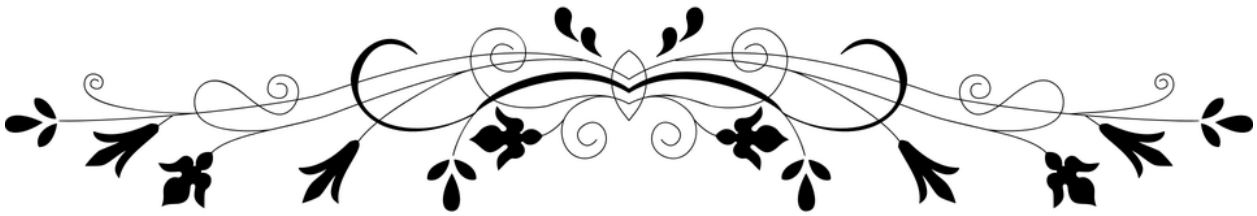

CHAPTER 2

Review of Literature

2. Review of Literature

In this chapter, early work reported on the synthesis and characterization of hydrogels is discussed. Additionally, different types of approaches used to develop hydrogels are also discussed. The primary focus of this chapter is to highlight the hydrogel history, critical discoveries, physical and chemical methods used to synthesize hydrogel. The significant drawbacks of physical hydrogels and problems associated with Chitosan are also discussed. Based on this critical review aim and objective of the current thesis have been formulated.

The first work on the hydrogel has been reported by Wichterle and Lim in 1960 with the crosslinking polymer based on copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA)[22].

Refojo (1965) showed that Glyceryl methacrylate-based hydrogel could be used for ophthalmic applications. They found a linear relationship between water content and the refractive index of the hydrogel [23].

Merrill et al. (1970) has synthesized Polyvinyl alcohol-heparin hydrogel using glutaraldehyde as a crosslinker for their potential application as diffusion membrane in hemodialysis[24].

Voldbich et al. (1975) developed Poly (glycol Monomethacrylate) based hydrogel for plastic operation of the nose. These hydrogels have been used in humans to fix L-shaped grafts during plastic surgery of the nose [25].

Peppas et al. (1980) formulated glutaraldehyde crosslinked Polyvinyl alcohol hydrogel. These specific hydrogels have been tested as injection material for the treatment of vocal cords [26].

Beekhuis et al. (1985) studied hydration stability of intracorneal hydrogel implanted in the corneal stroma. They observed that hydrogel having water content up to 69.5% are stable in the corneal environment[27].

Shalaby et al. (1990) have developed hydrogel using 1- Vinyl-2- Pyrrolidone as monomer and functionalized albumin as a crosslinker for gastric drug delivery system. The developed hydrogels were degradable in the presence of the pepsin enzyme [28].

Cascone et al. (1995) demonstrated growth hormone delivery using a blend of natural polymer and synthetic polymer. They have used Hyaluronic acid/ Poly(acrylic) sponge, Collagen /Polyvinyl alcohol hydrogel, and Hyaluronic acid / Polyvinyl alcohol hydrogel as a matrix to deliver growth hormone [29].

Risbud et al. (2000) reported hydrogel composite based on Polyacrylamide and Chitosan using N, N methylene bisacrylamide. They have demonstrated the potential of these hydrogels as a drug delivery matrix for the delivery of amoxicillin[30].

Tripodo et al. (2005) synthesized based on Inulin for pH-responsive drug delivery. During the synthesis, the Inulin derivative was prepared by reacting Inulin with methacrylic anhydride. The Inulin- methacrylic anhydride derivative was further treated with UV to get crosslinked network. A model drug Ibuprofen was loaded into these hydrogels, and its release profile was studied [31].

Dadsetan et al. (2010) developed stimulus-sensitive negatively charged hydrogel based on Oligo (poly (ethylene glycol) fumarate) and Sodium Methacrylate. These hydrogels were sensitive to both pH and ion. They have used the developed hydrogel for the delivery of doxorubicin. Drug delivery from these hydrogels occurs through an ion-exchange mechanism [32].

Lu et al. (2015) have demonstrated the potential of hydrogel for cell encapsulation. They have synthesized hydrogel composed of Chondroitin Sulfate multiple aldehydes and N-Succinyl Chitosan. To perform a cell encapsulation study, they have loaded Hela cells into these hydrogels. These hydrogels are resistant to mechanical damage [33].

Hydrogels based on synthetic polymer and natural polymers have been used for soft contact lens, drug delivery systems, tissue engineering, implants and wound dressing applications. These materials are preferred for controlled drug delivery applications, because it shows some astonishing properties such as its biocompatibility with soft tissue, and uniform distribution of drug in the hydrogel matrix. Beside this, we can control the physical and chemical behaviour of hydrogel network. Recent research on hydrogel has inclined to develop more biocompatible and non-toxic materials for various bioengineering applications [22]. Hydrogel can be defined as a three dimensional crosslinked polymer network having hydrophilic components and resists dissolution in water[34].Hydrogel network can be developed by either physical crosslinking or chemical crosslinking. Sometimes, both physical crosslinking and chemical crosslinking techniques are used simultaneously to develop hydrogel network. Their mechanical strength depends on various factors such as network morphology, chemical nature and equilibrium swollen state [35].

Hydrogels can be developed by either natural polymer or synthetic polymer. Synthetic polymers are preferred to develop hydrogel with respect to natural polymer due to its low cost and versatility[36].Among natural polymers, Chitosan is a copolymer composed of N-acetyl-D-glucosamine and D-glucosamine. It is produced by alkaline deacetylation of chitin. Chitosan shows some drawbacks such as poor tensile strength, and water insolubility [37].Chitosan lactate is water soluble derivative of chitosan [38]. Polyvinyl alcohol is a synthetic polymer and has been widely tested for various biomedical

applications such as artificial skin, artificial cartilage, wound dressing and drug delivery matrices due to its astonishing properties such as mechanical property and biocompatibility[39]. Therefore, chitosan lactate and polyvinyl alcohol-based hydrogel composite will enable the formation of new material which is water-insoluble as well as mechanically robust, viscoelastic, and biocompatible as well.

2.1 Development and characterization of physically crosslinked hydrogel

Cascone et al. have reported the synthesis of Poly (Vinyl alcohol) based physical hydrogel using freezing thawing method. The major purpose of this work was to develop hydrogel based 'Polymeric hybrid tissue'. To develop this hydrogel, they have used two kinds of solvents namely culture media and water. Beside this, in order to achieve polymeric hybrid tissue, hydrogels were loaded with 3T3 cells (mouse fibroblast cell line) and human umbilical vein endothelial cell. In order to know cell adhesion potential of these hydrogels, these hydrogels were seeded with 3T3 Cell line and human umbilical vein endothelial cell. But the major problem of these hydrogels is poor cell adhesion and cell growth over its surface[40].

Vrana et al. developed polyvinyl alcohol/ Chitosan hydrogel by the physical method using the freeze-thaw technique. They have studied the impact of the freeze-thaw cycle on developed hydrogel on cell behavior. They have observed significant improvement in developed hydrogel properties such as protein adsorption, wettability, surface texture upon changing freeze-thaw cycles[41].

Using the freeze- thaw method, Mathew et al. reported hydrogel composite from Polyvinyl alcohol (PVA) and Chitosan (water-soluble). They have studied various properties of these hydrogels by scanning electron microscopy and mechanical property. In this method, the freeze thaw method crosslinked only PVA, not Chitosan. To crosslink

Chitosan, PVA Chitosan membrane were further dipped in sterile coagulation bath (containing 70 gram KOH, 1-liter water, and Na_2 SO_4). This makes synthesis process more complex. Besides, hydrogels prepared by this method displayed poor mechanical strength[42].

Guanghua et al. developed PVA/ Chitosan hydrogel by the physical method through the freezing-thawing method. The swelling study of these hydrogels was performed in simulated intestinal and gastric fluid. The good miscibility of PVA and Chitosan was confirmed by FTIR, SEM, and DSC techniques [43].

Pei et al. synthesized composite hydrogel from PVA, Chitosan, and alginate hydrogel. The developed hydrogel has displayed excellent light transmittance, higher water transmission. In addition to this, they have loaded ornidazole, and its release was studied. Based on these characterizations, they have suggested that these hydrogels may be used as wound dressing material [44].

Tang et al. proposed the development and characterization of temperature sensitive PVA/Chitosan/ hydroxyapatite hydrogel to facilitate protein delivery. The synthesis of the hydrogel was carried out using *in situ* and *ex situ* routes. The hydrogel exhibited lower crystallinity, partial substitution by carbonate and similar structural properties as that of biological appetites. The rheological analysis suggested enhanced strength of the *in situ* synthesized hydrogel as compared to pure chitosan/PVA gel. Comparison of the efficacies of the hydrogels containing different percentage of hydroxyapatite revealed that the hydrogel containing 0.1 mM hydroxyapatite prepared using *in situ* procedure had lowest degree of swelling and slowest release rate of protein. Based on the results, the authors suggested that the developed hydrogels can be suitable candidates for applications like protein delivery, artificial bones and tissue engineering. Since the

composite hydrogels were developed by PVA, Chitosan and hydroxyapatite, the resultant hydrogels will be non-homogenous in nature. But, due to the inorganic nature of hydroxyapatite, these hydrogels are not suitable for topical drug delivery[45].

Bahrami et al. developed hydrogel by blending PVA and Chitosan, followed by crosslinking with glutaraldehyde. Mechanical study revealed that crosslinking has improved the tensile strength of hydrogel. But, elongation of the hydrogel has been reduced due to crosslinking. Contact angle measurement for all the samples were performed in dry state and wet state. All the samples exhibited lower contact angle in wet state with respect to dry state. Water uptake studies of all the samples were performed. Water uptake study revealed that, water uptake study increases with increase in the PVA concentration. Beside this, Crosslinking reduced the water uptake capacity of these samples. The developed PVA Chitosan-based hydrogel needs to dip into 1 N NaOH and Saturated Na_2SO_4 to separate residual chemicals, followed by washing with deionized water to separate unreacted chemicals, alkali [46].

Levic et al. synthesized PVA / SA cross-linked by freeze-thaw method for encapsulating d- limonene. This network is further treated with calcium to induce ionic interaction. But these hydrogels can be used for food processing purpose. Limonene was encapsulated into physical hydrogel composed of sodium alginate and Polyvinyl alcohol. The purpose of encapsulation is to protect the aroma of limonene. But at a low concentration of limonene (1% w/w), no leakage of aroma was found, but at a higher concentration (5% and 10% w/w), leakage of aroma was observed. This is the major problem observed with these hydrogel composites.[47].

Yang et al. developed polymeric membrane from PVA and Chitosan by using radiation techniques. These polymeric membranes were further subjected to freezing-thawing. The effect of both techniques was observed in the physicochemical properties of a hydrogel,

such as enhanced swelling behavior and improved thermal property concerning membrane developed by the freeze-thawed hydrogel. These hydrogels have also shown improved properties concerning hydrogel prepared by freeze-thawed and subsequent irradiation. They have also demonstrated that hydrogel membranes prepared from irradiation alone are not suitable for wound dressing due to their insufficient mechanical property. Apart from this, the PVA / Chitosan hydrogel prepared by freeze-thaw followed by irradiation exhibited antibacterial property against E Coli bacteria as a function of Chitosan concentration [48].

In 1999, Cascone first reported the composite hydrogel from PVA and Chitosan by using the freezing-thawing technique. They have also developed hydrogel composite from Polyvinyl alcohol and water-soluble Chitosan. The variation in thermal behavior and morphology of developed hydrogel were studied by changing the concentration of Chitosan. Based on the obtained result, they explained the formation of a less regular structure of composite hydrogel membrane with the rise in Chitosan concentration. Therefore, the obtained hydrogel matrix was more porous [49].

Kim et al. reported the development of PVA and sodium alginate loaded with nitrofurazone for wound healing applications. To develop these hydrogels, they have used the freeze-thawing method. Based on the obtained result, they have shown that with the increase in the concentration of sodium alginate, thermal stability, elasticity, and swelling capacity of composite hydrogel were improved. But they have observed a decrease in the mechanical property of composite hydrogel with the increase in the concentration of sodium alginate [50].

Hwang et al. synthesized hydrogel composite from PVA and dextran. To develop these hydrogels, they have used the freeze-thawing method. The variation in physicochemical

of these hydrogels was studied with the change in the amount of dextran. But due to the addition of dextran, the mechanical strength and thermal stability of composite hydrogel have reduced. With the increase of dextran, some of the hydrogel properties have improved, such as elastic behavior, water vapor transmission, swelling capacity, and porosity. This enhancement was observed due to an increase in hydrophilic behavior and miscibility of dextran with polyvinyl alcohol. Further, the wound healing potential of polyvinyl alcohol/ dextran hydrogel loaded with gentamicin was evaluated with respect to polyvinyl alcohol/ dextran hydrogel without drug. A significant improvement in wound healing was observed with drug-loaded composite hydrogel [51].

Peppas et al. reported PVA crosslinked hydrogel by the freezing-thawing method. They developed semicrystalline PVA gel by subjecting freezing-thawing to PVA solution. The obtained network was due to the induction of crystallite in polymer chains of Polyvinyl alcohol [52].

Ma et al. developed physical hydrogel using the above approach based on Polyvinylpyrrolidone / PVA as an implant material for cartilage replacement. Due to its three-dimensional network and water content, hydrogels exhibit natural tissue-like properties. The mechanical property and tribological property of these hydrogels were evaluated. The high mechanical strength of these hydrogels was observed due to the formation of the hydrogen bond. Therefore, it behaves like viscoelastic material similar to native cartilage. Although the developed hydrogels are suitable materials for cartilage replacement based on their viscoelastic behavior and coefficient of friction, their attachment with host tissue has not been evaluated[53].

Takamura et al. developed PVA based drug-releasing matrix by the freeze-thaw method. They have evaluated the impact of drug release from PVA hydrogel with pluronic L -62 or sodium alginate. The physical strength of these hydrogels was evaluated. It has been

observed that the physical strength of these hydrogels increases with the addition of sodium alginate. But drug release behavior reduces with the addition of sodium alginate to the composite hydrogel [54].

Sung et al. reported a hydrogel system based on PVA and Chitosan loaded with minocycline. To induce cross-linking in these hydrogels, the freeze thaw method was used. Due to the incorporation of Chitosan, porosity, water vapor transmission rate, swelling capacity, and elasticity of composite hydrogel have increased. PVA/Chitosan hydrogel has displayed its potential as better wound dressing material concerning gauze (control).Composite hydrogels were developed by blending PVA and Chitosan. Hydrogel devoid of Chitosan has displayed higher strength with respect to the composite hydrogel. But the incorporation of Chitosan into composite hydrogel resulted in a decrease in the strength of hydrogel. [55]

Huang et al. reported a new approach to develop wound dressing material from PVA and glucan without using a crosslinker. They developed hydrogel by blending both polymers and dried at 110 ^{o}C. Since there was no formation of a covalent bond between Polyvinyl alcohol and glucan in film, it can quickly release glucan and facilitate healing. The major drawback associated with these hydrogels is that a higher amount of glucan may result in delayed wound healing. Besides this, the tensile strength of the membrane decreases with an increase in the concentration of glucan in the composite membrane[56].

Hassan et al. synthesized PVA-based physical hydrogel using the freezing-thawing method. The morphological changes of these hydrogels were studied for a 6-month duration upon subjecting to swelling at 37 ^{0}C. During the synthesis of these hydrogels, various parameters were considered, such as molecular weight of PVA, the concentration of the solution, and the freezing-thawing cycle. Upon changing the freezing-thawing cycle, variation in crystallite in the resultant gel was observed. A higher initial

concentration of PVA induces higher crystallinity and aqueous stability. Higher molecular weight PVA produced thicker crystals [57].

Ricciardi et al. evaluated the mechanical property of PVA hydrogel prepared by the freeze-thawing method. During this study, two types of samples were used, 2-month-old samples and rehydrated samples. The change in viscoelastic behavior of these hydrogel samples was studied. Based on the result, it was observed that gel stiffness decreases due to aging and rehydration of samples [58].

Liu et al. developed physical hydrogel by blending PVA with three different polymers as gelatin, starch, and Chitosan. They have used two approaches synergistically, freezing-thawing and coagulation synergistically, to synthesize hydrogel. Further, various physicochemical properties such as swelling, mechanical, and degradation behavior of these hydrogels were studied. The degradation study of these hydrogels in PBS has displayed improvement towards degradation. This occurs due to the coagulation of polymeric chains of the hydrogel. Protein adsorption of these hydrogels was studied. Vascular endothelial cells were proliferated comprehensively over these hydrogels. Difficulty in performing cell culture study of these hydrogels was observed due to the less stable network of the hydrogel. The easy degradation of hydrogel results in a change of pH of culture media. Therefore low adhesion of cells was observed with hydrogels[59]. Liu et al. also developed physical hydrogel by blending PVA and gelatin by freeze-thaw followed by coagulation. In this work, they also evaluated the differential scanning calorimetry and dynamic mechanical, thermal analysis of these hydrogels. In this study, they explored the role of polymer fraction in rheology and the tensile behaviour of hydrogel. By observing the result, it has been pointed out that the thermal property of PVA remains unaffected with the addition of gelatin. Therefore, it can be stated that PVA crystallites remain unaffected with the addition of gelatin. The crystallinity, melting

temperature, ultimate strength and glass transition temperature of these hydrogels increases with increasing thermal cycles. Upon coagulation, the glass transition temperature of these hydrogel reduces. But upon coagulation of these freeze-thaw hydrogels, reduction in crystallinity of these hydrogel composite was observed [60].

Guan et al. synthesized physical hydrogel from PVA, chitin nanowhisker and hemicelluloses using the freezing-thawing process. In this hydrogel system, all the polymers were held together by intermolecular hydrogen bonding. The hydrogel network matrix was made mainly from PVA and hemicelluloses. This matrix also possesses a homogeneously distributed chitin nanowhisker. Due to the incorporation of these nanowhiskers into the matrix, thermal stability, degree of crystallinity, and compressive strength has improved. SEM study of these hydrogels exhibited irregular raptured lamellar structure. Therefore, it may hinder the transport of molecules through it [61].

Amin et al. developed physical hydrogel based on PVA and Chitosan. They have utilized the freeze-thaw technique to develop these hydrogels. They have loaded bee venom to these hydrogels, and its pharmacological activity, such as wound healing capacity, was evaluated. The in vivo study performed on diabetic rat has confirmed that the bee venom loaded hydrogel has accelerated wound healing. The anti-inflammatory activity of such hydrogels was evaluated. Besides this, a Skin irritation test and microbial penetration test were also performed. The swelling ratio, tensile property, surface pH and gel fraction of these hydrogels were evaluated. The Chitosan used in this wound dressing formulation is not soluble in water. The major problem of developed hydrogel formulations is highly acidic. These formulations may cause skin irritations. Therefore, this formulation can be used after neutralization[62].

Kamoun et al. synthesized physical hydrogel based on PVA and sodium alginate through the freeze-thawing method. To develop hydrogels, aqueous solutions of both polymers were used and mixed in a specific ratio. The gel fraction, water uptake, elasticity and elongation at break of these hydrogels were evaluated. SEM study of PVA hydrogel without sodium alginate displayed a smooth surface. With the addition of sodium alginate, PVA based composite hydrogel displayed tiny pores on their surface. The number of pores in these hydrogel increases with increasing sodium alginate into the composite hydrogel. The release study of ampicillin from these hydrogels was performed. Besides this, the mechanical property, protein absorption, hemocompatibility of these hydrogels was evaluated. The elongation at break and tensile strength of PVA-Sodium alginate membrane decreases with a rise in the concentration of sodium alginate in the hydrogel membrane [63].

Mohsen et al. developed PVA / Chitosan-based hydrogel for the delivery of antibiotic. They have synthesized these hydrogels by freezing-thawing cycle, without using any crosslinking agent. Besides this, all the polymers used to develop these hydrogels do not exhibit toxicity and carcinogenic effect. Therefore, it can be considered a suitable material for biomedical use. They have loaded sparfloxacin as a model drug, and its release profile was studied. The assessment of various physicochemical properties such as gel fraction, in vitro degradation, swelling capacity, and mechanical properties of these hydrogels was performed. The antimicrobial study of these composite hydrogels revealed that antibacterial activity is due to the presence of positively charged Chitosan. PVA / Chitosan have displayed antibacterial activity against both gram-positive (Bacillus Subtilis, Staphylococcus aureus) and gram-negative bacteria (Escherichia coli, Klebsiella pneumonia and Pseudomonas areuginosa). From the dissolution study of these hydrogels, it can be concluded that dissolution of these hydrogels increases with an increase in

Chitosan in the PVA- chitosan hydrogel. This result confirms that the developed hydrogel has crosslinking with PVA molecules only. It may lead to the poor mechanical strength of the hydrogel[64].

2.2 Disadvantages of physical hydrogels

The most critical issue of these physical hydrogels is their stability during prolonged biomedical and pharmaceutical application. Therefore, it is essential to evaluate the stability of these physical hydrogels before long term applications. The most common problems associated with these hydrogels include degradation of PVA chains, melting of crystallites, generation of additional crystallites with time. These shortcomings may change the properties of physical hydrogels[65].

2.3 Development and characterization of chemically crosslinked hydrogel

Dimitrov et al. developed polyacrylic hydrogel by chemical crosslinking using macro diisocyanates as crosslinker. The drug release from these hydrogels was studied. From the result, it has been confirmed that drug release from these hydrogels depends on the pH of the medium. The chemical structure of MDI depends on the reactants (nature of diol) used to prepare MDI. Besides this, the quantity of MDI influences the rate of drug release from the hydrogel network [66].

Das et al. developed a biodegradable hydrogel composite from dextrin and polyacrylic acid using N, N′-methylene bis (acrylamide) as a crosslinking agent. The reversible nature of these hydrogels was confirmed by swelling and deswelling kinetics. Ornidazole and ciprofloxacin were used as a model drug to perform drug release studies from these hydrogels. In vitro biocompatibility of these hydrogels was evaluated by human mesenchymal stem cells. Biodegradation of these hydrogels was performed in buffer (pH

7) containing lysozyme enzyme. Although, the developed hydrogels have displayed their porous nature. But the size of pores present in the hydrogel is not uniform in size[67].

Vercruysse et al. prepared hyaluronic acid crosslinked hydrogel network using polyvalent hydrazide as a crosslinker. In this study, they have synthesized polyvalent hydrazide and characterized it. Although hydrogels have been developed using hyaluronic acid, they have not presented any biocompatibility assay to know its interaction with host tissue[68].

Bektas et al. reported the synthesis of methacrylate gelatin hydrogel by crosslinking through UV exposure. These hydrogels were found as a suitable substitute for cornea stroma damage. These hydrogels have presented some specific features, such as transparency and high mechanical strength. Besides, they also exhibited high cell viability after incubation with human corneal keratocyte. In vivo, the behaviour of these hydrogels was also tested in the rabbit eye. The in vivo study of these hydrogels has not produced any ulcer formation, inflammation and oedema in the transplanted region of the cornea. The integration of these hydrogels with corneal tissue was evaluated through the hematoxylin and eosin method. This assay revealed good integration with tissue with less foreign body reaction. Immunohistochemical analysis of developed hydrogels needs to be conducted to know its acceptance as a corona substitute[69].

Nagahama et al. reported the development of Chitosan/gelatin membrane. The excellent compatibility between these two polymers was confirmed by the XRD study. The decrease in crystallinity of the Chitosan membrane with the addition of gelatin indicated the amorphous behaviour of gelatin. They represented that the thermal stability of the gelatin-chitosan membrane is less concerning the Chitosan membrane. The difference in thermal stability is attributed to the amorphous behaviour of gelatin. Cell adhesion evaluation of these hydrogels was performed using MG-63 osteoblast cell [70].

Singh et al. prepared transparent Chitosan hydrogel using formaldehyde as a crosslinker. These hydrogels were sensitive to pH and temperature. These hydrogels displayed high swelling at high temperature and low pH**.** Due to crosslinking through formaldehyde, the thermal stability of these hydrogels has enhanced. FTIR spectra of these hydrogels exhibited a peak at 1566.8 cm^{-1} attributed to imine bond formation, confirms the crosslinking between Chitosan and formaldehyde. Due to crosslinking, the strength of the hydrogel membrane has increased. Crosslinking has reduced the swelling capacity of developed Chitosan hydrogel [71].

Beppu et al. reported crosslinking of Chitosan by glutaraldehyde. Further, the developed hydrogels were characterized by scanning electron microscopy and atomic force microscopy. XRD study of crosslinked Chitosan membrane exhibited a low degree of crystallinity with respect to uncrosslinked Chitosan membrane. TGA study of these hydrogels revealed early removal of water molecules from the crosslinked Chitosan membrane. The hydrophobic behaviour of the chitosan membrane increases due to crosslinking through glutaraldehyde [72].

Li et al. reported the synthesis of crosslinked Chitosan using glutaraldehyde as a crosslinking agent. Based on the XRD result, they found that the crystallinity of Chitosan reduces due to crosslinking. In the differential study, they found that the thermal stability of Chitosan is higher than crosslinked Chitosan. From the SEM study, they found that the surface of crosslinked Chitosan has a porous surface. They also reported that crosslinked Chitosan exhibit antimicrobial behaviour against Burkholderiacepacia. During the preparation of hydrogel, acetic acid was to dissolve Chitosan. Besides this, no biocompatibility analysis of these hydrogels was performed[73].

Li et al. developed electrospun nanofibre using Chitosan. They developed nanofibres with different concentrations of genipin. Tensile strength, in vitro degradation and swelling of these hydrogels, were evaluated. Besides this, cytocompatibility analysis of these hydrogels was performed using the L 929 cell line. For clinical application of these Chitosan-based fibers, more studies on degradation and biocompatibility would be needed[74].

Liang et al. reported the synthesis of thermosensitive hydrogel nanoparticle from carboxylatedmethoxypoly(ethylene glycol) grafted Chitosan. These hydrogels were evaluated by transmission electron microscopy and dynamic light scattering. Three drugs (5 Fluorouracil, Bovine serum albumin and dexamethasone) were loaded into these hydrogel nanoparticles, and their release pattern was studied. The cytocompatibility of these hydrogels was evaluated through MTT assay using mouse embryonic fibroblast cell line. The porosity of these hydrogels depends on the degree of substitution (DS). The porous hydrogel network developed by the specific concentration of polymers becomes compact upon increasing the degree of substitution. Therefore, drug release from these hydrogels is also affected by the degree of substitution [75].

Zhao et al. has observed stress relaxation behaviour in alginate hydrogel. Alginate hydrogel used in this study were crosslinked by two methods, namely ionic crosslinked and covalent crosslinking. Ionic crosslinked alginate hydrogel undergoes stress relaxation due to breaking, followed by the formation of new ionic linking. Simultaneously, covalently crosslinked alginate hydrogel undergoes stress relaxation due to the migration of water from the gel. In ionic crosslinked alginate hydrogel, stress relaxation does not depend on the size of the sample. In contrast, the stress relaxation of covalently crosslinked alginate hydrogel depends on the size of the sample [76].

Biswal et al. developed gelatin hydrogel by crosslinking through glutaraldehyde. These hydrogels were further characterized by the FTIR technique. These hydrogels were loaded by ciprofloxacin, and its release was studied. The cell proliferation of these hydrogels was performed using the MG-63 cell line. The mechanical property of these hydrogels was evaluated through a stress relaxation test using the Weichert model [77].

Stress relaxation studies are performed to understand the persistence of visco elastic nature of the hydrogel for long term. To evaluate the stress relaxation parameter of the hydrogel can be mathematically calculated and plotted against time. This graph between stress relaxations versus time, enabling one to know the time frame in which visco-elastic behavior is maintained. Besides, the formula presented in the thesis allows determining which composition will be best suited for the desired application[78, 79].Chen et al. also reported the stress relaxation of PVA-HA / PAA hydrogel using the following equation [80]

$$\%\, Stress\ relaxation\ = \frac{S0-St}{S0} \times 100$$

where S_0 = Initial stress of sample and S_t = Relaxation stress of sample after time t

Liu et al. reported the synthesis of scaffold using collagen and Chitosan. This scaffold was crosslinked by glutaraldehyde. Microstructural analysis of scaffold was performed using scanning electron microscopy. The cell viability of these scaffolds was studied through the MTT technique using dermal fibroblast cells. A decrease in the swelling ratio of the scaffold was observed due to an increase in the concentration of glutaraldehyde. The swelling pattern also changes due to the structure collapsing of the scaffold. From the swelling analysis of these scaffolds, it can be concluded that swelling of the scaffold depends not only on the concentration of crosslinker but also on the three-dimensional

structure of the scaffold. Before clinical applications, a more detailed degradation study of these hydrogels need to be performed[81].

Fookes et al. reported Chitosan/ β glycerophosphate-based hydrogel for oral delivery of fluoride. These hydrogels can be used for the treatment of osteoporosis treatment. This hydrogel system was found stable till 48 hours upon exposure to pH 4 and pH 7 buffer solutions. The controlled release of sodium fluoride from these hydrogels was observed for 6 hours. In vivo studies of sodium, fluoride hydrogels were performed using rat. Effect of API on hydrogel system has not been performed. Besides this, API's effect in water solution for a longer duration (more than 6 hours) needs to be studied[82].

Vignesh et al. developed Chitosan / hyaluronic acid hydrogel containing deferoxamine (DFO) loaded PLGA nanoparticle. The developed hydrogel system was found to produce an angiogenesis effect. To develop deferoxamine nanoparticle, a double emulsion solvent diffusion approach was used. Scanning electron microscopy confirmed the spherical shape of the nanoparticle. DFO release from the developed hydrogel was observed for ten days. Free DFO and DFO NP was loaded into these hydrogels were injected subcutaneously into mice, and its angiogenesis effect was observed. During the preparation of hydrogel, Chitosan was dissolved in acetic acid solution. Therefore, it is mandatory to neutralize this solution to minimize the effect of acetic acid[83].

Campos et al. reported microparticle using PVA and Chitosan as precursor. These microparticles were crosslinked through glutaraldehyde. In order to prepare these microparticles, the water in emulsion technique was used. These techniques involve two steps- step (i) Generation of the two-phase system by dispersing polyvinyl alcohol and Chitosan solution in vegetable oil results into formation of tiny droplet of polymer solution step (ii) hardening of these drops using glutaraldehyde, results in the construction

of the micro particle. The cytocompatibility of these microparticles was evaluated using mouse peritoneal macrophages. During the synthesis of crosslinked microparticles, Chitosan was dissolved in acetic acid: methanol solvent. The presence of acetic acid in microparticles may limit its wide applications[84].

Wang et al. prepared PVA / Chitosan-based hydrogel crosslinked by glutaraldehyde. These hydrogels were biodegradable and pH-sensitive. They presented that the pH-sensitive nature of these hydrogels is due to the presence of an ionic pendant group. They have classified water present in hydrogel into four different types- category ‘a’, category ‘b’, category ‘c’ and category ‘d’. Category ‘a’ water means the water molecules associated with polarized ionic group of polymer. Category ‘b’ means water molecules associated through hydrogen bonding. Category ‘c’ involves the presence of water molecules in the cages surrounded by hydrophobic groups. Category‘d’ involves the water molecules imbibed by capillary pores of the material. Category d water is also known as bulk water. Category ‘a’ and category ‘b’ water is collectively termed as bound water. Category ‘c’ and category‘d’ water is called intermediate water and free water, respectively. The water content of hydrogel is determined gravimetrically [85].

Wang et al. also reported the development of a biodegradable semi-interpenetrating polymeric network using PVA and Chitosan. FTIR study of swollen polyvinyl alcohol / Chitosan exhibited at 1643 cm^{-1} indicated the generation of imine bond (C=N) due to interaction between aldehyde groups glutaraldehyde and amino group of Chitosan. This peak was disappeared after three days, indicating the instability of the Schiff base (C=N). The toughness of these hydrogels has improved with an increase in the concentration of polyvinyl alcohol The major drawback associated with these hydrogels is the leaching of PVA from PVA-Chitosan hydrogel in an acidic environment. It shows that PVA is physically crosslinked within the hydrogel network[86].

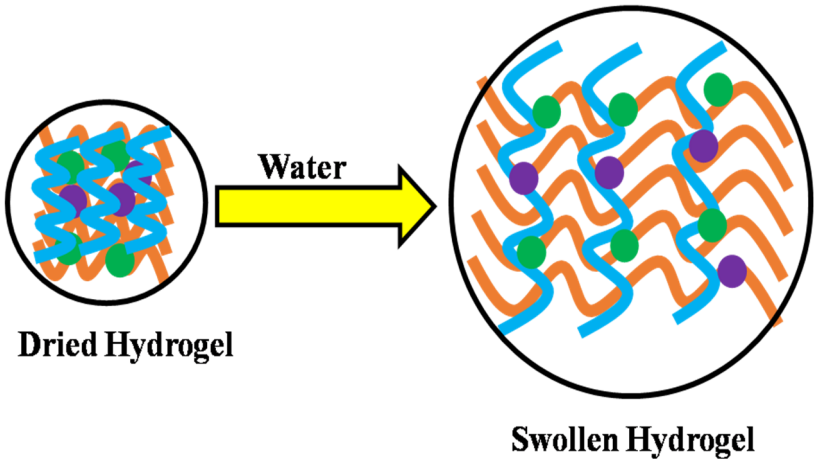

Figure 2.1- Schematic representation of swelling of hydrogel in water (Green and blue dots represent hydrophilic group of polymers).

Altinisik et al. synthesized PVA / Chitosan hydrogel using tartaric acid as a crosslinker. They have performed enzymatic degradation of these hydrogels using lysozyme enzyme in buffer solution (pH 7.4**)** at 37 ^{0}C temperature. They have evaluated the swelling property of hydrogel using the following mathematical equation.

$$\% S = \frac{\text{ms} - \text{md}}{\text{md}} \times 100$$

where m_s = mass of swollen sample and m_d = mass of dried sample

In this study, they have loaded amoxicillin as a model drug, and its release study was performed by UV-Vis spectrophotometer. They have also evaluated thermal stability, crystallinity, surface topography, and structural analysis of the hydrogels [87].

Khan et al. prepared hydrogel using low viscous Chitosan and PVA. To develop these hydrogels, glutaraldehyde was used as a crosslinking agent. The swelling behavior of hydrogels was evaluated using different pH buffers. They reported that hydrogel composite having a higher content of PVA shows a high swelling rate in higher pH buffer.

They have loaded diphenhydramine drug into hydrogel by swelling method, by keeping dried hydrogel in the drug solution. They have evaluated drug percentage drug loading of the hydrogel by weight method using the following formula.

$$Drug\ loading\ \% = \frac{\mathrm{WD} - \mathrm{Wd}}{\mathrm{Wd}} \times 100$$

where W_d and W_D represent the weight of dried hydrogel and weight of the drug-loaded hydrogel respectively. Further, the drug release kinetics study of these hydrogels was studied using different kinetic models[88].

Similarly, Osman et al. also reported the approach to calculate percentage drug loading efficiency of 5 aminosalicylic acid drug from the levan-pNIPA based hydrogel using the following mathematical expression [89].

$$5 - ASA\ (wt\%) = \frac{((\mathrm{W2} - \mathrm{W1}) \times (0.7/\ 1000))}{\mathrm{W1}} \times 100$$

where W_2= Weight of swollen gel and W_1= Weight of dried gel

Yang et al. prepared hydrogel blended membrane using PVA and Chitosan. To develop these membranes, formaldehyde was used as a crosslinker. The FTIR result revealed that the absence of hydroxyl group in composite membrane and the formation of new functional groups such as ether linkage and acetal ring in the new composite membrane. These functional group changes occur due to the interaction of formaldehyde and hydroxyl group associated with PVA. Another important observation was the decrease in crystallinity of PVA due to crosslinking with formaldehyde. This has been confirmed by thermogravimetric analysis, differential scanning calorimetry, and dynamic mechanical analysis. During the preparation of these PVA/ Chitosan hydrogels, an incompatibility issue was observed. Since pure PVA is a crystalline polymer. But upon treatment with formaldehyde, the crystalline fraction of PVA decreases. Besides this, the separation

between crystalline fraction and an amorphous fraction of PVA also decreases. This is confirmed by an increase in the damping peak of PVA crosslinked hydrogel[90].

Islam et al. developed blend hydrogel from PVA and Chitosan using TEOS (tetraethoxysilane) as crosslinker. TEOS was used as a crosslinker, because TEOS is nontoxic. The significant outcome of this work was that the developed PVA/ Chitosan hydrogels were pH-sensitive. These hydrogels displayed more swelling in a neutral solvent system concerning an acidic and alkaline environment. Owing to higher swelling in a neutral environment, these hydrogels were considered as potential matrices for drug delivery. Dexamethasone was loaded into these hydrogels, and its release study was observed in the simulated intestinal and gastric fluid by UV visible spectrophotometer. FTIR results of this study revealed the appearance of a peak at 1080 $cm^{-1}$and 1020 cm^{-1} is due to the presence of siloxane bond generated by TEOS. Besides this, the porous nature of these hydrogels was confirmed by scanning electron microscopy. These hydrogels were developed by dissolving Chitosan in acetic acid. No in-vitro biocompatibility test has been performed using these hydrogels[91].

Costa junior et al. reported hydrogel synthesis using PVA and Chitosan as the precursor. These hydrogels were crosslinked through glutaraldehyde. XRD study of these hydrogels revealed the decrease in chitosan crystallinity due to the formation of a crosslinked network via glutaraldehyde. Besides this, chemical etching was used on these hydrogels through sodium hydroxide solution (0.5 M) for 5 minutes to separate uncrosslinked PVA. These hydrogels were subjected to SEM analysis to observe voids. The void formation was observed in uncrosslinked PVA/ Chitosan composite. Whereas, Crosslinked PVA/ Chitosan composite membrane was intact after etching and did not display any void on their surface. FTIR result of these hydrogel composite exhibited broad peaks in the range between 3200 cm^{-1} to 3550 cm^{-1} confirms the formation of intramolecular and

intermolecular hydrogen bonds. Schiff base formation due to crosslinking between PVA and Chitosan was approved by the band's appearance at 1550 $cm^{-1}$and 1634 cm^{-1} in the FTIR spectra. PVA / Chitosan crosslinked hydrogel displayed a reduction in swelling due to crosslinking and increased Chitosan concentration. Apart from this, no in-vitro degradation study and viscoelastic study have been performed using these hydrogels[92].

Abdelaal et al. reported the development of PVA and Chitosan-based hydrogel using two different approaches, namely Chemical crosslinking and irradiation. The swelling behavior of these hydrogels was studied in simulated gastric fluid and simulated intestinal fluid. These hydrogels exhibited pH-responsive behavior. 5 Fluorouracil was loaded as a model drug to know the drug release profile of these hydrogels. FTIR spectra of glutaraldehyde crosslinked Chitosan hydrogel displayed a peak at 1642 cm^{-1} indicates the formation of an imine bond. Similarly, PVA / Chitosan chemically crosslinked hydrogel exhibited a peak around 1646 cm^{-1} is due to crosslinking formation between PVA and Chitosan through hemiacetal or acetal formation. Simultaneously, the decrease in the O-H band was observed in radiation-induced crosslinked PVA/ Chitosan hydrogel. This result suggests that radicals are formed in macromolecules resulting in crosslinking of PVA chains. PVA and chitosan crosslinked through irradiation. Therefore it promotes more self-crosslinking of PVA molecules. Due to self-crosslinking, a reduction in equilibrium swelling of hydrogels was observed[93].

Zu et al. developed hydrogel composite from PVA and Chitosan through chemical crosslinking. To crosslink these polymers, glutaraldehyde was used as a crosslinker. Nano insulin was loaded into these hydrogels, and its release pattern was evaluated by high-performance liquid chromatography. Scanning electron microscopy of freeze-dried hydrogel was performed. SEM study of the hydrogels revealed the porous nature of hydrogels. Besides this, pore size was also affected due to the crosslinking. The pore size

of these hydrogels reduces with an increase in the extent of crosslinking. The excellent miscibility of both polymers during hydrogel formation was confirmed by X-ray diffraction and differential scanning Calorimetry. In order to use this formulation for clinical applications, in vitro biocompatibility analysis of these hydrogels must be performed. Besides this, a viscoelastic study needs to be performed to get information about the viscoelastic nature of these hydrogels [94].

Alhosseini reported the development of electrospunnanofibre scaffold using PVA and Chitosan. Owing to the presence of larger pores in the fibers, these scaffolds could be used for nerve tissue repair. The degradation study of this scaffold was evaluated by keeping them in PBS buffer (pH 7.4) followed by its weight measurement in the different time intervals. The degradation of these scaffolds was calculated by the following equation[95]

$$Degradation\ Index\ = \frac{\mathrm{Wo} - \mathrm{Wt}}{\mathrm{Wo}} \times 100$$

where W_o and W_t is the initial and weight after time t respectively.

Mansur et al. developed crosslinked hydrogel network using PVA / Chitosan. FTIR Study of Chitosan blends exhibited an increase in the intensity of imine band (C=N) and decrease in the intensity of amine band, confirms the formation of crosslink network through glutaraldehyde. The swelling study of these hydrogels has displayed the impact of crosslinking on the swelling of developed hydrogel. With the increase in the concentration of glutaraldehyde, swelling of Chitosan blend hydrogel has reduced. The decrease in swelling of hydrogel is attributed to the formation of the more rigid network through crosslinking. In vitro biocompatibility of these hydrogels were evaluated through MTT assay using VERO cell [96].

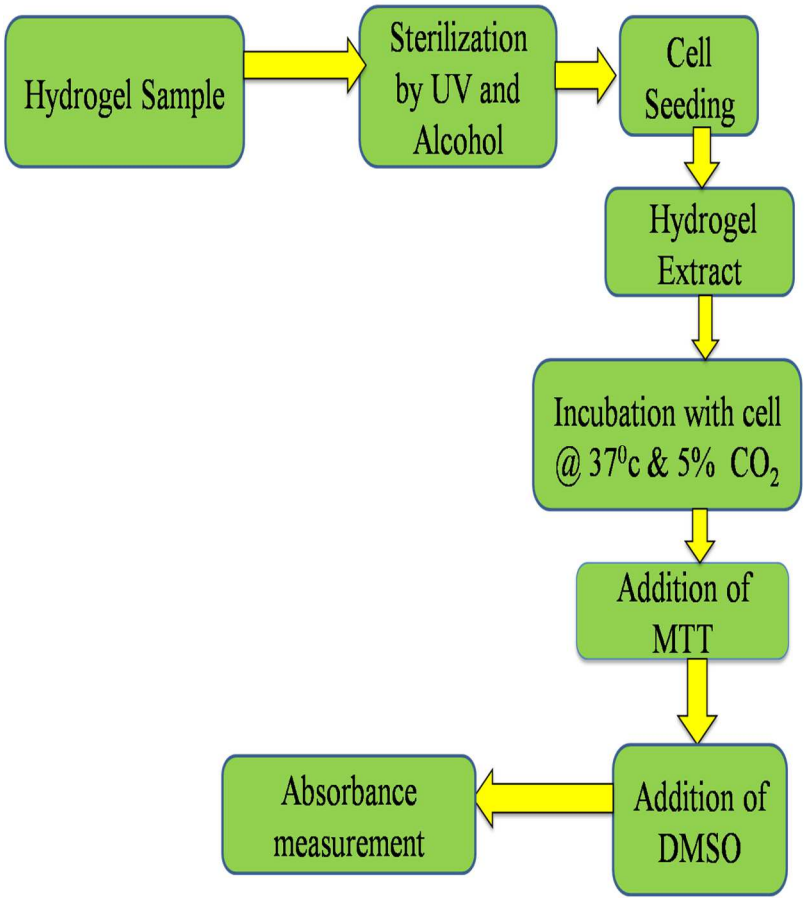

Figure 2.2- Schematic representation of MTT assay of hydrogel sample.

2.4 Problems associated with Chitosan

Chitosan is soluble in acidic pH solutions[97, 98]. It possess primary amino group with pKa value 6.3. Therefore, at acidic pH amino group of chitosan is protonated and get dissolved upon formation of positively charged chitosan [99].Cho et al. reported that Chitosan-based formulations prepared using acidic solvent might affect the biocompatibility of the system. Because residual acid present in the drug delivery system adversely affects drugs' potency through accelerating degradation of drugs [98]. Similarly, Sogias et al. have reported that acid soluble Chitosan, upon administration in

neutral or basic pH, undergoes precipitation and causes harmful effects [100]. Zhou et al. have reported that removal of residual acid from acid soluble chitosan is difficult due to the attractive force between carboxylate ion of acid and ammonium ion of Chitosan. They also reported that a small quantity of residual acid might cause toxic effects upon application to wound or tissue[101].

2.5 Problem statement

As discussed in the literature mentioned above survey, numerous work has been reported on Polyvinyl alcohol and Chitosan for various bioengineering applications. They have used physical and chemical methods to develop hydrogels. In these reports, most of the authors have utilized acid-soluble Chitosan to develop hydrogels. The major drawback associated with acid-soluble Chitosan is acid residue in the final product, which could adversely affect biocompatibility by leaching to the nearby tissue [98, 100, 101]. Besides, acid-soluble Chitosan-based biomedical products need to be neutralized before their last use in clinical applications[102, 103]. These shortcomings motivated us for further research based on water-soluble Chitosan. Very little work has been reported to date on water-soluble Chitosan. Therefore, our current research work is inclined to develop a hydrogel system based on water-soluble Chitosan. The potency of these hydrogel systems was evaluated after incorporating Chitosan (water-soluble) into polyvinyl alcohol. These hydrogels were developed through a chemical approach.

2.6 Aim and objectives of present work

The major objectives of our research is as follows-

- **Objective 1: Polyvinyl alcohol / Chitosan Lactate hydrogel for controlled drug delivery**

  - Synthesis of Polyvinyl alcohol / Chitosan Lactate hydrogel
  - Characterization of hydrogel by XRD, FTIR, DSC, TGA, SEM, AFM.
  - Water holding capacity of hydrogels.
  - In- vitro biocompatibility study of hydrogels.
  - Incorporation of Ciprofloxacin drug into the hydrogels and its in- vitro release study
  - Antimicrobial study and In vitro degradation study.
  - Stress relaxation study of hydrogel
  - Contact angle study of hydrogels.

- **Objective 2: Polyvinyl alcohol / Chitosan Oligosaccharide hydrogel for controlled drug delivery**

  - Synthesis of Polyvinyl alcohol / Chitosan Oligosaccharide hydrogel
  - Characterization of hydrogel by XRD, FTIR, DSC, TGA, SEM, AFM.
  - Water holding capacity of hydrogels.
  - In vitro biocompatibility Study of hydrogels.
  - Incorporation of Lomefloxacin drug into the hydrogels and its in vitro release study
  - Antimicrobial study of hydrogels

- Stress relaxation study of hydrogels
- Contact angle study of hydrogels.