Chapter 2
Microbial Polysaccharides as Advance Nanomaterials

Abstract The microorganisms offer great amounts of polysaccharides in the presence of additional carbon source. Certain polysaccharides serve as storage compounds. The polysaccharides excreted by the cells, called as exopolysaccharides, are of industrial importance. The exopolysaccharides may be reported in association with the cells or may remain in the medium. The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups such as carboxyl, which can function as polyelectrolytes, are commercially more important. These emerging microbial polysaccharides are recently explored as nano-materials for diverse biomedical applications. This chapter emphasize on nano-applications of microbial polysaccharides in diverse discipline of biomedical science.

Keywords Microbial • Polysaccharides • Nanoparticles • Drug delivery

2.1 Introduction

Polysaccharides are non-toxic, natural, and biodegradable polymers that envelop the surface of most cells and play significant functions in a variety of biological mechanisms e.g. immune response, adhesion, infection, and signal transduction. Studies on the optional treatments applied by diverse cultures all the way through the history exposed the fact that the utilized plants and fungi were rich in bioactive polysaccharides with established immune-modulatory activity and health encouraging effects in the treatment of inflammatory diseases and cancer. Therefore significant research has been directed on illuminating the biological activity mechanism of these polysaccharides by structure-function analysis. In addition to the attention on their applications in the health and bio-nanotechnology sectors, polysaccharides are also employed as stabilizers, thickeners, bioadhesives, probiotic, and as emulsifier, and gelling agents in food and cosmetic industries, biosorbent and bioflocculant in the environmental sector. Polysaccharides are either isolated from biomass capital like algae and higher order plants or derived from the fermentation broth of bacterial or fungal cultures. For economical and sustainable production of bioactive polysaccharides at commercial scale, inspite of plants and algae, microbial sources are favored because they facilitate fast and high yielding production procedures under

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Table 2.1 Classification of polysaccharides

Polysaccharides	Complete class
Microbial Polysaccharides	**Bacterial polysaccharide:** bacterial cellulose, dextran, bacterial hyaluronic acid, xanthan, emulsan, β-d glucans, curdlan, alginate, gellan and pullulan, scleroglucan and schizophyllan. bacterial hyaluronic acid, kefiran, exopolysacharide, xanthan gum, dextran, welan gum, gellan gum, diutan gum and pullulan
	Fungal polysaccharides: Chitin, scleroglucan, lentinan, schizophyllan krestin, galactofurinase
	Yeast polysaccharide: Zymosan, glucans, glycogen, mannan
Mammalian Polysaccharides	Glycosaminoglycans (hyaluronic acid or hyaluronan, Chondroitin sulphate), gelatin and heparin sulfate, chitin and chitosan
Others	B-1,3-glucans derived from a variety of natural sources (such as yeasts, grain, mushroom or seaweed), poly-gamma-glutamate (aminoacid polymer)

completely controlled fermentation conditions. Microbial production is attained within days and weeks in contrast to plants where production takes 3–6 months and highly experiences from geographical or seasonal differences and ever growing issues about the sustainable utilization of agricultural lands. In addition, production is not only independent of solar energy which is indispensable for production from microalgae but also favorable for employing various organic resources as fermentation substrates. In relation to recent reports, the global hydrocolloid market dominated by algal and plant polysaccharides like starch, carrageenan, galactomannans, pectin, and alginate is predictable to arrive at 3.9 billion US dollars by 2012. Intervening these traditionally used plant and algal gums by their microbial counterparts entails new strategies and significant development has been made in discovering and developing new microbial extracellular polysaccharides (exopolysaccharides, EPSs) that enjoy novel industrial importance. Recent review explored four EPSs, namely, xanthan, pullulan, curdlan, and levan, as biopolymers with exceptional potential for a variety of industrial sectors. Nevertheless, when evaluated with the synthetic polymers, natural origin polymers still symbolize only a small portion of the current polymer market, typically owing to their costly production processes. Thus, a lot of inputs have been devoted to the progress of cost-effective and eco-friendly production processes e.g. studying the possible use of cheaper fermentation substrates. Tables 2.1 and 2.2 demonstrate complete class of microbial polysaccharides (Fig. 2.1).

The microorganisms can offer great quantity of polysaccharides in the existence of surplus carbon source. A number of these polysaccharides serve as storage compounds. The polysaccharides excreted by the cells, known as exopolysaccharides, are of great commercial importance. The exopolysaccharides may be originate in association with the cells or may stay in the medium. The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups e.g. carboxyl, which can utilize as polyelectrolytes, are commercially more significant.

Table 2.2 Commercially significant microbial polysaccharides and its applications

Polysaccharide	Source(s)	Description	Application(s)
Dextrans	Leuconostoc mesenteroides, Acetobacter Sp., Streptococcus mutans	Dextrans are among the oldest known complex bacterial polysaccharide made of many glucose molecules, composed of chains of varying lengths (3–2000 kda)	Blood plasma expander Used in the prevention of thrombosis (as adsorbent). In the laboratory for chromatographic and other techniques involved in purification, widely used in foods, cosmetics and biotechnology, wound dressing
Xanthan	Xanthan gum is a polysaccharide secreted by the bacterium Xanthomonas campestris	It is composed of pentasaccharide repeat units, comprising glucose, mannose, and glucuronic acid in the molar ratio 2:2:1	In food industry for stabilization and gelling and viscosity control, in oil industry to enhance oil recovery, in the fabrication of tooth pastes and paints
Pullulan	Pullulan is a polysaccharide polymer produced from starch by the fungus Aureobasidium pullulans	It consisting of three maltotriose units, also known as α-1,4-;α-1,6-glucan, connected by an α-1,4 glycosidic bond	Biodegerable polysaccharide used in food packing and coating
Gellan	Gellan gum produced by the bacterium Sphingomonas elodea (formerly Pseudomonas elodea)	Gellan gum is a water-soluble anionic polysaccharide. It is composed of repeating unit of the polymer is a tetrasacharide, which consists of two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid	In food industry as thickner and solidifying agent
Recombinant hyluronan	rDNA technology	It is an anionic, nonsulfated glycosaminoglycan distributed in nature	Clinical significance in cancer, wound repair, inflammation, granulation and organization of the granulation tissue matrix, cell migration, skin healing, fetal wound healing and scarring, for cosmetic uses
Curdlan	Curdlan is produced by non-pathogenic bacteria such as Agrobacterium biobar. The production of curdlan by Alcaligenes faecalis is being developed to be used in gel production as well	Curdlan is a linear beta-1,3-glucan, a high-molecular-weight polymer of glucose. Curdlan consists of β-(1,3)-linked glucose residues and forms elastic gels upon heating in aqueous suspension	As a gelling agent in cooked foods and form strong gel above 55 °C, for immobilization of enzymes

(continued)

Table 2.2 (continued)

Polysaccharide	Source(s)	Description	Application(s)
Scleroglucan	Scleroglucan is produced by fermentation of the filamentous fungus *Sclerotium rolfsii*	Scleroglucan is a water soluble, nature-derived polysaccharide	Used for stabilizing latex paints, printing inks and drilling muds
Bacterial Alginate	The bacteria Pseudomonas aeruginosa and Azotobacter vinelandii have been shown to secrete exocellular polysaccharides similar to the alginic acid from algae	Alginic acid, also called algin or alginate, is an anionic polysaccharide distributed widely in the cell walls of brown algae and several bacterial strains where through binding with water it forms a viscous gum	In food industry as thickening and gelling agent, used as ion exchange agent, and used for the immobilization of cells and enzymes
β-Glucans	Naturally occurring in the cell walls of cereals, yeast, bacteria, and fungi	Comprised of a group of β-D-glucose polysaccharides with considerably varying physicochemical properties dependent on source. Typically, β-glucans form a linear backbone with 1–3 β-glycosidic bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals	Used in various nutraceutical and cosmetic products, as texturing agents, and as soluble fiber supplements, but can be problematic in the process of brewing.
Levan	Levans are a group of fructans; polymers of fructose forming a non-structural carbohydrate, which in the case of levans can themselves link together to form super-molecules comprising even hundreds of thousands	Synthesized by levansucrase from Pseudomonas syringae	Approach for food supplements to provide safe and efficient delivery of microelements
Emulsan	Produced by Acinetobacter calcoaceticus	Emulsan is a polyanionic heteropolysaccharide bioemulsifier	In oil industry to enhance oil recovery and in cleaning of oil spills

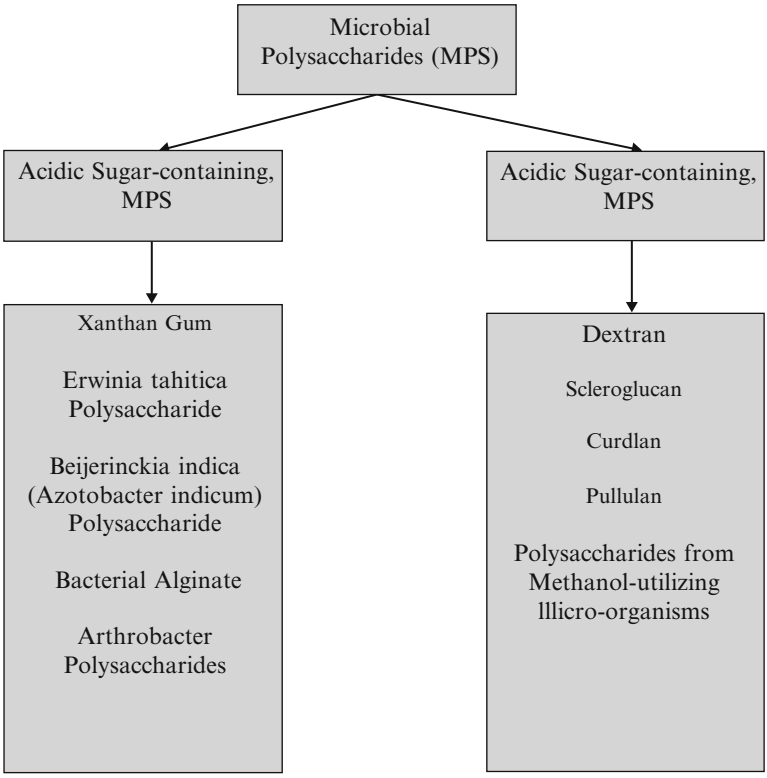

Fig. 2.1 Microbial polysaccharides

2.2 Microbial Polysaccharides: General Applications

Microbial polysaccharides have great commercial significance. They are engaged in the stabilization of foods, and development of various industrial and pharmaceutical compounds. The commercial importance of a polysaccharide depends on its potential to modify the flow characteristics of solutions (technically known as rheology). Polysaccharides can enhance the viscosity and, are therefore useful as thickening and gelling agents. Microbial polysaccharides are of immense significance in oil industry. Via conventional methodologies, only 50 % of the oil can be extracted. And the rest is either trapped in the rock or too viscous to be forced out. It is now likely to recover such oils also by a procedure known as microbial enhanced oil recovery (MEOR). This can be achieved by means of injecting surfactants and viscosity decreasing biological agents (i.e. the microbial polysaccharides e.g. xanthan and emulsan).

2.3 Microbial Polysaccharides Production

The production of polysaccharides positively occurs in the surplus amount of carbon substrate in the growth medium while limiting nitrogen supply. A carbon/nitrogen ratio of around 10: 1 is acknowledged to be positive for optimal polysaccharide production. The production process is usually carried out by batch culture fermentation. Via manipulating the nutrient flow, differential production of polysaccharides can be attained. By means of limiting nitrogen flow in the medium, typically neutral polysaccharides are produced. When amount metal ions are inadequate, acidic polysaccharides are principally synthesized. Molecular oxygen supply of approximately 90 % saturation is perfect for excellent growth and polysaccharide synthesis.

2.4 Biosynthesis of Polysaccharides

Microorganisms are proficient in synthesizing a large number of polysaccharides. The pathways for their biosynthesis are similar to the procedures that take place for the formation bacterial cell wall. It is anticipated that there are well over 100 enzymatic reactions, directly or indirectly involved in the synthesis of polysaccharides. Initially with glucose, suitable sugars (by transforming glucose to others) are included in the formation of polysaccharides.

2.5 Polysaccharides Recovery

Since the polysaccharide production enhances, there arises a noticeable increase in viscosity of the culture broth. The polysaccharides can be precipitated by acids, salts, or organic solvents, and recovered via utilizing appropriate techniques.

2.6 Microbial Polysaccharides vs Plant Polysaccharides

Owing to immense competition between microbial and plant polysaccharides for industrial applications, various advance techniques and methodologies have been explored by several researchers to explore their structural backbone and their associated biological functions. Development of plant polysaccharides is comparatively cheap, while it is uncontrolled and takes place for a short span in a year. On the contrary, microbial polysaccharides production is well regulated and can be sustained throughout the year. Nevertheless, fermentation procedures for fabrication of cheap (from plant sources) polysaccharides are not advisable.

2.7 Microbial Polysaccharides: General Features

Among the various microbial polysaccharides, approximately 20 are of industrial significance. As previously mentioned, the commercial worth of a polysaccharide is usually based on its rheological features specifically its capacity to modify the flow properties of solutions. A preferred record of commercially significant polysaccharides, the microorganisms employed for their synthesis, and their applications are mentioned in the Table 2.2. A number of the significant characteristics of individual microbial polysaccharides are shortly explained hereunder.

2.7.1 Xanthan

Xanthan or more often known as xanthan gum was the foremost polysaccharide presented commercially. It is a well investigated and most extensively used hexopolysaccharide (Fig. 2.2).

It has molecular weight in the range of $2–15 \times 10^4$ Da. The central repeating unit of xanthan is a pentasaccharide containing mannose (Man), glucose (Glc) and glucuronic acid (GIcA) with acetate (Ac) and pyruvate (Pyr) as represented below. Fundamentally, xanthan is a branched polymer with β $(1 \rightarrow 4)$ linked glucan (glucose polymer) backbone bound to a trisaccharide (Man, GIcA, Man) side chain on alternate glucose residues. The mannose has either acetate or pyruvate groups. The number of acetate or pyruvate molecules in xanthan is variable and is based on the bacterial strain used. The culture environment and the recovery procedures also affect the amount of pyruvate and acetate residues. It is assumed that the viscosity of xanthan gum is affected by the contents of pyruvate and acetate. Xanthan gum is employed as a food additive for the fabrication of soft foods. It is also employed in oil industry for increasing oil recovery. In addition, xanthan is functional for the fabrication of tooth pastes and water based paints. For xanthan biosynthesis, the monomers are linked to a carrier lipid molecule and then moved to an increasing polymer chain. The triggered monosaccharide nucleotides flow energy for the configuration of glycosidic bonds between neighboring units. The biosynthesis of other exopolysaccharides is similar with that of xanthan. Dextran production

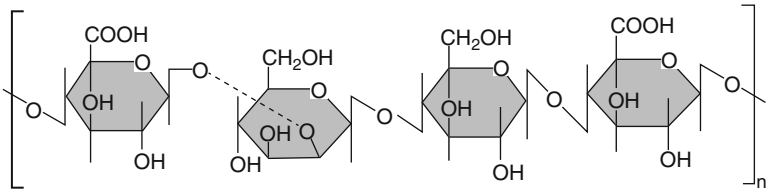

Fig. 2.2 Xanthan gum

Table 2.3 Drug delivery applications of xanthan gum

Polysaccharide involved	Size	Application	Ref
Xanthan gum	Nanoparticles	Lysozyme delivery (protein/ polysaccharide NP)	[1]
Xanthan gum, locust bean gum	Nanoparticles	Silicon dioxide Delivery	[2]
Locust bean gum, xanthan gum	Microparticles	Celecoxib drug delivery	[3]
Xanthan gum	Microparticles, NPs	Sustained release of antiseptic agent (Chlorhexidine)	[4]
Chitosan–Xanthan Gum starch–xanthan gum galactomannan from Schizolobium parahybae and xanthan	Liposomes	Pulmonary Delivery of Rifampicin for controlled drug delivery	[5]
	Hydrogels		[6]
	Hydrogels	Curcumin delivery	[7]

however is much simpler as described later. Xanthan is commercially developed from the Gram-negative bacterium, Xanthomonas campestris. The culture medium generally consists of 4–5 % carbohydrate (glucose, sucrose, corn starch hydrolysate), 0.05–0.1 % nitrogen (ammonium nitrate, urea, yeast extract) and salts. The pH is maintained approximately at 7.0, and the fermentation is take place by batch culture for 2–3 days. Precipitation of xanthan in the culture broth is usually achieved by isopropanol or methanol and these agents also kill the microorganisms. The precipitated xanthan can be dried and employed for commercial reasons. Table 2.3 describes drug delivery applications of xanthan gum.

2.7.1.1 Microwave Irradiation in the Grafting Modification of the Xanthan Gum

Xanthan gum is a polysaccharide synthesized by the fermentation of the bacterium Xanthomonas campestris [8]. This organism is reported in nature on the leaf surfaces of green vegetables, particularly the cabbage family. The gum is employed as a food additive and rheology modifier. The %G of poly(acrylamide) on xanthan gum under microwave-initiated grafting has been reported to be much higher in contrast to ceric-induced conventional grafting (62.87 %G) [8]. The grafting yield was found directly proportional to the microwave power and exposure time. The swelling of the xanthan gum was reported to differ contrariwise with the %G, while erosion varied directly with the %G. The microwave-assisted graft co-polymerization was employed as an effective tool to alter the release features of xanthan gum by the grafting of acrylamide on xanthangum.

2.7.2 Dextrans

Dextrans are glucans (polymers of glucose) chemically containing $1 \rightarrow 6$ glycosidic linkages, having molecular weights range between 15,000 and 500,000 kDa (Fig. 2.3). Currently dextrans are used as blood plasma expanders, for the prevention of thrombosis and in wound dressing.

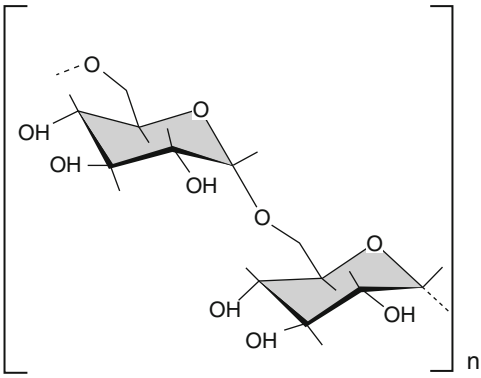

Fig. 2.3 Structure of Dextran

Moreover, dextrans are also functional in the laboratory analytical techniques for purification of biomolecules. Dextrans can be produced by a extensive variety of Gram-positive and Gram-negative bacteria. For an example *Leuconostoc mesenteroides* and Streptococcus mutans are utilized for the synthesis of dextran. On the contrary to other exopolysaccharides (which are synthesized within the cells), dextrans are synthesized by extracellular enzyme in the medium. The enzyme which is responsible for this synthesis is dextransucrase (a transglucosidase) which take action on sucrose and carried about polymerisation of glucose residues, and concurrently releases free fructose into the medium. The commercial production is achieved by means of lactic acid bacterium, *L. mesenteroides* by a batch fermentation process. In addition to sucrose, the culture medium comprised of organic nitrogen source and inorganic phosphate. The crude dextran formed is precipitated by alcohol and then subjected to acid hydrolysis. Recently, the alcohol precipitated polymeric dextran is directed to enzymatic hydrolysis by using exo- or endo-a dextranases to get dextrans of desired molecular weight. The resulting dextrans can be fractionated and dried. It is also feasible to employ a cell free system for the synthesis of dextrans. The extracellular enzyme dextrasucrase can convert sucrose into dextran in a cell-free nutrient solution. This reaction is optimum at pH 5.0–5.5 and temperature 25–30 °C. Table describes drug delivery applications of dextrans.

Cationic polysaccharides are extensively investigated in various areas such as water treatment, food, cosmetic, papermaking, chemical, and petroleum industries. The combination of cationic polysaccharides with anionic polymers can results in interpolyelectrolyte complexes with hydrogel-like structures further expanding the application of the former. Incomplete oxidation of dextrans with periodate, to form adialdehyde in the oxidized sugar unit and subsequent reductive amination employing diverse polyamines of interest such as spermine or quaternary monoammonium derivatives; these products were employed for gene delivery. A corresponding synthesis is found for schizophyllan, a β-glucan. Utilizing alike approach, the hydroxyl in the 6 position of a β-glucan from oat was oxidized with paraformaldehide and then underwent a reductive amination with ammonium acetate and $NaBH_4CN$ (Fig. 2.4). The kinetic of the reaction of polysaccharides, with epichlorohydrin and various tertiary amines was investigated, by quantification of reagents

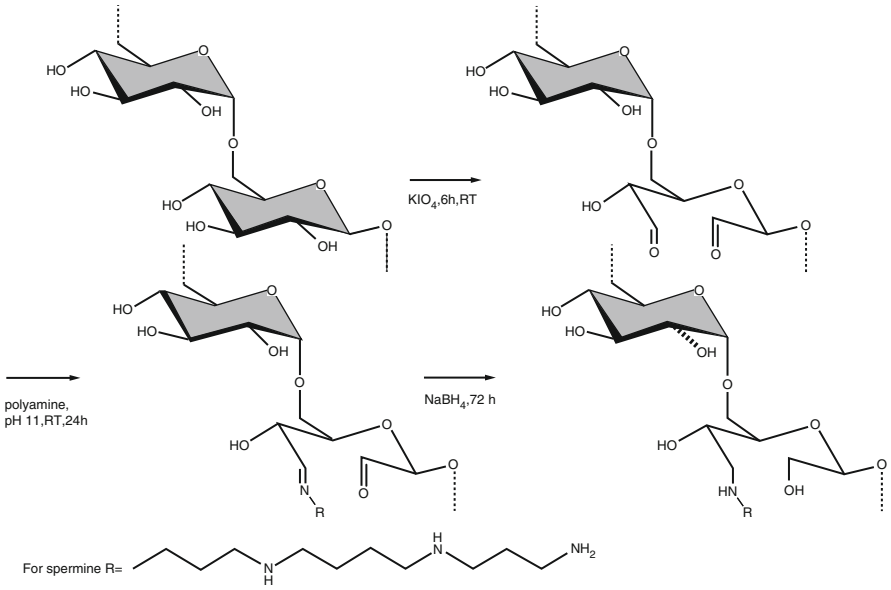

Fig. 2.4 Periodate partial oxidation and subsequent reductive amination of dextran

and products in the reaction mixture with or without the presence the polysaccharide, at steady intervals. They investigated that the use of cyclic amines such as1-methyl-imidazol or 1,4-diazabicyclo[2,2,2]octan as "catalysts" together with another tertiary amine, used for the substitution, was not necessary and led to a cationic polysaccharide with mixed and uncontrolled chemical composition. After 30 min of reaction at 40 °C, 80 % of epichlorohydrin had reacted with the amine, reaching a 94 % after 3 h. Initially, the original epoxy groups remained unchanged, but they started to decline at 24 h (Fig. 2.4). The paramount solvent for this reaction was water and an equimolar amine: epichlorohydrin ratio contributed satisfactory results.

2.7.2.1 Hydrophobically Modified Dextran

In the report by Nichifor et al. [9], dextran molecular weight close to 30,000 g/mol was covalently bound to bile acids (cholic and deoxycholic acids) through ester linkages. Bile acids are natural products consisting of a facially amphiphilic steroid nucleus with a hydrophobic b-side and a hydrophilic a-side [9, 10]. When these compounds are chemically bound to a water-soluble polymer the resulting amphiphilic polymer might exhibit a better compatibility with biological systems and interact favorably with proteins, enzymes or lipids [11].

2.7.2.2 Dextrans: Colon-Specific Drug Delivery

Dextrans are polysaccharides with a linear polymer backbone with principally 1,6--D-glucopyranosidic linkages. They are derived from bacterial cultures of Leuconostoc mesenteroides. These glycosidic linkages are hydrolysed by moulds, bacteria and also by the mammalian cells. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Dextranases activity of the colon is revealed by anaerobic gram-negative intestinal bacteria especially the Bacteroides. Dextran has also been reported to be degraded in human feces owing to bacterial action. Numerous drug-dextran prodrugs in which the drug molecule is associated to the polar dextran macromolecule remain intact and unabsorbed from the stomach and the small intestine but when the prodrug arrive into the colonic microflora containing as much as 1011 Bacteroides per gram it is acted upon by dextranases which cleave the dextran chain randomly and at the terminal linkages releasing the drug, free into the colon. Growing attention is being focused on dextran prodrugs. Major attempt was carried out by Harboe et al. [12] who conjugated naproxen to dextran by ester linkage. Dextran ester prodrugs of ketoprofen and naproxen using dextran with molecular weight (MW) 10,000–500,000 were reported for their releasing the drug specifically in the colon region of pig. The release of naproxen was upto 17 times higher in the cecum and colon homogenates of pig than in control medium or homogenates of SI. A series of prodrugs, naproxen-dextran, ketoprofen-dextran and ibuprofen-dextran have been tested in vitro and in vivo in pigs. They suggested this prodrug system as a active system for site specific delivery presenting high bioavailability of the drug but still no absorption of the prodrug into the circulation. Also, this system could offer protection to the drug in the upper GIT and selective regeneration in the cecum/colon. This method delivers drug particularly to the colon and can be employed for colon targeting. There are various additional nano-applications of dextran explored (Table 2.4).

2.7.2.3 Cationization of Dextrans

There are various modified forms of dextran available (Fig. 2.5). Nichifor et al. have widely reported about the fabrication of amphiphilic cationic dextrans, formerly crosslinked with epichlorohydrin or not, by replacing them with groups alike to the mentioned ones however in which one of the quaternary ammonium substituent's was an alkyl chain between C2 and C16 instead of methyl [24]. In this circumstance the reagent active for cationization was a 2,3-epoxypropyl alkyl dimethyl ammonium chloride derived from epichlorohydrin and a dimethyl alkylamine [24]. Products with a DS between 0.18 and 0.94 were derived. The kinetic of the reaction of polysaccharides, with epichlorohydrin and various tertiary amines was investigated [24], by quantification of reagents and products in the reaction mixture with or without the presence the polysaccharide, at regular intervals. They reported that the use of cyclic amines e.g. 1-methyl-imidazol or 1,4-diazabicyclo [24] octan as "catalysts" together with another tertiary amine, used for the substitution, was not

Table 2.4 Drug delivery applications of dextrans

Polysaccharide involved	Size	Application	Ref
Dextran sulphate	Silver nanoparticles	Antimicrobial activity	[13]
Dextran	Zwitterionic pH/redox nanoparticles	For enhancing tumor intercellular uptake of doxorubicin	[14]
Chondroitin sulfate and dextran sulfate	Nanoparticles	Delivery of chloramphenicol to treat intracellular Salmonella infections	[15]
Chitosan–dextran sulfate	Nanoparticles	For controled delivery of bioactive molecules and cells in bone regeneration	[16]
IgA-Loaded Chitosan–Dextran Sulfate	Nanoparticles	Enhanced Immune Response	[17]
Dextran	Nanoparticles	Delivery of doxorubicin	[18]
Dextran sulfate	Superparamagnetic iron oxide nanoparticles	As a contrast agent for atherosclerosis	[19]
Dextran	Graphene oxide gold nanoparticles	For sensitive detection of concanavalin A	[20]
Dextran	Nanoparticles	Targeted delivery of cisplatin for breast cancer growth and metastasis	[21]
dextran/chitosan shell	Nanoparticles	BSA/chitosan core—Doxorubicin loading and delivery	[22]
Poly(DL-lactide-co-glycolide)-grafted dextran	Nanoparticles	To enhance antitumor effect of adriamycin	[23]

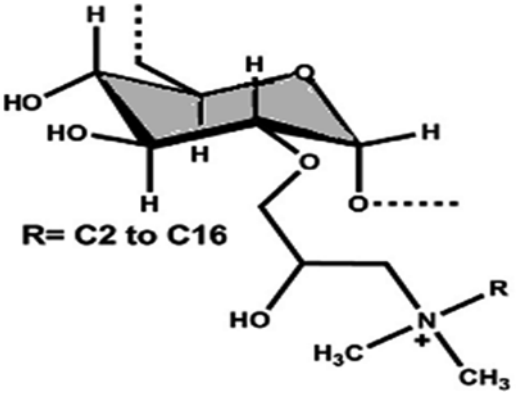

Fig. 2.5 Dextran modification via cationic substitution which present alkyl chains of varying length

essential, and led to a cationic polysaccharide with mixed and uncontrolled chemical composition. After 30 min of reaction at 40 °C, 80 % of epichlorohydrin had reacted with the amine, reaching a 94 % after 3 h. Originally, the original epoxy groups remain unchanged, nevertheless they started to decline at 24 h. The finest solvent for this reaction was water and an equimolar amine: epichlorohydrin ratio furnished satisfactory results.

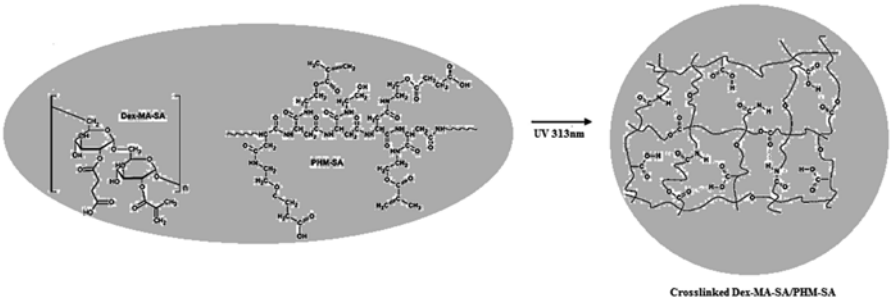

Fig. 2.6 Cross-linking, succinic and methacrylated derivative of dextran with a methacrylated and succinic derivative of poly(N-2-hydroxyethyl)-dl-aspartamide (PHEA), PHM-SA

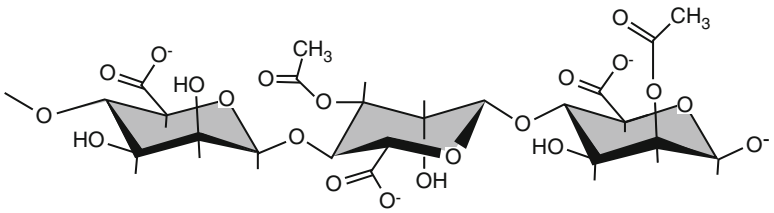

Fig. 2.7 Structure of alginate

There are various derivatives explored by using several cross linking agents. In earlier studies methacrylated and succinic derivative of dextran with a methacrylated and succinic derivative of poly(N-2-hydroxyethyl)-dl-aspartamide (PHEA), PHM-SA were prepared as mentioned in Fig. 2.6. These and many alike methodologies have revolutionize the dextran applications in diverse areas of biomedical science.

2.7.3 Bacterial Alginate

Alginate is a linear polymer composed of mannuronic acid and glucuronic acid (both of them being uronic acids) in a proportion ranging from 4: 1 to 20: 1. Some of the mannuronic acid residues are acetylated (Fig. 2.7).

Alginate is commercially produced by Gram-negative bacteria, Pseudomonas aeruginosa and Azobacter vinelandii. The type of organism used and the culture conditions determine the relative proportion of mannuronic acid and glucuronic acid residues and the degree of acetylation in alginate. Alginates with high contents of mannuronic acid are elastic in nature while those with high concentration of glucuronic acid are strong and brittle. Algal (seaweed) alginates are also polymers of mannuronic acid and glucuronic acid, and comparable in structure with bacterial alginates. However, algal alginates lack acetylation. For commercial purposes,

Table 2.5 Drug delivery applications of bacterial alginate

Polysaccharide involved	Size	Application	Ref.
phenylalanine ethyl ester-alginate conjugate	Nanoparticles	Vitamin B2 delivery	[25]
Alginate	Gold nanoparticle	To encourage cellular interactions	[26]
Alginate	Silk sericin loaded nanoparticles	To promote anti-inflammatory efficacy	[27]
Polyvinyl alcohol/sodium alginate	Nanoparticles	Antibacterial activity	[28]
biodegradable graft copolymer sodium alginate-g-poly (N,N-dimethylacrylamide-co-acrylic acid)	Gold nanoparticles	Anti micro bacterial application	[29]
Chitosan/alginate	pH-sensitive core-shell nanoparticles	For efficient and safe oral insulin delivery	[30]
Alginate	Calcium carbonate hybrid noparticles	For combination chemotherapy	[31]
Polyvinyl alcohol/sodium alginate	Silver nanoparticles	Antibacterial	[28]
Nanoparticles of chitosan-alginate	Chitosan-alginate	Improvement of crocin stability	[32]

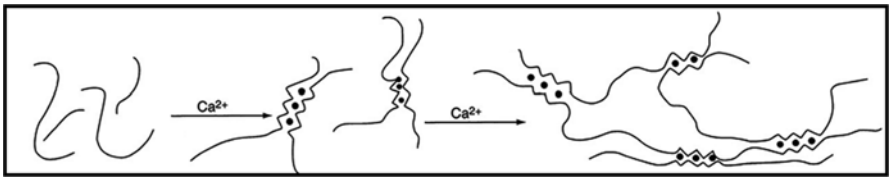

Fig. 2.8 Divalent mediated sodium alginate beads formation

seaweed alginates are more commonly used than bacterial alginates. This is mainly because bacterial alginates are relatively unstable and get easily degraded. Alginates are useful as thickening agents in food industry, and for immobilization of cells and enzymes. Table 2.5 describes drug delivery applications of bacterial alginate. Aliginate in the presence of divalent ion like Ca^{2+} form gel which explore its application in various immobilization experiment (Fig. 2.8).

2.7.4 Scleroglucan

Scleroglucan is a glucose polymer (glucomer). It is a neutral polysaccharide with β 1 → 3 glucan backbone and single glucose (Glc) residue branches (β 1 → 6 linkage) (Fig. 2.9).

The branching occurs at a regular sequence at every third glucose unit in the polymer backbone chain. Scleroglucan is a fungal heoxpolysaccharide. It is commercially produced by Sclerotium glucanicum, S. rolfsii and S. delphinii. Scleroglucan is useful

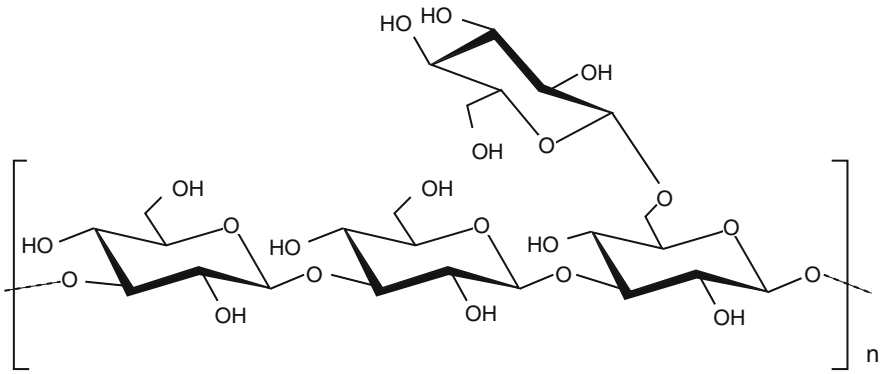

Fig. 2.9 Structure of Scleroglucan

for stabilizing latex paints, printing inks and drilling muds. Scleroglucan is recently explored as biocompatible material by incorporating an aqueous ferrofluid in poly(vinyl alcohol) and scleroglucan (SCL) hydrogels, loaded with theophylline as model drug for release studies. Rheological results showed that higher storage modulus and a more compact structure are obtained by incorporating the ferrofluid into the hydrogels. Schizophyllan is a natural b-(1-3)-D-glucan polysaccharide produced by the fungus Schizophyllum. Modified schizophyllan forms stable complexes with antisense oligonucleotides, and when studied in different melanoma and leukaemia cell lines, the cytotoxicity was found to be negligible [33]. Schizophyllan is a new potential candidate for an antisense oligonucleotides carrier [34].

2.7.5 Gellan

Gellan is a linear heteropolysaccharide. The repeating unit of gellan is composed of two glucose, one glucuronic acid and one rhamnose molecules (Fig. 2.10). Gellan is produced by Pseudomonas el odea. A deacetylated gellan which forms firm and brittle gels under the trade name Celrite has been developed by a reputed company in USA (Kalco Inc). Gellan is used in food industry. Even at a low concentration, it is a thicker. Table 2.6 describes drug delivery applications of gellan.

2.7.6 Pullulan

Pullulan is an α-glucose polymer (α-glucan) with α $1 \rightarrow 4$, and a few α, $1 \rightarrow 6$ glycosidic bonds. Pullulan is produced by using the fungus, Aureobasidium pollulans (Fig. 2.11). Hydrophobically modified pullulan (Fig. 2.12): Various cholesterol-bearing pullulans with different molecular weights from the parent pullulan and

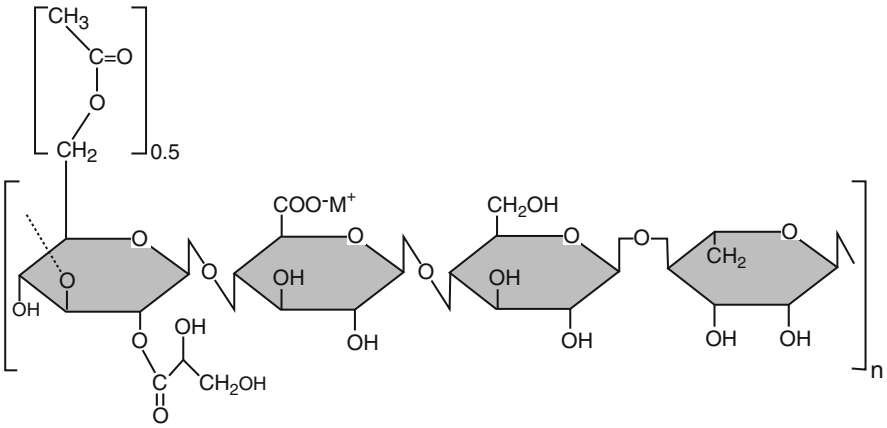

Fig. 2.10 Structure of gellan

Table 2.6 Drug delivery applications of gellan

Polysaccharide involved	Size	Application	Ref.
Gellan	Gum/titanium dioxide nanoparticle	For the cleaning and disinfection of parchment	[35, 36]
P123/TPGS mixed micelles and gellan gum	In situ gel systems	For ophthalmic delivery of curcumin	[37]
Hexadecyl gellan amphiphilic	Nanoparticles	In vivo lipid-lowering potential	[38]
Gellan gum methacrylate and laponite	Nanocomposite hydrogel	Biomedical applications	[39]
Gellan	Gel beads containing magnetic nanoparticles	Containing magnetic nanoparticles	[40]
Gellan gum blended PEI nanocomposites as gene delivery agents:	PEI nanocomposites	As gene delivery	[41]
Gellan gum	Aqueous dispersions/its nanohybrids	Reducing and stabilizing agent and	[42]
Quaternized gellan gum	Nanoparticles	Antibacterial activity (for controlled release of ciprofloxacin with potential dermal applications)	[43]

different DS from the cholesteryl moiety were synthesized by Akiyoshi et al. [44] and formed stable and monodisperse self-aggregates (20–30 nm) by intra- and/or inter-molecular self-aggregation in a diluted aqueous solution [45]. The cholesterol-bearing pullulan self-aggregates are regarded as a hydrogel nanoparticle, in which pullulan chains are cross-linked noncovalently by associating cholesteryl moieties. The sizes of the self-aggregates decreased with an increase in the DS of the

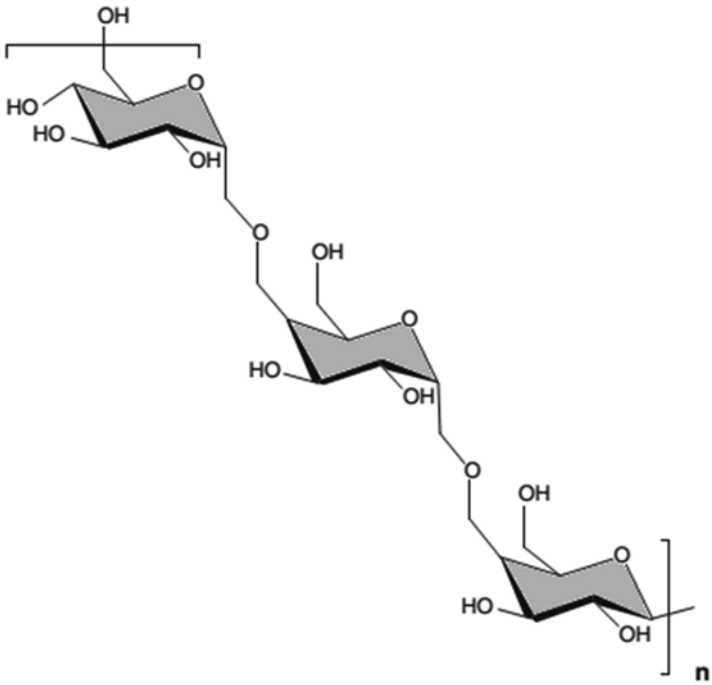

Fig. 2.11 Structure of pullulan

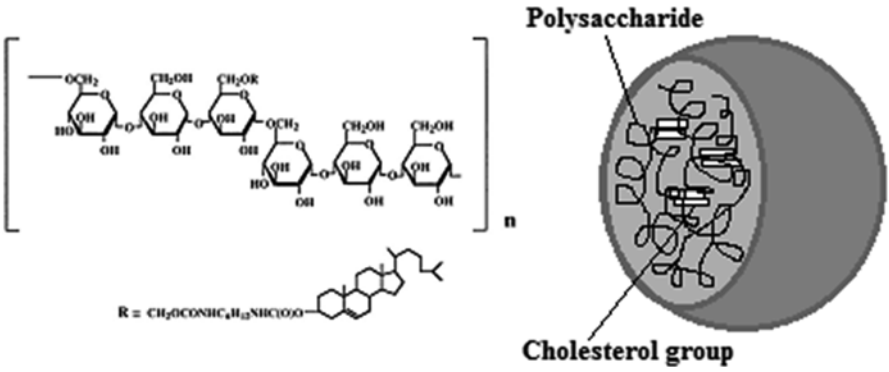

Fig. 2.12 Structural features of cholesterol modified pullulan and nanogels formed by self-assembly

cholesteryl moiety, whereas the aggregation number of cholesterol-bearing pullulans in one nanoparticle was almost independent of the DS [46].

It is estimated that about 70 % of glucose (the substrate) is converted to pullulan during fermentation, although the time taken is rather long (5–7 days). Pullulan is mainly used in food coating and packaging. Table 2.7 describes drug delivery applications of pullulan.

Table 2.7 Drug delivery applications of pullulan

Polysaccharide involved	Size	Application	Ref.
Pullulan–spermine	Magnetic nanoparticles	Plasmid EGFP-p53 delivery	[47]
Pullulan	pH-responsive nanoparticles & charge-reversible pullulan-based shells	Carriers of anticancer drugs for combination therapy	[48]
Pullulan	Nanoparticles	For transmucosal protein delivery	[49]
Pullulan	Silver nanoparticles	Antimicrobial activities	[50]
Pullulan stabilized gold nanoparticles	Pullulan stabilized gold nanoparticles	For cancer targeted drug delivery	[51]
Pullulan	pH-sensitive nanoparticle	Carrier for adriamycin to overcome drug-resistance of cancer cells	[52]
Pullulan	pH-sensitive pullulan-based nanoparticle	Carrier of methotrexate and combretastatin A4 for the combination therapy against hepatocellular carcinoma	[53]

2.7.6.1 Cationic Modified Pullulan

Pullulan is a natural water-soluble polysaccharide with a repeated unit of maltotriose condensed through the α-1,6 linkage and is non-toxic, non-mutagenic non-immunogenic, and noncarcinogenic in nature [54]. Stable complexes were produced from cationic modified pullulan/DNA as temperature-sensitive gene carriers [55]. Thomsen et al. [56] used cationic non-viral gene carriers prepared from pullulan and spermine for conjugation with P-DNA and to transfect rat brain endothelial cells (RBE4s) and human brain microvascular endothelial cells (HBMECs). The HBMECs and RBE4s were successfully transfected with the fluorescent reporter gene pHcRed1-C1, with good transfection efficiency and low cytotoxicity. Secretion of hGH1 protein was detected after in vitro transfection of HBMECs with pullulan–spermine complexed with pCMV6 Entry GH1. Thus, the pullulan–spermine delivery system may be used as a method to deliver DNA to brain endothelial cells and to use these cells as factories for protein secretion. Similarly, complexes were also studied for liver targeting gene expression. Thakor et al. [57] studied in vitro transfection using conjugated pullulan–spermine/pDNA anionic complexes in rat sensory neurons. Complexes were found to be stable for 1 week and to protect the DNA from degradation. In vitro transfection of rat sensory neurons occurred at different spermine nitrogen: DNA phosphateratios, but the efficiency was highest for anionic complexes (anioplexes). Anioplexes did not exhibit any measurable cytotoxicity upto 20 lg ml^{-1} DNA. The transfection efficiency was also maintained in the presence of serum and antibiotics. This suggests that pullulan–spermine/DNA anioplexes are an effective gene delivery technology, particularly for neurons. Cationic pullulan, dextran and mannan complexed with pDNA were also tested in cellular models and in vivo mice models. The cationized pullulan is reported to a promising

non-viral carrier of pDNA for mesenchymal stem cells. Similarly, cationic pullulan was also reported for gene delivery applications targeted at liver cells. Cationic groups were introduced by reacting various amounts of glycidyl trimethyl ammonium chloride with pullulan. The cationic derivatives readily formed polyionic complexes with DNA. The nanoplexes were taken up by the liver cells in a time dependent manner. They were found to have excellent blood compatibility, and in vitro transfection on HepG2 cells demonstrated good transfection efficiency. The high solubility and chain flexibility of pullulan may be contributory factors to its blood compatibility [58]. PEI-conjugated pullulans were also developed and investigated for possible use in gene delivery applications. The pullulan–PEI conjugate seems to be a promising gene delivery vector that shows good hemocompatibility and low toxicity without compromising the transfection efficacy of PEI [59].

2.7.6.2 Cholesterol-Bearing Pullulans

Various cholesterol-bearing pullulans (Fig. 2.12) with different molecular weights from the parent pullulan and different DS from the cholesteryl moiety were synthesized by Akiyoshi et al. [55–57] and formed stable and monodisperse self-aggregates (20–30 nm) by intra- and/or inter-molecular self-aggregation in a diluted aqueous solution [55]. The cholesterol-bearing pullulan self-aggregates are regarded as a hydrogel nanoparticle, in which pullulan chains are cross-linked noncovalently by associating cholesteryl moieties. The sizes of the self-aggregates decreased with an increase in the DS of the cholesteryl moiety, whereas the aggregation number of cholesterol-bearing pullulans in one nanoparticle was almost independent of the DS [57].

2.7.7 Curdlan

Curdlan is a β-glucose polymer (β-glucan). The glucose residues are held together by β 1 → 3 glycosidic bonds (Fig. 2.13).

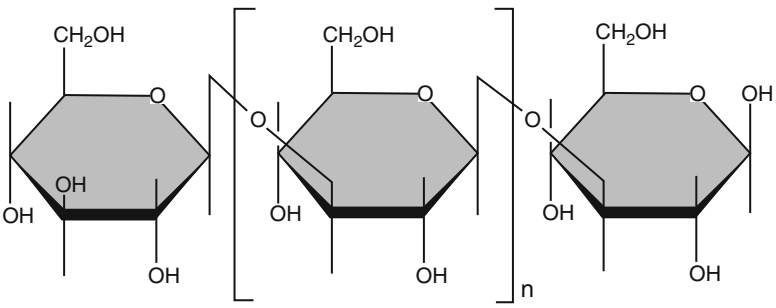

Fig. 2.13 Structure of curdlan

Table 2.8 Drug delivery applications of Curdlan

Polysaccharide involved	Size	Application	Ref
Curdlan	Nanoparticles	Cell Type-Specific Delivery of RNAi	[60]
Curdlan-conjugated PLGA	Nanoparticles	Enhancement of macrophage stimulant activity and drug delivery capabilities	[61]
Curdlan-capped gold	Nanoparticles	To enhance interaction with protein	[62]
Carboxylic curdlan-deoxycholic acid	Self-aggregated nanoparticles	Carrier of doxorubicin.	[63]
Curdlan	Nanoparticles	For intracellular siRNA delivery	[64]
Carboxymethyl curdlan-capped silver nanoparticles		Application in surface enhanced Raman scattering	[65]
Cholesterol-conjugated carboxymethyl Curdlan	Self-assembled nanoparticles	Carrier for epirubicin.	[66]
Curdlan derivatives	Self-assembled hydrogel nanoparticles	Anti-cancer drug delivery	[67]

The exopolysaccharide curdlan is commercially produced by employing Alcaligenes faecalis. Curdlan-like polysaccharides are also produced by other microorganisms such as Agrobacterium rhizogenes and Rhizobium trifolii. Curdlan forms strong gels when heated to above 55°C. Therefore, it is used as a gelling agent for cooked foods. In addition, curdlan is also employed for immobilization of enzymes. Table 2.8 describes drug delivery applications of curdlan.

2.7.8 Levan Polysaccharides

Levans are a group of fructans; polymers of fructose forming a non-structural carbohydrate, which in the case of levans can themselves link together to form super-molecules comprising even hundreds of thousands (Fig. 2.14).

Levans are synthesized in approximately all bacterial versions of fructan production, as well as being possible to produce by fracturing soybean mucilage. Levan, fructose-composed biopolymer of bacterial origin, has potential in biotechnology due to its prebiotic and immunostimulatory properties. It was suggested that the combination of levan and nutritionally important microelements in the form of NPs serves as a first step towards a novel "2 in 1" approach for food supplements to provide safe and efficient delivery of microelements for humans and support beneficial gut microbiota with nutritional oligosaccharides [68].

2.7.9 Bacterial Polysaccharides

Recently, considerable development has been made in exploring new bacterial polysaccharides that exhibit novel and highly functional properties, e.g. the association between the exclusive properties of xanthan gum (the foremost microbial

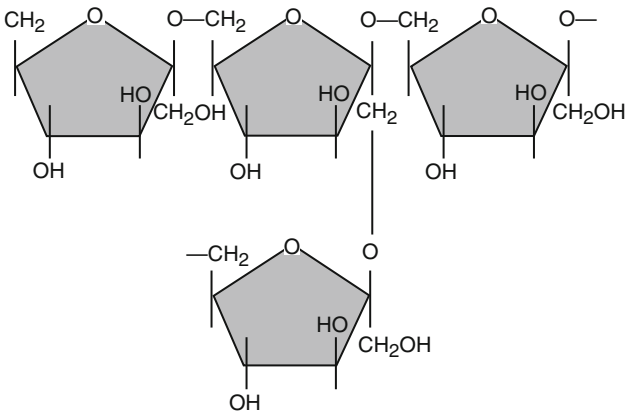

Fig. 2.14 Structure of levan

polysaccharide of commercial significance) and its use in major food, industrial, and oil field applications is conferred. Moreover, gellan gum (the extracellular polysaccharide produced by *Pseudomonas elodea*) can be employed in microbiological media and in gelled and structured food products [69]. Three other industrially useful bacterial polysaccharides have been explored:

- S–130, the extracellular, high viscosity polysaccharide produced by a strain of *Alcaligenes*, has excellent suspending and heat stability features functional in oil field drilling, work over and completion, and enhanced oil recovery fluids.
- S–194 has outstanding suspending features and remarkable compatibility with salts, making it significant in agricultural applications, specifically flowable pesticides and liquid fertilizers.
- S–198 has exceptional stability to shear and has potential application in the growing market of water–based lubricants.

2.7.10 Gellam, Guar and Xanthan Gums

Gellan gum, a linear anionic polysaccharide comprising tetrasaccharide glucose:glucuronic acid: rhamnose. The industrial polysaccharide is deacetylated with alkali, which facilitates the presence of free carboxylic groups. The initial heating of concentrated solutions followed by the cooling facilitates alteration in the chain conformation from coil to helix, results in ordered junction zones, and therefore to temperature-reversible gels [70]. Much stronger physical hydrogels that can be derived in presence of di and trivalent ions, are the foundation of in situ gelling ophthalmic and oral formulations. The mechanism of gelation encompasses the formation of double helical junction zones, followed by the aggregation of double helical segments to form a 3D network by complexation with cations and hydrogen bonding with water. The subsequent gels are pH and ionic strength sensitive [70–72]. Beads of gellan ionically crosslinked with Al^{3+} only or co-crosslinked

with glutaraldehydes welled more and released faster glizipine at pH 7.4 than at pH 1.3. Enhanced release at pH 7.4 is triggered not only since the ionization of carboxylic acid groups, nevertheless also owing to reduction of aluminum ions by ion exchange with sodium ions of the medium. This concluding mechanism did not ensue in the case of chemically crosslinked beads, which displayed more sustained release at pH 7.4. Related release pattern was reported for beads of gellan crosslinked with calcium ions. Networks with other polysaccharides have been fabricated taking advantage of the prospect of using gellan as cross-linker. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) has been used to activate the carboxylic groups of gellan, so as to react with the hydroxyl groups of scleroglucan. The hydrogels presented pH and ionic strength sensitivities, owing to the protonation state of the carboxylic groups of gellan and scleroglucan and the screening role of the ions to the COO−. Investigations of the outcome of Na^+, Ca^{2+} and pH on the release rate of theophylline revealed that high Na^+ or Ca^{2+}/COO− ratio and low pH results in the release rate, owing to the cross-linking effect of the ions that stretches a further strength to the complete network, or to the protonation of the COOH that allows the co-cross-linking, correspondingly [73]. Alike outcomes were examined for scleroglucan crosslinked with 1,6-hexanedibromide. The presence of the salt in the medium results in shrinking of the network and, though the interaction between the COO− groups of scleroglucan and theophylline became weaker, a substantial reduction in the drug release was reported [74, 75]. The nonionic polysaccharide guar gum that can be altered with anionic and cationic substituent's to attain pH-sensitive release [76, 77]. Carboxymethyl derivatives crosslinked with barium cations can shield proteins from the acidic pH of the stomach and regulate the release in simulated intestinal conditions [78]. If the microbeads are crosslinked with trivalent ions, such as Al^{3+} or Fe^{3+}, instead of divalent ions e.g. Ba^{2+}, Ca^{2+} or Cu^{2+}, a slower drug release can be achieved at pH 7.4, achieving around 100 % released in 20 h. Conversely, cationic guar gums crosslinked with EGDE have high attraction for oppositely charged drugs, mainly at acid pH. Nevertheless, at alkaline pH or in the presence of salts the interactions are broken, and the drug is released. Hydrogels of xanthan gum have also shown to be pH and ionic sensitive. Crosslinked xanthan networks have been fabricated using two strategies. In the first one, cyclic trisodium trimetaphosphate was employed in an alkaline medium for sustaining the coil conformation [79]. In the next approach, adipic acid dihydrazide and a soluble carbodiimide in acidic conditions were employed. Both hydrogels performed decidedly different each other. Release of methylene blue from the hydrogels crosslinked with cyclic trisodium trimetaphosphate depended on the swelling of the network; namely, higher amounts of methylene blue were released in serum-mimicking than in acid medium. This performance is recognized to the ionization of the phosphate groups at alkaline pH, which results in electrostatic repulsive forces that in turn cause a rise in the swelling ratio. At acid pH, the protons screen the most of the phosphate anions, which results in reduction of the repulsive interactions and of the swelling. The modification in the conformations also plays a role in the reduction of the swelling at acidic pH. Via distinction, networks crosslinked with adipic acid dihydrazide released methylene blue faster in acidic pH than in the

saline solution. This is because of the low amount of carboxylic groups free in the helix conformation after the cross-linking, which also hinders the helix to coil transition. Examining for a different approach to control drug release, sodiumdodecyl sulfate (SDS) was added to co-networks of alginate and xanthan. Sodiumdodecyl sulfate is a well-known anionic surfactant, chiefly used to enable the wetting and to enhance the apparent solubility of drug. Nevertheless, after ionotropic crosslinking SDS is supposed to organize to the crosslinker cation, becoming more hydrophobic.

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