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Sachinkumar Vasantrao Patil
Ashokrao Mane College of Pharmacy Pethvadgaon Kolhapur MS India
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Sachin Patil

Polysaccharides are polymers of simple sugar building blocks. Crosslinked polysaccharides are having many applications in pharmaceuticals. Different methods are available for crosslinking of polysaccharides. Different chemical and physical methods are summarized and discussed. Chemical crosslinking of polysaccharide is highly versatile method with good mechanical stability. However, the crosslinking agents used are not only affects the integrity of substances but are often toxic compounds, which have been extracted from gel before they can be applied. Such adverse effects are avoided with the use of physically crosslinked gels.

Introduction

Polysaccharides are polymers of simple sugar building blocks. Polysaccharides functions as thickeners, stabilizers, suspending agents, gelling agents, film formers, aerating agents, flocculants, binders, emulsifiers, lubricants, texturing agents and structuring agents[1,2]. Polysaccharides are primarily used to thicken or gel water and are frequently classified into two groups thickeners and gelling agents[3]. Gelling agents are more difficult to use than thickeners and their use is complicated by the fact each requires its own set of procedure to produce satisfactory gel[4]. Thus polysaccharides are hydrated in an aqueous environment thereby creating the gel structure called hydrogel. Aqueous solutions of hydrophilic polymers at low or moderate concentrations, where no substantial entanglement of chains occurs, normally show Newtonian behavior. On the other hand, once crosslinking between different polymer chains are introduced, the so obtained network shows visco-elastic and sometimes pure elastic behavior. As the term implies, crosslinks have to be present to avoid dissolution of hydrophilic polymer chains into aqueous phase[5-13]. A great variety of methods for crosslinking has indeed been used. Since it is advantageous for many applications that the hydrogels are biodegradable, labile bonds are frequently introduced in the gel. These bonds can be present either in polymer backbone or in the crosslinks used to prepare gel. The labile bonds can be broken under physiological conditions either enzymatically or chemically; in most of cases by hydrolysis[13]. Crosslinked polysaccharides were used for coating and/or embedding medicinal active substances or drug compositions. The invention furthermore relates to a drug which contains an active substance which acts in the large intestine or an active substance which is broken down on passing through the stomach or small intestine could be coated with or embedded in one of the crosslinked polysaccharides[14]. In this review methods of crosslinking of polysaccharides are described and discussed. Chemical and physical methods are used for crosslinking of polysaccharides. In physical crosslinking dissolution is prevented by physical interaction which exists between different polymer chains. In chemical crosslinking covalent bonds are present between different polymer chains.

Physical crosslinking:

In recent years there has been increase in interest in physical crosslinking. The main reason is that use of crosslinking agents is avoided. These agents cannot only affect the integrity of substances to be entrapped e.g. proteins, cells, but these agents are often toxic compounds which have to be removed or extracted from the gel before they can be applied. In physical crosslinking, polysaccharides forms crosslinked network with counterion at the surface. High counterion concentration would require longer exposure times to achieve complete crosslinking of the polysaccharides. For physical crosslinking different methods have been investigated. Crosslinking by ionic interaction: Alginate is a well-known example of polysaccharide that can be crosslinked by ionic interactions. Alginate is a polysaccharide with mannuronic and glucuronic acid residues can be crosslinked by calcium ions[15]. Crosslinking can be carried out at room temperature and physiological pH. Therefore alginates gels can be frequently used as matrix for the encapsulation of living cells[16] and for the release of proteins[17]. The release of proteins from the alginate microparticles obtained by spraying a solution of sodium alginate into an aqueous solution of calcium chloride can be modulated by coating the particles with cationic polysaccharide for example chitosan[18,19]. Polycations can be crosslinked by anions. Chitosan is an amino-polysaccharide obtained by alkali induced deacetylation of chitin. Chitosan based hydrogels were obtained by croslinking with glycerol-phosphate disodium salt[20]. Interestingly, in presence of this salt, chitosan solution remains liquid below room temperature, but quickly gel when heated. Biological materials, proteins and chondrocytes, were incorporated prior to the injection within the thermogelling solution.[20] Carrageenan a polysaccharide composed of 1,4-linked-α-D-galactose and 1, 3 linked-β-D-galactose with a variable portion of sulfate groups, forms a gel with potassium ions, but also shows gelation under salt-free conditions. However, gels prepared in the presence of metallic ions were substantially stronger than those obtained under salt-free conditions[21]. Ionically crosslinked chitosan hydrogels are formed by complex formation between chitosan and polyanions, such as dextran sulfate or polyphosphoric acids[22]. Doxorubicin was encapsulated in nanoparticles of the chitosan hydrogels. This particle showed a minimal burst release and retained good in vitro cytotoxicity due to released drugs. Alginate was ionically crosslinked by immersing in aqueous solution of calcium acetate of different molarities at time. Permeability of acetaminophen in hydrated calcium alginate film was studied. Permeability depends upon the concentration of calcium acetate solution and immersion time. The highest limiting permeability was observed in film prepared using the highest concentration of calcium acetate for greater time. It is suggested that this is result of rapid croslinking of sodium alginate at the surface which in turn shows the diffusion of calcium ions into the film[23]. Gellan gum is a linear anionic polysaccharide produced by the microorganism *Pseudomonas elodea*[24]. Hydrophilic drug, propranolol hydrochloride was encapsulated in gellan gum beads in an aqueous environment by the ionotropic gelation method. Optimal preparation conditions allowed a very high incorporation efficiency (92%). Storage conditions (wet or dry state) and the time of storage did not modify the release of the drug[25]. Cross-linked calcium-gellan beads containing diclofenac sodium as model drug, using full 3^3 factorial design were prepared. Drug quantity, pH of cross-linking solution, and speed of agitation were selected as variables for factorial design. Studies indicate that, in addition to drug:polymer ratio, pH of cross-linking solution and speed of agitation also influence the formation and properties of calcium-gellan beads[26]. Thus ionotropic gelation with gellan gum seems to offer new opportunities in the field of bioencapsulation and could be useful for the encapsulation of fragile drugs. Also crosslinked chitosan and alginate films are promising for controlled drug delivery applications. Crosslinking by crystallization: Poly (vinyl alcohol) (PVA) is a water soluble polymer. When aqueous solutions of PVA are stored at room temperature they gradually form a gel with, however, a low mechanical strength. Interestingly, once aqueous solution of this polymer undergoes freeze-thawing process a strong and highly elastic gel is formed[27]. Addition of alginate to the PVA solution before freeze-thawing, the gel properties could be modulated. With increase in concentration of alginate, the mechanical strength of gel increased which was associated with a

decrease in release of model drug[28]. Hydrophobised polysaccharides: Examples of polysaccharides reported in literature used for preparation of physically crosslinked hydrogels by hydrophobic modification are chitosan, dextran, pullulan and carboxy methyl curdlan[29-35]. This compound forms monodisperse hydrogel nanoparticle of 20-30 nm with high water content upon dialyzing a solution from DMSO against PBS buffer. Various proteins such as α chymotrypsin, BSA and insulin have been incorporated. As example, the hydrophobic antitumour drug adriamicin (ADR) was taken up inside the particles by simply mixing the pullulan suspension with ADR[31]. Slow release was observed at pH 7.4, which increases at lower pH of the medium due to increased solubility of drug. The stability of the drug was increased, however due to poor cellular uptake of the complexes and / or slow release of ADR, the invitro cytotoxicity against HeLa cells appeared rather low. Rapid complexation was also observed for insulin approximately 10 molecules of insulin were taken up inside the pullulan particles within 10 minutes after mixing[32]. The resulting microenvironment protected the protein effectively against the thermal denaturation aggregation and enzymatic degradation. Dissociation of insulin occurred on addition of BSA, which may provide a basis for invitro release of the insulin. Indeed the in vivo activity of insulin loaded nanoparticles was similar to that free insulin after i.v. injection. By covalent attachment of lactoside or galactoside to the pullulan backbone, nanoparticles were obtained which show binding to the receptor β-D-galactose-specific lectin $RCA_{120,}$ which provides a possibility for a cell-specific targeting. In vitro they were more effectively internalized than conventional nanoparticles by cells expressing galactose-specific receptors such as rat hepatocytes and HepG2 cells, in contrast to cells which lack such receptors[33]. Moreover, biodistribution studies showed enhanced accumulation in the liver. Self assembled hydrogels nanoparticles have also been prepared from carboxy methyl curdlan, a polysaccharide having anti-tumor activity, by substitution with a hydrophobic sulfonyl urea[35]. Release of the anti-cancer drug all-*trans* retinoic acid from these nanoparticles showed first order kinetics. An increased drug-loading and degree of hydrophobic modification resulted in a slower release. Upon conjugation of lactobionic acid to the CM-curdlan, specific binding to HepG2 cells was observed in vitro. Another example of a hydrophobized polysaccharide is the water soluble glycol chitosan substituted with palmitoyl chains. They assemble into unilamellar polymeric vesicles in the presence of cholesterol[36]. These polymeric vesicles are found to be biocompatible and haemocompatible and capable of entrapping water-soluble drugs[37]. Highly porous solid materials are obtained after freeze drying, which are hydrated without swelling to 20 x its dry weight in alkaline buffer[38]. In contrast to most chitosan based materials, the hydration decreased in acidic environment, which was attributed to the higher erosion rate at low pH. Chitosan has been grafted with PL(G) by Qu et. al., in which the hydrophobic polyester side chains are responsible for the hydrophobic interactions in water[39, 40]. Reversible water uptake of the materials was observed when the pH was switched between 2.2 and 7.4, the lowest pH providing the highest swelling due to charge repulsion by protonation of the free amine groups on the polymer backbone. The maximum water uptake in the protonation form depends on the glycolic acid / lactic acid content. Other chitosan hydrogels that respond to external charges like pH or temperature have been prepared by grafting with poly(acrylic acids) (PAAc)[41] or poly(*N*-isopropylacrylamide) (PNIPAAm)[42] In addition to chitosan, also carboxy methyl dextran has been grafted with PNIPAAm chains aming at thermosensitive hydrogels[43]. Cloud point measurements were conducted in this case, indicating a phase transition at around 38°C, but no data on hydrogel characteristics have been reported yet.

Chemical crosslinking:

Chemical crosslinking of polysaccharide is highly versatile method with good mechanical stability. During crosslinking counterions diffused into the polymeric and crosslinking agent reacts with polysaccharides forming either intermolecular or intramolecular linkages. Factors which affect chemical crosslinking are concentration of crosslinking agent and crosslinking time. The high concentration of crosslinking agent induces rapid crosslinking. Like physical crosslinking high

counterion concentration would require longer exposure times to achieve complete crosslinking of the polysaccharides[44]. Chemical crosslinking is carried out by following methods.

Crosslinking by radical polymerization:

Chemical crosslinking can be carried out by radical polymerization in presence of crosslinking agent. In particular dextran is used as building block for (degradable) hydrogels. Dextran is a bacterial polysaccharide, consists essentially of -1,6 linked D-glucopyranose residues. The low molecular weight fractions of dextran (Mw between 40 and 100 kDa) have been used as plasma expander[45] which has resulted in a good documentation of pharmacological activities and side effects of dextran. Dextran has therefore been investigated for the delivery of drugs, proteins and imaging agents[46]. Moreover, due to presence of dextranase in colon, dextran based gels are under investigation as a colon delivery system[47]. Research on polymerizable dextran was pioneered by Edman et al.[48] who reacted dextran dissolved in water with glycidylacrylate. A hydrogel was formed after the addition of an initiator system containing of *NNNN* tetramethylene-diamine and ammonium peroxydisulfate to an aqueous solution of the acryldextran also containing *NN* methylenebisacrylamide. Enzymes were immobilized with almost full retention of their activity by an emulsion polymerization technique in microspheres of polyacryldextarn.[48, 49]. A method has been developed to synthesize methacrylated dextran. Dextran is dissolved in a suitable aprotic solvent (DMSO) after which derivatization with glycidylmethacrylate (GMA) catalyzed by 4-(N-N-dimethyl amino) pyridine, is carried out. Almost quantitative incorporation of GMA was found and the degree of substitution can be fully controlled. A detailed analysis by NMR[50] and by the mass spectroscopy of the products obtained after enzymatic degradation[51] revealed that under the selected conditions the reaction of GMA and dextran was a transesterification resulting in a dextran derivative with the methacyrlate group directly attached to the dextran chain. The synthetic procedure developed was also suitable to derivatize other compounds with methacrylate groups, among which are inulin[52] and sucrose[53]. Meth(acrylate) groups can also be introduced in water-soluble polymers using (meth)acryloyl chloride,[54,55] methacrylic anhydride,[56] and by the subsequent reaction of dextran with bromoacetyl bromide and sodium acrylate[57]. Moreover, using enzymes as catalyst, (meth) acrylic groups have been introduced in mono and disaccharides, which can be used for synthesis of hydrogels[58, 59, 60]. The synthesis is carried out in anhydrous pyridine and products are normally obtained in a high yield. In contrast to chemical methods, enzymatic synthesis results in a very good regeoselectivity. A polymerizable dextran derivative was obtained by reaction of dextran with maleic anhydride. These dextran derivatives can be converted into a hydrogel by UV-induced polymerization of the vinyl groups. The gels were not degradable under physiological conditions. They did, however, exhibit a strong pH dependant swelling behavior due to the presence of carboxylic acid groups in the network[61]. In synthesis of methacrylated dextran derivatives the polymerizable groups are connected via a) hydrolytically sensitive group(s) to a dextran backbone. The hydrolysable groups are either a carbonated ester or lactic acid groups. After polymerization these derivatives the gel degraded under physiological conditions due to the presence of (carbonate) ester groups in the crosslinks, yielding dextran, lactic acid and short fragments of Poly(2 hydroxy ethyl methacrylate) (pHEMA) as degradation products[62]. Chitosan undergoes crosslinking by radical induced polymerization in presence of potassium persulfate at 60°C, leading to extensive crosslinking of fragmented chains on subsequent cooling at 4°C. As a result a possible conformation change leading to higher crystallinity, as evidenced by IR, X-ray and C_{NMR} was observed. Chitosan crosslinking leads to formation of permanently covalent network, which may allow the free diffusion of water/bioactive material and also enhance the mechanical properties. Chemical crosslinks are formed by irreversible covalent links as in covalently crosslinked chitosan. Thus allow drug delivery to be efficiently controlled[63].

Crosslinking by aldehyde:

In order to establish crosslinking, rather drastic conditions have to be applied (low pH, high temperature etc.). This has especially been investigated for the preparation of crosslinked amine containing polysaccharides[64]. Because glutaraldehyde is a toxic compound that even at low concentration shows cell growth inhibition, alternatives has been developed. Crosslinking of gelatin using polyaldehydes obtained by partial oxidation of dextran has been reported[65]. These gels were designed for application in wound treatment and epidermal growth factor (EGF) was incorporated to promote wound healing. The release rate of EFC decreased with increase in storage time which was ascribed to the ongoing processes of both chemical crosslinking and physical structuring of the hydrogel matrix. Poly(aldehydeguloronate) obtained by oxidation with periodate of partially depolymerized alginate, can be crosslinked with adipic acid dihydrazide and converted into a hydrogel. The swelling and degradation of the gel could be controlled by the amount of adipic acid dihydrazide[66]. These hydrogel films have therefore potential to act as a delivery matrix for sustained release of drug at wound sites[67]. The procedure for the preparation of crosslinked chitosan microspheres coated with polysaccharides or lipid for intelligent drug delivery system is reported. The microspheres were prepared with an inverse emulsion of 5-FU or its derivative solution of hydrochloric acid of chitosan in toluene containing SPAN 80. Chitosan was crosslinked with Schiff's salt formation by adding glutaraldehyde toluene solution. At the same time, the amino derivatives of 5-FU were immobilized, obviously resulting in an increase in the amount of drug within the microspheres. The microspheres were coated with anionic polysaccharides (e. g. carboxymethylchitin, etc.) through a polyion complex formation reaction. The results revealed that the coating layers of microspheres were effective barriers to 5-FU release[68].

Crosslinking by addition reaction:

Polysaccharides can be crosslinked with 1,6-hexa-methylenediisocyanate or 1,6-hexanedibromide and many other reagents. The network properties can be easily tailored by the concentration of the dissolved polysaccharide and the amount of crosslinking agent. The crosslinking reactions are preferably carried out in organic solvents, because water can also react with the crosslinking agent. Further, since the crosslinking agents are generally speaking very toxic, the gels have to be extracted extensively to remove traces of unreacted agents. Once these matrices are aimed for the release of pharmaceutically active agent, they have to be loaded after the gel formation and extraction process. This means that protein molecules can be loaded in meshes of the gels which are larger than the protein and these systems therefore show typically first-order release. This often results in a limited duration of the release. Finally, between the polymer chains, linkages are established which are stable. This means that degradation only occurs once the polymer backbone is degraded by enzymes[69, 70, 71].

Crosslinking by condensation reaction:

A very efficient reagent to crosslink polysaccharides with amide bonds is *NN*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide. In order to obtain alginate gels with better mechanical properties than the ionically crosslinked gels, Mooney et al. developed a method to a covalently crosslink this polymer. Alginate and PEG-diamines were crosslinked using *N*-ethyl carbodiimide. The mechanical properties could be controlled by the amount of PEG-diamine in the gel and molecular weight of PEG[72].

Characterization of crosslinked polysaccharides:

Characterization of crosslinked polysaccharides were done by infrared spectroscopy, X-ray diffractometry, scanning electron microscopy and by water uptake methods[44, 63].

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About Authors:

Sachin Patil
Department of Pharmaceutics, Shree Santkrupa College of Pharmacy, A/P Ghogaon, Tal. Karad, Dist. Satara.
Email: sachinpatil79@rediffmail.com

D. R. Jadge
Shree Santkrupa College of Pharmacy, A/P Ghogaon, Tal. Karad, Dist. Satara. 415111.