Chapter – I

Introduction
Natural polymers such as polysaccharides, proteins, DNA and RNA, derived from living organisms are referred to as ‘Biopolymers’. In general, biopolymers have the property to degrade upon the action of microorganisms into CO₂ and water and are hence biodegradable. A biomaterial has recently been described as an interactive non-drug material that has a benign function and has the ability to establish a suitable contact with the surrounding tissues without causing undesired adverse effects. Therefore, these materials are essentially biocompatible and bio-functional and can be used and adapted for biomedical applications like biomedicine, tissue engineering, dentistry/dental materials, controlled drug delivery, surgery, artificial body organs, biomedical implants which performs, augments or replaces a natural function. The usefulness of biomaterials is further described as in neural prostheses, cardiovascular materials, blood or bone substitutes, orthopedic prostheses, plasma- and cytapheresis. Biomaterials can be natural or man-made that accordingly comprises whole or part of a living structure.

Polymeric carbohydrates i.e. ‘Polysaccharides’, are the important class of natural biological polymers and are supposed to be the first biopolymers formed on this earth from the most renewable resources. Polysaccharides, referred to as ‘glycans’ are present in almost all the living organisms in one or other form and constitute approximately three quarters of dry weight. They are the condensation products of monosaccharide units, linked through glycosidic bonds, to form linear molecular chains, have high molecular weight with general formula \((\text{C}_n\text{H}_{10}\text{O}_n)_n\), where \(n\) varies between 40-3000. They perform diverse roles in the physiology of plants, animals, and microorganisms. They are complex carbohydrates with structural diversity that results in different functional attributes viz. structural polysaccharides that form structural frame of living organisms (cellulose, carrageenan, chitosan and chitin) and storage polysaccharides that store energy (starch) thus making them most efficient and useful class of polymers. As surface material, they partially protect tissues from desiccation and as gums they are exuded from plants to seal and protect wounds. As thickeners, they serve a physical or mechanical role in animals and as specific substances; they are of importance in blood group specificity and in other immunological reactions.

Broadly polysaccharides are classified as:

1.1. **Storage polysaccharides**: They are energy storage polysaccharides which are present in seeds, stems, tubers, rhizomes (starch) and liver (glycogen) etc. By far the
most common storage polysaccharides found in plants, which serve primarily as reserve foods, are commonly branched or a mixture of linear and branched polysaccharide as in the case of starch. In animals, the structurally similar glucose polymer is the more densely branched glycogen, sometimes called 'animal starch'. Glycogen's properties allow it to be metabolized more quickly, which suits the active lives of moving animals.

1.2. **Structural polysaccharides:** They provide protective walls or lubricate cell coatings. Cellulose, the prime example of structural polysaccharides, almost always linear molecules, are used in the cell walls of plants and other organisms providing physical structure and strength and are claimed to be the most abundant organic molecule on earth. These find significant role in the paper and textile industries, used as a feed stock for the production of rayon (via the viscous process), cellulose acetate, celluloid and nitrocellulose. Chitin, chitosan, chondroitin sulfates, hyaluronic acids etc. are some other few examples of structural polysaccharides.

1.3. **Hetero polysaccharides:** They contain two or more different types of monosaccharide units and occur in both straight and branched chain forms. Connective tissue polysaccharides, glycol proteins, glycol lipids and plants gums like pectin, lignin and mucopolysaccharides are few examples.

1.4. **Homo polysaccharides:** It has one type of monosaccharide and subdivided into straight chain and branched chain, depending upon the arrangement of the monosaccharide units.

Variety of naturally occurring polysaccharides that are derived from renewable resources are available for use in various applications including pharmaceutical and biomedical applications because of their inherent biological and chemical properties such as non-toxicity, bio-compatibility, being benign to mammalian tissues, biodegradability, high chemical reactivity, poly functionality, chirality, chelation with cost effectiveness and easy availability. Therefore, polysaccharides and polysaccharide-based polymers are foreseen as one of the potent materials for developing advanced materials for food and health benefits.

Polysaccharides show excellent adsorption behavior due to the flexibility of polymer chain, presence and high chemical reactivity of hydroxyl, acetamido and primary amino functional groups, present in each glucose unit along the polysaccharide chain. In an
aqueous environment, polysaccharides are known to get hydrated to create a hydrogel like structure. Polysaccharides such as starch, chitosan sodium alginate, carrageenan and cellulose have been shown to form hydrogels upon graft copolymerization of vinyl monomers. It is also well known that for utilization in the diversified fields such as biomedicine, pharmacy, agriculture etc., specific properties like those of hydrogels are required. Hydrogels have been widely studied in various biomedical, agricultural applications, for their favorable absorbent and biological properties like biodegradability and biocompatibility.

Hydrogels are a special class of polymers with three dimensional networks of cross-linked polymer chains that can absorb water up to thousand times of their weight within their porous cross-linked networks. They can be prepared from almost any water soluble polymer, covering a wide range of chemical compositions and physical properties. The polymeric chains in hydrogels contain acidic or basic groups bound to them which are responsible for absorption of water. The acidic groups on the chains deprotonate at high pH, whereas the basic groups protonate at low pH. Hydrogels possess certain other reactive functional groups along the polymer backbone, which often make them sensitive to the conditions surrounding environment due to which they own the title of “intelligent or smart material". Hydrogels are synthesized using different techniques such as free radical copolymerization or cross-linking and have been significantly studied for their properties and swelling behavior. In recent years, considerable research has been done on the characterization and swelling behavior of hydrogels, prepared by simultaneous free radical copolymerization and cross-linking in the presence of an initiator and a cross-linking agent.

From among the available wide range of polymers, on the basis of sustainability and nature of the source, there are both natural occurring and synthetic polymers which extend from natural biopolymers to common synthetic polymers like plastics that are used in nearly every industry. Synthetic polymers, with wide range of properties and uses are manmade and are produced commercially on a very large scale. Both, natural and synthetic polymers provide ample variety of properties leading to their full exploitation in everyday life and can be produced with a wide range of stiffness, strength, heat resistance, density and even price. The continuous exploration in the field of science and technology of polymers has uplifted their role in the society making them requisite part of everyday life.
Amino polysaccharides, a class of structural polysaccharides, derived from microorganisms or animals, are also used in biomedical applications and act as supply of dietary fiber. Chitin and chitosan, are the two most important and abundant amino polysaccharides that finds many useful properties in foods and have also been utilized as food additives. They show great affection towards lipids and therefore, affect lipid absorption. They have also been widely used in biomedical applications like wound dressing, [16, 17] antifungal-antibacterial [18-20] etc. Their ability to absorb oxygen is helpful in the treatment of wounds and burns. Hyaluronic acid, chondroitin sulfate and heparin belong to glycosaminoglycans, a class of polysaccharides, present in the connective tissues of mammals which are known to successfully accelerate wound healing and tissue regeneration in both laboratory animals and humans (hyaluronic acid, in particular). Although, the exact mechanism of wound healing process is not clearly understood but the oligomeric metabolites of N-acetyl glucosamine and glucosamine are considered to be responsible. The functionalities, like N-acetyl glucosamine and glucosamine that are present in many glycosaminoglycans are also present in chitin and chitosan, therefore, accelerated wound healing has also been reported for these substrates. Chitosan and its derivates has also been used to aid in the regeneration of both periodontal tissue and bone, [21] and a continuing effort has been taken up in fabricating chitin/ chitosan into sutures and other implantable medical devices. The use of chitosan and chitin as fibers and films has been also reviewed. [22]

Chemically cross-linked nano-structured chitosan/PVA blends were successfully synthesized and tailored for potential use in biomedical applications. [23] A lot of biodegradable polymers (synthetic as well as natural) can be used for tissue engineering. Synthetic polymers like poly (vinyl alcohol), poly(ethylene glycol), etc and natural polymers such as chitin, chitosan, collagen, etc. have been used for this purpose. [24, 25] The α-chitin/gelatin composite membranes were used to study bioactivity enzymatic biodegradation, swelling, etc. these were found to be biocompatible with MG63 osteoblast-like cells. The biocompatibility and bioactivity results of these membranes indicate that these can be used for tissue engineering. [26] Carboxymethyl derivatives of chitin and chitosan have shown as good adsorbents for metal ions, as carrier for drug delivery systems, in wound healing, as anti-microbial agents, have anti-tumor activities, used in tissue engineering and as components in cosmetics and food. [27] Hydrogels based on chitosan and gelatin has been used on a large scale for tissue engineering and other
biomedical applications. The chitosan-based wound dressing material, synthesized by Ong et al. showed good homeostatic and antimicrobial properties. Chitosan grafted with 1-Lactide revealed biocompatible and biodegradable behavior, useful in tissue engineering.

De Souza et al. synthesized chemically cross-linked chitosan and PVA blended hydrogels and investigated their swelling behavior in simulated body fluid. The hydrogel exhibited adequate cell viability, non-toxicity and appropriate properties which can be modified for potential use in tissue engineering. Synthesis and swelling behaviour of pH sensitive graft copolymer hydrogels of chitosan and a mixture of AAc and hydroxyethyl methacrylate was investigated by Sadeghi. The pH-responsive hydrogels based on carrageenan and sodium alginate were prepared by Hosseinzadeh using chemically cross-linking method. Cross-linked chitosan-poly(N-isopropylacrylamide) (PNIPAAm) hydrogels having interpenetrating network system were synthesized by Alvarez-Lorenzo et al. and the hydrogels were found to have markedly increased drug loading capacity in comparison to the pure PNIPAAm hydrogels.

In recent years, biological dressings derived from natural products have been mostly used because of their great biocompatibility. Kumar et al. studied the synthesis of chitin hydrogel/nano ZnO composite and evaluated it for wound dressing antibacterial bandage material. The high energy cross-linked pH-sensitive hydrogels created from carboxymethylated chitin and carboxymethyl chitosan (CM-chitosan) displayed excellent mechanical properties and good swelling in water. Recently, Park et al. developed a new biodegradable glycol chitin-based thermoresponsive hydrogel scaffold that quickly transforms into a durable hydrogel after its application as a mild viscous solution at room temperature under physiological conditions.

Thus, we find that technology from daily commodities to high level scientific inventions stands on the domains of dynamic research and the materials which are efficiently and scientifically viable are the outcome of the research and development in the field of polymers. These materials are also adaptable for being economical. From being a part of the household commodities, they have grabbed their place in the medical, transportation, textiles and industrial fibers, pharmaceutics, biomedicine, aerospace, agriculture, separation science, adhesives, coatings, etc. They play an integral role in therapeutics as drug delivery systems and bio-sensors, have been extensively used as components of
devices in medical fields such as inhalers and catheters, inert bio prostheses or transdermal patches, in testing devices and for bio regulation. In textile industry, they have been used for improvement in flammability, wet ability and the most important, the aesthetic requirements. They have also been used in insulating, conducting and semi-conducting fields, as films in separation science etc. Engineering and technology has been a subject of continuous research in the field of polymer, physical and chemical sciences and the entire spectrum of needs and application possibilities await the polymer of future.\[40\]

The significant use of hydrogels in the areas of biomedicine, agriculture, food chemistry etc. has raised the interest to further explore their synthesis, properties and applications. Both natural as well as synthetic polymers are suitable for such specific applications. Natural polymers being biocompatible with usually have high molecular weights, are suitable for some applications but natural polymer-based hydrogels are mechanically weak and undergo some uncontrolled degradation. On the other hand, synthetic polymers with precise molecular weight can be synthesized to form hydrogels in which cross-link density, hydrophobic and hydrophilic balance and degradation kinetics can be easily controlled. However, synthetic hydrogels may be potentially toxic, which needs to be minimized by purification. The use of less toxic reagents, in situ gelation, UV photo cross-linking strategies and bio inspired gelation based on endogenous proteins and enzymes are few methods for minimizing toxicity.

In view of the above, taking into considerations the properties of both the natural and synthetic polymers, the blending of the two polymers would boost the properties of the hydrogel, rejecting the negative aspects of the individual polymers. It is for this reason that it was thought significant to develop copolymers from natural polymeric backbones like chitosan, chitin and synthetic polymer, PVA with further functionalization through grafting of hydrophilic monomers to induce/ enhance properties like those of hydrogels and utilize the products in various applications.

It is, therefore, pertinent to discuss the structure and properties of each of the polymers used, the chemistry and importance of the grafting process and a brief review on the grafting of different monomers, monomer mixtures onto both natural and synthetic polymeric/ copolymeric backbones and their use in various applications.
1.5. Chitin:

Chitin, \( \text{(C}_8\text{H}_{13}\text{O}_5\text{N})_n \), was first isolated from mushrooms in 1811 by Henri Braconnot.\textsuperscript{[41]} It is the second most abundant natural biopolymer in the world after cellulose\textsuperscript{[42-44]} and constitutes about 106-107 tons in marine biomass alone. It is present as a part of the arthropod exoskeleton in combination with protein and calcium carbonate, containing between 20-60% of chitin. It mainly functions as the hard exterior of arthropods and protecting the soft interiors of these organisms from their harsh environment. It acts as structural support for organisms and prevents fluid loss in these organisms. Since the isolation of chitin, chitin and its derivatives have been used in various industries because of the presence of multiple functional groups. It has been found applicable for a number of biological applications like food and medical field,\textsuperscript{[45-48]} agriculture\textsuperscript{[49]} and aquaculture,\textsuperscript{[50, 51]} dental\textsuperscript{[52, 53]} and cosmetics,\textsuperscript{[54,55]} wastewater\textsuperscript{[56, 57]} and membranes.\textsuperscript{[58, 59]}

In its unmodified form, chitin appears to be translucent, hard and flexible. Structurally, it is a polysaccharide consisting of \( \beta-(1\rightarrow4) \) 2-acetamido-2-deoxy-D-glucose repeat units, some of which are deacetylated (Fig.1). The degree of deacetylation usually varies between 8 - 15% and depends on the species and the method used for isolation and purification. Chitin is not one polymer with a fixed stoichiometry, but is a class of polymers of N-acetyl glucosamine with different crystal structures and degrees of deacetylation, with fairly large variability from species to species. On the basis of crystallinity of the structure, chitin is found in two different forms i.e. \( \alpha \)-chitin and \( \beta \)-chitin. \( \alpha \)-Chitin, with alternating anti-parallel polysaccharide strands, is found in the crab and shrimp skeletal while \( \beta \)-chitin with parallel strands of polysaccharides is found in skeletal of squid pen. The extent of crystallinity and rigidity in \( \alpha \)-chitin is more than that in \( \beta \)-chitin. Chitin with two parallel chains alternating with an anti-parallel strand of polysaccharide, constitute \( \gamma \)-chitin and is found in fungi.

![Fig.1 Structure of Chitin](image-url)
The highly crystalline structure of chitin, which is due to the strong hydrogen bonding among its functional groups like acetamido, hydroxyl and carbonyl groups, makes it insoluble in common organic solvents. Recently, it has been found that under mild conditions, chitin gets dissolved in calcium solvent system and this solvent system can be used to prepare chitin based hydrogels. \(^{61-63}\) \(\alpha\) and \(\beta\)-Chitin (0.1% w/v) when suspended in water can give its hydrogel which is dried between filter papers for 20h at room temperature to convert respectively to \(\alpha\)- and \(\beta\)-chitin membranes. \(^{64, 65}\) The \(\alpha\)- and \(\beta\)-chitin hydrogel membranes have the properties like swelling, enzymatic degradation, etc and can be used as biomaterials in applications such as tissue engineering. \(^{62, 64}\) The membranes and scaffolds can also be easily prepared with the use of chitin gel. A gel-like membrane, useful as a carrier for medications to be applied to wounds, was prepared by dissolving chitosan in an acid–water–glycerol solution which when neutralized forms a gel upon standing. \(^{66}\)

Chitin has an overall positive charge that makes it to bind to negatively charged objects, such as skin and proteins. It is insoluble in water and organic solvents. Chitin has extensive medical uses; the use of chitin during sutures makes the process shorter and less painful and increases the healing by 50%. It has also been used for artificial blood vessels, antibacterial sponges and dressings. Histo-chemical and immune-histochemistry of chondrocytes-sponge composites with \(\beta\)-chitin sponges reveals cartilaginous structure with high absorption efficiencies of about 98%. \(^{67}\) The presence of internal hooks in chitin helps to remove impurities in water and hence act as a water purifier. Chitin provides protective layer to the seeds against growth of the fungus thus helping in farming. Other than these, chitin has also been used in a large number of applications to separate materials, as food additives and as cosmetics.

It is a biodegradable, \(^{68, 69}\) biocompatible and non-toxic biopolymer with anti-inflammatory and antibacterial properties and has been used for a number of biomedical applications like wound healing, \(^{27, 70}\) wound dressing and as tissue supporting scaffolds. \(^{71-73}\) The scaffolds of \(\alpha\)-chitin/ nanosilver and \(\beta\)-chitin/ nano-silver composites were found to have antibacterial, blood clotting, cytotoxic activities, swelling and wound healing properties and therefore these can be used as wound dressing materials. \(^{74, 75}\) The bioactivity study of \(\beta\)-chitin scaffolds, prepared through
lyophilization technique, using simulated body fluid (SBF) solution, indicates the presence of calcium phosphate and hence these can be used for tissue engineering applications.\textsuperscript{[76]} The scaffolds of $\alpha$-chitin/ nano-silica composites showed increased bioactivity and biocompatibility and in vitro study of the bioactivity, swelling ability and cyto-toxicity of the scaffold was found to be biocompatible with MG63 cell line indicating that these scaffolds can be used in bone tissue engineering.\textsuperscript{[77]} Hydroxyapatite is a natural component of bones and teeth and being osteoconductive and osteopedic in nature it can also be used in orthopedics and dentistry.\textsuperscript{[78, 79]} Chitin does not have good mechanical properties but addition of hydroxyapatite increases its mechanical strength and then it can be used for repair and reconstruction of bone as a bone substitute.\textsuperscript{[80]}

1.6. Chitosan:

Most of the natural polymers like cellulose, carrageenan, agar-agar, starch, dextrans and many others are either neutral or acidic in nature but chitin and chitosan are highly basic polysaccharides because they have primary amino groups in their structure. Chitosan, a deacetylated derivative of chitin with a degree of deacetylation of 75% or above,\textsuperscript{[81]} is a linear natural polysaccharide derived from the shrimp shells and other sea crustaceans. Highly concentrated (40%) alkali solution or heating powdered chitin in fused calcium hydroxide at 180 °C for 30 min are the processes used for deacetylation of the N-acyl groups of chitin. The extent of deacetylation determines the number of free amino moieties that distinguishes the two polysaccharides, chitin and chitosan. With the increase in temperature or strength of alkaline solution, the degree of deacetylation of chitin can be increased. The ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-glucopyranose structural units determines the extent of deacetylation of chitin to chitosan. When this ratio is less than 1, then we treat the biopolymer as chitosan but when it is more than 1, then we regard it as chitin. It also affects the biodegradability and immunological activity of chitosan.\textsuperscript{[82]}

Fig.2 represents the structure of chitosan in which the glucosamine and N-acetylg glucosamine units are linked by $\beta$-(1→4) glycoside bonds.
The extent of deacetylation, distribution of acetyl groups along the chain and molecular weight of chitosan determines its solution properties. [83-85] The physical characteristics properties of chitosan like viscosity, elasticity, tear strength and solubility, thus depends on its molecular weight and degree of deacetylation. It is insoluble in basic and neutral medium because of inability of free amino groups to get protonated while in acidic medium it is soluble as chitosonium salt with protonated free amino groups. The polycationic amines on its surface interact with anionic carbohydrates, lipids and proteins present on the surface of bacteria and hence inhibit the growth of bacteria. It is found to be soluble in 1% or 0.1M acetic acid and the required amount of acid depends on the amount of chitosan to be dissolved. [86] Recently a method has been developed to solublize chitosan in water using glycerol-2-phosphate at a neutral pH. [87-90] The solution so formed has been found to be stable at room temperature which upon heating to about 40.1°C changes to gel.

Chitosan has two advantages over chitin. It is more soluble in comparison to chitin due to positive charge on C-2 of the glucosamine monomer at a pH less than 6.5 and hence is a better antimicrobial agent than chitin. [91] In neutral pH, it loses its charge and gets precipitated from the solution. Chitin requires highly toxic solvents such as lithium chloride and dimethylacetamide to be dissolved while chitosan gets readily dissolved in diluted acetic acid. Secondly, chitosan has more free amino groups that can actively participate as sites of attachments of different functionalities leading to the modification of chitosan for better applicability. The antibacterial effect of many derivatives of chitosan like acid free water soluble chitosan and quaternary N-alkyl chitosan [92] has been reported.

Chitosan is a pseudoplastic material and acts as an excellent viscosity-enhancing agent in acidic environments. These properties of chitosan help in wound healing and can also be used in slow drug release system. The water-soluble salts with inorganic and organic
acids include glyoxylate, pyruvate, tartarate, malate, malonate, citrate, acetate, lactate, glycolate and ascorbate. Chitosan and its chito-oligosaccharides produced by enzymatic and acidic hydrolysis of chitin have been found useful in biological applications due to high biocompatibility, muco-adhesive nature, blood clotting ability and non-toxicity of chitosan. Chitosan can be employed in formation of hydrogels, films and fibers that can be utilized in biomedical applications. These have been found useful in applications like wound dressing and drug delivery systems, and the food and chemical industries. It acts as antimicrobial films to cover fresh fruits and vegetables. It contains cations and so can suppress the growth of microorganisms and bacteria that possess anions and hence classified as a bio-polymer. The water soluble derivatives of chitosan also show antimicrobial activity. The mechanism of antimicrobial activity of chitosan is expected to cause cell lysis, breakdown of cytoplasmic membrane barrier then it forms chelates with trace metal cations. Other applications include cell immobilization and drug delivery, in dental applications, in adhesion bandages for surgery, industrial, pharmacy, cosmetics and biotechnology. Chitosan can absorb enzymes, anionic polysaccharides and metal ions, as well as associate with food processing wastewaters. Hence it also has applications in separation and purification processes.

Thus, chitosan has been regarded as a source of potential bioactive material and has attained increasing commercial interest but it also has several limitations such as processability, inadequate water solubility under physiological conditions, fragile nature, less stable because of its sensitivity to pH and hydrophilic nature to be utilized as biomaterial. Therefore, improving these shortcomings is the key challenge that needs to be addressed for elevation of chitosan as a biomaterial. It is one of the most facile polymers that can be altered structurally by modifying the reactive functional amino and hydroxyl groups that will add new biological activities to the polymer and give useful hydrogel matrices. There is, therefore, a growing interest in the chemical modification of chitosan in order to improve its solubility in different media and widen its applications. The stability of chitosan can be improved by using various type of techniques like, cross-linking or blending by reagents. Chitosan get cross-linked with glutaraldehyde through covalent bonds which decreases its solubility. Other reagents, such as, epichlorohydrin, diisocyanate or 1, 4-butanediol diglycidyl ether have also been used for cross-linking chitosan.
Some investigations have discussed the thermal behaviour of chitosan and chitosan derivatives, including chitosan, chitosan Schiff bases, chitosan-metal ion complex, chitosan/ poly(3-hydroxybutric acid) blends and chitosan-lactic/ glycolic acid. Chitosan blended with PA6 (chitosan/PA6) polymer blend exhibits deficient thermal degradation characteristics. This investigation first used FTIR to observe the hydrogen bond effect between chitosan and PA6. Many studies are reported in the literature on the blends of chitosan with a variety of polymers such as chitosan/polyamide 6, chitosan/cellulose acetate fibers, chitosan/cellulose using a common solvent, chitosan/polyethylene glycol, chitosan/polyvinylpyrrolidone and chitosan/polyvinyl alcohol to obtain polymer with improved properties. The blend of oxidized starch and chitosan has also been cross-linked. The free amino groups and the hydroxyl groups present on the units of chitosan are available for modification through the binding of different reactive functional molecules leading to different chitosan chemical derivatives, modification by graft copolymerization giving grafted chitosan and chitosan composites have been reported. De Souza et al. synthesized chemically cross-linked chitosan and PVA blended hydrogels and investigated their swelling behaviour in simulated body fluid. The hydrogel exhibited adequate cell viability, non-toxicity and appropriate properties which can be modified for potential use in tissue engineering. Chemically cross-linked nanostructured chitosan/PVA blends were successfully synthesized and tailored for potential use in biomedical applications. Amphiphilic chitosan-polyactic acid graft copolymers with polymeric micelles forming ability that can be used as a promising delivery carrier for the entrapment and controlled release of hydrophobic drugs were synthesized by Wu et al. A semi-interpenetrating network of poly(ethylene oxide) and chitosan cross-linked with glyoxal was synthesized. Physically cross-linked PVA/chitosan composite hydrogels was prepared by cyclic freezing/thawing techniques, in the simulated gastric (pH 1.0) and intestinal (pH 7.4) media and the microstructure and swelling behaviour of the hydrogels were investigated.

1.7. Poly vinyl alcohol:

Polyvinyl alcohol is a crystalline synthetic substance having atactic structure. Hermann and Haehnel were the first to synthesize polyvinyl alcohol from polyvinyl acetate by its hydrolysis using potassium hydroxide in alcohol in 1924. The ester groups of polyvinyl
acetate are replaced with hydroxyl groups when it is treated with methanol and aqueous sodium hydroxide for the commercial preparation of polyvinyl alcohol. Vinyl acetate, the raw material for the synthesis of polyvinyl alcohol, is initially polymerized and then partially hydrolyzed which leads to the substitution of some of the ester groups with hydroxyl groups. In the presence of aqueous sodium hydroxide, the substitution process is completed. PVA is then precipitated by saponification of the resulting solution by adding suitable saponification agent. The precipitates are then separated and dried after washing. The completion of saponification indicates the completion of hydrolysis of polyvinyl acetate. The general reaction for the synthesis of polyvinyl alcohol from polyvinyl acetate using ethanol may be represented as follows:

$$[\text{CH}_2\text{CH(OAc)}]_n + \text{C}_2\text{H}_5\text{OH} \rightarrow [\text{CH}_2\text{CH(OH)}]_n + \text{C}_2\text{H}_5\text{OAc}$$

The structure of polyvinyl alcohol is as follows:

$$\left[ \begin{array}{c} \text{CH} \\
\text{CH} \\
\text{OR} \\
\end{array} \right]_n$$

where R = H or COCH$_3$

The extent of hydrolysis and polymerization determines the functional uses and physical properties of polyvinyl alcohol. On the basis of hydrolysis, polyvinyl alcohol is of two types: (i) partially hydrolyzed polyvinyl alcohol and (ii) fully hydrolyzed polyvinyl alcohol. The number of ester groups in PVA are responsible for its various properties. The partially hydrolyzed polyvinyl alcohol can be utilized in foods. On observing the structure of polyvinyl alcohol, it has been found that it has 1,3-diol linkages along with small percentage of 1,2-diol linkages which depends on its methods of preparation from vinyl ester. \[^{137}\]

$$\text{PVA with 1,3-diol linkages} \quad \text{PVA with 1,2-diol linkages}$$

Polyvinyl alcohol is, thus, a synthetic polymer which has neither odor nor taste and is colorless or cream colored granular powder. It is completely soluble in water but partially soluble in alcohol and does not get dissolved in organic solvents. It gets
hydrolyzed up to 86.5 to 89% and has molecular weight of the range of 26,300 to 30,000. The presence of inter and intra molecular hydrogen bonding in PVA results in higher melting point (about 230 °C). It is a highly flexible substance with great tensile strength. It does not combine with oxygen and aromatic substances therefore, can be used to resist them. Since PVA has high affinity for water, its properties depend on the humidity. In humid atmosphere the tensile strength of PVA decreases. But water increases the plasticity of PVA and thus its tear strength and elongation. PVA can be compressed negligibly. Its Poisson’s ratio lies between 0.42 and 0.48.\(^{138}\)

It is cheap, non-toxic material, completely biodegradable, non-carcinogenic and biocompatible polymer\(^{139,140}\) and is used for various purposes. It is therefore, also used as component of cosmetics, bacterio-static agent and other externally applied medicines.\(^{141,142}\) It has excellent chemical resistance, film forming tendency, emulsifying and adhesive properties. It does not react with oils and greases so can be used to resist these. PVA films, thus, are used to protect food supplements and dry food from moisture uptake. It has also been used as packaging covers for laundry detergents because it can be modified as water soluble films. PVA films are also used in sunglasses and in other optical applications. As an adhesive, it is used in the preparation of solid board and in making spiral winding when mixed with boric acid. In the form of glues with polyvinyl acetate, it can be used as thickener and modifier and has been used for coating papers and as release liners. It has been modified as plastic supporting sheets which are biodegradable and have been used in making certain products of female hygiene and adult incontinence. It is used as protective colloid in the preparation of dispersions of polyvinyl acetate and aids emulsion polymerization.

In polyethylene terephthalate bottles, PVA has been used as a barrier for carbon dioxide. In the form of films, PVA is used in water transfer printing. Amphiphilic membranes from PVA can be used for enzyme immobilization.\(^{143}\) Cross-linked PVA membranes have good swelling properties and can be useful in sustaining drug release.\(^{144}\) It is widely used for the biomedical applications such as cartilage replacement,\(^{145}\) pharmaceutical release agents,\(^{146}\) reconstructive surgery,\(^{147}\) drug-delivery systems,\(^{148}\) contact lenses,\(^{149}\) skin replacement material, artificial muscle and vocal cord reconstruction\(^{150}\) and other industrial applications. It has been used to treat dry eyes in the form of eye drops and as lubricating solution for contact lens. PVA, in the form of
fiber, is used in concrete as strengthening agent. With nitric acid it is used to prepare an ester polyvinyl nitrate. Fibres of vinylon are prepared with the use of PVA in Japan. It is also used as surfactant to synthesize nano-beads encapsulated with polymer. Chemical resistant gloves can be prepared using PVA.

PVA because of the inherent properties of hydrophilicity, bio-compatibility, non-toxicity, good film forming ability, long-term temperature and pH stability, \(^{151}\) is considered as a better candidate in the class of biomaterials and hydrogels in particular and can be used in biomedical applications. \(^{152}\) Cross-linked PVA-maleic acid based hydrogels have been reported for colon targeted drug delivery \(^{153}\) while PVA cross-linked with glutaraldehyde hydrogel discs have been reported for the release of glipizide, an oral antidiabetic drug \(^{154}\) and controlled release of sulfosalicylic acid electrically. \(^{155}\) PVA hydrogels are permeable to aqueous alcoholic solution. \(^{156}\) PVA based hydrogels have limited applications due to poor mechanical strength. Polymer blending is a simple yet attractive method to overcome this limiting factor and it provides improved physical and chemical properties to the hydrogels. Grafting and cross-linking are the two methods commonly employed to modify and improve the functional properties of the hydrogels. Bio-artificial polymeric hydrogels, based on blends of PVA with biological macromolecules like hyaluronic acid, dextran and gelatin have improved biocompatibility than pure PVA hydrogels \(^{157}\) and have been extensively assessed as efficient matrices for uploading and release of human growth hormone and other macromolecular drugs. \(^{158}\) The hydrogels have high tensile strength, flexibility and are fully degradable. Because of its properties, it has been used as cross-linked polymer.

Cross-linking is a highly inventive method to build and modify polymers with improved properties, such as mechanical, thermal and chemical stability. \(^{159}\) PVA is a linear polymer and can easily be cross-linked by irradiation \(^{160,161}\) or bifunctional group containing chemical agents such as glutaraldehyde, \(^{162}\) boric acid, \(^{163}\) and hexamethylene disocyanate, \(^{164}\) maleic acid, tartaric acid or citric acid etc. Because of its unique structural properties, PVA can also be cross-linked without the use of a chemical agent. Varshosaz et al. \(^{165}\) synthesized cross-linked PVA hydrogel and studied its swelling and drug release behaviour. Modification through graft copolymerization is another well studied method for improving upon the properties of the polymers both natural and synthetic. It involves covalent attachments of graft chains onto a polymer.
surface avoiding their delamination and assures the long term chemical stability of introduced chains, in contrast to physically coated polymer chains. The pH sensitive grafted hydrogel was synthesized by gamma radiation induced copolymerization of AAc onto PVA by Arnaouty et al. \cite{166} Panda et al. \cite{167} carried out the graft copolymerization of AAc onto PVA using a Ce (IV)-glucose redox system in an aqueous sulphuric acid medium under nitrogen atmosphere. An acrylic acid or methacrylic acid grafted PVA membrane was prepared for CH$_3$OH/ H$_2$O separation by Shantora and Huang. \cite{168} The pH-sensitive poly (vinyl alcohol-g-methacrylic acid) and poly (vinyl alcohol-g-acrylic acid) hydrogels were prepared by gamma ray irradiation and their application as drug carriers for oral drug delivery taking insulin as the model drug was studied by Park et al. \cite{169}

1.8. Graft Copolymerization:

Over many years, polymer surface modifications have been studied in various fields of industrial applications using different innovative techniques including chemical and physical processes. Physical processes take advantage of surface segregation, radiation of electromagnetic waves and oxidation with gases while chemical modifications use wet treatment, blending, coating, grafting and metallization. Grafting has advantages over other methods in several points, including easy and controllable introduction of graft chains with a high density and exact localization of graft chains to the surface with the bulk properties unchanged.

Generally, grafting has been used as an important technique to improve the functional properties of polymers due to the combined additive physical and chemical properties of both the polymers used in the formation of the graft copolymer. Thus, graft copolymer is a blend of properties of the parent polymer and the grafted polymer and is designated as specialty polymers with improved physical and chemical properties not inherent of either of the individual polymers. The choice of the monomer to be grafted to the main polymer backbone is in the direction to give a product with desirable tailor made properties that can be made to use in various technically important fields. Zhu et al. \cite{170} commented that polymer grafting allows the conversion of commodity polymers to value-added specialty polymers. Instances given are: grafting of highly charged ionic polymers onto high molecular weight polyacrylamide to give better flocculation effects, grafting of elastomers onto polypropylene to enhance impact strength for applications in
the automobile industry and grafting of malic anhydride oligomers onto polyolefins to improve the adhesion of the base polymers to metals and glass fibres.

1.9. Graft Copolymers:

Graft copolymer is one of four main classes of copolymers (alternate, random, block and graft) in which the branches of a homopolymer or a copolymer are attached on to the backbone of a different preformed homopolymer or a copolymer. Successful synthesis of different graft copolymers such as thermoplastic elastomers, emulsifiers, for making stable blends or alloys etc have been carried and are being used for long.

Random and alternating copolymer, prepared by polymerizing two kinds of monomers (M₁ and M₂) possess better than the constituent homopolymers but have not been found suitable for utilization as specialty polymers. The most interesting characteristics of sequencing copolymer i.e. both block and graft copolymer, on the other hand, is that these exhibit many characteristic properties of each constituent homopolymer. The formation of graft or block copolymers with sufficiently large polymeric sequences of diverse chemical compositions sometimes open the way for meeting out more specialized requirements. Conditions are usually designed so as to retain the desirable properties of the individual block or graft components eliminating the undesired properties.

The main purpose of preparing graft copolymers is to modify the properties of natural and commercially available synthetic polymers. There is a little perturbation and therefore, the bulk properties of polymer molecule undergo little changes. The side chains disturb the regularity of the lattice and the crystalline content of such polymers becomes smaller with increasing degree of branching. Grafting can significantly affect mechanical properties of polymers such as their tensile strength, impact strength, extensibility etc. When grafting is carried out on a crystalline polymer and crystallinity disrupted, the strength of the material may act to reinforce the structure. However, when crystallinity is not disturbed, then continuous increase in strength can be obtained with the increase of graft content. [171]

Graft copolymers are therefore, specialty polymers having branches of different polymeric chains which can lead to desirable properties, providing means of blending
and introducing special properties such as dye ability, crease resistance or water repellency that are not inherent properties of the parent backbone. Because of this added advantage, modification of wide variety of synthetic and natural polymers utilizes graft copolymerization.

The overall graft copolymerization reaction involves three main steps that can be represented by the following chemical equations.

Initiation

\[
\begin{align*}
PH & \rightarrow P' \quad \text{(1)} \\
M & \rightarrow M' \quad \text{(2)} \\
\hat{P} + M & \rightarrow PM' \quad \text{(3)}
\end{align*}
\]

Propagation

\[
\begin{align*}
M' + nM & \rightarrow M_n \quad \text{(4)} \\
PM' + nM & \rightarrow PM'_{n+1} \quad \text{(5)}
\end{align*}
\]

Termination

\[
\begin{align*}
P' + M' & \rightarrow M_n \rightarrow P \quad \text{(M)}_{n+1} \quad \text{(6)} \\
PM'_{n+1} + PM'_{n+1} & \rightarrow PM_{2n+2} \quad \text{(7)}
\end{align*}
\]

Where \(P'\) is the primary radical on the polymeric backbone, \(M\) is the monomer unit and \(PM'\) is the initial chain. \(PM'_{n+1}\) are the graft growing chains. Any parameter that affects one or more of these steps causes a variation in the degree of grafting. However, attention is essentially confined to the first step where high energy radiation is often considered as an alternative to other initiation methods.

The formation of Graft copolymers can takes place via one of the following two processes:
1.9.1. **Grafting onto:**

It is one of the simpler methods that involve synthesis of a graft polymer with well defined side chains. Typically a monomer of a lower molecular weight, polymerized in situ (eq.4) is copolymerized onto the active site, generally the free radicals (eq.6), generated on the preformed polymers using chemical or radiations as initiating systems. The concentration of monomer with respect to the polymer and the copolymerization method determines the number of chains that are grafted. With change in the concentration of the monomer with time, the formation of the graft is affected and random placement of different number of branches is observed and this has important effects on the physical properties of the grafted copolymer. Grafting onto polyethylene, polypropylene, cellulose, starch has been achieved involving this process.

1.9.2. **Grafting from:**

In the grafting-from method, the preformed polymer backbone is activated with the generation of free radical sites capable of initiating functionality (eq.3). The monomer is initiated by the active sites of the polymer and the formation of the graft copolymer takes place. The lengths of each grafted chain may be different due to kinetic and steric hindrance effects. Free radical polymerization, anionic grafting, cationic grafting, atom-transfer radical-polymerization have been used in the synthesis of grafting from copolymers. The reactions involving “grafting from” method have been conducted from polyethylene, polyvinyl chloride, and polyisobutylene.

1.10. **Methods of synthesis of graft-copolymers:**

1.10.1. **Physico-mechanical method:**

In this method, the active sites on the polymer backbone can be generated by swelling the polymer in a suitable solvent. Penetration of the solvent through the polymer causes rupture of bonds leading to the formation of free radicals. Freezing of polymer–monomer mixture also generated active sites in the same way. Application of stress to polymeric backbone causes segmental motions and molecular flow which may lead to bond scission and consequent formation of free radicals on the backbone polymer. The free radicals may also be generated by mastication and milling of backbone polymer used for grafting.
1.10.2. Chemical activation:

Conventional radical initiators, \[^{179, 180}\] various redox systems, metal chelates, \[^{181}\] Lewis acids \[^{182}\] and strong bases have been used successfully for chemical grafting. Misra et al. have successfully grafted a wide variety of monomers onto wool, \[^{183}\] cellulose, \[^{184}\] and starch \[^{185}\] by the use of redox systems and metal chelates as graft initiators. Free radicals can also be generated on cellulose by direct oxidation. \[^{186}\]

1.10.3. Radiation induced grafting:

The field of graft copolymerization has been totally revolutionized by the use of radiations. This method is based upon the principle that when electromagnetic radiations are passed through matter, intensity of these radiations decrease and the absorbed radiations activate the polymeric backbone.

Various low and high energy radiations are capable of generating active sites on polymeric backbone where grafting can occur.

1.10.3.1. Low energy radiation grafting: Photo-chemical grafting:

When a chromophore on a macromolecule absorbs light, it goes to an excited state, which may dissociate into reactive free-radicals, whence the grafting process is initiated. If the absorption of light does not lead to the formation of free-radical sites through bond rupture, this process can be promoted by the addition of photosensitizers, e.g. benzoin ethyl ether, dyes, such as Na-2,7 anthraquinone sulphonate or acrylated azo dye, aromatic ketones (such as benzophenone, xanthone) or metal ions/ \( \text{UO}_2^+ \). That means the grafting process by a photochemical technique can proceed in two ways: with or without a sensitizer. \[^{187, 188}\]

1.10.3.2. High energy radiation grafting:

Gamma rays from Co-60 are more advantageous over those from Ce-137 and has been widely used due to higher energy emission, (1.25 MeV compared to 0.66 MeV for Cs-137), simplicity, high penetrating power, longer half life period (5.3 years), ease of preparation and low cost.
Graft copolymerization, using γ-rays as a source of initiation can be affected by any of the following methods:

1.10.3.2.1. Simultaneous/Mutual irradiation method:

Simultaneous irradiation is the simplest irradiation technique for preparation of graft copolymers. In this method a polymer backbone is irradiated in the presence of a monomer available in different forms i.e. vapour, liquid or in bulk solution. Irradiation is carried out either in air or inert atmosphere (e.g. N₂) or preferably under vacuum, leading to the formation of active free radicals on both the polymer backbone and the monomer units. The radicals on the polymer backbone offer the site for grafting. Diffusion of monomer into the polymer plays an important role in the direct radiation method, as it is by this means that the monomer reaches the active sites within the polymer.

1.10.3.2.2. Pre-irradiation Method:

Pre-irradiation method involves a combination of two steps: (i) irradiation of the polymer backbone leading to the formation of peroxides or hydroperoxides and (ii) contact of the irradiated polymer backbone with monomer. Two different situations may arise, depending upon whether peroxide or hydro-peroxide is formed in the irradiated polymer.

1.10.3.2.3. Grafting by trapped radicals:

The presence of trapped radicals has been detected in many irradiated polymers. When a polymer backbone is irradiated, a number of free radicals remain trapped in the rigid polymer matrix. These trapped free radicals can survive for a considerable length of time and can act as active sites to initiate graft copolymerization. [189, 190]

1.10.4. Plasma activation method:

Activation by plasma generates active sites on polymer surface owing to electron bombardment and the species so generated can be used in the modification of polymer backbone. The advantage of plasma modification over the gamma radiation is that the changes are confined to a limited depth of few nanometers leaving the bulk properties of the polymer unchanged.
1.10.5. Intercross-linking of two different polymers:

In this method two preformed polymers of different types are cross-linked to give the graft copolymer instead of polymerization of a monomer in the presence of the preformed polymer. Reaction between the two polymers depends on the structures of both (a) cross-linking and (b) degrading type vinyl polymers.

When a mixture of two polymers is irradiated or chemically reacted, it may lead to a graft copolymer through inter cross-linking, the following reactions are expected to occur:

1.10.6. Enzymatic grafting:

The enzymatic grafting method is quite new and involves enzyme for initiation of the chemical/ electrochemical grafting reaction. For example, tyrosinase is capable of converting phenol into reactive o-quinone, which undergoes subsequent non-enzymatic reaction with chitosan. Enzymatic grafting on a poly(dicarbazole-N-hydroxysuccinimide) film was reported by Cosnier et al.

1.10.7. Microwave assisted grafting:

Microwave assisted free radical polymerization is also emerging as a novel technique in the field of polymer science to get grafted biomaterial with or without catalyst. The polymerization under microwave irradiation of acrylamide and acrylic acid has been investigated with the purpose of developing polyelectrolytes for waste water treatment. Under microwave irradiation grafting and homopolymerization have been studied
without initiators \cite{193} or in the presence of very low concentration of initiator. \cite{194} The formation of the graft copolymer is achieved either using microwaves directly \cite{195} or in combination with the conventional initiators. \cite{196}

1.11. Brief review of Literature:

Natural and synthetic polymers because of their structural variations offer a wide range and scope of reactions which provide an attractive option to discover and devise new synthetic strategies to develop new polymeric materials. Using these strategies, number of natural and synthetic polymers have been modified to give materials with improved properties.

Modification through copolymerization, specifically graft copolymerization, has been an area of great interest as retaining the inherent properties of the polymer to be modified polymer, additional properties of the grafted polymers are supplemented and the modified polymer attains a wider scope of its applicability. Ramakrishna et al. \cite{197} prepared carbohydrate polymeric blend of chitosan and PVA by water in oil emulsion and achieved controlled release of Acebutolol HCl in pH 7.4 media. Porous PVA-chitosan based hydrogels for tissue engineering in enhancing chondro genesis of implanted cells was synthesized by Abbas et al. \cite{198} Francisco et al. \cite{199} used rice husk ash in the preparation of high performance chitosan and poly(AAc) based superabsorbent gels. These superabsorbent hydrogels showed good swelling properties and responsive behaviour in relation to both pH and salt solution. Dai et al. \cite{200} prepared N-succinyl chitosan/ alginate network and investigated release of nifedipine drug from the composite. Biodegradable gelatin-chitosan films incorporated with essential oils were synthesized by Gómez-Estaca et al. \cite{201} and the films were tested for antimicrobial properties in fish preservation. Chitosan-gelatin composites and films were prepared by Pereda et al. \cite{186} and investigated for antimicrobial activity. Hari et al. \cite{202} developed Chitosan-alginate micro-particle system and studied the controlled release of bioactive peptides including insulin. The hydrogels of chitosan and poly (AAc) with interpenetrating polymeric networks were synthesized and characterized by Lee et al. \cite{203} Electro-sensitive interpenetrating polymer network hydrogels of PVA and chitosan were prepared by Kim et al. \cite{204}
Chitin cross-linked hydrogels, obtained through physical or chemical cross-linking using epichlorohydrin (ECH) in the NaOH/urea aqueous system, showed that the chemically cross-linked chitin hydrogels exhibit rapid gelation process and possess uniform porosity, low crystallinity, good mechanical strength and high swelling ratio with excellent biocompatibility. The hydrogels created from gamma rays cross-linked carboxymethylated chitin derivatives exhibited excellent mechanical properties, good swelling in water and displayed characteristic pH-sensitive properties in their swelling behaviour. Synthesis of novel superabsorbent hydrogels with good biodegradability was obtained from chitin and succinic anhydride in the presence of 4-dimethylaminopyridine as esterification catalyst. Superabsorbent hydrogels with good biodegradability (91% within 7 days) and highest absorbency (345 g/g-polymer) were prepared from chitin dissolved in lithium chloride and N-methyl-2-pyrrolidinone and esterification cross-linking with 1,2,3,4-butanetetracarboxylic dianhydride (BTCA). Using chitin regenerated hydrogel (RG) and swelling hydrogel (SG) with N-acetyl-D-(+)-glucosamine, GlcNAc membranes of chitin/ gelatin have been prepared. The characteristics like swelling, mechanical, enzymatic degradation and cell studies for growth of NIH/3T3 fibroblast cells, of the chitin/ gelatin membranes synthesized with or without (GlcNAc) were studied. From the results it was observed that the chitin/gelatin membrane synthesized with or without GlcNAc showed high elongation and stress and these membranes can be employed for tissue engineering applications.

Alginate/ phosphorylated chitin blend films were synthesized in solvent water and studied for bioactivity using biomimetic technique in simulated body fluid solution. The blend films were also found to adsorb successfully the Ni^{2+}, Zn^{2+} and Cu^{2+} ions. Chitin/ silk fibroin nano-fibrous scaffolds were prepared by electro-spinning method and used to fabricate a biomimetic nano-structured bicomponent scaffolds. From the cytocompatibility and cell behaviour on the chitin/ silk fibroin blend or hybrid nano-fibrous scaffolds it has been observed that these nano-fibrous scaffolds can be used as tissue engineering scaffolds.

Pal et al. successfully synthesized and characterized PVA-gelatin hydrogel membranes for biomedical applications. Gamma radiation induced preparation and characterization of PVA-gelatin hydrogels for 3-D cell culture was carried out by You et al. Mogdhaddam and Eslahi synthesized nano-composite based on polyaniline/ PVA/ Ag with antibacterial activity.
Graft copolymerization of various vinylic monomers onto the natural and synthetic polymers and their composites is also well known. The use of acrylic acid as monomer has very fair reputation as non-toxic and biocompatible materials. \(^{[215]}\) and acrylic acid graft copolymers have widely been used for drug delivery systems, flocculation and settling of aqueous suspension, paper treating, resins and as gelling and stabilizing agent for soil and mud. \(^{[216]}\) Grafting of functional monomers such as AAc directly confers ionic (cationic) characters to the polymer backbone. \(^{[217]}\) Various initiators (azoisobutytritrile, potassiumpersulfate and benzoyl peroxide) were used for graft copolymerization of AAc on starch obtained from potato, thus depicting reactive susceptibility of AAc towards grafting. \(^{[218]}\) Novel nano-porous superabsorbent hydrogel based on poly (AAc) grafted onto Salep were prepared and their swelling behaviour were studied by Pourjavadi et al. \(^{[219]}\) Hydrophilic poly (AAc) and PVA based hydrogels have enhanced potential to deliver the drug to the colon. \(^{[220]}\) Lu et al. \(^{[221]}\) synthesized PVA/ poly(AAc) hydrogel coatings for improving electrode–neural tissue interface. Hydrogel PVA/ poly(AAc) inter penetrating networks were synthesized and tailored as coatings for poly(dimethyl siloxane) based natural electrodes with the aid of plasma pretreatment. Taleb et al. \(^{[222]}\) used gamma radiations as a source of initiation, cross-linking and to graft PVA and methacrylic acid (MAAc) onto gelatin to produce PVA/MAAc/ gelatin copolymer which can be used as antibiotic drug carrier. Byun et al. \(^{[223]}\) studied the release of drug indomethacin from PVA and poly (AAc) hydrogels, prepared by thermal cross-linking method. Level of gelation was studied by varying the amount of poly (AAc).

AAc and MAAc were graft polymerized onto chitosan by Shantha et al. \(^{[224]}\) using ceric ion. pH sensitive Chitosan-g-poly(AAc-co-HEMA) copolymers were synthesized and their swelling behaviour was investigated by Sadeghi. \(^{[225]}\) Nesrinne and Djamel \(^{[226]}\) prepared pH sensitive cross-linked poly(AAc-co-AAm) by chemical method. An interesting work of corrosion inhibition was carried out by Geethanjli et al. \(^{[227]}\) using poly (AAm) and poly (AAc) grafted pectin with maximum of 85% corrosion inhibition. Verma et al. \(^{[228]}\) grafted N-hydroxymethyl acrylamide on to k-carrageenan using peroxomonosulfate and glycolic acid system as redox system and used them for metal ion uptake and flocculation studies. Flocculation tendency of grafted k-carrageenan was found to be good than k-carrageenan. Sodium acrylate and acrylamide were grafted upon biopolymer alginate by Sadeghi et al. \(^{[229]}\) Swelling properties of Chitosan-g-poly (AAm)
was studied by Pourjavadi et al. \textsuperscript{[219]} prepared by varying the concentration of AAm and MBA concentration under inert atmosphere. 1-Lactide was grafted onto chitosan by Skotak et al.\textsuperscript{[230]} and the graft copolymer was found to be biocompatible and biodegradable for use in tissue engineering. Mi et al. \textsuperscript{[231]} fabricated chitosan membrane having sponge like character for use in wound dressing applications. Adsorption of brilliant green from aqueous solution onto cross-linked chitosan graft copolymers was investigated by Ozkahraman et al.\textsuperscript{[232]} Novel poly (MAAc) cross-linked pre-gelled starch graft copolymer, synthesized by Mostafa et al. \textsuperscript{[233]} were used for the removal of basic dyes from aqueous medium. Devi et al. \textsuperscript{[234]} synthesized blood compatible hydrogel composed of starch, acrylamido ethyl propane sulfonic acid and acrylamide and evaluated them for their use in cell and tissue engineering applications.

Kurita et al. \textsuperscript{[235]} and Ren et al. \textsuperscript{[236]} have carried out graft copolymerization of AAm, AAc and methyl methacrylate (MMA) onto powdery chitin using CAN as redox initiator. The Chitin-g-poly(ε-caprolactone) (Chitin-g-PCL) copolymer was synthesized through ring-opening polymerization method and the copolymer was characterized by different analytical techniques. \textsuperscript{[237]} Nano-composite scaffolds of β-chitin hydrogel/ nano-bioactive glass ceramic was synthesized which was found to be biocompatible for periodontal bone regeneration. \textsuperscript{[238]} Chitin hydrogels based on poly(vinyl pyrrolidone) grafted chitin were found to have powerful chelating potential for Ni\textsuperscript{2+} and Cd\textsuperscript{2+} ions and can thus be effectively used for their removal from wastewater. \textsuperscript{[239]} Chitin-MAAc hydrogels were prepared and in vitro cytotoxicity assay revealed that the hydrogel was non-cytotoxic with cells able to adhere and proliferate well on the hydrogel. \textsuperscript{[240]} A new biodegradable glycol chitin-based thermo-responsive hydrogel scaffold was developed which converts to a hydrogel after its application as a mild viscous solution in order to investigate its effects on the proliferation and odontogenic differentiation of colony-forming human dental pulp cells (hDPCs) in the presence of enamel matrix. \textsuperscript{[241]} β-Chitin/ nBGC (bioactive glass ceramic nanoparticles) composite scaffolds, prepared by lyophilization technique, were found to have enhanced porosity, swelling, bioactivity and degradation in comparison to the control scaffolds. The composite scaffolds were non-toxic and can be used for the treatment of periodontal bone defects. \textsuperscript{[242]} β-Chitin hydrogel/ nZnO composite bandages with controlled swelling and degradation was fabricated and evaluated for blood clotting ability as well as platelet activation, antibacterial activity. \textsuperscript{[243]} A readily water soluble chitin-methacrylate hydrogel was
prepared and used for biodegradability studies with enzyme Lysozyme. The hydrogel was found to be non-cytotoxic during the in vitro studies. Chitin was graft copolymerized with methyl methacrylate (MMA) using tributylborane (TBB) as initiator at ordinary temperature in water. \[^{[244]}\] Graft polymerization of styrene onto chitin macro-initiator has been carried out by Kadokawa et al. \[^{[245]}\] to synthesize chitin-graft-polystyrene via atom transfer radical polymerization (ATRP). Sukhlaaied and Riyajan \[^{[246]}\] used PPS as an initiator for the synthesis of graft copolymer of carrageenan and PVA and observed that percentage of grafting decreases with increase in carrageenan content. Grafting of binary mixture of acrylic and vinylic monomers onto polymeric blends of PVA and gelatin was carried out by Bajpai et al. \[^{[247, 248]}\]

Thus, copolymerization of different polymer bases having different properties and grafting of monomers having varied functionalities onto the synthesized copolymeric bases may lead to the availability of new tailor made copolymers with broad spectrum of applications. Since, chitosan, chitin and PVA are eco-friendly, non-toxic, biodegradable and biocompatible and have many possible applications in food, pharmaceuticals and other industries, modification of these substrates through copolymerization to give bipolymeric backbones viz. Chitosan-chitin and chitin-PVA copolymer which can further be modified by graft copolymerization of hydrophilic monomers. The modified polymers have properties due to both the base and the grafted polymers and thus can find use in different applications.
1.12. Aim and Objectives

Natural polymers such as polysaccharides, proteins, DNA and RNA, derived from living organisms are referred to as ‘Biopolymers’. Polymeric carbohydrates i.e. ‘Polysaccharides’ are important class of natural biological polymers and are supposed to be the first biopolymers formed on this earth from the most renewable resources. They show excellent adsorption behavior due to the flexibility of polymer chain, presence and high chemical reactivity of hydroxyl, acetamido and primary amino functional groups present in each glucose unit along the polysaccharide chain and are known to get hydrated to create a hydrogel like structure in an aqueous environment. Graft copolymerization of polysaccharides such as starch, chitosan sodium alginate, carrageenan and cellulose with vinyl monomers have been shown to form hydrogels.

Chitin and chitosan are two important natural amino polysaccharides, which find utility as useful properties in food and food additives, in biomedical field etc. Chitin is present as a part of exoskeleton of arthropods and some other organisms and functions as structural support. Chitosan is a deacetylated derivative of chitin, with a high degree of deacetylation (75% or above), with improved properties and hydrophilicity. Chitin is a biodegradable, biocompatible and non-toxic biopolymer with anti inflammatory and antibacterial properties and has been used for a number of biomedical applications like wound healing, wound dressing and as tissue supporting scaffolds. Chitosan and its chito-oligosaccharides produced by enzymatic and acidic hydrolysis of chitin have been found useful in biological applications due to high biocompatibility, muco-adhesive nature, blood clotting ability and non-toxicity of chitosan. Chitosan can be employed in formation of hydrogels, films and fibres that can be utilized in biomedical applications. These have been found useful in applications like wound dressing and drug delivery systems and the food and chemical industries.

Synthetic polymers are manmade and have wide range of properties and uses on commercial scale. Polyvinyl alcohol, PVA, is a cheap, non-toxic, biodegradable, non-carcinogenic, biocompatible and hydrophilic synthetic polymer and can be used in a variety of applications. It also possesses properties such as mechanical strength, elasticity, adhesive, emulsifying and water retention capacity. It is biodegradable that makes it useful as an environmental friendly polymer. It is used in waste water treatment
by removal of metal ions from water, production of films, wound dressing agent and in biomedical field, as a scaffold supporting material for tissue engineering application, artificial organs and contact lenses etc. It is also used as component of cosmetics, bacterio-static agent and other externally applied medicines.

In view of the above, taking into considerations the properties of both the natural and synthetic polymers, the blending of the two polymers would boost the properties of the hydrogel, rejecting the negative aspects of the individual polymers. It is for this reason that it was thought significant to develop copolymers from natural polymeric backbones like chitosan, chitin and synthetic polymer, PVA with further functionalization through graft copolymerization of hydrophilic monomers to induce/enhance properties like those of hydrogels and utilize the products in various applications.

The present work has been carried with following objectives:

1. To modify chitin by copolymerization with chitosan and with synthetic polymer i.e. PVA by chemical method, using APS as radical initiator, to give Chs-co-Chi and Chi-co-PVA copolymers.

2. To optimize the reaction conditions for the synthesis of different copolymers, determined on the basis of percentage swelling as a function of time.

3. To carry out graft copolymerization of AAc, AAm and their binary mixture, (AAc + AAm), onto Chs-co-Chi and Chi-co-PVA copolymeric bases by chemical method using APS as radical initiator.

4. To evaluate the optimum conditions for affording maximum swelling percentage of their respective AAc, AAm and (AAc + AAm) grafted Chs-co-Chi and Chi-co-PVA copolymers.

5. To study the swelling behaviour of pristine Chs-co-Chi and Chi-co-PVA copolymers and their respective AAc, AAm and (AAc + AAm) grafted copolymers in acidic pH (2.8) and basic pH (7.2).

6. To study the pH sensitivity and pulsative behaviour of pristine Chs-co-Chi and Chi-co-PVA copolymers and their respective AAc, AAm and (AAc + AAm) grafted copolymers in acidic pH (2.8) and basic pH (7.2).
7. To study the swelling behaviour of pristine Chs-co-Chi and Chi-co-PVA copolymers and their respective AAc, AAm and (AAc + AAm) grafted copolymers in aqueous saline solution (0.9%).

8. To characterize the pristine Chs-co-Chi and Chi-co-PVA copolymers and their respective AAc, AAm and (AAc + AAm) grafted copolymers by using various characterization techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), Particle Size Analysis and Thermogravimetric analysis (TGA).

9. To utilize the pristine Chs-co-Chi and Chi-co-PVA copolymers and their respective AAc, AAm and (AAc + AAm) grafted copolymers in waste water treatment as adsorbents for removal of metal ions and dyes from their respective aqueous solutions and as flocculants for treating aqueous coal suspension solution.

10. To utilize the pristine copolymers, Chs-co-Chi and Chi-co-PVA and their respective AAc, AAm and (AAc + AAm) grafted copolymers in biomedical applications as drug carrier for glucosamine sulphate.

11. To utilize the pristine copolymer, Chs-co-Chi as a support to immobilize amylase and study their hydrolytic activity with respect to hydrolysis of starch.
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