Polysaccharides
DOI 10.1007/978-3-319-03751-6_31-1

Chemically Modified Natural Polysaccharides to Form Gels

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Abstract

Polysaccharides have been utilized for a wide variety of industrial, cosmetic, food, and medical applications. The presence of functional groups on polysaccharides has been exploited for chemical modification to prepare polymers with unique properties. Various polysaccharides form hydrogels through physical or chemical cross-linking, and many of them possess environmentally responsive properties, known as smart hydrogels. Polysaccharide-based smart hydrogels are ideal for biomedical and pharmaceutical applications due to their inherent biocompatibility, degradability, and environment sensitivity, such as pH, temperature, and specific biomolecules.

Keywords

Polysaccharides; Chemical modification; “Smart” hydrogels; Chemically cross-linked; Physically cross-linked

1 Overview on Polysaccharides

Polysaccharides are polymeric carbohydrates in which a large number of carbohydrate repeating units are linked together by glycosidic bonds. In nature these are typically used for energy storage (e.g., starch and glycogen) or for mechanical structure (e.g., cellulose and chitin). Polysaccharides can be divided into homopolysaccharides (homoglycans) having the monosaccharide repeating unit and heteropolysaccharides (heteroglycans or hemicelluloses) having different types of repeating units. In general, these polysaccharides can be either linear by containing, for example, only 1,4-β-glycoside bonds (e.g., cellulose) or branched by containing a mixture of 1,4-β-glycoside bonds as well as other bonds such as 1,6-α-glycoside bonds (e.g., glycogen). Polysaccharides may also contain sugars which are missing an oxygen from a given position (deoxy sugars), sugars with amine units (amino sugars), and sugars with sulfate/sulfonate moieties (sulfate/sulfonate sugars). The presence of the amine and sulfate/sulfonate groups allows for other chemical modifications. Table 1 briefly highlights a few examples of different types of polysaccharides.

A hydrogel is a network of polymer chains which absorb water but do not dissolve due to either physical or chemical cross-links. As polysaccharides are already polymeric in nature and possess a plurality of nucleophilic moieties (hydroxyls and sometimes amines) along their backbone, they are easily converted into hydrogels. This, along with their typically low price and natural biodegradability, makes them attractive starting materials for forming various hydrogels. Linear homoglycans

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DOI 10.1007/978-3-319-03751-6_31-1

Table 1 Types of polysaccharides and examples

Linear homoglycans	Branched homoglycans	Linear heteroglycans		Branched heteroglycans
Amylose/cellulose	Amylopectin	Alginate	Chitin	Galactomannans
Chrysolaminarin	Dextran	Gellan	Chitosan	Guar gum
Curdlan	Schizophyllan	Laminarin	Carrageenan	Locust bean gum
	Scleroglucan	Pullulan	Chondroitin sulfate	Xanthan gum
		Welan		
		Xylan		

From references Pigman et al. (1950), McNaught and Wilkinson (1997), Matthews et al. (1999), McMurry (2000); BeMiller (2007), Varki et al. (2009), Heinze et al. (2012)

are typically used for mechanical stiffness and strength in hydrogel applications (Chang et al. 2009, 2010; Sannino et al. 2009). Branched and linear heteroglycans, such as locust bean gum, xanthan gum, and others, tend to form supermolecular complexes based on their respective chain entanglement which naturally leads to strong gel formation even without modifications beyond a simple heating–cooling cycle (Fernandes 1991; Lundin and Hermansson 1995; Mannion et al. 1992; Urayama et al. 2008). Notably, aminoglycans possess an amine unit which acts as a stronger nucleophile than hydroxyl units, allowing for a greater flexibility in chemical modifications. Additionally, the amine unit is weakly basic, making the polymer pH sensitive (Qu et al. 2000; Risbud et al. 2000; Wang et al. 2004). Figure 1 shows examples of polysaccharide structures.

2 Chemical Modifications

With a plurality of nucleophilic groups ranging from alcohols to amines as well as highly reactive acid moieties, polysaccharides represent an ideal platform for a wide array of chemical modifications. However, there are some drawbacks which must be considered for chemical modification of polysaccharides. First, polysaccharides inherently have poor solubility in organic solvents, and thus, they must be processed, at least initially, in highly polar solvents or water. Second, the presence of a multitude of nucleophilic moieties makes it rather difficult to control the extent of modification, i.e., degree of substitution. Third, polysaccharides are susceptible to hydrolysis, although fairly resilient.

2.1 Ether Conjugation

Most chemical modifications of polysaccharides generally rely on the plurality of nucleophiles present along the chain in the form of hydroxyl or amine units. Several hydrogel-forming modifications, particularly of water-insoluble cellulose, have been used to generate water-soluble/gel-like cellulose derivatives for many years. Most of these reaction require cellulose to be activated by conversion to alkali cellulose (cellulose which has been treated with sodium hydroxide) prior to subsequent conjugation (Fig. 2). Once formed into alkali cellulose, the material can be transferred into a variety of derivatives including carboxymethylcellulose (formed by reacting alkali cellulose with sodium monochloroacetate), hydroxyethyl cellulose (formed by reacting alkali cellulose with ethylene oxide), hydroxypropyl cellulose (formed by reacting alkali cellulose with propylene oxide), and methylcellulose (formed by reacting alkali cellulose with methyl chloride). Additionally, combinations of modifying agents, e.g., propylene oxide and methyl chloride, can be used to generate polysaccharides with multiple modifications, e.g., hydroxypropyl methylcellulose. These ether derivatives of cellulose are popular for use as thickeners and gums, as they form physical hydrogels (BeMiller 2007).

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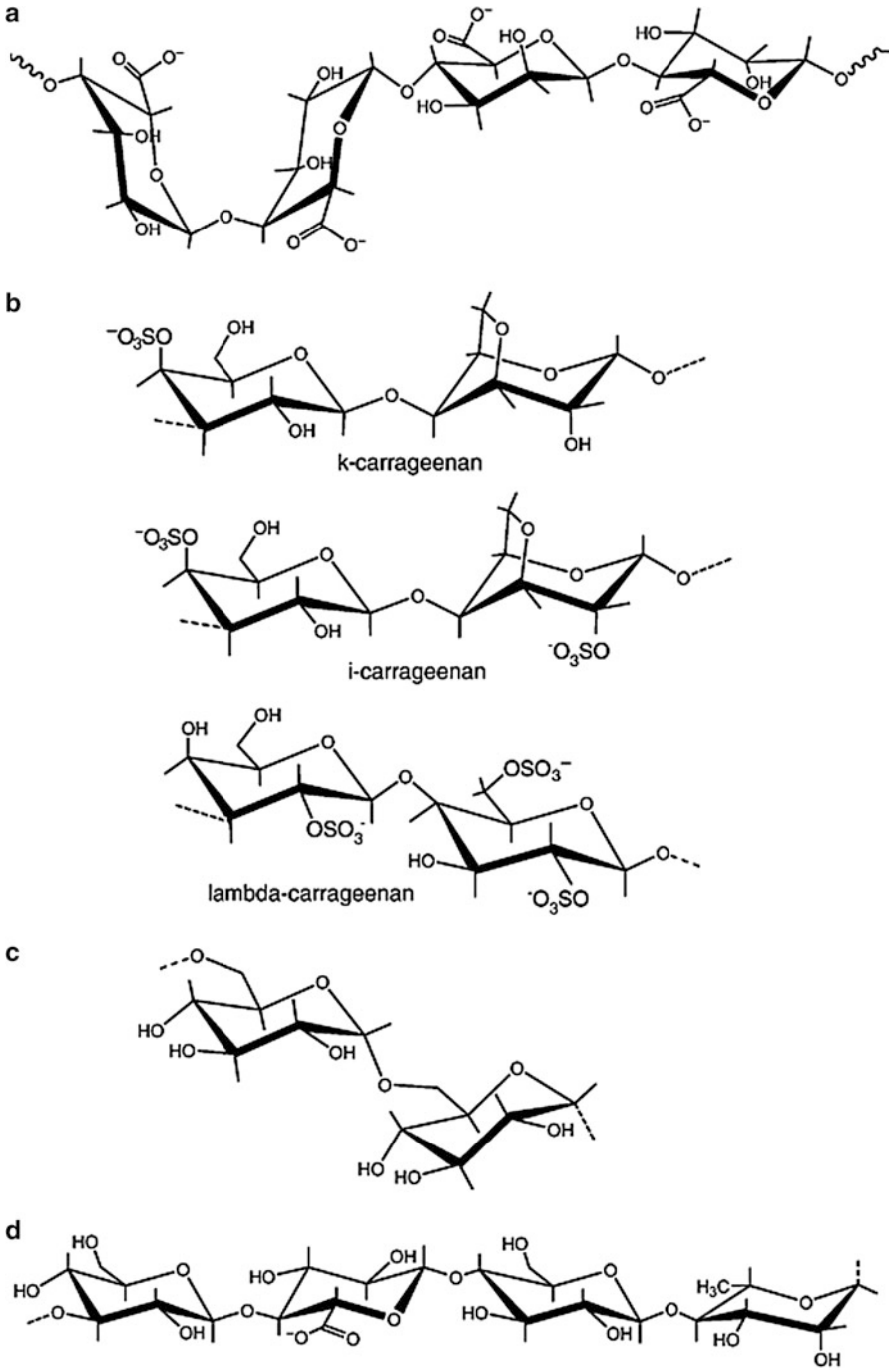

Fig. 1 (continued)

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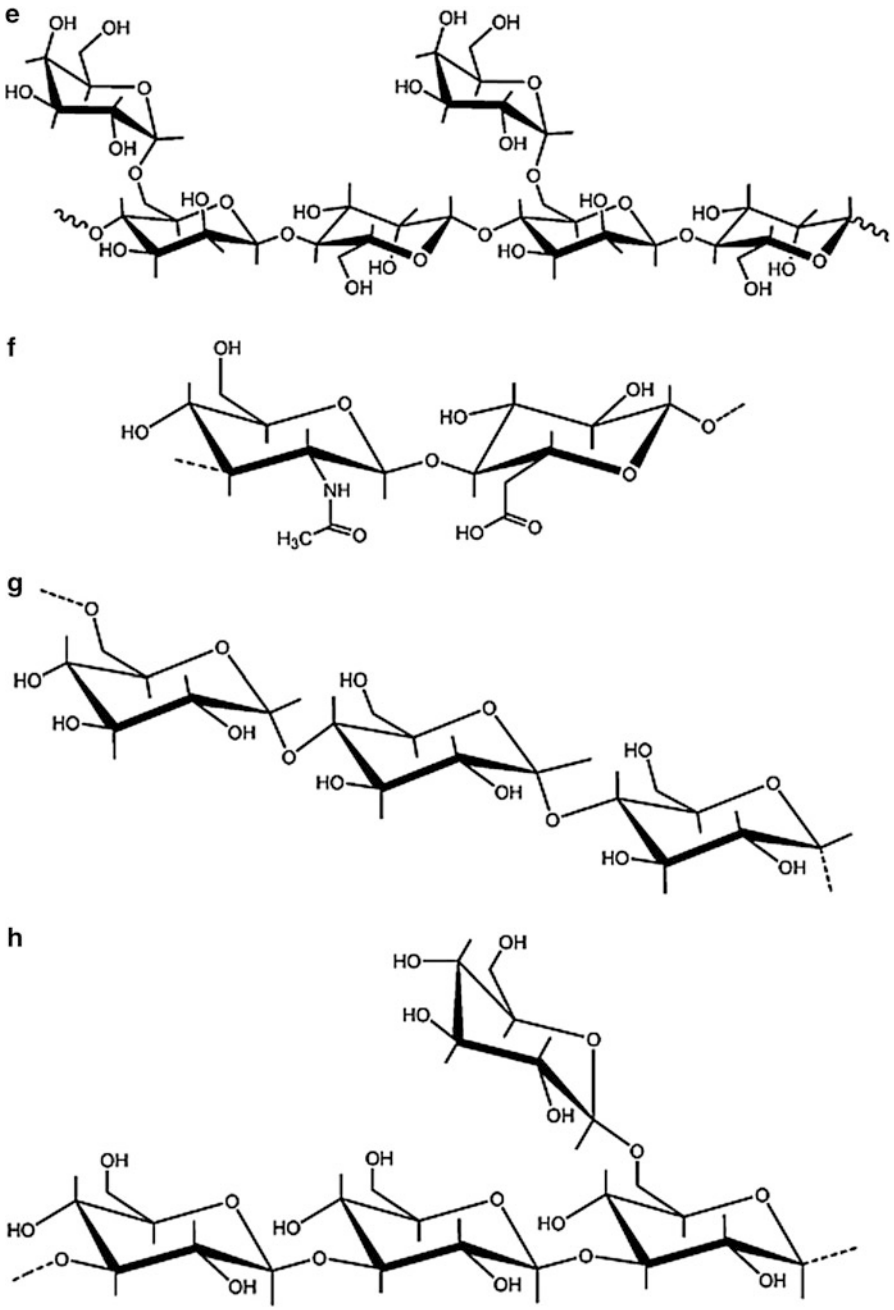

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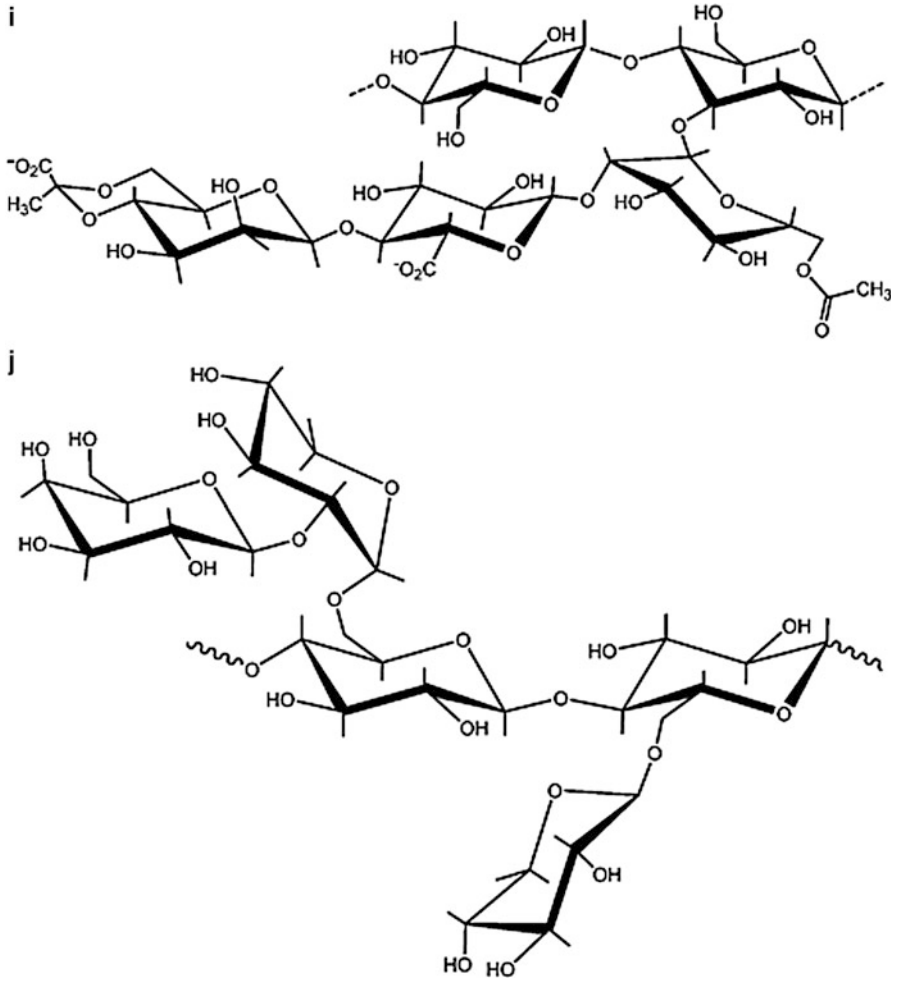

Fig. 1 Chemical structures of alginate (**a**), carrageenan (**b**), dextran (**c**), gellan (**d**), guar gum (**e**), hyaluronic acid (**f**), pullulan (**g**), scleroglucan (**h**), xanthan (**i**), and xyloglucan (**j**) (Reproduced with permission from Coviello et al. (2007))

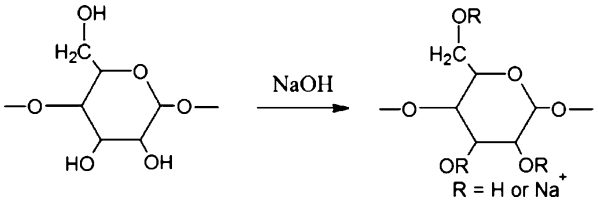

Fig. 2 Conversion of cellulose to alkali cellulose

Carboxymethylcellulose (CMC, Fig. 3a), also known as sodium carboxymethylcellulose to reflect its deprotonated state, notably has high water solubility and is utilized traditionally in foods and other household applications as a thickener and emulsifier. Due to the presence of the carboxymethyl moiety, this material has a pKa of roughly 4.0 (Abu-Ghoush et al. 2009) and can be converted between protonated and deprotonated forms by changing solution pH. Naturally it is fully water soluble and usually does not form a hydrogel. CMC, however, has the capacity to form hydrogels by ionic interaction with multivalent cations such as iron (III) and calcium (Yakup Anca 2000; Davidson et al. 2013). Additionally CMC hydrogels can be achieved by a multitude of further

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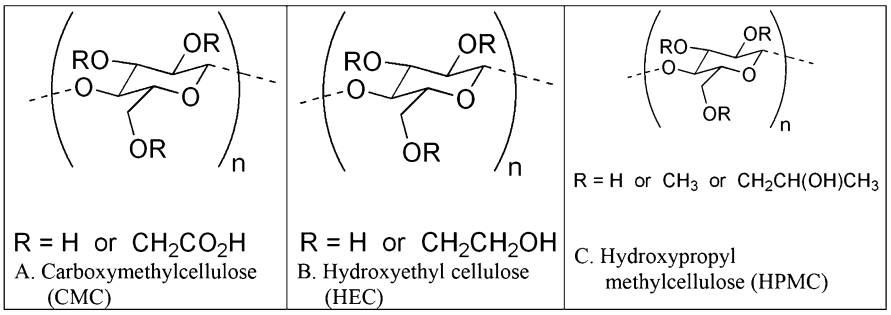

Fig. 3 Examples of chemically modified cellulose. (**a**) Carboxymethylcellulose (CMC). (**b**) Hydroxyethyl cellulose (*HEC*). (**c**) Hydroxypropyl methylcellulose (*HPMC*)

chemical modifications including reactions with dialdehydes (Rokhade et al. 2006), divinyl sulfone (Sannino et al. 2004), and diepoxies (Kono et al. 2013), as well as with interaction with radiation (Wach et al. 2001) and other methods. CMC hydrogels tend to display pH sensitivity due to the presence of the carboxymethyl units. In deprotonated state, i.e., at high pH, CMC hydrogels swell more rapidly due to both higher hydrophilicity of the anionic COO^- and the lack of hydrogen bonding which occurs in the protonated state (Barbucci et al. 2000). Figure 3 also shows the repeating units of hydroxyethyl cellulose (HEC) and hydroxypropyl methylcellulose (HPMC). Methylcellulose, despite the hydrophobic modification, has improved water solubility over cellulose, because the modification prevents the polysaccharide chains from forming crystalline domains that prevent solubility of native cellulose.

Methylcellulose possesses a thermal sensitivity and exhibits a lower critical solubility temperature (LCST) between 40 °C and 50 °C (Ruel-Garipy and Leroux 2004). LCSTs of HPC and HPMC are known to be ~42 °C (Winnik et al. 1992) and 69 °C (Joshi 2011), respectively. HEC does not display a thermal sensitivity (Kan et al. 2004). An additional ether-type addition of cellulose is ethyl (hydroxyethyl)cellulose (EHEC). It forms into a gel upon cooling; however, when mixed with surfactants such as sodium dodecyl sulfate (SDS) or cetyltrimethylammonium bromide (CTAB), the solution becomes a reverse thermogel capable of transitioning around body temperature (Ruel-Garipy and Leroux 2004). Ether-modified celluloses are highly stable and can be utilized for further chemical modifications (Lee et al. 2005).

3 Chemically Cross-Linked Polysaccharide Hydrogels

Polysaccharide hydrogels can be generated by chemically cross-linking the polysaccharide using permanent covalent bonds. This can be done in a one-step process by direct addition of a cross-linking agent or can be done in a two-step process in which the polysaccharide is modified to contain activated groups (such as vinylic groups) and then subsequently cross-linked in a second step. The benefits of the first method are speed and ease of a "one-pot"-type reaction, though the second type offers more flexibility as the activated polysaccharide can be subsequently combined with other monomers or components to create complex multicomponent hydrogels with varying properties.

3.1 Direct Cross-Linking Methods

There are several methods and conditions under which polysaccharides can be cross-linked directly to form into chemically conjugated hydrogels. One method which utilizes no chemicals is the application of high-energy ionizing radiation. This method has been utilized previously to cross-link

DOI 10.1007/978-3-319-03751-6_31-1

water-soluble polysaccharide derivatives, such as carboxymethyl starch, carboxymethylcellulose, carboxymethyl chitin, and carboxymethyl chitosan, by exposing paste-like suspensions of each to electron beams or gamma irradiation. In this condition, water hydrolysis products create free radicals that interact with the polysaccharide chains which can induce chemical reactions between the chains leading to cross-linking (Yoshii et al. 2003). Recently, gamma radiation was applied to solutions of carboxylated locust bean gum to create superabsorbent hydrogels. It should be noted that the cross-linking reaction by exposure of polysaccharide to radiation competes against degradation of the polysaccharide which places some limits on this method in terms of exposure and cross-linking degree (Hayrabolulu et al. 2013).

Chemicals which may be applied to directly cross-linking polysaccharide primarily include multifunctional moieties which are reactive towards nucleophiles. One example of this is cross-linking by dialdehydes. Figure 4 shows a schematic overview of an aldehyde–nucleophile reaction commonly used for cross-linking. Typical aldehydes and nucleophiles react in a rapid and spontaneous manner at room temperature allowing for simple one-step reactions. Typically, this has been done with low molecular weight dialdehydes. For example, chitosan is reacted with a variety of phthalaldehydes that cross-link preferentially with the amines (Hirano and Takeuji 1983). Another example is the reaction of glutaraldehyde with alginate to form hydrogels to control delivery of pesticides for agricultural uses. Alginate does not contain amine units but the aldehyde reaction can also occur with other nucleophiles such as alcohol units (Kulkarni et al. 2000).

In addition to low molecular weight dialdehydes, large multifunctional aldehydes can be utilized to achieve this reaction. Recently, an interesting variation of this chemistry was used to create an in situ forming aldehyde-linked hydrogel. In this technique, chitosan was reacted with succinic anhydride to add carboxylic acid moieties along the backbone. This allowed the chitosan to remain soluble at higher pH. Separately, hyaluronic acid was reacted with sodium periodate in the dark to generate aldehyde units along the backbone of the hyaluronic acid (Fig. 5). When solutions of these polymers were combined, they reacted spontaneously to form a strong cross-linked hydrogel. This

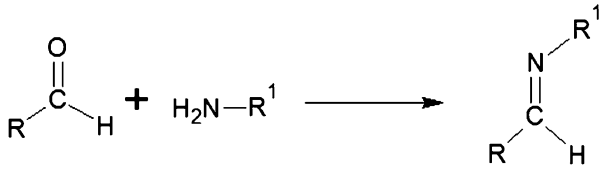

Fig. 4 Example of aldehyde reaction with a nucleophile

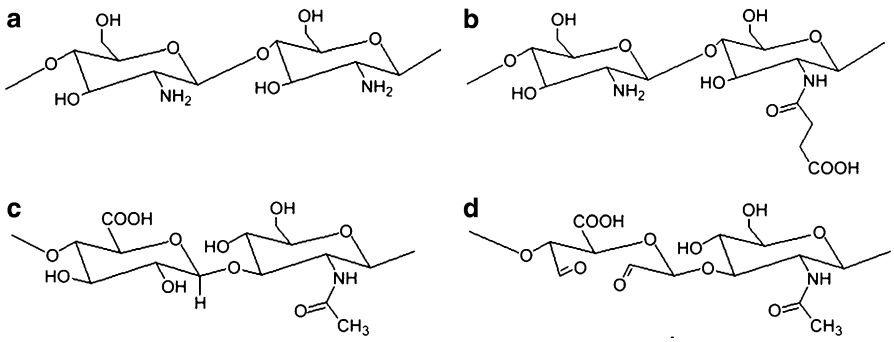

Fig. 5 Chemical structures of chitosan (**a**), *N*-succinyl-chitosan (**b**), hyaluronic acid (**c**), and aldehyde hyaluronic acid (**d**) (Reproduced with permission from Tan et al. (2009))

reaction, also known as a Schiff base reaction between and amine and an aldehyde, proceeded rapidly in 1–4 min to form a hydrogel system which is biodegradable and biocompatible enough to support growth of bovine chondrocytes on the surface of the hydrogel (Tan et al. 2009). Similarly, aldehyde-converted dextran was reacted with chitosan to form a chemically cross-linked hydrogel. Bovine serum albumin (BSA) was released in a controlled manner from the matrix showing promise for potential use as a drug delivery material as well as for tissue engineering and cell culture (Cheng et al. 2013).

Another method of cross-linking polysaccharides to form hydrogels is to apply epoxide moieties to the polysaccharide. In the presence of nucleophiles, epoxide rings undergo ring opening to conjugate to the nucleophile. Due to ring strain, epoxides are highly susceptible to nucleophilic attack, and thus, this reaction proceeds quite easily even at room temperature with no catalysts. An example schematic of this reaction is shown in Fig. 6. Examples of this cross-linking reaction include reactions involving amine-bearing polysaccharides, such as chitosan, with ethylene glycol diglycidyl ether or epichlorohydrin (Wan Ngah et al. 2002). In this case, the amine serves as the nucleophile. Additionally, alcohol units can be used as the nucleophile in reactions, for example, between poly(ethylene glycol) diglycidyl ether and hyaluronic acid (Collins and Birkinshaw 2008). In general, the amine group is a much more suitable nucleophile for this and other reactions. The hydroxyl reaction may occur but is very slow unless the pH is quite high (Lawal et al. 2011).

Polysaccharides can be cross-linked as well by utilizing difunctional isocyanates to form polyurethanes. A generalized schematic of this reaction is shown in Fig. 7. Similar to previous items, the urethane moiety (R-N=C=O) is susceptible to reacting with nucleophiles. It has a higher reactivity towards alcohols, allowing for easier reactions along this route. There are, however, some drawbacks for reacting polysaccharides directly with isocyanates, as this reaction typically does require increased temperatures, specific solvents, and catalysts. This reaction is also highly susceptible to the presence of water which leads to degradation of the isocyanates into carbon dioxide gas. Due to polysaccharides' crystallinity, poor solubility, tendency to contain water, susceptibility to temperature, and acid/alkaline, native polysaccharides are poorly suited for conversion into PEU hydrogels. Typical modifications include depolymerization or alkylation, although this often leads to plastics instead of thermogels (Donnelly et al. 1991). Despite this, there are examples of hydrogels generated utilizing this chemistry typically when mixed with other alcohol/amine-bearing ingredients. For example, urethane reactions have been utilized to cross-link mixtures of chitosan, poly (ethylene glycol) (PEG), and alcohol-terminated polydimethylsiloxane (Rodkate et al. 2010). Additionally, hyaluronic acid has been cross-linked utilizing star-shaped polyethers with isocyanate groups on the distal arms to form biodegradable hydrogels for drug delivery applications (Dhanasingh and Groll 2012).

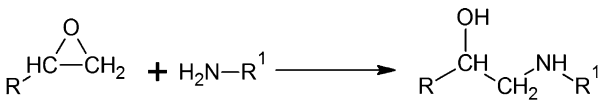

Fig. 6 Reaction between amine and epoxide

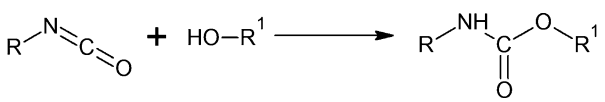

Fig. 7 Generalized isocyanate reaction to form urethane linkage

DOI 10.1007/978-3-319-03751-6_31-1

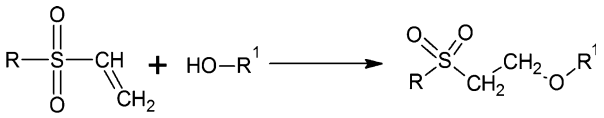

Fig. 8 Generalized vinyl sulfone–alcohol reaction

Another method of cross-linking is by utilizing divinyl sulfone. A generalized schematic of this reaction is shown in Fig. 8. Divinyl sulfone operates as a Michael's type reaction acceptor and is reactive preferentially towards nucleophiles such as amines and thiols. Vinyl sulfone can also react with alcohols at high pH. This reaction has been done with hyaluronic acid to form hydrogels (Collins and Birkinshaw 2008). This vinyl-sulfone-linked hyaluronic acid has been investigated for drug delivery as well as for use as biocompatible coatings and tissue scaffolds. In addition to direct divinyl sulfone, a variety of sulfone-terminated PEGs have been used for this as well. The presence of the hydrophilic PEG spacer leads to an overall lower cross-linking density which allows for greater swelling and faster drug release (Hahn et al. 2004). HPC has also been reacted with divinyl sulfone at high pH (pH~12) to form a temperature-responsive hydrogel which shrinks upon warming. This system was investigated for its potential to serve as an actuator or "artificial muscle" (Hinkley et al. 2004). In addition to divinyl sulfone and PEG/vinyl sulfone, vinyl sulfone macromers have also been produced. To create this, mercaptoalkanoic acid was modified with divinyl sulfone to yield vinyl sulfone alkanoic acid which was subsequently conjugated to dextran using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) as catalyst. This formed dextran/vinyl sulfone with varying degrees of substitution was reacted with thiol-terminated PEGs linear and star shaped. This in situ reaction formed dextran–PEG hydrogels rapidly which were biodegradable over the course of 3–21 days based on the degree of substitution (Hiemstra et al. 2007). These examples highlight some of the capabilities of this versatile reaction mechanism.

3.2 Activated Cross-Linking Methods

The above sections highlight a variety of methods for generating polysaccharide hydrogels directly in a "single-step" reaction. This chemical method is robust and simple. There is often a requirement for the hydrogel to include other monomers or be reacted in a certain manner. Thus, it becomes desirable to first convert the polysaccharide into an activated form. Typically, this takes the form of attaching vinyl groups onto the polysaccharide which can subsequently participate in radical chain reaction. Several of the linking chemistries are similar to those described above but will be highlighted briefly with an emphasis on their functionality.

3.2.1 Epoxide–Acrylate

One common method is to react polysaccharide with epoxide–acrylates to generate a vinylically reactive macromer. The initial reaction typically involves an epoxy reaction between glycidyl methacrylate and glycidyl acrylate with a polysaccharide. The epoxide ring opens against one of the polysaccharide nucleophiles (alcohol or amine) binding the vinyl group to the nucleophile. Since this reaction occurs easily at room temperature with no need for a catalyst, the vinyl group remains unreacted, allowing for the subsequent material to be purified and mixed with other reagents easily. Examples of this include the grafting of glycidyl methacrylate onto cashew gum (Guilherme and Reis 2005), galactomannan (Reis et al. 2003), dextran (Hennink et al. 1996; De Smedt et al. 1995), and hyaluronic acid (Leach et al. 2004). This allows these macromers to be subsequently reacted into semisynthetic hydrogels. Recently, this technique has been applied to synthesize biocompatible

cryogels for cell growth and seeding. The components used were low molecular weight hyaluronic acid–methacrylate generated by reacting glycidyl methacrylate with autoclaved hyaluronic acid (having reduced chain length) as well as full molecular weight hyaluronic acid–methacrylate. Dextran–methacrylate was synthesized by dissolving dextran in dimethyl sulfoxide (DMSO) and reacting with glycidyl methacrylate utilizing 4-(dimethylamino)pyridine as a catalyst. Solutions of the thus formed polysaccharide–acrylates were then dissolved in water and degassed by sparging/ultrasound. They were then frozen and irradiated directly with electron beam irradiation to achieve cross-linking. The resultant cryogels were found to be highly biocompatible and readily allowed for ingrowth of 3T3 cells (Reichelt et al. 2014).

3.2.2 Vinyl-Oyl Chloride

Vinyl groups can also be attached to polysaccharide using vinyl-oyl chlorides, such as acryloyl chloride and methacryloyl chloride, both of which are readily commercially available. The acyl chloride moiety is highly reactive towards any nucleophile including alcohol and water, and thus, care must be taken to ensure that the reaction is performed under anhydrous conditions. The resultant formation of HCl limits the reaction from proceeding, and so typically this reaction is performed in the presence of an HCl scavenger such as triethylamine (Fig. 9) (Tran et al. 2011). This reaction has been previously applied to acrylate starch which had been previously modified to contain sulfate groups to provide hydrophilicity. This reaction was performed at 60 °C in DMF utilizing pyridine as an acid scavenger. The resultant modified starch was subsequently combined with a comonomer, acrylic acid, and initiated via ammonium persulfate/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine redox pair utilizing a blowing agent sodium bicarbonate to simultaneously increase the rate of reaction by raising pH and generate carbon dioxide gas bubbles. The formed superporous hydrogel (SPH) was capable of absorbing up to 200 times its weight in water (Kuang et al. 2011).

3.2.3 Carbodiimide Conjugation

Conjugation of polysaccharides can also be accomplished via carbodiimide-mediated chemistry. A carbodiimide is any reagent with the N=C=N moiety typically close to the middle of the reagent. Popular carbodiimides include *N*,*N*′-dicyclohexylcarbodiimide (DCC) (preferably in hydrophobic solvents) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (preferable in aqueous or polar solvents). The reaction initiates with the acid end cap of the material reacting with the carbodiimide to form an unstable *O*-acylisourea intermediate. The intermediate proceeds to react with nucleophiles (preferentially with amines over hydroxyls) forming an amide bond with a side product being an insoluble organo-urea which is typically removed by filtration (Fig. 10) (Monagle

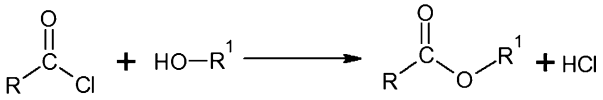

Fig. 9 Generalized acyl chloride–nucleophile reaction. If "R" is acrylate or methacrylate, the resultant molecule is capable of participating in radical chain polymerization

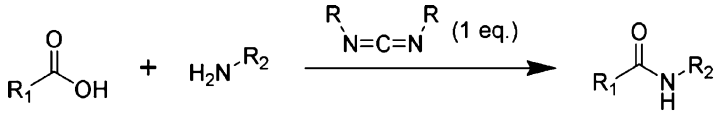

Fig. 10 Example of generic carbodiimide-mediated reaction between an acid and an amine

DOI 10.1007/978-3-319-03751-6_31-1

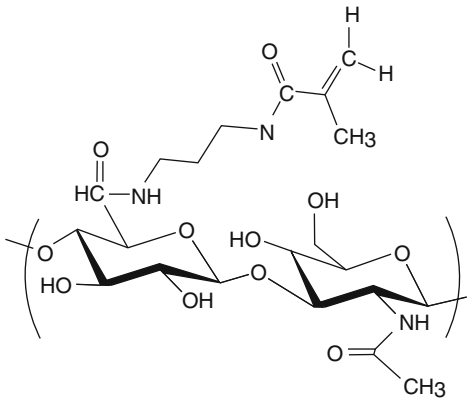

Fig. 11 Hyaluronic acid conjugated to methacrylate units by carbodiimide-mediated attachment (Reproduced with permission from Kim and Park (2002))

1962). This chemistry has been applied to forming polysaccharide gels by reacting hyaluronic acid with *N*-(3-aminopropyl)methacrylamide hydrochloride utilizing EDC as a carbodiimide reagent. The resulting hyaluronic acid derivative (Fig. 11) had chemical conjugates between the carboxylic acid units of the hyaluronic acid and the aminopropylmethacrylamide, allowing for subsequent cross-linking with poloxamer diacrylate to form a thermally sensitive, chemically cross-linked hydrogel which served well as a scaffold for controlled release of human growth hormone (Kim and Park 2002).

3.3 Enzymatic Methods

One of the more unusual chemistries recently applied to adding vinyl reactive groups is the utilization of biological enzymes to catalyze the conjugation of vinyl acrylate onto dextran in order to activate it for subsequent use in radical chain-initiated reaction. Solutions of proleather and lipase were utilized to catalyze the reaction between dextran and vinyl acrylate. The subsequent dextran acrylate was then cross-linked to form hydrogels which showed good biocompatibility and biodegradability (Ferreira et al. 2002, 2004).

4 Physically Cross-Linked Polysaccharide Hydrogels

Physically cross-linked polysaccharide hydrogels are formed through chain entanglement, ionic interactions, hydrogen bonding, and hydrophobic interactions.

4.1 Chain Entanglement

Polymer chains of polysaccharides can intertwine to form physical cross-links. The “gum” series of polysaccharides form chain-entangled hydrogels upon simple heating and cooling of their solutions. Xanthan gum is a heteropolysaccharide composed of a 1-4-linked β-D-glucose backbone. This backbone has a trisaccharide side chain composed of two mannoses and one glucuronic acid at every other residue. Locust bean gum is a 1-4-linked β-D-mannan backbone with 1-6-linked α-D-galactose side groups. When respective solutions of these two gums are mixed and heated, the chains gain motility, allowing for them to intertwine. Upon cooling a gel is formed which is highly resilient to redissolution except for at extremely high temperatures (Higiro et al. 2006). These kinds of gels are typically used in the food industry as viscosity modifiers for a wide variety of products as well as for

forming edible films and other substances (Aydinli and Tutas 2000). Although chain entanglement is not typically a desired method of physical cross-linking in semisynthetic polysaccharides, it is often a contributing factor along with other modes of physical cross-linking.

4.2 Ionic Interaction

Several anionic polysaccharides react to form hydrogels directly by interaction with multivalent cation metals, such as calcium (Olivas and Barbosa-Canovas 2008; Betigeri and Neau 2002), iron (Kroll et al. 1996), and others. Cationic polyglucosamines have the capacity to react with polyanions, such as sodium tripolyphosphate(Betigeri and Neau 2002), to form gels. Most notable examples of this kind of cross-linking include cross-linking interaction between sodium alginate and calcium chloride as well as the cross-linking interaction between chitosan and sodium tripolyphosphate. These ionic interactions have the capacity to form hydrogels very quickly, so fast that discrete beads of alginate/calcium or chitosan/tripolyphosphate can be formed by simply dripping the polysaccharide solution into the polyvalent ion solution.

4.2.1 Alginic Acid–Calcium

Alginic acid (Fig. 1a) is a linear copolymer comprised of blocks of (1-4)-linked β-D-mannuronate (M) and the C-5 epimer α-L-guluronate (G) residues, respectively. Alginic acid is extracted from the cell walls of brown algae and used for a wide variety of medical, food, and industrial purposes as a water-absorbent gum/thickener. When processed at high pH utilizing sodium hydroxide or potassium hydroxide, it is converted to sodium alginate or potassium alginate. In this form, it is freely water soluble at low concentrations (Remminghorst and Rehm 2009). When a solution of alginate interacts with calcium, the sodium or potassium exchanges with calcium. Divalent calcium ions interact with two carboxyl groups of alginate to form ionic cross-links and a gel (Fig. 2). This versatile chemistry can be applied for a wide variety of applications including drug delivery (McLennan et al. 2000) and medical devices/tissue scaffolds (Christensena 2011; Livnat et al. 2005).

4.2.2 Chitosan/Tripolyphosphate

An analogous situation occurs with a cationic polysaccharide (chitosan) which interacts with tripolyphosphate to form a hydrogel (Vimal et al. 2013). Chitin is found naturally as the structural component in the exoskeleton of crab, shrimp, and insects. This material is readily available in bulk at an extremely low price from commercial fishing industry. Shell pieces are chopped/ground and then soaked in hydrochloric acid and deproteinized typically by hot alkali treatment with refluxing sodium hydroxide. This, along with oxidization procedures using oxidizing agents (e.g., KMnO4 + oxalic acid + H2SO) to remove pigmentation, allows for chitin to be isolated from shells. Subsequently, chitin can be converted to chitosan by deacetylation of the chain using alkali treatment (Abdou et al. 2008). Chitin can also be extracted from fungal sources as well (Rane and Hoover 1993; Zikakis 1984). Chitosan is inherently water insoluble at neutral and high pH due to the deprotonated form of the amine units along the chain. When exposed to low pH (typically $pH < 5$, easily achieved with even 1 % acetic acid), however, the chitosan becomes protonated and fully soluble in water. When this chitosan solution is exposed to a multivalent anion, such as sodium tripolyphosphate which is dibasic, a chemical cross-link is formed between the chains, creating a polysaccharide hydrogel held by ionic influences (Mi et al. 1999). This hydrogel can occur with other materials to create mixed hydrogels as well as has the capacity to have anionic materials, such as alginate, absorb onto the hydrogel due to ionic attraction. These capabilities make this type of hydrogel desirable for microparticle drug delivery systems (Ko et al. 2002). The mechanical strength, in the absence of reinforcement, of chitosan/tripolyphosphate is not as high as that of other

ionic polysaccharide hydrogels such as calcium ion-cross-linked alginate. For this reason, it is not typically used for devices or other larger structures (Shu and Zhu 2000).

4.2.3 Other Ionic Polysaccharides

Although chitosan and alginate are the most popular materials for generating ionic polysaccharide hydrogels, any polysaccharides which have a plurality of charges along its backbone can be used for this purpose. Carrageenan (Fig. 1) is a family of polysaccharides which have anionic sulfonate (SO^{3-}) groups present along their backbone. These anionic sulfonate groups have also been used as sites for ionic cross-linking with divalent and trivalent counterions as well as by interaction with other natural polymers such as gelatin. Gellan gum forms thermoreversible gels in the presence of divalent counterions. Scleroglucan, when derivatized to introduce carboxylic acid units, can be cross-linked by calcium ions to form hydrogels (Coviello et al. 2007). Ionically cross-linked polysaccharide hydrogels are inherently sensitive to both pH and ionic strength of the media. Since it is the balance between ions that generates these gels, changes in pH can shift the protonation degree of the ions. When applied in vivo or in a sink system over time, the low molecular weight cross-linking ion (e.g., calcium and tripolyphosphate) leaches out of the system, and eventually the hydrogel redissolves.

4.2.4 Polyelectrolyte Complex

A polyelectrolyte complex forms when two polymers having opposite charges interact. This is a popular method of forming an ionic gel with polysaccharides as they typically contain or can be modified to contain opposing charges. These ionic interactions are stronger than other interactions, such as van der Waals or hydrogen bonding. Polysaccharide polyelectrolyte complexes are formed by interacting a polycation (e.g., chitosan) with an anionic polysaccharide. Anionic polysaccharides include alginic acid, hyaluronic acid, chondroitin sulfate, carboxymethylcellulose, dextran sulfate, pectin, and xanthan. Polysaccharide polyelectrolyte complexes can also be formed with proteins, such as gelatin, fibroin, albumin, keratin, or collagen, or anionic synthetic polymers such as polyacrylic acids (Bhattarai et al. 2010).

4.3 Hydrogen Bonding

Hydrogen bonding is a strong electromagnetic attraction between hydrogen and an electronegative atom such as oxygen or nitrogen. As shown in Fig. 12, hydrogen bonding in polysaccharides is common due to the presence of several participatory groups such as alcohols, carboxylic acids, and amines (Blackburn 2004). Hydrogen bonding is an environmentally sensitive process. For hydrogen bonding to occur, the hydrogen-bearing groups must be appropriately protonated for carboxylic acid or deprotonated for amine (Fig. 12) and at an appropriate temperature with a low salinity for a strong

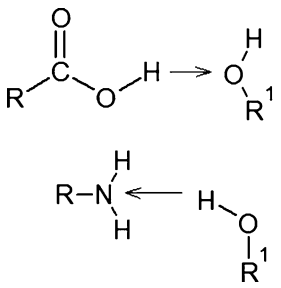

Fig. 12 Examples of hydrogen bonding of carboxylic acid and amine with alcohol units. *Arrows* indicate hydrogen bonds

DOI 10.1007/978-3-319-03751-6_31-1

hydrogen bond to form. Agarose only forms hydrogen bonds at relatively low temperatures, and chitosan only forms these bonds at pH higher than 5 (Francis Suh and Matthew 2000). Semisynthetic polysaccharides, such as carboxymethylcellulose, also undergo hydrogen bonding in a pH-sensitive manner (Barbucci et al. 2000). Hydrogen bonding is sensitive towards other components in the solution, such as urea, which disrupt this form of physical bond and reduce its capacity to gel a material (Moon et al. 2012).

4.4 Hydrophobic Physical Cross-Linking

One effective way of forming a hydrogel is to attach hydrophobic units to the chain for hydrophobic association. The hydrophobic association is primarily due to the lack of attraction between water and the hydrophobic moieties rather than any attraction among hydrophobic moieties (Chandler 2005). The presence of an excess of hydrophobic moieties will simply yield a water-insoluble polymer (Miyamoto et al. 1995). One good example of this is cellulose acetate with high levels of acetate conjugation. They are water-insoluble plastics used for a variety of films, photographic and consumer purposes (Kamide 2005). The thermal sensitivity is strongly tied to the relative content of hydrophobic and hydrophilic portions of the polymer, and thus, it can be controlled by adjusting the hydrophobic content of a polysaccharide. Methylcellulose, which has thermogelation properties but with a relatively high transition temperature, can be modified to contain additional stearate (C18 fatty acid) groups along the chain. Stearoyl chloride was reacted with methylcellulose in the presence of triethylamine (which serves as an HCl scavenger). In another method, stearic acid was reacted with methylcellulose utilizing carbodiimide-mediated chemistry and dimethylaminoethylpyridine (DMAP) as a catalyst. Both of these conjugation techniques were successful in generating hydrophobically modified cellulose (Lee et al. 2005) (Fig. 13). Similarly cellulose has been reacted with hydrophobic modifiers, such as 1,2-epoxydecane and butyl isocyanate, to form thermogelling derivatives based on hydrophobic associations (Miyamoto et al. 1995). Additionally reacetylated chitosan can serve as a thermogel based on hydrophobic modifications as well (Li et al. 2013).

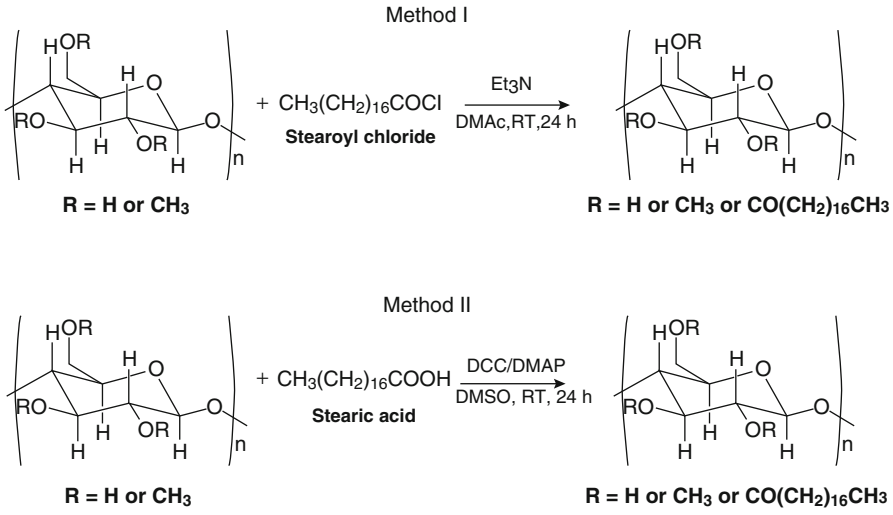

Fig. 13 Synthetic methods for generating stearate-modified methylcellulose (Reproduced with permission from Lee et al. (2005))

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5 Stimuli-Responsive Polysaccharide Hydrogels

An increasingly popular field of research is into "smart" or "environmentally sensitive" hydrogels. This is a popular field both for polysaccharide hydrogels and hydrogels in general. The phrase "smart" typically refers to hydrogels which undergo a change in their physical bonds or attraction towards water due to a change in the environmental factor, such as pH, ionic strength, temperature, light, and presence of specific molecules, such as glucose or enzymes. In some instances, the phrase is applied to hydrogels which undergo chemical changes such as those which are sensitive towards enzymatic hydrolysis of a specific enzyme which may be utilized for drug delivery.

Stimuli-responsive polysaccharide hydrogels display a phase transition behavior. There are two (or three for un-cross-linked polymers) different phases these responsive hydrogels can exist in as defined by the Flory–Huggins theory (Bromberg and Ron 1998). The polymer–solvent interaction creates osmotic pressure ($\Delta\pi_{mix}$) attracting the polymer chains to the solvent, thus driving the polymer to either expand or, if there are no other bonds holding it together, to ultimately dissolve. Additionally, there are polymer–polymer interactions related to the strength of the polymer chain attraction for each other as an elastic force ($\Delta\pi_{elast}$). These forces in a given polymer system are always in balance, and the following equation applies:

$$\Delta\pi = \Delta\pi_{mix} + \Delta\pi_{elast} = 0$$

In a general hydrogel system, the opposing forces balance and thus define the swelling of the hydrogel. However, in a responsive system, the relative balance of these forces is affected by a specific stimulus. As this occurs, the system transitions through three stages (Harsh and Gehrke 1991):

1. Precipitate stage ($\Delta\pi_{mix} \ll \Delta\pi_{elast}$): There is maximum polymer–polymer interaction with minimal polymer–solvent interaction, and thus, the polymer is precipitated from the solution and behaves in a hydrophobic manner resisting water infiltration.
2. Gel stage ($\Delta\pi_{mix} \sim \Delta\pi_{elast}$): The opposing interaction forces are close to balance with one another. The polymer chains retain enough interaction to hold the hydrogel together as one piece but still be highly hydrated with water. Depending on the system, this transition may be very sharp or broad.
3. Solution/fully swollen stage ($\Delta\pi_{mix} \gg \Delta\pi_{elast}$): The polymer–solution interaction is maximized, and the polymer either completely dissolves (in the situation where the physical bonds were holding it together) or in the case of a chemically cross-linked hydrogel expands to reach their maximum swelling.

This general theory applies to the interaction of physical bonds with the environment, but with several exceptions and nuances. For example, altering the relative attractive forces is a reversible change, whereas breaking of chemical bonds by enzymes or formation of chemical bonds by light is an irreversible change. Additionally, the method in which these interactions change depends drastically on the system being altered. Several types will be detailed and highlighted with examples below (Qiu and Park 2001).

5.1 Enzymatic/Special Molecule Sensitivity

In general, polysaccharide hydrogels display enzymatic sensitivity in that they are degraded by the enzyme which is a nonreversible change to the polymer backbone. A good example of this is the degradation of dextran-based hydrogels by dextranases leading to increased release of the drug payload. This method was used as a means to develop a hydrogel for controlled protein delivery

based on enzymatic degradation rate (Hennink et al. 1997). One valuable aspect of this application is towards colon-specific drug delivery where dextranases are present in higher concentrations than elsewhere in the body (Hovgaard 1995). Additionally "dual-sensitive" hydrogels have been made by forming interpenetrating polymer networks (IPNs) consisting of oligopeptide-terminated PEG and dextran. This requires the presence of both papain and dextranase to degrade the hydrogel and release the drug under very specific conditions.

There are a wide range of hydrogels which display sensitivity towards a variety of biomolecules including glucose, peptides/proteins, nucleic acids, and other materials (Miyata et al. 2002). Glucose sensitivity can be achieved by incorporation of concanavalin A (Con-A), which is a carbohydrate-binding protein with a strong affinity for glucose. A dextran–PEG-based cross-linked hydrogel incorporating Con-A was made to provide glucose sensitivity. As part of forming this, hydrogel dextran was modified using glycol methacrylate to form dextran–methacrylate, and Con-A was modified by Michael's addition with ethylene glycol acrylate–methacrylate. These were reacted with PEG-dimethacrylate to form a chemically cross-linked hydrogel. In the absence of glucose, Con-A is attracted to the dextran chain and binds to this chain. When glucose is present, however, it competes for this binding and as such increases the swelling of the hydrogel, thus making the hydrogel as a whole sensitive towards glucose. The goal of this type of research is to generate an intelligent pump which releases certain quantities of insulin based on the glucose content in the blood (Yin et al. 2010).

5.2 pH Sensitivity

Some hydrogels exhibit pH sensitivity primarily due to the presence of ionizable groups. Several natural polysaccharides, such as alginic acid and chitosan, already have ionizable groups available and display pH sensitivity. Weak acid, such as –COOH, present in hydrogels ionizes at high pH and thus swells to a greater degree or dissolves. On the other hand, hydrogels containing weak bases, such as amines, become ionized at low pH for higher swelling or dissolution. In all cases, the sensitivity towards pH is related to the materials' acidity constant which defines the equilibrium state of the ionized fraction at a given pH. The equilibrium constants for weak acids and weak bases can be written as shown in Table 2. K_a and K_b are the acidity constant and basicity constant of the weak acid and weak base, respectively. A weak acid at low pH (where $[H^+]$ is high) is primarily in the protonated (non-ionized) form which is not water soluble (or not swellable). At high pH, it is primarily in the deprotonated (ionized) form which is water soluble (or swellable). A weak base dissolves (or swells) at low pH, but become water insoluble (or not swellable) at high pH. Due to the wide range of pH, it is common to convert the K_a and K_b values into pK_a and pK_b, the sum of which for a conjugate acid/base pair equals 14 in an aqueous system. One simple and practical implication of pH sensitivity with natural polysaccharides is that chitosan is normally insoluble in water at neutral pH. It dissolves quite easily, however, in dilute acids (e.g., 0.1 M acetic acid) due to ionization of the amine units along its backbone (Rinaudo et al. 1999).

Polyelectrolyte complex beads formed by mixing chitosan and alginate have been utilized to control the release of bitter gourd extract in a pH-dependent manner (Lin et al. 2014). Sometimes the pH-sensitive portion of the hydrogel is not from the polysaccharide. Radical chain polymerization of

Table 2 Equilibrium constants for weak acids and weak bases

Weak acids	Weak bases
$HA \leftrightarrow H^+ + A^-$	$B + H_2O \leftrightarrow BH^+ + OH^-$
$K_a = \frac{[H^+][A^-]}{[HA]}$	$K_b = \frac{[BH^+][OH^-]}{[B]}$

acrylic acid was performed in the presence of tragacanth gum. During this reaction, the initiator abstracts a hydrogen atom from tragacanth gum chain, and poly(acrylic acid) initiates from this point leading to a grafted polymer. Due to the presence of the plurality of –COOH units along the poly (acrylic acid) chain, the system displayed a reduced swelling and drug release at low pH (Singh and Sharma 2014).

5.3 Thermal Sensitivity

A gel can react towards changes in solution temperature in two primary ways. The first is referred to as the "normal" thermogel response in which heating of the solution improves overall polymer solubility. The increase in temperature reduces chain entanglement and hydrogen bonding, leading to either dissolution (for a physically cross-linked gel) or increased swelling for a chemically cross-linked gel. This type of response is common for gels made from gelatin or mixtures of xanthan and locust bean gum (Acharya et al. 2010; Carafa et al. 2011). Typically, however, when one refers to a thermogel or thermally sensitive hydrogel, this is not the response they are indicating. Responsive thermogels are generally considered to be "inverse" thermogels. In this situation, heating of the solution leads to a change in the water–polymer entropy or physical bonding that leads, counterintuitively, to gelation or shrinking of the hydrogel at increased temperature. As temperature increases, hydrogen bonding decreases while hydrophobic interaction increases.

Generally, the process of thermogelation is considered to be driven by entropy relative to the water–polymer attraction system. When water molecules are associated with the polymer chain, they are highly organized leading to a decreased entropy relative to the entropy of free water. The attractive force of water–polymer binding energy is an enthalpic term (ΔH_f, heat of fusion). The energy of the system as a whole can be generally described by the Gibbs free energy equation as follows:

$$G = H - TS$$

where G, H, S, and T are Gibbs free energy, enthalpy, entropy, and temperature, respectively. At low temperatures, the entropy drive is reduced, and the heat of fusion (ΔH_f) dominates so that the water molecules prefer to be associated with the polymer chain and the physically cross-linked hydrogel dissolves freely while a chemically cross-linked hydrogel swells to its maximal extent. As temperature increases, the total energy of the system, as described by Gibbs free energy of the system, favors the entropic term rather than the enthalpic term, and the water molecules prefer to be in unorganized form in free solution rather than bound to the polymer chain (Oesterhelt et al. 1999; Harris 1992; Wang et al. 1997). The condition at which these two forces are in balance is referred to as the lower critical solubility temperature (LCST), and it is at that point that the material gels to a solid. The nucleophilic groups along the polysaccharide chain bond strongly to water. Thus, the requirement to generate a thermogel is simply to modify the polysaccharide to introduce hydrophobic moieties (Li et al. 2010; Kuang et al. 2006; Zhai et al. 2012).

Several examples have already been discussed, including methylcellulose, HPMC, etc. Chitosan, dextran, pullulan, and carboxymethyl curdlan have been hydrophobically modified to form thermogels (Lee et al. 2005; Hennink and van Nostrum 2012; Jeong et al. 2002). Polysaccharide hydrogels can also be generated relying on an additional, typically synthetic polymer for providing the thermogelation property. Examples of this include graft polymers of alginate with poloxamer (a known synthetic thermogel containing blocks of polypropylene oxide and polyethylene oxide) (Chen et al. 2011) and hyaluronic acid with poly(*N*-isopropylacrylamide) (PNIPAM) (Mortisen et al. 2010). In both these situations, the polysaccharide provides general hydrophilicity as well as

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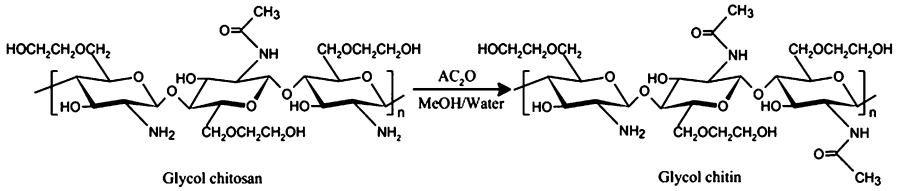

Fig. 14 Reacetylation reaction of glycol chitosan with acetic anhydride (Image reproduced with permission from Li et al. (2013))

other desired properties (biodegradability, biocompatibility), while the thermogel (poloxamer or PNIPAM) provides the thermogelling property.

Thermal gelation LCST is sensitive towards other components present in the solution. For instance, some materials (NaCl, KF, $(NH_4)_2SO_4$, and poly(ethylene glycol)) lower the LCST due to their water structure formation properties (Lee et al. 2005). Conversely, the addition of hydrotropic agents, materials which reduce water structure formation such as salicylate, diethylnicotinamide, urea, etc., will raise the LCST. This property can be controlled by the degree of hydrophobic modification. For example, methylcellulose has thermal sensitivity with LCST of >45 °C. This makes the methylcellulose useful for some applications, such as food preparation as a batter viscosity modifier (Sanz 2005), but physiologically not applicable as a tissue scaffold or drug delivery gel. To lower the LCST of methylcellulose, additional hydrophobic groups may be added. Stearate (C18) groups were introduced to methylcellulose of 14,000–40,000 Da and a degree of methyl substitution around 1.6–1.9. Stearates were conjugated onto a methylcellulose chain at 0.3 or 1.6 mol%. The original methylcellulose could not thermogel at 33–37 °C, regardless of NaCl concentration, but that the stearate-modified methylcellulose could gel easily at these conditions (Lee et al. 2005).

Chitosan can also be converted into a thermogel by carefully controlling the degree of deacetylation. A highly controlled thermogelling glycol chitin was generated by reacetylating the chitin chain to contain the desired quantity of acetylation units (Li et al. 2013). This was done by reacting glycol chitosan with acetic anhydride at room temperature for varying reaction times to achieve varying degrees of reacetylation (Fig. 14). Glycol-modified chitosan has enhanced water solubility over conventional chitosan due to glycol unit which allows the polymer to dissolve at high pH. Further modification to add the acetyl units achieves a material which carefully balances the hydrophobic interactions leading to gel formation and the hydrophilic attractions which lead to solution formation. The formed hydrogel was found to be rapidly gelling allowing for gelation upon injection into a warm body. With higher degrees of acetylation, the polysaccharide was more susceptible to degradation in the presence of lysozymes. The material displayed good biocompatibility and allowed for controlled release of doxorubicin, a chemotherapeutic agent, over 13 days. Overall this research showed that there is good promise for modified chitosan to be utilized as thermogels in the fields of drug delivery and tissue engineering. A great deal of these properties (biodegradability, biocompatibility) can be attributed to the polysaccharide itself which lends the use of these materials to medical applications.

5.4 Light Sensitive

Light-sensitive hydrogels refer to the hydrogels with reversible sensitivity towards light without light-induced chemical cross-linking. A typical strategy to form a light-sensitive hydrogel is to utilize a thermally sensitive polymer and then incorporate a material which generates heat from light such as melanin (Ninh et al. 2014) or graphene oxide (Lu et al. 2014). Photosensitive moieties, such

DOI 10.1007/978-3-319-03751-6_31-1

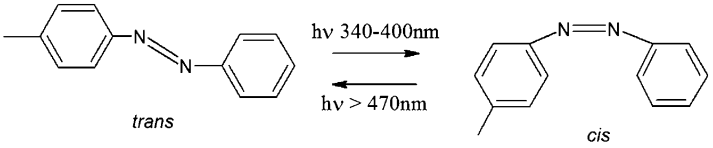

Fig. 15 The cis/trans conversion of [4-(phenylazo)phenyl]carbamate upon exposure to light Yashima et al. (1995)

as [4-(phenylazo)phenyl]carbamate which switched between cis and trans upon exposure to either UV light or light >470 nm, can be grafted to cellulose/amylose (Fig. 15) (Yashima et al. 1995). This light-sensitive moiety has been utilized to modify HPMC which, in the presence of cyclodextrin, forms a photosensitive hydrogel. Another method of inducing light sensitivity is to conjugate a light-inducible zwitterion, such as spiro benzopyran or spiro napthoxazines, which undergoes reversible ring opening upon light impingement. This method has been utilized to convert methylcellulose, dextran, and other polysaccharides into a form that had varying water/organic solubility based upon light exposure. More information can be found in a review article (Wondraczek et al. 2011).

6 Conclusion

The possibilities for generating hydrogels out of polysaccharide precursors are boundless. The inherent biocompatibility/biodegradability as well as the presence of a plurality of nucleophilic groups along the polysaccharide backbone provides many benefits. Polysaccharides can be modified, allowing for both chemical and physical hydrogels. Several natural as well as modified polysaccharides display sensitivity to environmental conditions such as temperature, pH, salinity, and light, allowing for the generation of environmentally responsive or "smart" polysaccharide hydrogels. The many capabilities of polysaccharide hydrogels are useful for applications to industrial, cosmetic, food, and healthcare fields.

Acknowledgment

This work was supported by the Showalter Research Trust Fund and the National Institutes of Health through CA129287 and GM095879.

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