Chapter 1

Introduction

Introduction

1.1 Water-Soluble Polymers

Water-soluble polymers are used primarily to disperse, suspend (thicken and gel), or stabilize particulate matter. These functions make water-soluble polymers suitable for a wide variety of applications including water treatment, paper processing, mineral processing, formulation of detergents, textile processing, the manufacture of personal care products, pharmaceuticals, petroleum production, enhanced oil recovery and formulation of surface coatings[1]. However, they can perform many of the following functions as shown in Fig 1.1.
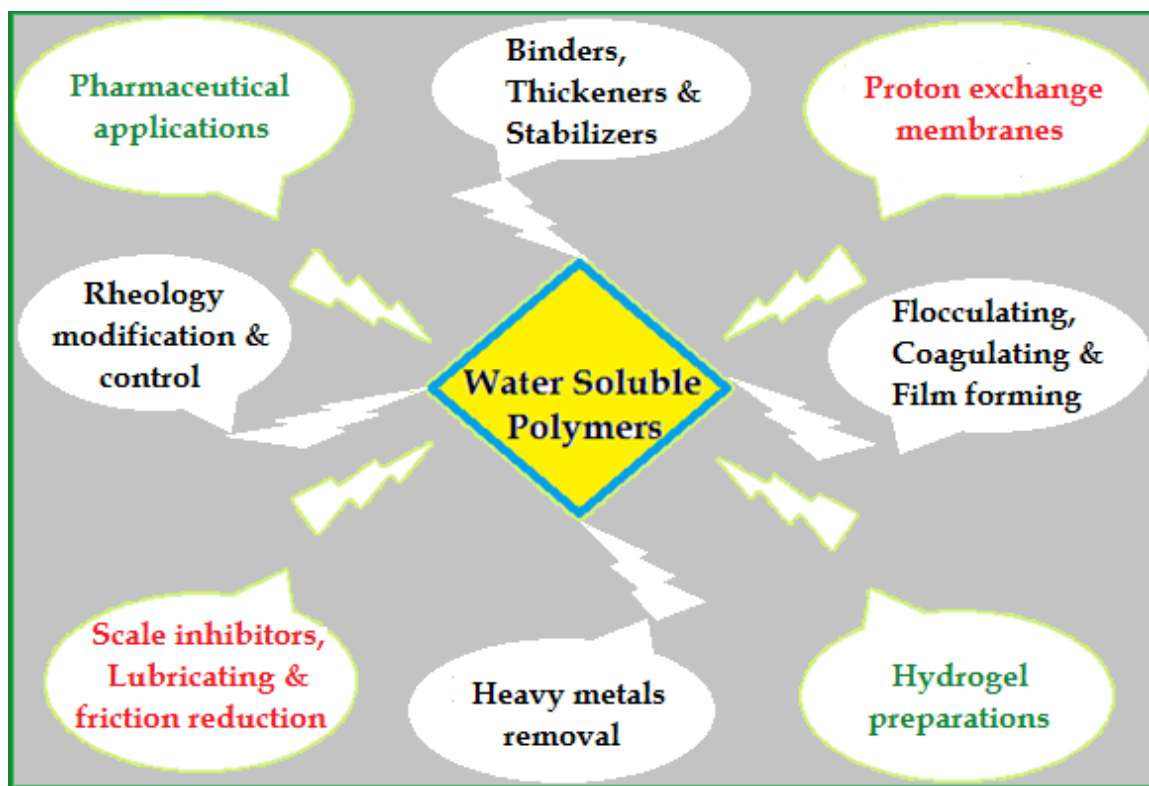

Figure 1.1 Different Functions/Applications of Water-soluble Polymers

These polymers often perform more than one function in any given application. The world's highest growth is particularly in segments such as adhesives, building products, paper, textiles and water treatment[2]. The aggregate volume consumption of

these polymers is increasing at an average annual rate of 3.0–4.0%. The following pie chart (Fig 1.2) shows world consumption of water-soluble polymers[3].
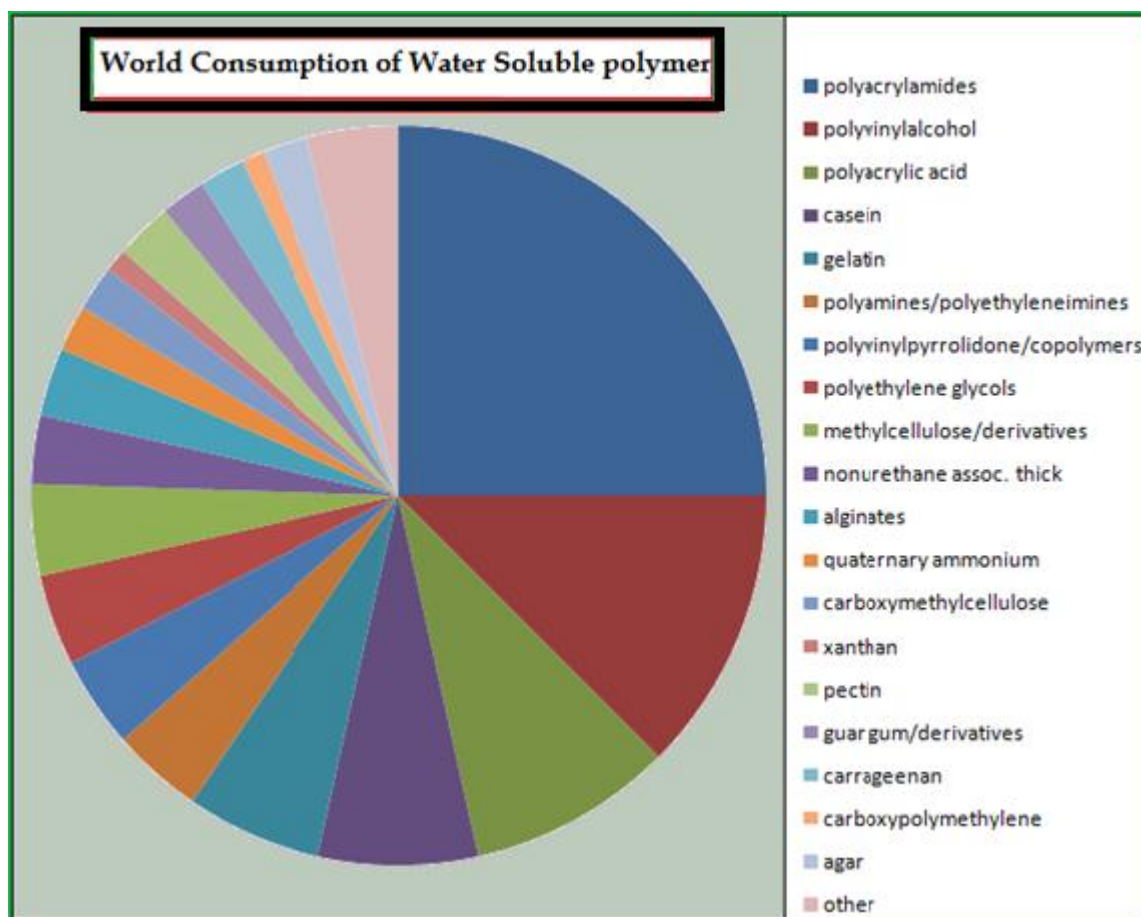

Figure 1.2 World Consumption of Water-soluble Polymers

These polymers have been synthesized to suit specific needs like development of drug delivery systems. Polymers can be classified based on any of the following categories: (1) source (Natural, semi synthetic, synthetic); (2) structure of polymer (Linear, Branched chain, Crosslinked or Network polymers); (3) type of polymerization (Addition, condensation polymers); (4) molecular forces (Elastomers, Fibres, Thermoplastic, Thermosetting); (5) Chain growth polymerization (Free radical governed); (6) degradability (biodegradable, non-biodegradable).

1.1.1 Synthetic Water-Soluble Polymers

Synthetic water-soluble polymers are substances that dissolve, disperse or swell in water and, thus, modify the physical properties of aqueous systems in the form of

gellation, thickening or emulsification/stabilization. These polymers have repeating units or blocks of units; the polymer chains contain hydrophilic groups that are substituent's or are incorporated into the backbone[4]. Some of the common synthetic water-soluble polymers are:

a) Poly(ethylene glycol) (PEG)

b) Polyvinyl pyrolidone (PVP)

c) Polyvinyl alcohol (PVA)

d) Poly(acrylic acid)(PAA)

e) Poly(acrylamide)

f) N-(2-Hydroxypropyl) methacrylamide (HPMA)

g) Divinyl Ether-Maleic Anhydride (DIVEMA)

h) Polyoxazoline

i) Polyphosphates

j) Polyphosphazenes

1.1.1.1 Polyacrylic acid (PAA)

Polyacrylic acid is a biodegradable Water-soluble polymer with various industrial applications, including as a super adsorbent (e.g., in disposable nappies), in water treatment, *etc.*[5]. Poly(acrylic acid) (PAA) copolymers modified with block-copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) have a wide range of medicinal applications as their components are considered pharmaceutically safe. The unique property of polyacrylic acid is that it exists as a liquid at pH 5 and as a gel at pH 7. Permeation of cations into the gelled polymer converts the gel back to a liquid. It has been found to be ideal for ocular delivery of ribozymes to the corneal epithelium as a drug delivery vehicle[6].

Hydrophobically modified poly(acrylic acid) (HMPAA) shows some interesting rheological properties in semidilute aqueous solutions, such as interchain aggregation followed by an increase in the apparent molecular weight and enhanced viscosity as well as shear sensitivity. HMPAA is prepared by modification of PAA in its acidic form by alkylamines in an aprotic solvent in the presence of N, N′-dicyclohexylcarbodiimide (DCCD).

Polyacrylic acid based polymers are mainly used for oral and mucosal contact applications such as controlled release tablets, oral suspensions and bioadhesives. It is

also used as a thickening, suspending and emulsion stabilizing agent in low viscosity systems for topical applications. For bioadhesive applications, high molecular weight acrylic acid polymer crosslinked with divinyl glycol are extensively formulated in a variety of drug delivery systems for mucosal applications. Buccal, intestinal, nasal, vaginal and rectal bioadhesive products can all be formulated with such polymers[7].

The monomer by which PAA synthesized is acrylic acid:
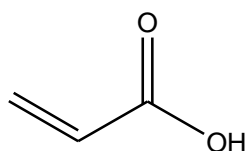
Acrylic Acid   *Relative molecular mass:* 72.06 g/mol

Acrylic acid is a colourless liquid with an irritating acrid odour at room temperature and pressure. Its odour threshold is low (0.20-3.14 mg/m). It is miscible in water and most organic solvents. Acrylic acid is commercially available in two grades: technical grade (94%) for esterification, and glacial grade (98-99.5% by weight and a maximum of 0.3% water by weight) for production of water-soluble resins. Acrylic acid polymerizes easily when exposed to heat, light or metals, and so a polymerization inhibitor is added to commercial acrylic acid to prevent the strong exothermic polymerization. The inhibitors that are usually used in acrylic acid preparations are the monomethyl ether of hydroquinone (methoxyphenol) at 200 ± 20 ppm, phenothiazine at 0.1% and hydroquinone at 0.1%. Methylene blue at 0.5 to 1.0% and N, N'-diphenyl- p-phenylenediamine at 0.05% is also used.

Poly (acrylic acid) has been synthesized by many different ways by using different polymerization methods, initiator system and solvent systems. In 1974 and 1978, several types of initiators were used in anionic polymerization of TBA, include s-butyllithium, 1,1-diphenyl-3-methylpentyllithium (DPPL), t-butyl α-lithioisobutyrate (BLIB), diethyl α-tetrabutylammonium ethylmalonate (BAEM), and t-butyl α-tetrabutylammonium isobutyrate (BAIB).
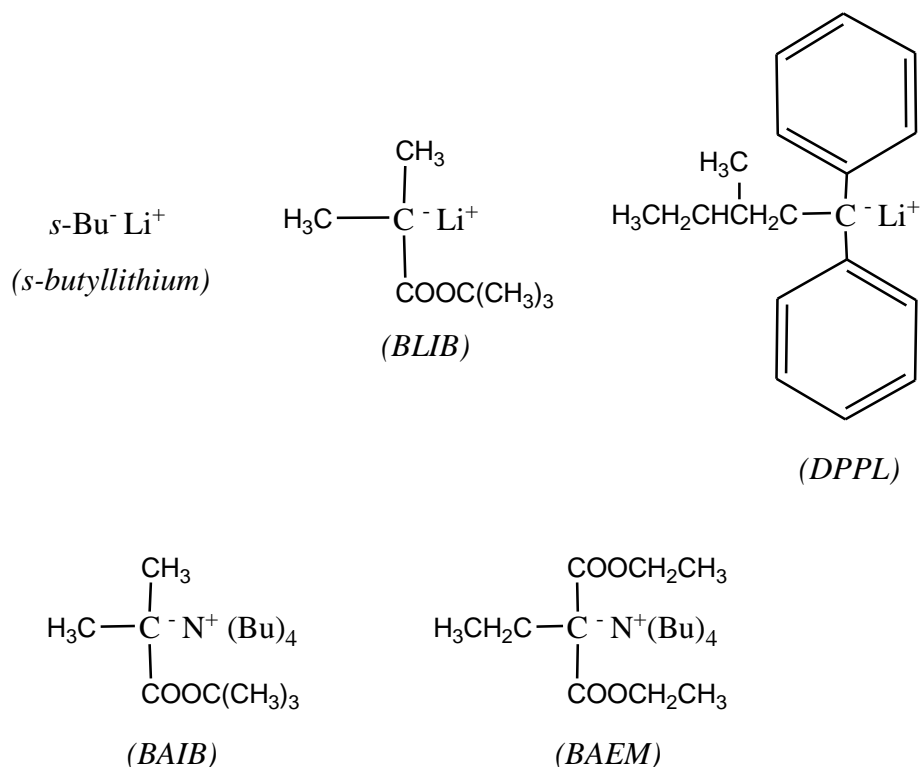
DPPL was prepared by the reaction of *s*-butyllithium with 1,1-diphenylethylene in THF. BLIB was synthesized in an all glass reactor under high vaccum by the reaction of *t*-butyl isobutyrate with lithium diisopropylamide which was prepared by the reaction of *n*-butyllithium with diisopropylamine. BAEM and BAIB were similarly synthesized by the reaction of diethyl ethylmalonate and *t*-butyl isobutylate with NaH in THF, followed by the addition of tetrabutylammonium bromide[8,9].

In 1987, PAA was synthesized by anionic polymerization of t-butyl acrylate (TBA), followed by hydrolysis. In anionic polymerization of acrylate monomers the labile α-hydrogen may cause a serious problem in addition to the presence of the carbonyl group. In fact, proton abstraction by both initiators and propagating chain ends often occurs during the polymerization[10].

In 1988, Reetz[11] also reported that the metal-free, stable tetrabutylammonium salts of –SR (where R=*n*-butyl, phenyl, $(CH_3)_3SiOCH_2CH_2$), -CR(COOR')$_2$ (R, R'=CH_3, CH_3CH_2), and –C(CH$_3$)-(CN)$_2$ were excellent initiators in the polymerization of *n*-butyl acrylate at room temperature. Then in 1990, a striking effect of LiCl was observed which suppressed above/such reactions in the polymerization of TBA. The polymers with predictable MWs and relatively narrow MWD were obtained quantitatively by adding LiCl to the polymerization system[12].

Yasuda et al[13] have reported that the polymerization of acrylate monomers catalyzed by rare earth metal complex proceeds in a living manner to afford high MW polymers with extremely narrow MWDs.

In 1990, PAA of relatively low MW was synthesized in the presence of Cu^{2+} ions using hydrogen peroxide as initiator. It has been determined experimentally that the optimal polymerization conditions were when hydrogen peroxide amount was 1.0 wt % relative to the monomer at 90°C[14,15]. In 1995, it was observed that nitroxide decomposition in an acidic medium renders nitroxide-mediated polymerizations of AA difficult[16,17]. In 1999-2000, PAA was synthesized by radical polymerization in heterogeneous systems (inverse suspension[18,19]/miniemulsion polymerizations[20]) as particle state as well as homogeneous system (solution polymerization). In 2001 Ladaviere et al[21] synthesized PAA via ATRP through polymerization of tBA by using methyl 2-bromopropionate (MBP) as an initiator, PMDETA as ligand, and CuBr/CuBr$_2$ as catalysts. Direct polymerization of acrylic acid via ATRP produced a polymer with many carboxylic acid functional groups capable of complexing the copper ions used during the propagation steps, therefore the direct route was modified to incorporate a "capped" version of acrylic acid, namely tert-butyl acrylate, which does not interfere with the copper ions. In order to remove the *tert*-butyl "caps" the hydrolysis of the ester groups via trifloroacetic acid (TFA) was employed.
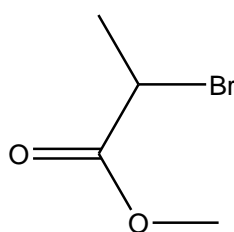

Methyl 2-bromopropionate
(MBP)

In 2003, Loiseau et al[22] synthesized PAA by controlled radical polymerization with reversible addition-fragmentation chain transfer by using trithiocarbonic acid dibenzyl ester and trithiocarbonic acid bis(1-phenylethyl) ester as chain transfer agents (CTA). This polymerization was controlled for low ratios [AA]:[CTA]. However, at higher conversion, and for high [AA]:[CTA], transfer to solvent occurred. At the end of the polymerization, chain transfer to solvent becomes important, even in solvents that are not well-known for their capacity to transfer (e.g., dioxane and methanol). After

neutralization a large proportion of chains were terminated by a proton and not by a thiol. They also have demonstrated that chain transfer to polymer occurs for higher molecular weight polymers only.

Trithiocarbonic acid dibenzyl ester
(CTA)

*Trithiocarbonic acid bis(1-phenylethyl)ester*
(CTA)
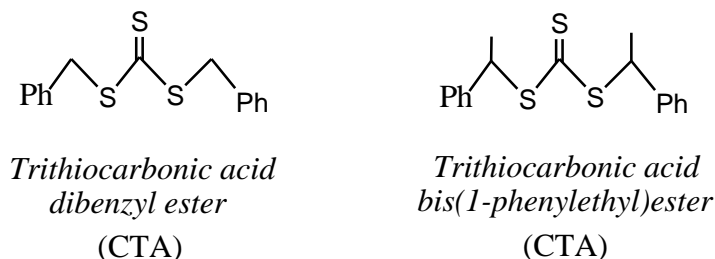
In 2005 Mishra et al have reported synthesis of PAA by using benzoyl peroxide as an initiator in toluene as a solvent. Later have prepared its sodium and potassium salts and have studied their corrosion scale inhibition efficiencies[23].
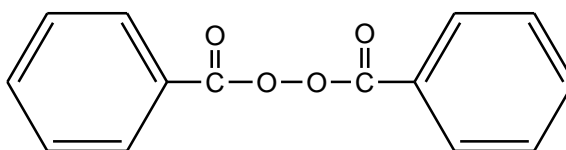
Benzoyl Peroxide

In 2005 Moulay et al have reported synthesis of PAA by solution polymerization method using sodium thiosulfate and potassium persulfate as a redox initiator and thioglycolic acid as an inhibitor[24].
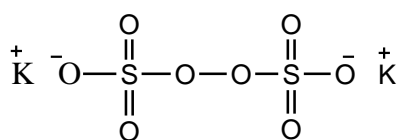
Potassium peroxydisulfate

Ionic liquids have attractive properties such as ionic conductivity, thermal stability, nonflammability, and nonvolatibility, which are considered to give environmentally friendly solvents[25-28]. The physical properties of ionic liquids have been extensively investigated [29,30]. It has been reported that by using ionic liquids as solvents via radical polymerization provides higher polymerization rates and higher molecular weights than in bulk or organic solvents due to a reduced termination rate because of the high viscosity

of the ionic liquids and also as a result of an increase in the propagation rate coefficient in some cases [31-36].

In 2010, Minami et al have synthesized PAA particles by dispersion polymerization of acrylic acid in ionic liquid N,N-diethyl-n-methyl-N-(2-methoxyethyl)ammonium bis(trifluoromethane sulfonyl)amide ([DEME][TFSA]) at 70°C with low hydrolysis grade (35.4%) poly(vinyl alcohol) as stabilizer. These PAA particles were easily extracted from the ionic liquid to water, and the PAA particles had a cross-linked structure during the polymerization without cross-linker[37].

Redox polymerizations are usually carried out in aqueous solution, suspension, or emulsion more rarely in organic solvents. This type of initiation is referred to as redox initiation, redox catalyst, or redox activation. Advantage of redox initiation is that radical production occurs at reasonable rates over a very wide range of temperatures, depending on the particular redox system, including initiation at moderate temperatures of 0-50ºC even lower. This allows a greater freedom of choice of the polymerization temperature than is possible with the thermal homolysis of initiators. Main features of the redox polymerizations are:

(i) a very short induction period,

(ii) a relatively low activation energy (10-20 kcal/mol), as compared 30 Kcal/mol for the thermal initiation which enables the polymerization to be carried out at low temperature and thereby decreasing the possibility of side reactions which may change the reaction kinetics and the properties of the resulting polymer,

(iii) controlled with ease at low temperature and comparatively high molecular weight polymers with high yields can be obtained in a very short time, and

(iv) provide direct experimental evidence of the existence of transient radical intermediates generated in redox reactions, and enables identification of these radicals as end groups of polymers.

1.1.2 Natural Water-Soluble Polymers

a) Xanthan Gum

b) Pectins

c) Chitosan Derivatives

d) Dextran

e) Carrageenan

f) Cellulose Ethers

g) Guar Gum

h) Hyaluronic acid

i) Albumin

j) Starch or starch based derivatives

1.1.2.1 Chitosan Derivatives

Chitin and chitosan have been used extensively in many areas ranging from food processing to waste management, medicine, biotechnology and pharmaceutical industries. Chitosan in particular has been used widely in pharmaceutical applications as a formulation excipient because it is biodegradable, biocompatible and less toxic. It has been used as a mucoadhesive, oral absorption enhancer and in protein and gene delivery[38]. The main drawback with chitin and chitosan is that it is difficult to dissolve them in water and in neutral pH. So, water-soluble derivatives of chitosan and chitosan have been synthesized by various researchers by chemical modification. These chemical modifications result in the formation of hydrophilic chitin or chitosan which have more affinity to water or organic solvents[39]. Limited solubility of chitosan and chitin has been overcome by chemical modification. For example, carboxymethylation of chitosan results in formation of N-carboxymethylchitosan (N-CMC) which is soluble in wide range of pH[40]. Chitin and chitosan derivatives are also used in treatment of industrial effluents because of their affinity to metal ions. N-CMC has been used widely in pharmaceutical areas for achieving controlled release of drugs, orthopedic devices and connective tissue[41-46].

Chitosan, the structural supporting material of crustaceans, insects, etc, is the N-deacetylated derivative of chitin, a naturally abundant polysaccharide. The parent chitin (Fig. 1.4(a)) is insoluble in most organic solvents; chitosan (Fig. 1.4(b)) is readily soluble in dilute acidic solutions below pH 6.0 due to the quaternization of the

amine groups that have a pKa value of 6.3, making chitosan a water-soluble cationic polyelectrolyte (Fig. 1.3). Chitosan is a natural and low-cost biopolymer which has been considered for use in a wide range of applications[47, 48]. Membranes based on natural low-cost chitosan are easily formed and have high hydrophilicity, good chemical and thermal resistance. In addition, the free amine and hydroxyl groups on the chitosan's backbone, each possessing a lone pair of electrons available for complexation, are readily accessible reactive sites that allow the chitosan to be modified and incorporated into sophisticated functional macromolecular systems.
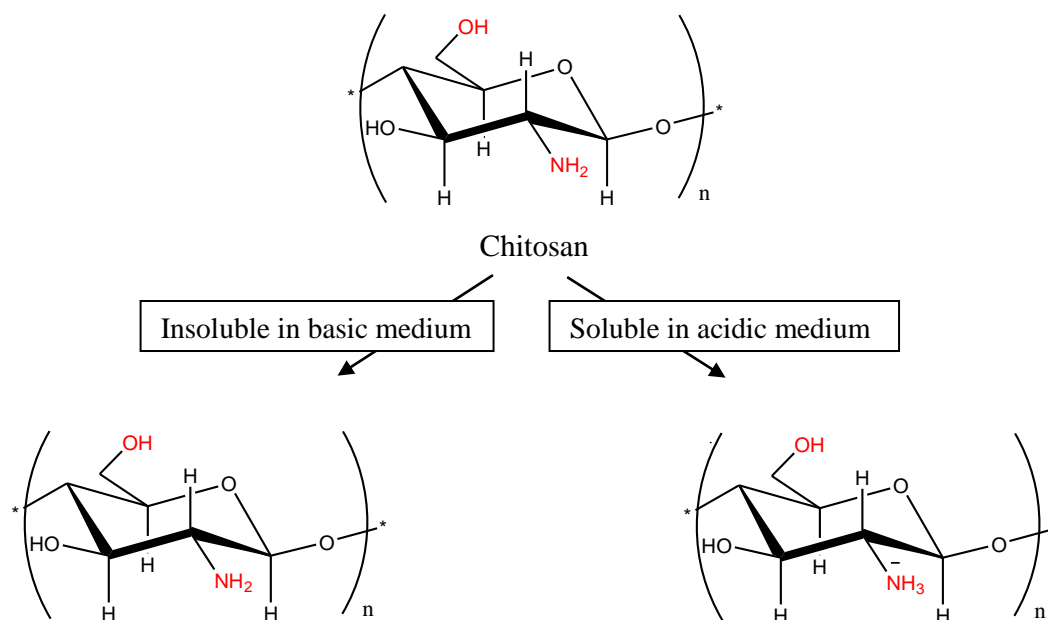

Figure 1. 3 Schematic illustration of chitosan's solubility in different medium

CS is potentially a useful membrane material due to its non-permeability to alcohol. However, in its native state, CS films exhibit very low conductivities and high degrees of swelling[49]. Although, high swelling levels in the membrane are a prerequisite for reaching high proton conductivities, at the changes associated with swelling impact upon the membrane's performance in terms of methanol permeability, dimensional stability and thermal stability. To solve the low conductivity and high swelling problems, CS was usually either ionically crosslinked with sulfuric acid[50,51] or incorporated into inorganic particles[52-55] and in addition, different Chitosan-based PEMs have been studied and have shown promising properties for application in the field of PEMFCs[56-59]

It is soluble in acetic acid & other mineral acids but insoluble in water and other common organic solvents. Due to its solubility problem its application has been

restricted, so to come over this problem there are so many efforts are going on. Out of which 6-amino-6-deoxychitosan (Fig. 1.4(c)) is its water-soluble derivative which is synthesized in a highly regioselective manner by converting its primary OH group into NH_2 group.

(a) Chitin   (b) Chitosan   (c) 6-amino-6-deoxy-chitosan
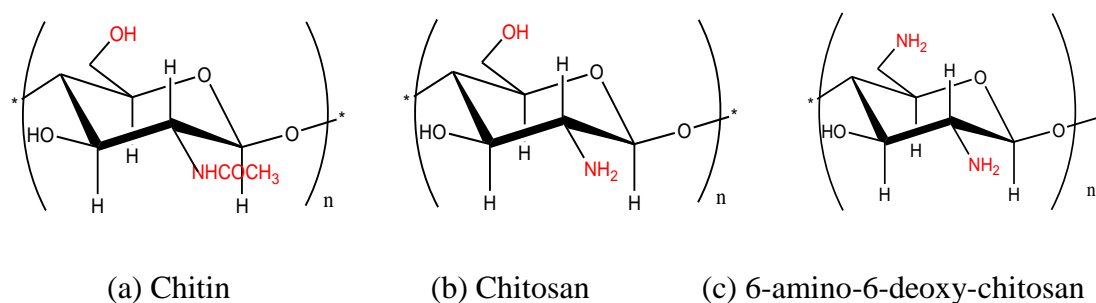
Figure 1.4 Chemical structures of chitin, chitosan and 6-amino-6-chitosan

In this thesis a novel hydrogel was synthesized by graft co-polymerization of acrylic acid on this modified chitosan, i.e., 6-amino-6-deoxy chitosan, whose complete description was done in a chapter 4. So far, the derivatives of chitosan has been derived are as follows:

1.1.2.1.1 Chitosan derivatives of importance

Many derivatives of chitosan till now have been derived by chemical modification, since it provides functional groups as primary amine and primary as well as a secondary hydroxyl groups in its monomers (Fig. 1.5).

Figure 1.5 Amenable functional groups in chitosan.

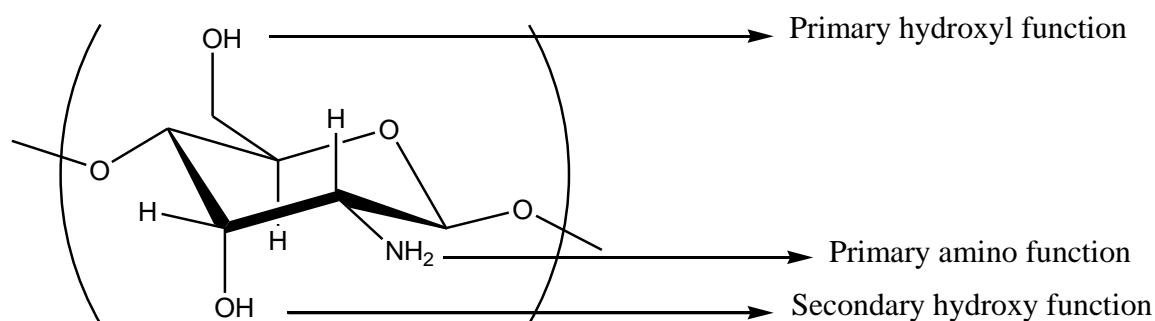The important examples of modified chitosans that hold prominent places in research are listed below:

1.1.2.1.1.1 Quaternized chitosan and N-alkyl chitosan

The quaternization of chitosan in different degrees of amino groups can be achieved with methyl iodide in alkaline solution of N-methylpyrrolidinone. For the protein and peptide drugs, chitosan and their salts as hydrochloride, glutamate are reported as an absorption enhancers. The trimethylation of chitosan also allows maintenance or improvement of the mucoadhesive properties of the starting chitosans dependently on quaternization degree. TMC with low-molecular-weight chitosan (DP < 20) have also been synthesized and evaluated to assess their potential as gene carriers in epithelial cell line by Thanou et al[60-64]. Amphiphilic N-octyl-N-trimethyl chitosan derivatives (N-trimethylated chitosan with octyl group on some monomers) have been investigated for its possible polymeric micelles formation, solubilization and controlled release of 10-hydroxycamptothecin[65]. Jia et al and Avadi et al reported the synthesis and antibacterial activity of quaternary ammonium salt, such as N,N,N-trimethyl chitosan, N-propyl-N,N-dimethyl chitosan and N-furfuryl-N,N-dimethyl chitosan and of N-diethylmethylamino chitosan[66,67]. Quaternized chitooligomers do posses antibacterial activity[68,69].

1.1.2.1.1.2 Highly cationic derivatives

Chitosan derivatives of dialkylaminoalkyl type with N-aminoethyl, N-diethylaminoethyl, N-dimethylaminoethyl, N-dimethylaminoisopropyl display significant cytotoxic activity and BACE1 inhibition property[70,71]. Xu et al synthesized a water-soluble derivative of chitosan, N-(2-hydroxyl) propyl-3-trimethylammonium chitosan chloride (HTCC) by its reaction between glycidyl-trimethyl-ammonium chloride. The HTCC was used for nanoparticle formation for protein delivery based on its ionic gelation process with sodium tripolyphosphate[72]. Treatment of chitin solution in solvents as DMA–LiCl or anhydrous pyridine with excess 1,6-diisocyanatohexane and exposure to water vapor for two days, produced flexible, opaque materials whose main characteristics include insolubility in aqueous and organic solvents, remarkable crystallinity, typical infrared spectrum, high N/C ratio, and degree of substitution but no thermoplasticity.

1.1.2.1.1.3 Hydroxyalkyl chitosans

Peng et al synthesized hydroxypropylchitosan and evaluated it as an antimicrobial whereas Dang et al evaluated it for its potential as a temperature sensitive injectable carrier for cells[73,74]. Self-assembled nanoparticles based on glycol chitosan were prepared as a carrier for paclitaxel, doxorubicin[75,76]. The long chain epoxides (1,2-epoxyhexane, 1,2-epoxydecane, and 1,2-epoxytetradecane) have been employed in homogeneous reaction with chitosan to obtain products having marked surface activity and foam-enhancing properties of chitosan.

1.1.2.1.1.4 Carboxyalkyl chitosans

Both, N-carboxyalkyl and O-carboxyalkyl chitosan derivatives have been prepared using different reaction conditions with monohalocarboxylic acid to attain the N versus O selectivity[77,78]. N-carboxyalkylation uses carboxyaldehydes in a reductive amination sequence[79]. By using glyoxylic acid, water-soluble N-carboxymethyl chitosan is obtained: the product is a glucan carrying pendant glycine groups[80]. N-Carboxymethyl chitosan is not only soluble in water, but has unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume and film, gel-forming capabilities also, all of which make it an attractive option in connection with its use in food products and cosmetics[81]. O-Carboxymethyl chitosan exhibits antibacterial activity and modified adhesive properties for instance, surface modification of tissue scaffolds of poly(lactide-coglycolide acid) with O-carboxy methylchitosan enhances chondrocyte adhesion; surface modification of Dacron vascular grafts enhances the blood compatibility[82].

Carboxymethyl chitosan and modified carboxymethyl chitosan at amino function with haexanoic, linoleic acid have been employed as a carrier for delivering drugs as gatifloxacin, camptothecin, ibuprofen, and adriamycin[83-86]. N-carboxyalkyl derivative was tested for antioxidant and antimutagenic activity[87].

Reaction of chitosan with acrylonitrile gives cyanoethyl chitosan whereas reaction of chitosan with ethyl acrylate in aqueous acidic medium gives N-carboxyethyl ester intermediate which can easily be hydrolyzed to free acid or used as an intermediate to substitute with various hydrophilic amines, without requiring protecting groups[88].

Lin et al synthesized N-carboxybenzyl chitosan by reductive amination sequence with 2-carboxy benzaldehyde and cross-linked with gultaraldehyde to develop pH-sensitive

hydrogel for colon specific drug delivery of 5-flurouracil[89]. Stable and self-sustaining gels are obtained from 4-hydroxyphenylpyruvic acid modified chitosan, i.e. tyrosine glucan in the presence of tyrosinase. No crosslinking is observed for chitosan derivatives of vanillin, syringaldehyde, and salicylaldehyde[90].

1.1.2.1.1.5 Sugar-modified chitosan

Initially, the sugar-bound chitosans (Fig. 1.6) had been investigated mainly for rheological studies; but since the specific recognition of cells, viruses, and bacteria by sugars was discovered, this type of modification has usually been used to introduce cell-specific sugars into chitosan. Hall and Yalpani were the first to report sugar-modified chitosan derivatives by reductive N-alkylation process[91].
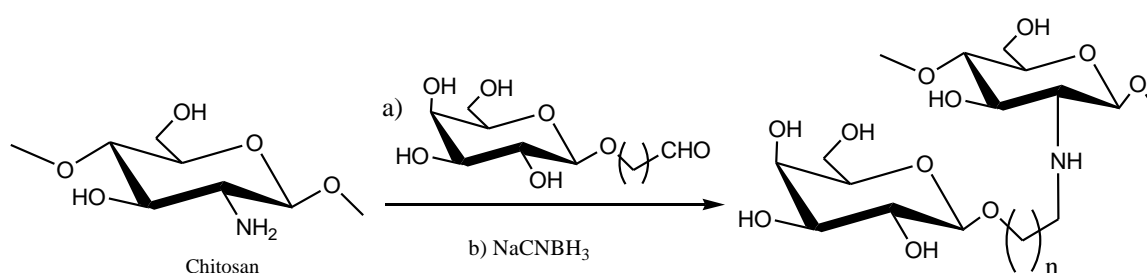

Figure 1.6 Example of sugar-modified chitosan

Stredanska and co-workers synthesized lactose-modified chitosan for a potential application in the repair of the articular cartilage by the same mode[92]. Moreover, lactosaminated N-succinylchitosan was found to be a good drug carrier for mitomycin C in treatment of liver metastasis[93]. The quaternized galaoctosylaed chitosan too hold the cellular recognition ability and possibility of gene delivery[94,95]. Hepatocyte cells is feasible because hepatocytes are the only cells that possess large numbers of high-affinity cell-surface asialoglycoprotein receptors that can bind to asialoglycoproteins and can internalize them within membrane bound vesicles or endosomes. Sashiwa et al prepared sialic acid bound chitosan as a new family of sialic acid containing polymers using p-formylphenyl-a-sialoside[96] by reductive N-alkylation[97]. Since sialic acid bound chitosan was insoluble in water, successive N-succinylations were carried out to obtain the water-soluble derivative N-succinyl-sialic acid bound chitosan.

The different type of spacer has been prepared on sialic acid or a-galactosyl epitope bound chitosans[98].

1.1.2.1.1.6 Cyclodextrin linked chitosan

Chitosans bearing cyclodextrin (CD) pendant were developed with an aim to combine unique characteristics of chitosan with the potential of CD to form non-covalent inclusion complexes with a number of guest molecules altering their physicochemical properties for improved drug delivery system, cosmetics, and analytical chemistry[99,100]. There are different means to link cyclodextrin to chitosan (Fig. 1.7).
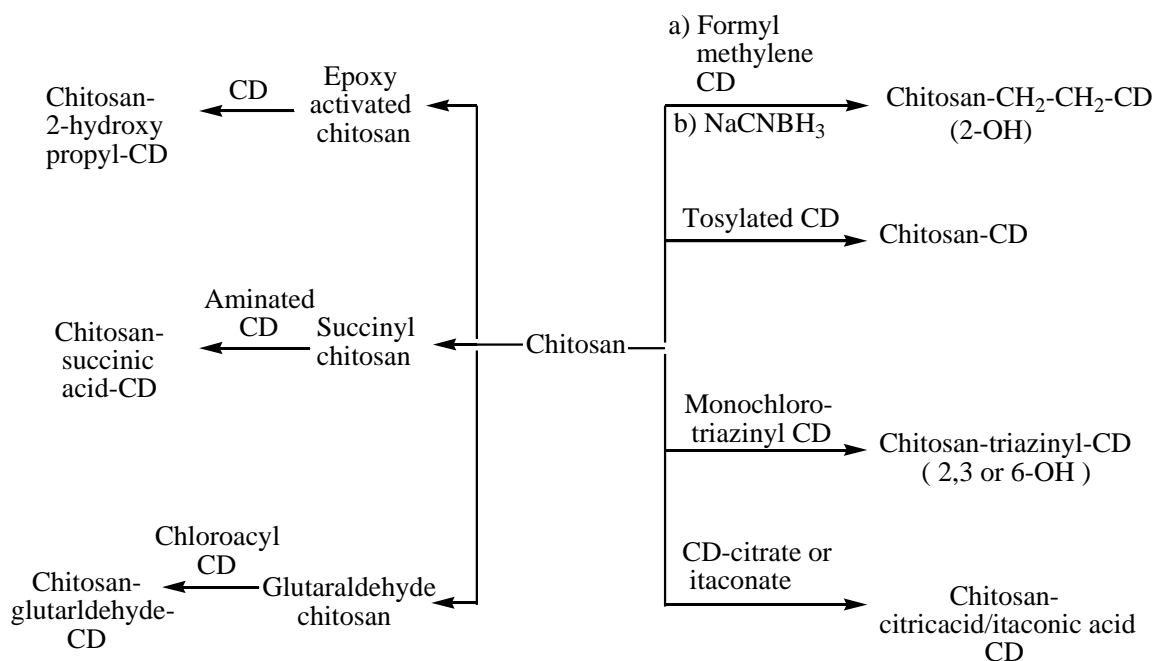
Figure 1.7 Example of cyclodextrin linked chitosan

Cyclodextrin linked chitosan: (1) by the reductive amination using formylmethylene CD, (2) by using tosylated CD, (3) by the nucleophilic substitution reaction using monochloro triazinyl derivative of CD, (4) via epoxy-activated chitosan, (5) by using redox aminated CD (mono-6-amino-mono-6-deoxy-b-cyclodextrin), (6) by the condensation of CD-citrate or itaconate with chitosan, (7) cross-liking of CD and chitosan by glutaraldehyde.

Chen and Wang[101] obtained CD-linked chitosan using tosylated β-CD and further evaluated the potential of β-CD for the release of I-131 in vivo and improved solubility. The CD-linked chitosan could also be prepared by the monochlorotriazinyl derivative of CD. Triazinyl moiety acts as a spacer[102]. This compound was used for

decontamination of waters containing textile dyes. They also reported analogous synthesis with β-CD-itaconate and chitosan along with its utility as ion exchange resin[103]. The β-CD linked chitosan using 1,6-hexamethylene diisocyanate as spacer was also prepared by Sreenivasan[104,105]. This material interacts with cholesterol and might be useful as an adsorbent. The spacer can be 2-hydroxypropyl moiety introduced by grafting β-CD onto chitosan using epoxy activated chitosan[106]. The spacer can be a reducing sugar derivative[107]. Aime et al[108] functionalized CD by means of a maleic spacer, whose free carboxyl group is subsequently activated with a carbodiimide to form amide linkages with amino groups of chitosan.

1.1.2.1.1.7 N-Acyl chitosan

Zong et al synthesized acyl chitosan (Fig. 1.8) with longer chains by reacting chitosan in pyridine/chloroform with hexanoyl, decanoyl, and lauroyl chlorides. These acylated chitosans with 4 degree of substitution per monosaccharide ring (disubstitution at amino and monosubtitution each at hydroxyl groups) exhibited an excellent solubility in organic solvents such as chloroform, benzene, pyridine, and THF.

On the other hand, the polymers belonging to the series of N-aliphatic- O-dicinnamoyl-chitosans displayed solubilities strongly related to the length of the flexible side chains. In general, increasing length of the flexible side chains reduced the solubility[109].

Mi et al prepared biodegradable N-acyl chitosan microspheres by water-in-oil (w/o) interfacial N-acylation method for controlled release of 6-mercaptopurine using acetic, propionic and n-butyric anhydrides as reagents for the interfacial N-acylation reaction[110].
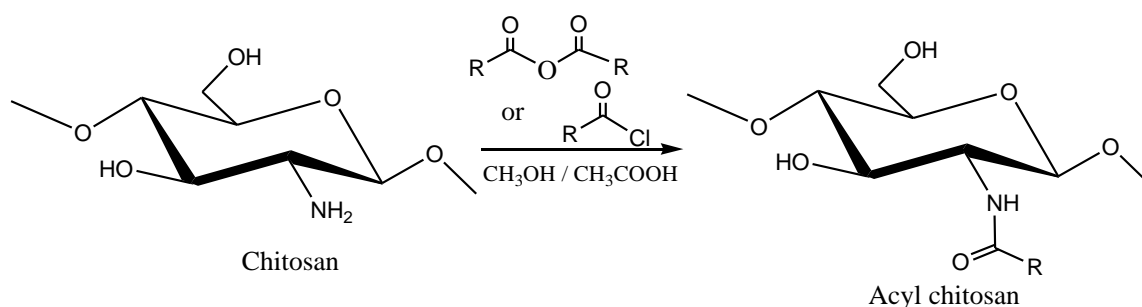

Figure 1.8 Synthesis of acyl chitosan

The release characteristics of the drug suggested that release is controlled by diffusion or by swelling followed by diffusion, depending on both the acyl chain length and the degree of acylation[111]. The acylated chitosan are being applied for stabilization of nanoparticles as iron oxide, and gold[112,113]. N-Succinyl-chitosan has unique characteristics in vitro and in vivo due to many carboxyl groups. For example, ordinary chitosan can be dissolved in acidic water but not in alkaline, whereas N-succinyl-chitosan with high degree of substitution exhibits the opposite behaviour[114]. N-Succinyl-chitosan can easily react with many kinds of agents due to $-NH_2$ and $-COOH$ groups in its structure. N-Succinyl chitosan, which can form self-assembly of well-dispersed and stable nanospheres in distilled water, shows great potential in the drug controlled release delivery[115].

Hu et al prepared N-acylated chitosan as N-acetyl, N-propionyl and N-hexanoyl with different degrees of substitution and evaluated in vitro for antibacterial activity. The results showed that intermolecular aggregation characteristic of N-acetylated chitosans with low DD may help in forming bridge to interact with bacterial cell[116]. The acylation can be achieved regioselectively at amino group by using protection as trityl group at the primary hydroxyl group. This approach was used to prepare N-chloroacyl 6-0-triphenylmethyl chitosan which can be further substituted or quaternized with amines as pyridine, imidazole, triethylamine, tributylamine, N-chlorobetainyl chloride[117-119].

1.1.2.1.1.8 O-Acyl chitosan

A hydrophobic moiety with an ester linkage into chitosan has two benefits: (i) hydrophobic groups contribute organo-solubility; (ii) the ester linkage is hydrolyzed by enzyme like lipase, etc. Although the selective O-acylation of chitosan in $MeSO_3H$ owing to the salt formation of primary amino group with $MeSO_3H$ was partly reported, the detailed chemical structure and the protecting effect of $MeSO_3H$ on amino group are not clear yet[120]. The preparation of O,O-didecanoylchitosan, O-succinyl chitosan was also reported through protected N-phthaloylchitosan as an intermediate[121-123]. One-pot synthesis for the O-acylation of chitosan in $MeSO_3H$ is also reported[124].

1.1.2.1.1.9 Thiolated chitosan

So far, four types of thiolated chitosans (Fig 1.9) have been generated: conjugates as chitosan–cysteine, chitosan–thioglycolic acid, chitosan–4-thiobutylamidine, and chitosan–thioethylamidine conjugate. Thiolated chitosan were synthesized by the derivatization of the primary amino groups of chitosan with coupling reagents bearing thiol functions.
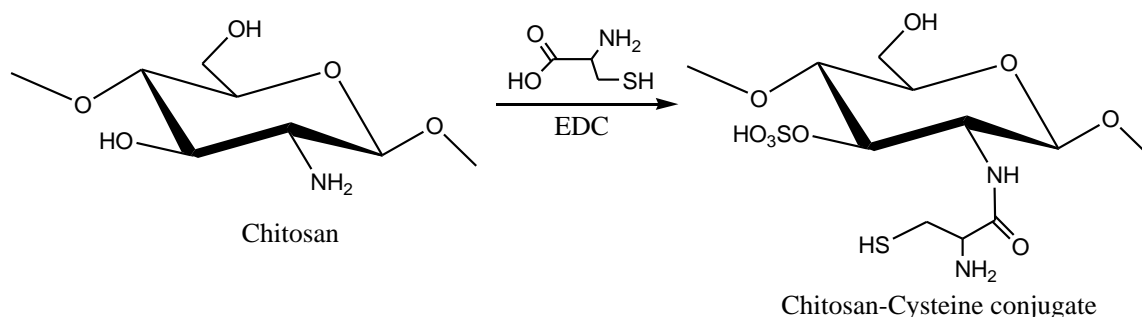

Figure 1.9 Synthesis of thiolated chitosan

Various properties of chitosan are improved by this immobilization of thiol groups allocating it to a promising new category of thiomers used in particular for the non-invasive administration of hydrophilic macromolecules.

(i) *Mucoadhesive properties*:

Chitosans offers mucoadhesive properties due to ionic interactions between the positive charged primary amino groups on the polymer and negatively charged sialic acid and sulfonic acid substructures of the mucus[125]. These mucoadhesive properties of chitosans can be significantly further improved by the immobilization of thiol groups on the polymer. The enhancement of mucoadhesion can be explained by the formation of disulfide bonds with cysteine rich subdomains of mucus glycoproteins, which are stronger than non-covalent bonds[126,127].

(ii) *Permeation-enhancing properties*:

The permeation of paracellular markers through intestinal mucosa can be enhanced 1.6–3-fold utilizing thiolated instead of unmodified chitosan. Chitosan possess the permeation-enhancing capabilities with increase in the paracellular route of absorption, which is important for the transport of hydrophilic compounds such as therapeutic peptides and antisense oligonucleotides across the membrane. The permeation-enhancing effect of chitosan can be strongly improved by the

immobilization of thiol groups. The uptake of the cationic marker compound rhodamine 123 was 3-fold higher in the presence of chitosan–TBA versus unmodified chitosans[128]. Thiolated chitosan possess not only the permeation-enhancing effect but also exhibit enhanced and sustained gene delivery[129].

(iii) *Cohesive properties, in situ gelling properties, (matrices for controlled release drug delivery system)*:

The reduced thiol functions on the chitosan backbone enable thiolated chitosans to form inter- as well as intra-molecular disulfide bonds resulting in cross-linking of the polymeric chains. Hence thiolated chitosans display, besides their strong mucoadhesive and permeation-enhancing properties, excellent cohesive property. This property provides a strong cohesion and stability of carrier matrices being based on thiolated chitosans and can guarantee a prolonged controlled release of embedded therapeutic ingredients. The usefulness of thiolated chitosans as carrier matrices for controlled drug release was demonstrated by means of model drugs, like insulin, clotrimazole, salmon calcitonin, fluorescein-isothiocyanate labelled dextran[130-133].

(iv) *Biodegradability*:

The biodegradability of thiolated chitosan has been demonstrated paving the way for its use as novel scaffold material[134]. Further studies in this direction were performed with L-929 mouse fibroblasts seeded onto chitosan–thioglycolic acid sheets. Results of this study showed that thiolated chitosan can provide a porous scaffold structure guaranteeing cell anchorage, proliferation and tissue formation in three dimensions[135]. Due to in situ gelling properties it seems possible to provide a certain shape of the scaffold material by pouring a liquid thiolated chitosan cell suspension in a mold. Furthermore, liquid polymer cell suspensions may be applied by injection forming semi-solid scaffolds at the site of tissue damage. Since low concentrated aqueous solutions of thiolated chitosan remain liquid when stored under inert conditions and are rapidly gelling under excess of oxygen, they seem to be promising candidates for such applications.

(v) *Enzyme inhibitory properties*:

Zinc-dependent proteases such as aminopeptidases and carboxypeptidases are inhibited by thiomers. The underlying mechanism is based on the capability of thiomers to bind zinc ions. This inhibitory effect seems to be highly beneficial for the oral administration of peptide and protein drugs[136].

1.1.2.1.1.10   Sulfated chitosan

The sulfur containing derivatives (Fig 1.10) were obtained by reacting chitosan with CS_2, formaldehyde and primary amine. By sulfation of chitosan some of the amino groups are converted to anionic centers and the polymer attains better polyelectrolyte properties which can be focused for developing potential drug carriers in the form of micelles or microcapsules[137,138]. Recently N-sulfonato-N,O-carboxymethylchitosan, polymer with anionic character has been evaluated positively in vitro and in vivo as absorption enhancer for the oral delivery of macromolecules as reviparin (low-molecular-weight heparin), mannitol, FITC dextran[139]. Apart from these valuable biological properties, chitosan sulfates exhibit high sorption capacities as expected, and are for of great advantage for metal ion recovery[140]. The sulfonic acid function was also introduced into chitosan by reacting with 5-formyl-2-furansulfonic acid sodium salt, under the mild conditions of the Schiff reaction that upon hydrogenation yielded N-sulfofurfuryl chitosan sodium salt dodging polymer degradation and O-substitution along with introduction of spacer in between chitosan backbone and sulfate group. For example, sulfonating agent 4-acetamidobenzene sulfonyl chloride reacts with –NH_2 or –OH (C6 position) groups leading to sulfanilamide derivatives of chitosan[141]. Chitosan sulfates have shown to possess anticoagulant and heamagglutination inhibition activities due to the structural similarity to heparin[142-146]. Other biological activities demonstrated by chitosan sulfates include antisclerotic, antiviral, anti-HIV, antibacterial, antioxidant, and enzyme inhibition activities[147-151].
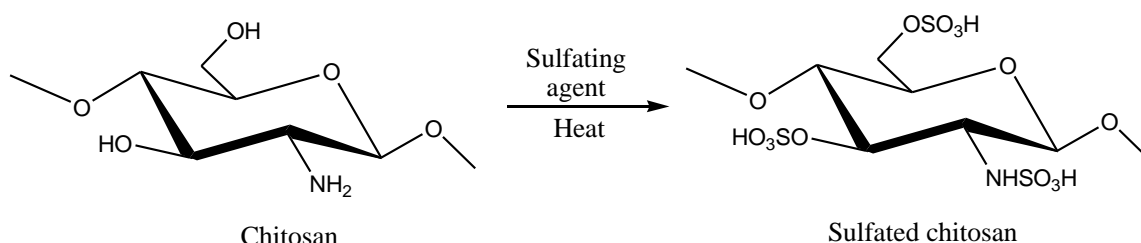
Figure 1.10 Example of sulfated chitosan

Various methods which involve combinations of sulfating agents and the reaction media have been used for the sulfation of chitosan. For sulfation of chitosan or derivatives of chitosan, various reagents being used include concentrated sulfuric acid[152], oleum[153], sulfurtrioxide[154], sulfurtrioxide/pyridine[155], sulfurtrioxide/trimethylamine[156,157], sulfur trioxide/sulfur dioxide, chlorosulfonic acid–sulfuric acid[158] and the most commonly used chlorosulfonic acid[159-161] in

homogeneous or heterogeneous conditions in the media as DMF, DMF–dichloroacetic acid, tetrahydrofuran, and formic acid[162] at different temperature ranges or under microwave irradiation[163]. Chitosan sulfates represent very important family of derivatives of chitosan which can demonstrate a range of biological activities.

1.1.2.1.2 Enzymatic modification of chitosan

The enzymatic grafting of phenolic compounds onto chitosan to confer water solubility under basic conditions has been reported[164]. The method takes help of tyrosinase which converts a wide range of phenolic substrates into electrophilic o-quinones which undergo two different subsequent non-enzymatic reactions with chitosan to yield either schiff bases or Michael type adducts. With tyrosinase chitosan in slightly acidic media the natural phenolic chlorogenic acid could be modified under homogeneous conditions with the modified chitosan being soluble under both acid and basic conditions, even when the degree of modification was low. The feasibility of using tyrosinase as a catalyst for grafting hexyloxyphenol onto the chitosan has been investigated successfully[165]. The spectral studies showed that hexyloxyphenol-modified chitosans have dramatically altered the physicochemical behavior. On the basis of contact angle measurements, the heterogeneous modification of a chitosan film was found to produce a hydrophobic surface due to the substituent. While homogeneously modified chitosan exhibited rheological properties characteristic of associating water-soluble polymers. Using the enzymatic strategy with tyrosinase enzyme, a dipepetide Tyr-Ala and peptide from casein hydrolysate were grafted on chitosan to get potential value added byproducts from food processing waste[166]. Another enzyme used for functionalization purpose is horseradish peroxidase. Using this enzyme, it was possible to graft the phenolic substrate dodecyl gallate onto the chitosan[167]. From the biochemically relevant quinones studied so far, it would seem possible to prepare materials of medical interest. For instance, menadione, a synthetic naphthoquinone derivative having the physiological properties of vitamin K is particularly prone to rapid reaction with chitosan, greatly modifying its spectral characteristics and increasing the surface hydrophobicity of treated chitosan films[168].

1.1.2.1.3 Graft copolymers of chitosan

Graft copolymerization is an attractive technique of modifying the chemical and physical properties of chitin and chitosan for widening their practical use. The properties of the resulting graft copolymers are broadly controlled by the characteristics of the side chains, including molecular structure, length, and number. There are a number of research works has been done to study the effects of these variables on the grafting parameters and the properties of grafted chitosan polymers

(i) *Graft copolymerization by radical generation*: These copolymers are frequently prepared by radical polymerization where in free radicals are generated first on the biopolymers backbone and then these radicals serve as macroinitiators for the vinyl or acrylic monomer.

(ii) *Copolymerization via polycondensation*

(iii) *Copolymerization via oxidative coupling*

(iv) *Cyclic monomer copolymerization via ring opening*

(v) *Copolymerization of preformed polymer by grafting onto method*

(vi) *Others*: In an attempt to improve the adhesion and growth of endothelial cells on chitosan, the cell adhesive peptide Gly-Arg-Gly-Asp (GRGD) was photochemically grafted to chitosan surface by first activating the peptide with a water-soluble functional moiety as N-succinimidyl-6-[40-azido-20-nitrophenylamino]—hexanoate to phenyl azido-derivatized peptides[169].

The chitosan grafts also display various biological properties, for example, antibacterial property by vinylimidazole chitosan antibacterial and superoxide scavenging (antioxidant) activity by maleic acid grafted hydroxypropyl chitosan and carboxymethyl chitosan. Above all, polylactide–chitosan graft holds tremendous potential as candidate in tissue engineering[170].

1.1.2.1.4 Chitosan–dendrimer hybrid

Sashiwa et al[171-174] established at first the synthesis of a variety of chitosan-dendrimer hybrids mainly by two procedures. In method I, the corresponding dendrimers bearing aldehyde and spacer are synthesized, and then these are reacted with chitosan by reductive N-alkylation. This procedure is advantageous because no cross-linking takes

place during the reaction. Sashiwa et al[175] synthesized a dendronized chitosan–sialic acid hybrid using convergent grafting of pre-assembled dendrons built on gallic acid and tri(ethylene glycol) (TEG) backbone. It is possible to generate more reactive dendrimers following method II, which uses commercial amino-dendrimers such as poly(amidoamine) (PAMAM) and poly(ethyleneimine) (PEI) dendrimers. However, method II suffers from the possibilities of cross-linking. As the construction of hybrid was difficult from original chitosan, a derivative, N-carboxyethylmethylester of chitosan as used as chitosan backbone. PAMAM dendrimers (G1–5) having a 1,4-diaminobutane core were attached to ester by amidation under conditions that prevent cross-linking[176]. Sashiwa et al has also reported the synthesis of polypropyleneimine dendrimer–chitosan hybrid[177]. Chitosan–dendrimer hybrids having carboxyl, ester, and PEG and various generations were also prepared using dendrimer acetal by reductive N-alkylation. The synthetic procedure could be accomplished by one-step reaction without organic solvent[178].

1.1.2.1.5 Cyclic-host bound chitosan

Tang et al[179] prepared the crown-ether bound chitosan with Schiff's-base-type and its reduced form (Fig 1.11).
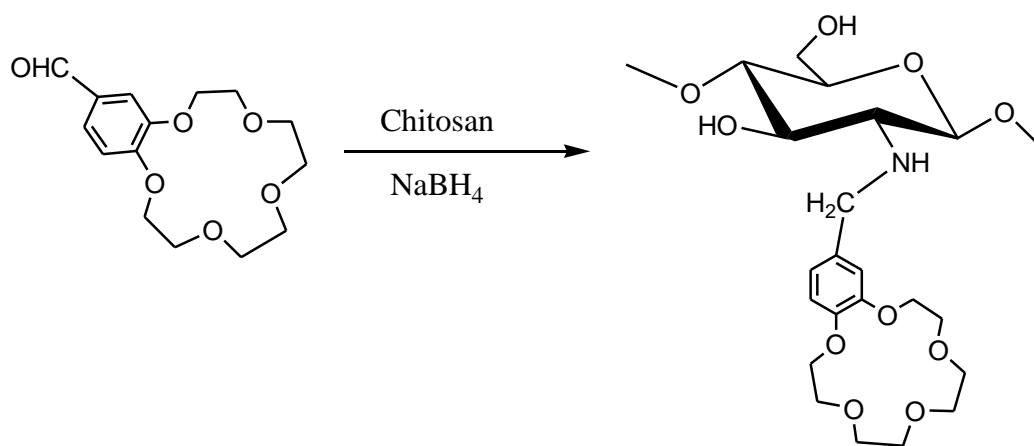
Figure 1.11 Example of cyclic host bound chitosan

As crown ethers have particular molecular structures and good complexing selectivity for metal ions. Crown ether-bound chitosans had not only good adsorption capacities for metal ions Pd^{2+}, Au^{3+}, and Ag^+, but also high selectivity for the adsorption of Pd^{2+} in the presence of Cu^{2+} and Hg^{2+}. Cross-linked types of crown-ether-bound chitosans were also reported[180]. These cross-linked derivatives have space net structures with embedded crown ethers, and each mesh has a certain space volume. When original

chitosan reacted with 4,40-dibromobenzo-18-crown-6-crown ether, the cross-linked product between 6-OH and NH_2 was obtained. However, this product would include heterogeneous cross-linking structure between 6-OH and 6-OH or NH_2 and NH_2. Benzylidene-protected chitosan (CTB) would produce a homogeneous cross-linking structure between 6-OH and 6-OH. These crownether bound chitosans would be useful for separation and preconcentration of heavy or precious metal ions in aqueous environments. Li et al[181] reported the first synthesis of calixarene-modified chitosan. Calixarenes have demonstrated outstanding complex ability toward ions, organic molecules, etc., and are considered the third best host molecules, after cyclodextrins and crown ethers. These derivatives did not dissolve in general organic solvent; however, they can easily be powdered and are thus better adsorbents than simple chitosan.

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