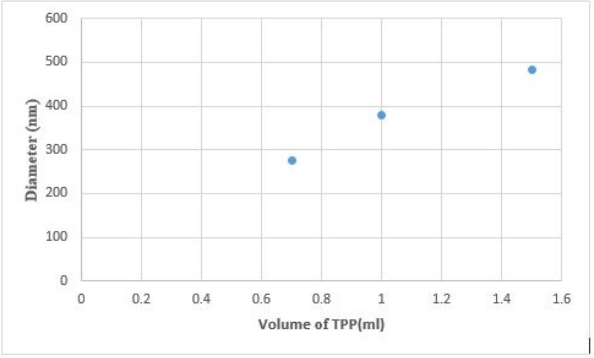

Figure 3.15: Size of Polylysine nanoparticles crosslinked with TPP

3.4.0.2 Zeta Potential

Zeta Potential of the PLNPs crosslinked with TPP were measured using the Malvern Zetasizer and the values are presented in the Fig 3.16.

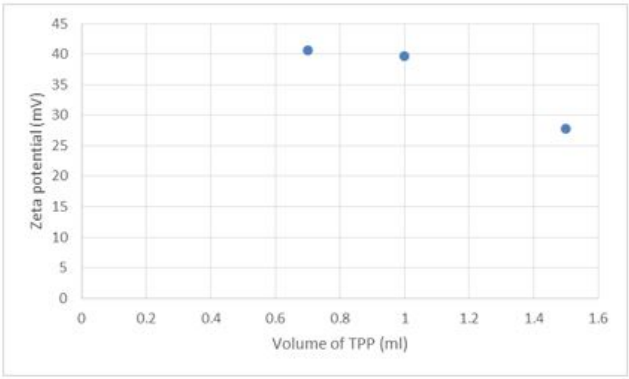

Figure 3.16: Zeta Potential of Polylysine nanoparticles crosslinked with TPP (2 mg/ml conc of TPP and PLL

As the amount of TPP was increased gradually, the zeta potential decreased showing the salting out effect. For the adsorption experiment we used the PLNPs that were synthesized using 1 ml of TPP. Even though the zeta potential is slightly on the lower side compared with the PLNPs formed using 0.7 ml of TPP, the yield of the sample was much better in the former. We also measured the zeta potential of the PLNPs that were synthesized using different concentrations of the cross-linker TPP and the values are shown in the Fig. 3-.17.

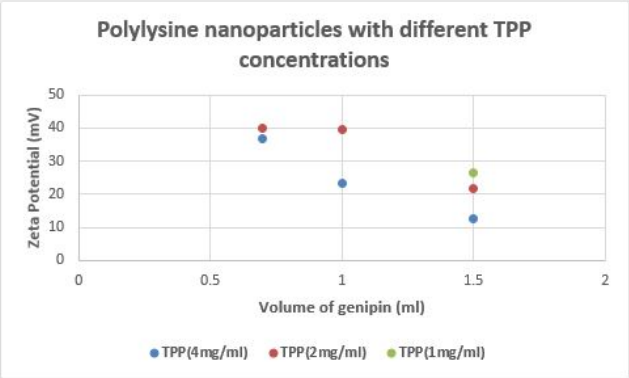

Figure 3.17: Zeta potential of PLNPs prepared using different concentrations of TPP

Highest values of the zeta potential were observed when the concentration of TPP used was 2 mg/ml indicating that the PLNPs suspensions are most stable. No formation of nanoparticles were observed when the volume of TPP used was 0.7 ml and 1 ml for the concentration of 1 mg/ml. There was no noticeable crosslinking between polylysine and TP when the polylysine solution used was acidic.

3.5 Characterization of PLNPs cross-linked with genipin

3.5.1 Size

Polylysine nanoparticles crosslinked with genipin were prepared by using plain polylysine solution (no acetic acid) and pre polymerized genipin. Volume of genipin used for cross-linking was varied from 0.02 ml to 1 ml and we observed the formation of nanoparticles as well as cross-linking even when the volume of genipin was as low as 0.02 ml indicating the strong binding abilities of genipin. The yield of nanoparticles was very less when the volume of genipin used was low (0.02ml, 0.04 ml, 0.06 ml and 0.08 ml) but as the amount of genipin was increased there was significant amount of yield after centrifuging.

Fig 3-14) PLNPs crosslinked with genipin (neutral pH conditions; pH = 7.3)

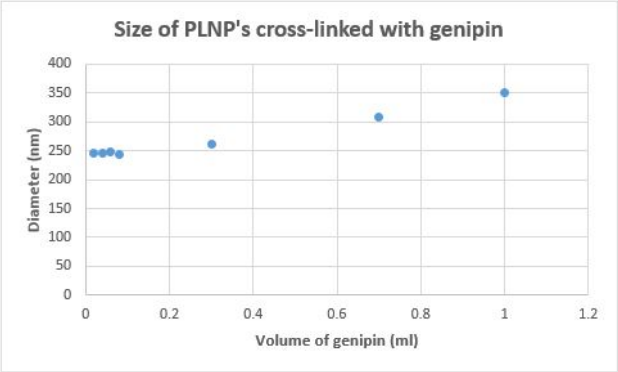

Figure 3.18: PLNPs crosslinked with genipin (neutral pH conditions; pH = 7.3)

For the lower volumes of genipin, the size of the nanoparticles was observed to be in the same range but as the amount of genipin was increased gradually, the size of the nanoparticles increased indicating the increase in the extent of cross-linking. We believe that the cross-linking of genipin with Polylysine occurs via aldol condensation under neutral conditions similar to that of chitosan but the validation of the claim with experimental evidence is beyond the scope of this study.

Volume of genipin (ml)	Diameter (nm)
0.02	245.1
0.04	246.7
0.06	247.5
0.08	244.7
0.3	262.1
0.7	308.4
1	350.1

Table 3.4: Zeta Potential of PLNP's with genipin

3.5.2 Zeta Potential

Fig 3-19 indicates the zeta potential of the genipin crosslinked polylysine nanoparticles using 2 mg/ml concentrations of the polymer and the cross-linker.

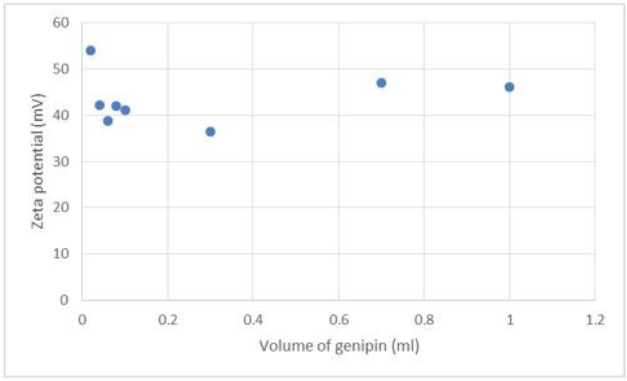

Figure 3.19: Zeta Potential of Polylysine nanoparticles crosslinked using genipin

Zeta potential values of the nanoparticles were in a similar range when low amounts of genipin were used but as the volume increased there was a slight increase in the zeta potential values. Overall, the values of zeta potential for PLL nanoparticles crosslinked with genipin were high indicating the stability of the solutions. We also synthesized PLL nanoparticles using genipin under acidic and basic conditions and the size and zeta potential values of the particles are listed in the table 3.5

Volume of genipin (ml)	pH of solution	Diameter (nm)	Zeta Potential (mV)
0.7	2.75	149.4	21.7
0.7	12.09	338.4	11.9
0.7	7.2	308.4	32.6

Table 3.5: Zeta potential of PLNP's with genipin under different pH conditions

The pH of the solution was adjusted accordingly by using concentrated acetic acid or 1 M KOH during the stirring and the solution was left overnight for it to reach equilibrium before being centrifuged the next day. From the zetapotential values obtained, the stability of the suspension was least at basic conditions compared to acidic and neutral conditions. This can be explained by the fact that at high pH conditions, none of the amine groups are protonated and there is least interaction between genipin and polylysine.

3.6 Characterization of Chitosan-polylysine nanoparticles using genipin

3.6.1 Size

Chitosan-polylysine gels crosslinked with genipin are being used in tissue engineering applications [54]. We wanted to synthesize the nanoparticles of chitosan-polylysine and we tried to achieve that using pre-polymerized genipin solution. Chitosan-polylysine solutions were mixed in the ratio of 10:1 and the final polymer solution concentration was around 2 mg/ml. Fig 3.20 shows the sizes of the CPNPs prepared using genipin.

Volume of genipin (ml)	Diameter (nm)
0.03	595.35
0.05	560.3
0.07	470.3
0.09	630.3
0.1	529.5
0.3	566.8
0.5	470.5
0.7	492.5

Table 3.6: Size of CPNP's synthesized using genipin

A schematic representation of genipin crosslinking with chitosan and polylysine is shown in the fol8lowing figure 3.21 [51]

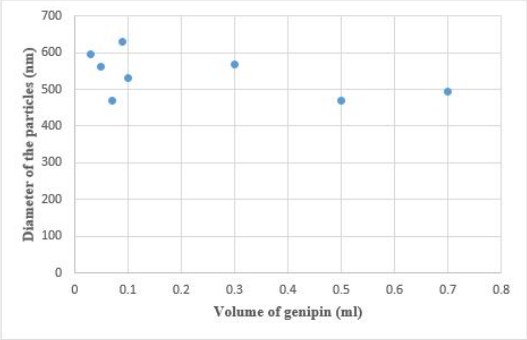

Figure 3.20: Size data of CPNPs crosslinked with genipin (2 mg/ml conc of the polymer)

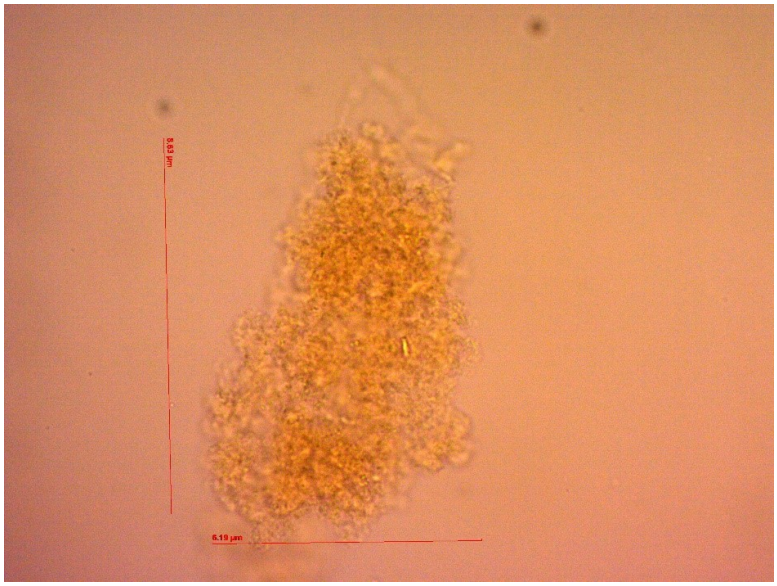

Figure 3.21: Chitosan-polylysiine particle cross-linked with genipin aggregate

Chitosan being abundant and polylysine being a good adsorbent for metal ions, mix nanoparticles of chitosan and polylysine can be tested for metal recovery applications.

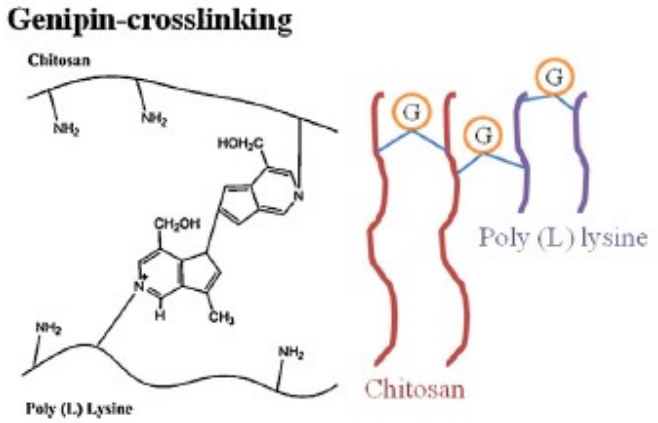

Figure 3.22: Genipin- Chitosan-Polylysine crosslinking network [54]

3.6.2 Zeta Potential

Table 3.7 lists the zeta potential values of CPNPs cross linked with genipin.

Volume of genipin (ml)	Zeta Potential (mV)
0.03	18.03
0.05	23.03
0.07	29.4
0.09	34
0.1	42.8
0.3	26.6
0.5	32.6
0.7	41.5

Table 3.7: CPNP's with genipin - Zeta Potential

3.6.3 CPNPs crosslinked with TPP

We also synthesized CPNPs by using different molecular weights of Polylysine ranging from 15,000 Da to 300,000 Da. The zetasizing data is shown in the table 3.8

CPNP (Mol wt of Polylysine)	Diameter (nm)	Zeta Potential (mV)
15,000-30,000 Da	193.6	26.23
30,000-70,000 Da	211.1	41.2
70,000-150,000 Da	188.3	33.6
150,000-300,000 Da	194.8	41.4

Table 3.8: CPNP's with TPP - Varying mol wts of Polylysine

Nanoparticles synthesized using the 30,000-70,000 Da and 150,000-300,000 Da had the highest Zeta potential values indicating the stability of the solution where as the sizes of the nanoparticles were around the same range for all the molecular weights.

3.7 Comparison of size and potential of various nanoparticles synthesized using genipin

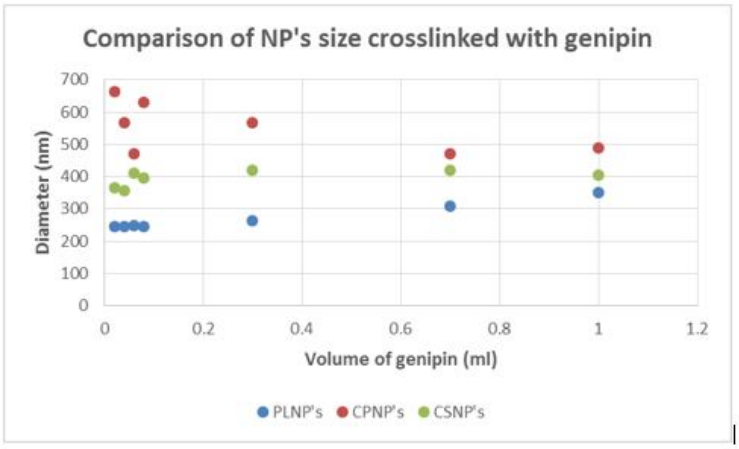

Figure 3.23: Particle size comparison of different nanoparticles crosslinked with genipin

From the synthesis data that we obtained, it can be noted that the chitosan-polylysine nanoparticles cross-linked with genipin had a larger size compared to the nanoparticles of pure polylysine or chitosan cross-linked with genipin when same amounts of cross-linker were used which is illustrated in the Fig 3.22. Genipin when cross-linked with two polymers had significantly higher size when compared to bonding with a single polymer.The pH of the chitosan-polylysine and chitosan nanoparticle suspensions was 3.5 where as the the pH of polylysine nanoparticle suspension was around 7.2 .

Fig 3.23 illustrates the values of zeta potential of different types of nanoparticles that were synthesized using genipin. Nanoparticle suspensions of polylysine and chitosan cross-linked with genipin had higher values of zeta potential compared to that of the mix nanoparticles and as the volume of genipin was increased, the values of potential gradually increased and then decreased after a certain limit

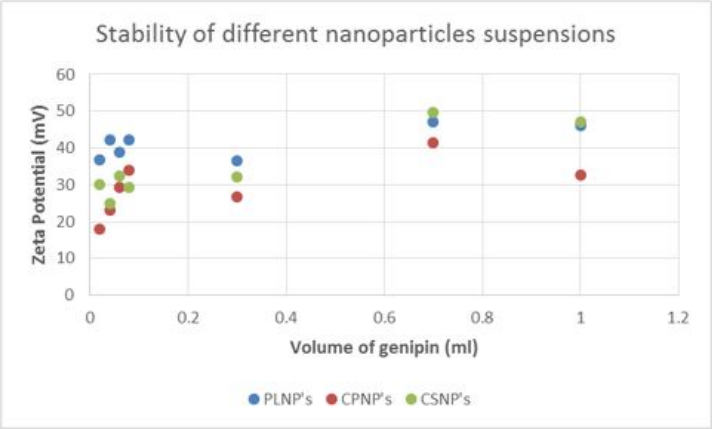

Figure 3.24: Zeta potential comparison of different nanoparticles crosslinked with genipin

All the nanoparticle suspensions had the highest zeta potential values when the volume of genipin used was 0.7 ml.

3.8 Equilibrium adsorption experiment

For the adsorption experiment, we used α- Poly-L-Lysine nanoparticles crosslinked with 1 ml of TPP because of their better yield and high values of zeta potential. To obtain polylysine nanoparticles laden with copper ions, we mixed 3 ml of copper sulfate solution to the nanoparticles and sonicated the mixture to uniformity. The pH of the nanoparticle solution after adding copper sulfate was adjusted to around 5.5 so that the amine groups of polylysine remain protonated and can take part in the adsorption. Higher pH favors the formation of copper hydroxide and to avoid it, we adjusted pH to be in acidic region. The mixture was sonicated till it was uniform in the same centrifuge test tube and was left under magnetic stirring for one week. The particles were centrifuged once again at 48,400 g (20000 rpm) for 20-30 minutes at the end of stirring. The supernatant was collected and was used to measure the intensity of the sample in MRI scanning. Even though ICPMS (Inductively coupled plasma mass spectroscopy) is the most common method used for determining the amount of adsorption, we tried using MRI to determine the amount of adsorption by measuring the

difference in intensities of the samples and standards. Initially, we used water samples as a control run to consider the effects of positioning on the magnetic field intensity and determined the intensities for the water run. Later we inserted the standard samples of Copper sulfate having concentration from 0.5- 50mM along with the corresponding supernatant solutions to calculate the intensities of the samples. The intensities of the standards and supernatants are listed below for the copper sulfate samples in the table 3.9

Sample name	Intensity
0.5s	1022.48
0.5sn	878.2
1s	1223.56
1sn	897.19
2s	1501.78
2sn	1088.45
5s	1843.05
5sn	1179.22
10	2015.73
10sn	1912.14
50s	2318.77
50sn	2209.75

Table 3.9: Intensities of standards and supernatants for MRI data

s denotes the standard sample (original concentration) and sn denotes the supernatant sample. The difference in intensities between the standards and supernatants was used to estimate the amount of adsorption. The calibration curve was developed using the intensities of the standard samples and the final concentration of the supernatants was estimated using the calibration curve.

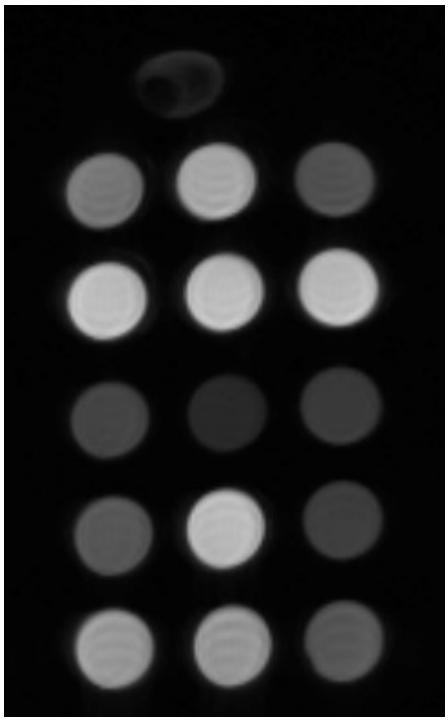

Figure 3.25: MRI scanning image of samples

Fig 3.23 indicates the positioning of samples inside the coil

	H2O	
2s	10s	2sn
50s	30s	50sn
0.5s	H2O	0.5sn
1s	30sn	1sn
5s	10sn	5sn

Figure 3.26: Samples positioning in the MRI scanner

From the final concentrations of the supernatants we estimated the amount of adsorption of copper that took place.

Standard conc (mM)	Standard intensities	Supernatant intensities	Supernatant conc (mM)
0.5	1022.484	878.19	0.27
1	1223.56	897.17	0.28
2	1501.78	1088.445	0.54
5	1843.05	1179.223	0.7344
10	2015.72	1912.13	8.37
30	2360.799	2209.754	22.48
50	2318.77	2343.60	35.06

Table 3.10: Supernatant concentration as calculated from calibration curve

Fig 3-20 denotes the calibration curve that was used for the calculation of supernatant concentrations.

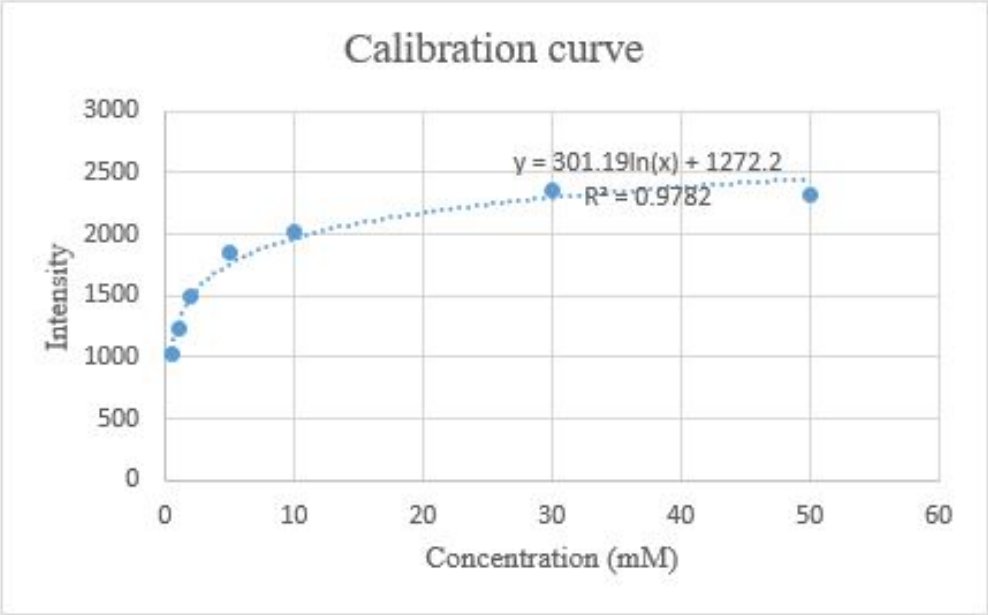

Figure 3.27: Calibration curve used for finding supernatant concentration

3.8.1 Particle adsorption capability

Freundlich and Langmuir isotherms are generally used to evaluate the amount of adsorption and they relate the total amount of metal adsorbed per unit mass of adsorbent, Q(mg/g), to the equilibrium metal ion concentration, Ce(mg/L). Langmuir isotherm fits a variety of data and it is represented as follows: [1, 16]

$$Q = \frac{Q_{max} K_s C_e}{1 + K_s C_e} \tag{3.1}$$

The linear form of the equation is represented by the following equation:

$$\frac{C_e}{Q} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_s} \tag{3.2}$$

Where Q_{max}(mg/g) is the maximum adsorption capacity of the adsorbent at monolayer coverage, and K_s is the Langmuir adsorption equilibrium constant related to the affinity of adsorptive sites for adsorbate at dilute concentration. The Langmuir isotherm assumes that adsorption layer is monolayer and adsorption occurs at specific homogenous adsorption sites resulting in identical energy adsorption at each site. Another assumption it takes into account is that the intermolecular forces decrease rapidly with increasing distance from the adsorptive sites. The equilibrium adsorptive capability of Polylysine nanoparticles crosslinked with TPP were investigated using the Langmuir isotherm where Q (mg/g) is the amount of Cu+2 adsorbed per unit mass of the adsorbent and Ce is the equilibrium concentration of the Cu+2 solution after adsorption. The experimental data of Q and Ce of 2 mg/mL of Polylysine nanoparticles crosslinked with 1 mL of TPP are plotted in the figure 3-25.

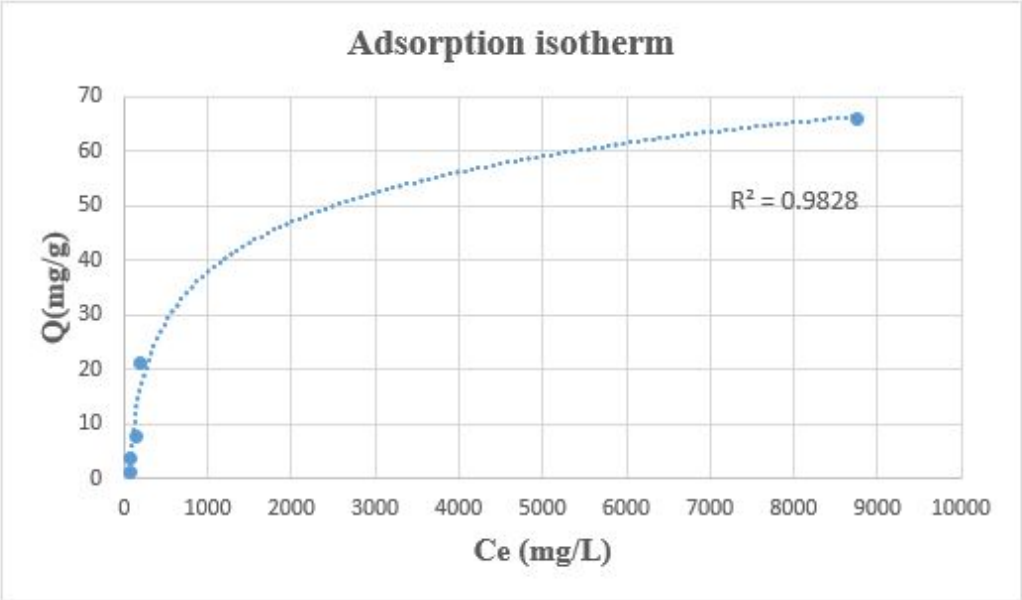

Figure 3.28: Langmuir isotherm relating Q and Ce

The linear form of the Langmuir isotherm model is shown in the following figure 3-26

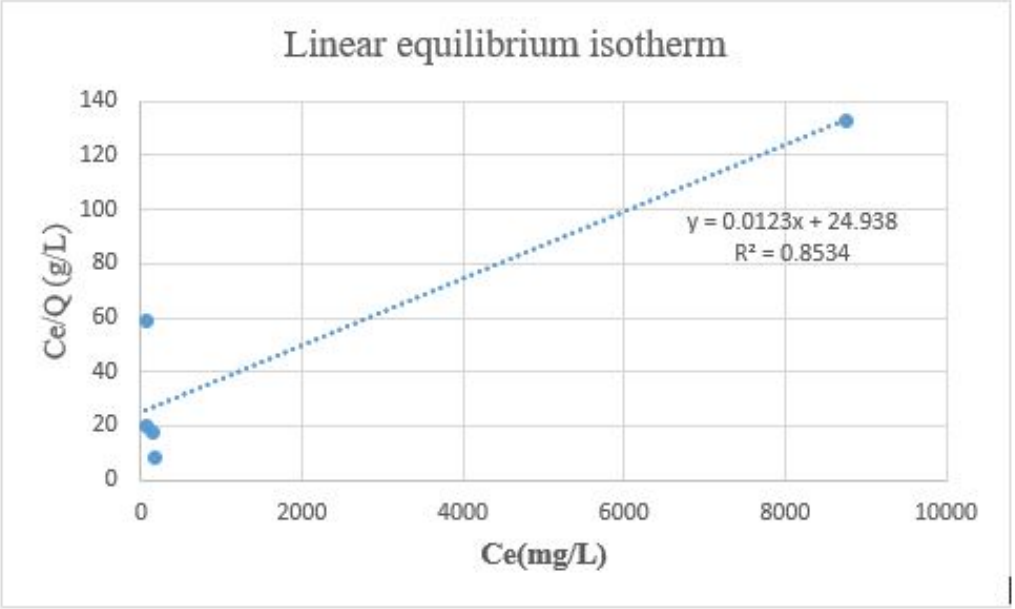

Figure 3.29: Linear Equilibrium isotherm

The linear form of the Langmuir isotherm is used to estimate the Q_{max} and K_s values to determine the adsorption capacity of PLNPs crosslinked with TPP. From the obtained equation, the value of Q_{max} for wet mass of the pellet was calculated to be 81.3 $\pm$ 30 μg/mg and the value of K_s was estimated to be around 0.0049 L/mg. From the obtained low K_s value, we can state that polylysine has a good affinity towards the Cu+2 ions. Compared to literature values, Q_{max} of α-polylysine in adsorbing hexavalent chromium is 42 mg/g [10], it shows that α-PLL is a very good adsorbent for divalent copper compared to chromium.

Polymer and adsorbed ion	$Q_{max}(wet, mg/g)$	K_s (L/mg)
Chitosan nanoparticles with divalent copper	4.24	0.0088
Alginate microbeads with divalent copper	18.32	0.0063
α-PLL solution with hexavalent chromium	42.6	0.0059
α-PLL nanoparticles with divalent copper	81.3	0.0049

Table 3.11: Adsorption capacities of various polymers

Even though ICPMS is the most commonly used technique for measuring the amount of adsorption, in this study, we used MRI scanning to verify if we can determine an estimate regarding the amount of adsorption using this technique. We believe further studies need to be conducted to validate the values and compare them with the literature values to determine the efficiency of this method.

Chapter 4

Conclusion and Future Work

We synthesized the nanoparticles of polymers chitosan, plylysine using TPP and genipin as the crosslinkers under different conditions and characterized them using zeta potential. Apart from TPP and genipin, we tried synthesizing the nanoparticles of the polymers using EDTA as the crosslinker by following similar protocol but were unsuccessful. All the different permutations of nanoparticles that we synthesized in this study can be further analyzed based on their colloidal stability and used for various applications. On the other hand genipin proved to be a very good cross linker with both Chitosan as well as Polylysine and the crosslinking took place even when the volume of genipin used was as low as 0.02 ml. Nanoparticle suspensions produced with genipin also had very high zeta potential values indicating the stability of the solution and all the different permutations can be tested for metal recovery applications. When bonded to chitosan, we could synthesize the CGNPs only under acidic conditions and there was formation of film under basic conditions. Regarding polylysine, even though abundantly available, one of the draw backs is that it is expensive compared to other polymers such as chitosan or alginate. The best way to utilize polylysine can be binding it with other polymers such as chitosan in an optimum ratio and then use the mix nanoparticles for various applications. This research concentrated on removing copper ion alone and this study can be further extended to other heavy metal ions. A lot of research can be done in this field to synthesize the best and most suitable nanoparticles for heavy metal recovery applications.

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