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Natural exopolysaccharides as novel excipients in drug delivery: A review

***Manjanna.K.M, [1]Shivakumar B., [2]Pramodkumar.T.M.**

**Department of Pharmaceutics, T.V.M. College of Pharmacy, Bellary, Karnataka*
[1]Department of Pharmaceutical Chemistry, S.C.S. College of Pharmacy, Harapanahalli
[2]Department of Pharmaceutics, J.S.S. College of Pharmacy, Mysore

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Abstract

Drugs are rarely administered as pure chemical substances alone and are almost always given as formulated preparations or medicines. Drug dosage forms contain many components in addition to the active pharmaceutical ingredient(s) to assist in the manufacturing process as well as to optimize drug delivery. Due to advances in drug delivery technology, excipients are currently included in novel dosage forms to fulfill specific functions and in some cases they directly or indirectly influence the extent and/or rate of drug release. Developments of several drug delivery systems are based on polymers act as excipients that do not change their chemical structure but these materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. The microbial Exopolysaccharides are water-soluble biomaterials secreted by a variety of micro-organisms during fermentation. These are chemically well-defined and have attracted worldwide attention as excipients due to their novel and unique physico-chemical properties. These Exopolysaccharides used multifarious industrial applications in foods, pharmaceuticals as emulsifiers, stabilizers, binders, gelling agents, lubricants and thickening agents. These are emerging as new sources of polymeric materials which are posses' special characteristics such as biodegradability, biocompatibility, bioadhesivity, mechanical and chemical resistance, swelling and gelling power which are gradually becoming economically competitive and as promising biomaterials for drug delivery This review discusses some of the important physicochemical properties, and applications of some novel Exopolysaccharides that are used or investigated as excipients in development of drug delivery systems.

Key words: Exopolysaccharides, Biodegradability, Biomaterials, Drug delivery systems.

Introduction

Pharmaceutical excipients are substances other than the API which have been appropriately evaluated for safety and are intentionally included in a drug delivery system. To aid in the processing of the drug delivery system during its manufacture, protect, support or enhance stability,

bioavailability or patient acceptability, assist in product identification, or enhance any other attribute of the overall safety, effectiveness or delivery of the drug during storage or use.[1]

The design of effective drug delivery systems has recently become an integral part of the development of new medicines. The goal is to provide a therapeutic quantity of medicine(s) to the proper site in the body in order to achieve the desired effect and maintain such effect for the entire period of treatment. Hence, research continuously keeps on searching for ways to deliver drugs over an extended period of time, with a well-controlled release profile. Developments of several drug delivery systems are based on Exopolysaccharides that do not change their chemical structure but these materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Moreover these modify, drug release to achieve the dosage forms to release the drug in a constant manner and maintain steady state plasma concentration for the entire period of treatment to reduce the dose related adverse effects. The recent rediscovery of polysaccharide based materials is also attributable to new synthetic routes for their chemical modification, with the aim of promoting new biological activities and/or to modify the final properties of the biomaterials for specific purposes. [2]

Exopolysaccharides (EPSs) are high molecular weight carbohydrate polymers that make up a substantial component of the extracellular polymers surrounding most microbial cells in the marine environment. Exopolysaccharides produced by a wide variety of microorganisms are water soluble gums which have novel and unique physical properties. Because of their wide diversity in structure and physical properties microbial Exopolysaccharides have found a wide range of applications in the food, pharmaceutical and other industries.[3] Some of these applications include their use as emulsifiers', stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening and suspending agents. These biopolymers are rapidly emerging as a new and industrially important source of polymeric materials which are gradually becoming economically competitive with natural gums produced from marine algae and other plants. The potential use of genetically modified microorganisms under controlled fermentation conditions may result in the production of new exopolysaccharides having novel superior properties which will open up new areas of industrial applications and thus increase their demand. In view of their unique and novel chemical and physical properties, microbial Exopolysaccharides are being used as gelling agents, emulsifiers, stabilizers, binders, coagulants, lubricants, film formers, thickening and suspending agents.[4-6]

2. Drug Delivery

The aim of our drug delivery research is to optimize the bioavailability of drug compounds by innovative formulation development. Drugs are rarely administered in their pure forms but are almost always given in formulated preparations. These can vary from relatively simple solutions to complex drug delivery systems through the use of appropriate additives or excipients in the formulations. It is the formulation additives that, among other things, solubilize, suspend, thicken, preserve, emulsify, modify dissolution, improve the compressibility and flavour drug substances to form various acceptable preparations or dosage forms.[7] Every drug needs a drug delivery system. Drug delivery defined as the use of appropriate additives or excipients in the formulations, administered into the body through different routes to provide varied and specialized pharmaceutical or pharmacological functions. The principle objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of large scale manufacture with reproducible product quality. Hence in the ultimate analysis, each and every dosage form irrespective of its final structure and nature is a

combination of the drug component and an assortment of different kinds of non-drug components. The design of dosage form, so that it has specific qualities and gives a specified type of action pattern, calls for an immense amount of scientific skill, matched with an equal amount of innovation and imagination. The chemical engineers have designed these different drug delivery systems by performing fundamental chemical engineering involves chemical reaction, chemical kinetics, mass transfer and the physicochemical properties of the polymer materials.[8] Today the population suffers from a variety of diseases and there are numerous dosage forms into which a drug substance can be incorporated for the convenient and efficacious treatment of a disease. Dosage forms can be designed for administration by all possible delivery routes to maximize therapeutic response, previously, the drug delivery systems were developed for the traditional routes of administration like oral topical, and parenteral routes but many novel drug delivery systems such as transdermal-through skin, nasal, ocular-through eye, pulmonary by lungs, have been developed from last few years for the purpose of the administration of drug to the body to make more effective and easy to administer.[9]

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated by using several excipients. Recently several technical advancements have been developed for a drug delivery. These techniques are capable of controlling the rate of drug release, sustaining the duration of therapeutic activity and /or targeting the delivery of drug to a tissue. These new strategies, often called novel drug delivery systems (NDDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. [10]

Over the past few decades, the rise of modern pharmaceutical technology and the amazing growth of the pharmaceutical industry have revolutionized the approach to drug discovery and development. The close association of people from various fields such as chemistry, biology, medicine and engineering in drug development research has led to the uncovering of the cellular and molecular basis for the action of many drugs. New drug delivery systems are discovered for the purpose of improving something new to treat or to cure the disease .We know that every drug delivery systems are necessary but has some disadvantages. By developing new drug delivery system we can do something better than the existing drug delivery systems for the patients.

2.1 Polymers in drug delivery

One of the most remarkable and useful features of a polymer's swelling ability manifests itself when that swelling can be triggered by a change in the environment surrounding the delivery system. Depending upon the polymer, the environmental change can involve pH, temperature or ionic strength and the system can either shrink or swell upon a change in any of these environmental factors of these sensitive systems. Drug release is accomplished only when the polymer swells and because many of the potentially most useful pH-sensitive polymers swell at high pH values and collapse at low pH values, triggered drug delivery occurs upon an increase in the pH of the environment. Such materials are ideal for systems such as oral delivery, in

which the drug is not released at low pH values in the stomach, but rather at high pH values in the upper small intestine [11]

2.2 Exopolysaccharides;

Exopolysaccharides are water soluble polymers and may be ionic or non-ionic produced by a variety of micro-organisms are mainly linear molecules to which side chains of varying length and complexity are attached at regular intervals. Examination of 'families' of microbial Exopolysaccharides which are closely related structures enables us to determine the effect of minor (or major) changes to structure on the physical properties of these macromolecules changes may also be seen in some of the biological properties of the polysaccharides, such as susceptibility to enzymes, interaction with antibodies, capacity and specificity of ion bonding. Acetyl groups frequently assert very marked effects on the properties of microbial polysaccharides.[12-13]

In recent years, there has been a growing interest in isolating new EPS producing bacteria, particularly from various extreme marine environments.[14] Many new microbial EPSs with novel chemical compositions, properties and structures have been found to have potential applications in fields such as adhesives, textiles, pharmaceuticals and medicine for anti-cancer, food additives, etc. Most EPSs produced by marine bacteria are linear hetero-polysaccharides consisting of three or four different monosaccharide's arranged in groups of 10 or less to form repeating units.[15] The monosaccharide's may be pentose's, hexoses, amino sugars, or uronic acids. EPSs possess different types of functional groups; most of EPSs are sulfated and high in uronic content, and this confers them a net negative charge and acidic properties at the pH of seawater (pH ~ 8)[16]

Microbial Exopolysaccharides have found a wide range of applications in the food, pharmaceutical and other industries due to their unique structure and posses' special characteristics such as biodegradability, biocompatibility, bioadhesivity, mechanical and chemical resistance, swelling and gelling power. Some of these applications include their use as emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening and suspending agents, drug release modifiers. These biopolymers are rapidly emerging as pharmaceutical industrially important and are gradually becoming economically competitive with natural gums produced from marine algae and other plants [17]

Microbial polysaccharides are favored in industry over natural polysaccharides from plant and marine sources because (i) they can be produced from well known, cheap and plentiful raw materials (ii) They have some peculiar rheological properties & resistance to hydrolysis at different temperature and pH conditions. (iii) They have superior ability to modify the properties of aqueous environments that is their capacity to thicken, emulsify, stabilize, encapsulate, flocculate, and swell to form colloidal suspensions, gels, films and membranes. These properties of Exopolysaccharides also account for their steadily increasing exploitation in the formulation of food products, biomedical products and cosmetic preparations. Therefore natural Exopolysaccharides as well as their derivatives, represent a group of biopolymer that are widely used in pharmaceutical formulations and in several cases plays a fundamental role in determining the mechanism and rate of drug release from the dosage form. [18] Among these macromolecules Scleroglucan, Gellan Gum, Pullulan, Cardulan, Xanthan, D-Xylose, Dextran are potentially useful for the formulation of modified release dosage forms.

2.3 Sources of Exopolysaccharides

Microbial polysaccharides, which serve different functions in a microbial cell, may be distinguished into three main types: (a) intracellular polysaccharides which may provide mechanisms for storing carbon or energy for the cell; (b) structural polysaccharides which are components of cell structures such as lipopolysaccharides and teichoic acids present as integral components of cell walls; and (c) extra cellular polysaccharides referred to in this chapter as exopolysaccharides. Depending on the microbial system, some exopolysaccharides form capsules around the cell thus becoming part of the cell wall, while others form slimes which accumulate outside the cell wall and have the ability to diffuse away into the liquid phase during the course of fermentation. As a result of Exopolysaccharides production the viscosity of the fermentations broth may undergo profound changes, starting out as a low-viscosity Newtonian fluid and ending up as a highly viscous non-Newtonian fluid. Those microorganisms that produce large amounts of polysaccharide slimes have the greatest potential for commercialization since these Exopolysaccharides may be recovered easily from the fermentation broth. The production of exopolysaccharides obtained from Corynebacterium viscous in a medium containing a C13-CI6 n-alkane mixture under aerobic conditions.[19-21]

3. Scleroglucan

Scleroglucan is a natural Exopolysaccharides produced by fungi of the genus sclerotium (Sclerotium Rolfsii) that has been extensively studied for various commercial applications and also shows several interesting pharmacological properties. The commercial product is termed scleroglucan; but it is also known with other names according to the company that produces the polysaccharide (eg: Actigum, clearogel, polytran FS Sclerogum)[22].Because of its peculiar rheological properties and its resistance to hydrolysis, temperature, and electrolytes, Scleroglucan has various industrial applications especially in the oil industry for thickening; drilling mud's and enhanced oil recovery. Other industrial use include the preparation of adhesives, water colors, printing inks, and liquid animal feed composition.[23]

In the cosmetic industry, Scleroglucan may be used in hair control compositions and in various skin care preparations, creams and protective lotions.[24] In pharmaceutical products Scleroglucan may be used as a Laxative in tablet coatings and in general to stabilize suspensions[25]. In the food industry numerous Japanese patents describe quality improvements of frozen foods, Japanese cakes, steamed foods, rice crackers and bakery products.[26] The use of Scleroglucan as an antitumor, antiviral and antimicrobial compound has also been investigated. Sclg has shown immune stimulatory effects compared with other biopolymers and its potential contribution to the treatment of many diseases should be taken into account in therapeutic regimens. Recently it has attractive properties of the polysaccharide in controlled drug release and especially in immunopharmaceutical applications. [27-28]

Chemical Structure:

Scleroglucan is a branched natural homopolysaccharide that gives only D-glucose after the complete hydrolysis. The polymer consists of a main linear chain of β-D- (1-3) - glucopyranosyl units; there is a β-D- glucopyranosyl unit (1-6) linked to every third unit.[29]. The structure was first elucidated by periodic oxidation analysis and later verified by methylated sugar analysis and 13C nuclear magnetic resonance (NMR) the chemical structure of the tetrasaccharide repeating unit of sclg as established by NMR analysis. Sclg chains in aqueous media feature a triple helical conformation of remarkable stability. The side chain units can be derivatized by means of selective, controlled periodate oxidation; the ensuring aldehyde groups oxidized to carboxylated groups thus yielding polyelectrolyte's of variable, controlled charge density that exhibit interesting conformation-dependent solution properties.

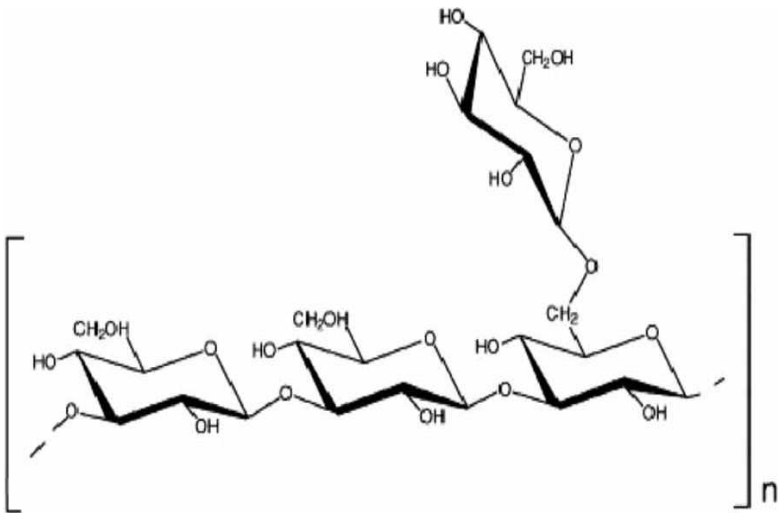


Figure: 1. Molecular structure of Scleroglucan

The carboxylated polymer (Sclerox) has been found to assume a single helical conformation in aqueous solution, the breakage of the triple helix occurs during the reaction leading to the aldehyde derivative until a degree of substitution equal or less than 40%, the stable conformation of the triple helix is retained, while introducing a percentage of aldehyde groups of the order of 50% or more the triple helix disentangles into single chains. Both Sclg and its oxidized derivatives have been proposed for the formulation of sustained drug release dosage forms.[30-31]

3.2 Solubility:

Scleroglucan disperses rather easily in water at room temperature due to the presence of β-D-(1-6)- glucopyranosyl groups that increase the solubility of the polysaccharide and decrease the ability to form the gels. Refined grades of Sclg dissolve readily in hot and cold water to form pseudo plastic solutions with shear thinning characteristics that tolerate high temperature, broad range of pH and a variety of electrolytes. [32] The viscosity of Scleroglucan solutions is affected only slightly by temperature variations. At 0.5 and 2%, it remains practically constant between 10 and 90^0C. At low temperatures, close to 7^0C, solutions of Sclg form thermo-reversible gels that may be caused by weakly interacting triple helix cross-linking mechanism. The viscosity of Sclg is unaffected over a pH range of 1 to 11 in addition of dimethyl sulphoxide in aqueous solutions of pH = 12.5 or higher or at temperatures above 90^0, the reduced viscosity, specific rotation and sedimentation co-efficient indicates disruption of the triple helical structure to a single random coil.[33] Sclg forms stable gels in the presence of chromium salts and borax at pH 10-11, and can be precipitated by the addition of quaternary ammonium salts under alkaline conditions.

3.3 Compatibility:

Rehydrated Sclg is compatible with electrolytes such as 5% Sodium chloride, 5% Sodium sulphate, 20% Calcium chloride and 10%disodium hydrogen phosphate. However, when the electrolyte concentrations are very high, solutions may gel and flocculate. Sclg is compatible, without synergism with most other thickeners such as locust bean gum, alginates, Xanthan and Carrageenan and cellulose derivatives and produces most favorable properties to modify the drug release in various pharmaceutical products [34].

3.4 Rheology:

Pseudo-plasticity or shear thinning is the salient characteristic of Scleroglucan solutions. This is evident in the gum solutions of 0.2% or lower but the flow becomes progressively more Newtonian as the Concentration decreases below 0.2%. Solutions containing less than 0.8% of Scleroglucan are not significantly thyrotrophic except at temperatures dropping to 10^0 and below. Due to high degree of Pseudo plasticity, gel states are not always clearly defined. Thus 1.2 – 1.5% solutions of purified gum form self-supporting sliceable gel at approximately 25^0 but at temperatures below 10^0, even much diluted solutions, from diffusely structured gels that tend to shrink and undergo synergism when left undisturbed for long periods of time. Such diffused gels disperse quickly with mild agitation. [35]

3.5 Suspending Properties:

A pseudo plastic flow system inherently combines a capacity for suspending fine particles with good pourability of suspension. Purified Scleroglucan at 0.1 – 0.2% effectively stabilizes 5-10% aqueous suspensions of fine powders such as Zinc-oxide, reprecipitated calcium carbonate and sulphamerazine. The viscosity of combinations of Scleroglucan with bentonite suspensions is markedly synergistic. Thus, while the apparent viscosities of 0.15% of purified gum and 5% bentonite are around 200 and 300 cps respectively, a combination of the two yields the viscosity of > 4000 cps. Although not a primary emulsifier in the sense of a surfactant Sclg enables very low energy dispersion during the formation of stable oil-in-water emulsions. In addition to the suspending action of the pseudo plastic system, prevention of coalescence seems to underlie this kind of stabilization [36]

3.6 Pharmaceutical Applications of Scleroglucan:

Pharmaceutical applications include the use in tablet coatings, ophthalmic solutions, injectable antibiotic suspensions and calamine lotion. Another important use of Scleroglucan is in the form of carboxylated derivative for use as a matrix for drug delivery in the form of tablets or films. For this purpose, Hydrogels obtained by the cross linking reaction between the polycarboxylated derivative of Scleroglucan and alkane dihalides were evaluated for the diffusion experiments and water up-take [37]. Here Scleroglucan offers advantages of controlled release as well as compatibility, biodegradability, and bioadhesiveness and thermal and chemical stability. The peculiar physicochemical properties of Scleroglucan suggested its suitability as a slow a remarkable swelling process that can slow down the diffusion of molecules previously loaded in the system. Furthermore, during the hydration process, the formation of a swelled layer slows down the penetration of the dissolution medium. This layer therefore represents the rate-limiting step of water penetration, which is very important for the release of model drugs. Coviello *et al* [38] reviewed the use of Scleroglucan and some derivatives in the field of pharmaceutics and in particular for the formulation of modified release dosage forms. The native Scleroglucan can be used for the preparation of sustained release tablets and ocular formulations; oxidized and cross linked Scleroglucan can be used as a matrix for dosage forms sensitive to environmental conditions. Another interesting approach is the preparation of a co-cross linked network using aqueous medium instead of the usual organic solvents and Gellan as cross-linker. At the beginning only physical entanglements between the different chains are effective; the network is subsequently “frozen” via chemical bonds, leading to a real physical-chemical gel with an improved stability. This new polymeric network showed a sustained release behavior that was better modulated than that obtained with the single polysaccharide [39].

Scleroglucan is one very clear example of a polymer whose microscopic structure corresponds to specific and peculiar properties of the macroscopic swelling behavior of the matrix. The

Exopolysaccharides can form gels in the presence of borate ions, showing a mixed network with both chemical and physical linkages between the cross-linker and the chains. According to the molecular dynamics approach, the rigid rods are almost forced to align and to form bundles in which the borate acts almost as a "Zipper". Cavities along the chains are thus formed that can allocate host molecules in function of their steric hindrance. This microstructure is obviously at the origin of the release profiles observed in vitro of different model drugs. It is also behind the unusual and anisotropic swelling found in the case of Scleroglucan/borax tablets.[40]

4. Pullulan

Pullulan is a water soluble, neutral linear polysaccharide consisting of α-1, 6-linked maltotriose residues. It is a fungal Exopolysaccharides produced from starch by Aureobasidium Pullulan. The early observation on this exopolymer was made by Bauer in 1938 and this Exopolysaccharides was named as Pullulan by Bender et al in 1959. Pullulan is biodegradable, impermeable to oxygen, non-hygroscopic and non-reducing. Pullulan is easily soluble in hot and cold water to make clear and viscous solution and also has high adhesion and film forming abilities. Pullulan films are thermally stable and possess anti-static and elastic properties and can be developed into compression moldings. These properties of Pullulan are attributed to the unique linking it possesses with structural flexibility. The principal advantages of Pullulan are that it is a nonionic polysaccharide and is blood compatible, biodegradable non-toxic, non-immunogenic, non-mutagenic and non-carcinogenic. [41-42] Pullulan is currently used extensively in the food industry. It is a slow digesting macromolecule which is tasteless as well as odorless hence it is used as a low-calorie food additive providing bulk and texture. Pullulan possess oxygen barrier property, good moisture retention and also it inhibits fungal growth. These properties make it excellent material for food preservation and are used extensively in the food industry. There is a twenty year history of safe use in Japan as a food ingredient and as a pharmaceutical bulking agent. FDA had estimated that daily intake of Pullulan would be up to 10 g per day for a person based on food categories and usage[43]

4.1 Description:

The name "Pullulan" was proposed by Bender, who was the first to describe the formation of this extra cellular polysaccharide by Aureobasidium Pullulan. It is essentially a linear polymer of repeating maltotriose units linked by ∝ - 1, 6 glycosidic bonds. Depending upon the culture conditions (duration phosphate concentration) under which this extracellular glycan is elaborated by Aureobasidium Pullulan, the molecular weight varies from about 10 to 3000 KDa. [44]

4.2 Chemical characterization

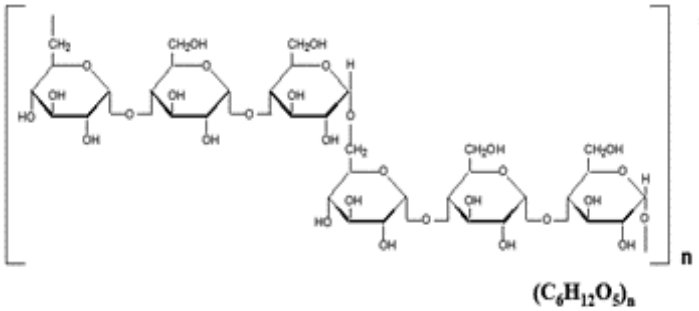


Figure 2. Molecular structure of pullulan

Pullulan is an essentially linear polysaccharide (glucan) consisting predominantly of repeating maltotriose units. The maltotriose units, which consist of three 1,4- linked glucose molecules, are linked by α-1, 6-glycosidic bonds. This repeating sequence forms a stair-step-type structure.

4.3 Physicochemical properties

Pullulan PI-20 is a white to off-white, tasteless and odorless powder. It is not hygroscopic. According to the specifications provided by the sponsors it contains less than 6% water. Pullulan dissolves readily in cold or hot water, but is insoluble in organic solvents, except dimethylformamide and dimethylsulfoxide. Aqueous solutions of Pullulan are viscous but do not form gels. The viscosity (10% w/w, 30^0C) of ten batches of Pullulan PI-20 was 132-179 mm^2/s. the viscosity of Pullulan solutions resembles that of gum acacia (gum Arabic) solutions, i.e. the viscosity of Pullulan is rather low in comparison with that of other soluble polysaccharides, such as guar gum. Differences in the pH or salt content do not substantially affect the viscosity of Pullulan solutions. The viscosity of an aqueous solution of Pullulan PI-20 decreases upon incubation with pullulanase. An aqueous solution of Pullulan PI-20 (10% w/w) has a pH of 5.0-7.0. Molecular weight gel permeation chromatograms of three batches of Pullulan PI-20 showed a molecular weight at the peak of the chromatogram of 173000-186000 Da with a number-average molecular weight (M_n) of 96900-101000 Da and a weight-average molecular weight (M_w) of 433000-479000 Da. Pullulan is structurally closely related to starch amylopectin and malt dextrin. All three carbohydrates consist of glucose units linked by α-1, 4 and α-1, 6-glucosidic bonds. Malt dextrin contains approximately 20% α-1, 6-glucosidic bonds and Pullulan approximately 30%. In comparison, corn starch contains 95% α-1, 4-glucosidic bonds and 5% α-1, 6-glucosidic bonds. Differences between pullulan and these glucans, besides the relative proportions of alfa-1, 4 and alfa-1, 6 bonds, are the tertiary structure of the molecule and the extent and mechanism of degradation of the materials in the human gut.[45]

4.4 Biomedical Applications of Pullulan

However, recently Pullulan is also being investigated for its biomedical applications in various aspects like targeted drug and gene delivery, tissue engineering, wound healing and also even in diagnostic imaging using quantum dots. Pullulan is highly water soluble; hence for drug delivery applications, mostly hydrophobized Pullulan is used as drug delivery carriers. These hydrophobized Pullulan molecules can form colloidally stable nanoparticles upon self-aggregation in water with monodispersity. Pullulan due to its film forming properties can entrap biological molecules and due to its excellent oxygen barrier properties these molecules remain stable with enhanced shelf-life. Pullulan can be chemically modified to produce derivatives with low solubility or a modified polymer that is completely insoluble in water. Pullulan derivatives are developed and their applications towards the above mentioned aspects were also studied by various groups [46]

Pullulan is now extensively studied for various applications in biomedical field. This is mainly due to its non-toxic, non-immunogenic and biodegradable properties. In comparison to a similar but more popular polysaccharide, Dextran, the degradation rate of Pullulan in serum is faster than that of Dextran. The degradation index is 0.7 after 48 hour incubation in comparison with 0.05 for Dextran. The degradation rate can be reduced or regulated by varying degrees of chemical modification. Some of the major areas in which Pullulan is investigated are discussed in the following section.

4.5 Tissue Engineering

Tissue engineering requires scaffold or artificial extracellular matrix that can accommodate cells and regulate their growth leading to three dimensional tissue regeneration. Na et al developed carboxymethyl Pullulan and conjugated it with heparin and investigated its properties towards tissue engineering applications. Heparin-conjugated Pullulan inhibited the proliferation of smooth muscle cells (SMCs). Heparin-conjugated Pullulan material can thus be used for the proliferation of vascular endothelial cells and to inhibit the proliferation of SMCs [47]

4.6 Surface Modification

Yet another promising application of this versatile polymer is the use in surface modification as evidenced by work by Hasuda et al. The authors synthesized photo reactive Pullulan, azidophenylpullulan (Az-pullulan) and photoimmobilised on polystyrene, polyethylene and silane coupled glass surfaces by micro patterning method. These surfaces which have different contact angles showed same contact angle for the entire surface. Interaction of these modified surfaces with proteins like albumin and cells having macrophage like properties which can adhere to various surfaces (RAW 264) was then studied. The authors observed that the adherence of both the cells and protein was more pronounced on the unmodified surface. The authors suggest that the Pullulan forms a hydrophilic non-ionic surface layer which reduces the protein adsorption. There was no cell adhesion also on to Az-pullulan modified surface. Hydrophilic surfaces reduce cell adhesion. Authors consider Az-pullulan as a promising candidate for bioinert surfaces due to reduced interaction with protein and cell.[48]

Targeted drug delivery with magnetic nanoparticles is possible with the use of external magnetic fields target the particle to the site of interest. Magnetic nanoparticles are also of interest in diagnostic imaging as well. But since the magnetic nanoparticles being hydrophobic gets easily destroyed or cleared from the circulation and these particles are also cytotoxic. Hydrophilic surface modification of these particles prolongs the half-life in the circulation.[49] Gupta et al coated prepared super paramagnetic iron oxide nanoparticles (SPION) and coated with Pullulan (Pn-SPION). They studied the effect of pullulan coating on the cytotoxicity and the cellular uptake of the nanoparticles. The cytotoxicity studies were done on fibroblasts and it was observed that with uncoated particles (SPION) the cell death was 60% and with Pn-SPION there were no cytotoxicity effects. Similarly cell adhesion test also showed that the attached cell number was decreased upto 64% in SPION but for pullulan coated it was comparable with the control cell population. The authors attribute the low toxicity of Pn-SPION to the hydrophilicity of Pullulan. By transmission electron microscopy the cellular uptake of the particles was also established. These Pullulan coated magnetic particles is thought to be useful for medical imaging like vascular compartment imaging, lymph node, receptor, perfusion and target specific imaging.[50]

4.7 Plasma Expander

Like Dextran, Pullulan was also explored as a potential blood-plasma substitute. Only highly water soluble polymers can be used as plasma expanders and Pullulan is highly water soluble. Blood plasma expander operates via the colloidal osmotic pressure induced by the macromolecules. It is reported that Pullulan to be used as a plasma blood expander should have a molecular weight of about 60kDa, and observed that Pullulan with higher molecular weight range increased venous pressure where as low molecular weight gets rapidly excluded from the organism followed by the development of secondary hemorrhagic shock. Therefore, the Pullulan for this particular purpose should be in the effective therapeutic molecular weight range free from low and high molar mass fractions. Shingel et al developed an anionically modified Pullulan via g-irradiation which was used as base for blood-plasma substitute.

Introduction of carboxyl and carbonyl groups increases the resistance of Pullulan to degradative action amylase. This substitute was studied in dogs under the conditions of experimental shock. An isovolumetric replacement with this new derivative of Pullulan resulted in a rapid recovery of the animal and the blood microcirculation was normalized. [51]

4.8 Molecular Chaperons

Chaperon like activity-able to catch and release proteins. Molecular chaperons selectively bind denatured proteins in order to prevent irreversible aggregation due to host-guest interaction. Then the host chaperon releases the protein in its refolded form. Water soluble polymers such as PEO were tried to increase the recovery yield of the native protein during refolding. These polymers block the exposed hydrophobic surface on the denatured proteins in such a way which just prevents the aggregation of proteins. Excessively strong binding to intermediate would prevent folding to the native confirmation.[52] Nomura et al developed hydrophobized Pullulan nanogels possessing properties of molecular chaperons. The complexes proteins were effectively released from the nanogels in their refolded forms in presence of cyclodextrins.They concluded that these amphiphilic nanogels selectively traps denatured proteins and cyclodextrin acts as a effectors' molecule to control the binding ability of host to proteins and that this nanogels system is a promising technique for protein refolding. The chaperon like property i.e. the binding and release of proteins in the active form is further being explored towards tissue engineering applications.[53]

4.9 Targeted Drug/ Gene Delivery and Imaging

4.9.1 Liver targeting

On the sinusoidal surface of the hepatocytes, asialoglycoprotein receptors are expressed abundantly which removes the asialo (galactoseterminal) glycoprotein's from the sinusoidal circulation by internalizing the bound protein via endocytosis. Kaneo et al has reported the strong binding of Pullulan to the asialoglycoprotein receptor with high affinity and the bound molecule is internalized to the hepatocytes via receptor mediated endocytosis. Pullulan accumulates in the liver in significantly higher amounts than other water soluble polymers. This property of Pullulan is widely exploited for targeted drug/gene delivery to liver. Interferon (IFN) is used as the conventional immunotherapy of hepatic virus C induced liver diseases. But the efficiency of current interferon therapies is reported to be clinically insufficient.[54] An attempt by Suginoshita et al was made to target IFN to liver by complexing it with Pullulan DTPA, They reported that intravenous administration of this IFN-DTPA-Pullulan conjugate in mice showed enhanced IFN activity than the free IFN. It was concluded from this study that this enhanced activity is due to the liver targeting ability of Pullulan and it seems to be a promising interferon therapy.[55]

4.9.2 Nanoparticles for Drug/Gene Delivery and Cancer Therapy

Recently the role of polysaccharides in developing controlled drug delivery systems has increased significantly and Pullulan is gaining lot of attraction towards this application. Self-assembling nanoparticles from hydrophobized Pullulan, pH sensitive derivatized Pullulan nanoparticles, and anionic/amphiphilic microparticles are some of the examples. Akiyoshi et al developed insulin delivery system of the size 20-30 nm by complexing the Hydrogel nanoparticles of cholesterol bearing Pullulan. These complexed nanoparticles were stable and protected insulin from the enzymatic degradation and suppressed insulin aggregation. It was proved *in vivo* that the biological activity of the complexed insulin remained intact. [56]

Carboxymethylation introduces negative charge in Pullulan and this derivative unlike Pullulan has low affinity for asialoglycoprotein receptors. The liver uptake clearance of pullulan was

decreased by more than hundred fold. This derivative was then investigated for application in chemotherapy. The authors conjugated doxorubicin, a known chemotherapy drug used in various cancers, via a peptide linker to carboxymethylated pullulan. Conjugating such small molecular drugs to polysaccharides make them inactive and is referred to as macromolecular prodrug. The conjugated drug to be pharmacologically active should get released from the prodrug. The conjugation reduces the free drug plasma concentration and drug exposure to other susceptible tissues. Compared to free drug these prodrugs have long half-life. This increased half-life will lead to passive accumulation of prodrugs in the tumor. This is because of the enhanced permeability of the prodrugs to tumor due to its leaky vasculature and the retention of these macromolecular conjugates due to decreased lymphatic drainage. Nogusa et al in their *in vivo* study the authors established that the conjugated drug was more effective than the free drug. They tried both the conjugate and free drug on murine carcinoma models, solid tumor (lung carcinoma and reticulosarcoma) and non-solid tumor (P388 leukemia). The conjugate was more effective in reducing the tumor volume and increasing the survival rate than the free drug. The conjugate but did not have any effect on leukemia cells indicating that it is effective only against solid tumors.[57]

Hydrophobically modified Pullulan is known to from self-assembled nanoparticles. Na et al developed pH sensitive self-assembled nanoparticles of succinylated Pullulan acetate/ sulfonamide (PA/SDM) conjugates which are responsive to even minute pH variations. These particles were of the size range <70nm and showed good stability but shrank and aggregated below pH 7.0. These particles were developed for targeting solid tumors and inflammatory regions where the extracellular pH (pH 6.5- 7.2) is lower than the normal tissues and blood. These particles were tested for loading and release properties with Adriamycin (doxorubicin). The drug release rate from the PA/SDM nanoparticles was pH-dependent and it was significantly enhanced below a pH of 6.8. The authors conclude that these pH-responsive PA/SDM nanoparticles may provide some advantages for targeted anti-cancer drug delivery due to the particle aggregation and enhanced drug release rates at tumor pH.[58]

Gene therapy is another area where the application of Pullulan is being explored. Gene therapy is thought to be a cure for various inherited disorders and cancer. Gene delivery is usually achieved by endocytic pathway. Efforts for gene therapy using virus have been performed but viruses are known to be immunogenic and can be hazardous. So attempts to develop non-viral vectors are taken and cationic derivatives of natural polymers are investigated towards this purpose. Recently Pullulan being biocompatible and non-toxic is investigated for gene delivery application. [59]. Hosseinkhani etal developed Pullulan derivative which has metal chelating residues and mixed with a plasmid DNA in aqueous solution containing Zn^{2+} ions to obtain the conjugate of Pullulan derivative and plasmid DNA with Zn^{2+} coordination. Pullulan is known for its specificity for liver and this property is exploited for liver targeting. The authors observed that the conjugate enhanced the level of gene expression at the liver parenchyma cells and the enhanced gene expression lasted for a period of 12 days after the injection. [60].

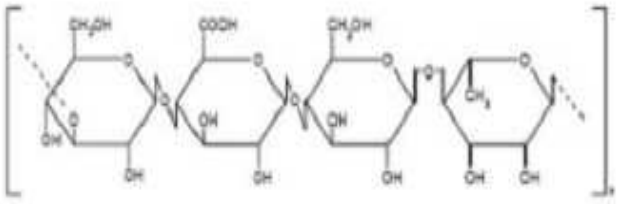
4.9.3 Medical Imaging

Recently nanotechnology is being investigated for successful and earlier detection of cancerous growths in the body. Quantum dots are nano-size semiconductor particles which has currently attracted lots of attention in the biological field. They are used as fluorescent probes for long-term cell-tracking as it is highly photo stable with strong fluorescence. Endocytosis of these QD's into the cell is usually low and for bioimaging purposes there should be detectable amount of the QD's. Recently there are numerous literatures reporting the efficiency of polysaccharides in the drug/gene delivery and imaging agents. Hasegawa et al developed cholesterol Pullulan

and amino-group-modified cholesterol Pullulan nanogels as a novel carrier to deliver QD into cells in comparison to conventional cationic liposome which has the disadvantage of forming aggregates once it is internalized in the cell. Nanoparticles were prepared by simple mixing with nanogels of cholesterol-bearing pullulan modified with amino groups and quantum dots. The size of these hybrid particles were about 38 nm. They reported that the intensity of fluorescence per cell of $CHPNH_2$ - QD nanoparticle was comparable to that of liposome-QD complex and particles with higher number of amino groups showed fluorescence upto 3-.4 times than that of the control. The authors conclude that the chemical modification of CHP by introducing cationic groups significantly enhances its cellular uptake and simultaneously the QD's better than the conventional cationic liposome's and that these nanoparticles could be a promising fluorescent probe for bioimaging.[61]

5. Gellan Gum;

Gellan gum is a bacterial Exopolysaccharides commercially prepared by aerobic submerged fermentation of Sphingomonas elodea. Gellan gum is a linear tetrasaccharide built up by →4)-L-rhamnopyranosyl-(α-1→3)-D-Glucopyranosyl-(β-1→4)-D-glucuronopyranosyl (β1→4)-D-glucopyranosyl-(β-1→ with O(2) L-glyceryl and O(6) acetyl substituent's on the 3-linked glucose. It consists of about 50,000 residues and it is normally de-esterifies by alkali treatment before use. Gellan gum forms a 3-fold double helix from two left-handed chains with the acetate residues on the periphery, and glyceryl groups and hydrogen-bonds stabilizing the interchain associations.[62]



[→3)-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1→]$_n$

Figure 3. Molecular structure of Gellan gum

5.1 Physicochemical Properties

5.1.1 Gelling Properties of the Gellan Gum.

Gelation of gellan solutions occurs abruptly upon heating and cooling of gellan gum solutions in the presence of cations. Such sol-gel transitions are considered as phase transition. The gelation of gellan gum is a function of polymer concentration, temperature, and presence of monovalent and divalent cations in solution. At low temperature Gellan forms an ordered helix of double strands, while at high temperature a single-stranded polysaccharide occurs, which significantly reduces the viscosity of the solution. The transition temperature is approximately 35^0C, but can range from 30-50^0C. Below transition temperature, a stiff structure is obtained (setting point), and results in gel formation. The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water. [63] Addition of monovalent or divalent cations during cooling markedly increases the number of slat bridges at junction zone, thereby improving the gelling potential of Gellan gum. Various

studies have been carried out to study the effect of different factors on the gel strength. Some of the important factors affecting gel strength are discussed bellow.

5.1.2 Acetyl content

Acetyl content is the most important factor affecting the gel strength. Gellan gum with different acetyl content gives gels with different properties. Native Gellan gum provides soft, elastic, thermo- reversible gels, and is very weak because of bulky acetyl and glyceryl groups that prevent close association between Gellan polymer chains in bulk-helix formation, and hinder compact packing of the cross-linked double helix. Deacetylated Gellan gum forms firm, brittle and thermo reversible gel because of the absence of acetyl and glyceryl groups.[64]

5.1.3 Type and concentration of ions

Ions have an impact on gel strength and brittleness. Gellan does not form gel in deionized water, but the addition of salts of calcium, potassium, sodium, and magnesium causes an increase in these two properties. Notably, divalent cations are more effective in achieving this; even in Gellan gels of very low concentration (0.2%,w/v), a high strength was achieved with a maximum at about 0.004% w/v calcium and 0.005%w/v magnesium. Similar gel strength can be achieved with 0.16% w/v sodium or 0.12% w/v potassium Gellan high salt concentration (1%w/v). It is important economically that strong gels can be obtained at low concentration of Gellan, with the incorporation of trace amount of salt [65]

5.1.4 Gel pH

Sanderson and Clark showed the gel strength to be enhanced within pH range of 3.5 to 8, which corresponds to the natural pH range of most foods. Change in pH does not alter the setting point of the gel, but affects melting temperature in some cases. For example, gels prepared with very low levels of monovalent ions melt at around 70^0C at neutral pH, but at pH=3.5 the melting temperature is slightly increased. This trend is not seen for divalent ions.[66]

5.1.5 Presence of hydrophilic ingredients

Addition of hydrophilic ingredients like sucrose (at about 10%, by mass per volume) tends to decrease the ion concentration required for optimal Gellan gel strength. Kasapis et al used transmission electron microscopy to examine the changing nature of a polysaccharide network with increasing levels of sugar. Mixtures of deacylated Gellan (<1%) with low (0-20%) and high (80-85%) levels of sugar were prepared and studied. Micrographs of the high sugar / Gellan gels produced clear evidence of reduced cross-linking in the polysaccharide network, which exhibits a transition from rubber to glass-like consistency upon cooling. [67]

Tang et al. studied the effects of fructose and sucrose on the gelling temperature, clarity, and texture properties of Gellan gels cross-linked with calcium or sodium ions. They reported the gelling temperatures of Gellan solutions to generally increase on the addition of sucrose, whereas addition of fructose up to 35% (by mass per volume) had no effect. Incorporation of fructose and sucrose markedly increased the gel clarity. Effect of sucrose on gel strength was found to be dependent on cations concentration. At low cations concentrations, sucrose strengthened the gels; but at high cations concentrations, sucrose weakened them. [68]

5.1.6 Temperature stability and flexibility of the melting point

Gellan gum is stable at higher temperatures and maintains its strength at 90^0C, whereas Xanthan gum looses 74% of its original strength after heating up to 90^0C. According to Sanderson and Clark, the melting temperature can be below or above 100^0C, depending on the conditions of gel formation. The most important factor responsible for the flexibility of the melting point is

concentration of cations in the gels because monovalent and divalent cations markedly increase the number of junction zones in gels and make them more resistant to temperature. Modification of the melting point can successfully replace other conventional thickeners / stabilizers, while used in much lower concentration. [69]

6. Effects of other natural hydrocolloids on the textural properties of gellan gum.

Various studies to find out the changes in the textural properties of Gellan gum when mixed with other food hydrocolloids have been carried out.

6.1 Sodium alginate

Sodium alginate dissolved in calcium chloride solution at 90^0C shows weak gel properties similar to those of ordered xanthan. The solutions show a sharp increase in rigidity on cooling, and convert to permanent gels on storage at low temperature. The gelsattain maximum hardness at about 40% calcium conversion (for alginate with a polyguluronate content of 58%), and their elasticity can be readily controlled by adjustment of Ca^{2+} concentration around this optimum value. Papageorgiou et al. observed that incorporation of moderate concentrations of Gellan (0.1-0.3%, by mass per volume, in combination with 2%, by mass per volume, alginate and 5 mM trisodium citrate, increased the strength of the gels, but did not significantly change their elasticity, indicating that the Gellan acts as strong 'filler' in an alginate matrix.[70]

6.2 Gelatin

Lau et al. carried out texture profile analysis on mixed gellan-gelatin gels to assess the effect of the ratio of the two components and calcium ion concentration. Hardness, brittleness, cohesiveness and springiness were measured. The results suggested that there was a weak positive interaction between Gellan and gelatin when no calcium was added; at higher concentrations, Gellan formed a continuous network and gelatin the discontinuous phase. Hardness was dependent on the concentration of Gellan gum in the mixture, whereas brittleness, springiness and cohesiveness were very sensitive to low levels of calcium (0-10 mM), but less sensitive to higher calcium concentrations and Gellan/ gelatin ratio. [71]

6.3 Carrageenan and Xanthan

Rodriguez-Hernandez and Tecante studied texture properties of Gellan – Carrageenan and gellan-xanthan mixtures in order to determine the contribution of both polysaccharides to the viscoelastic behavior of the mixture. Admixtures having a constant total concentration of 0.5% w/w with different proportions were prepared in presence of 0.01%w/v $CaCl_2$. It was observed that gel strength of 0.5% Gellan alone was the highest, and gel strength of the two-component gels decreased as the proportion of Gellan was reduced. Mixed gels having a Gellan concentration equal to or lower than 50% mass of the total concentration were less stiff and brittle, hence were more elastic[72].

6.4 Effect of chelatants on textural properties of Gellan gum

Camelin et al. studied the effect of various concentrations of sequestrates (sodium citrate, sodium metaphosphate, and EDTA) on Gellan gel setting temperature and rheological properties. Addition of EDTA between 0 and 0.8% (by mass per volume) progressively decreased the setting temperature. Citrate and metaphosphate decreased this parameter when added up to 0.4 or 0.6%, depending on Gellan gum concentration, eventually resulting in the absence of gel formation at room temperature for the 1.5% Gellan solution containing 0.4% citrate. This effect was accompanied by a significant decrease of gel strength, and might be attributed to the binding of divalent cations required for chain association during gelatinization by chelatants.[73]

7. Pharmaceutical Applications

In the presence of counter ions, this polymer is capable of forming gels that are particularly strong when formed with divalent ions. The degree of acylation also influences the strength of the resulting network. Indeed, when Gellan is acylated it forms soft, elastic, transparent and flexible gels while de-acylation leads to hard, non-elastic and brittle gels. The gels are thermo reversible, with a melting temperature, T_m, at about 50 ^{0}C, depending on the concentration and presence of cations that, stabilizing the gel, increase the T_m value. The polymer, initially used mainly as a food ingredient, has been widely investigated to devise novel ophthalmic formulations due to its ability to jellify in the presence of tear fluid cations thus providing drug ocular bioavailability. Hydrogels, in fact, show high patient compliance and *in situ* forming gels are even preferred since they are dropped as a solution in the eye where the transition into a gel actually takes place. Important parameters, like the gel strength, were studied to find a reliable indicator of the gel ocular bioavailability. *In vivo* experiments showed that only when the gel strength was within set limits, an appreciable increase in ocular bioavailability was obtained. The ocular contact time increased with increasing Gellan concentration; on the other side, the autoclaving process, carried out to sterilize the Gellan solutions, led to a significant reduction in the finished product of the gel strength due to a breakdown of the polymeric chains that was proportional to the autoclaving time.[74] Due to the decisive role of rapid gel formation in the use of *in situ*-gelling systems, contact times with different osmolalities were measured. As expected, gels formed with hypotonic solutions maintained their integrity for several hours.

Gels of gellan can be formed in the tear fluid even when the polymer concentration is very low and sodium proved to be the best gel-promoting ion *in vivo* though in physiological conditions the instilled drops are diluted, gels with a high elastic modulus can be formed. In fact, dilution of the tear fluid takes place upon instillation of a solution of salt free Gellan, but an elastic "skin" is immediately formed keeping the drops somehow compacted.[75]

Gellan has also been tested *in vivo* for the nasal uptake of fluorescein Dextran used as a model molecule. [76] The starting solution of Gellan behaves like a fluid but it forms a rigid gel when exposed to cations. Hence, it is suitable for nasal spray pumps with its initial low viscosity and the subsequent gelling upon contact with animal mucosa. A rapid gelation an also be expected in humans, the surface area of their nasal cavity being much larger than that of a rat. Furthermore, in comparison to plain solutions, lower doses can be administered because of the rapid gelation. Although with divalent cations (Ca^{2+}) the gels are much stronger, *in vitro* experiments demonstrated that a strong gel is formed in physiological conditions of 0.9% NaCl and the gel obtained *in vivo* is strong enough to remain in the nasal cavity for the required time interval, showing a slow clearance due to a higher local concentration. Gellan has also been tested for the encapsulation of biological components inside a polyion complex formed between Gellan and chitosan.[77] Apart from the sustained release, the use of capsules and microspheres offers several benefits. In particular, encapsulated substances can be protected, the small particle size enabling repetitive administration either orally or by injection as therapeutic bolus.

Selection of encapsulation method is crucial in achieving an encapsulation that allows enzymes and/or peptides to retain their catalytic activity or biological function. The method by complex coacervation is based on polyionic complexation through electrostatic interactions between cationic and anionic polymers resulting in the formulation of insoluble spherical capsules. It has been ascertained that Gellan-chitosan capsules retain proteins but release low-molecular weight substances across the capsule membrane. Upon loading into the capsules, an enzyme behaves like a free enzyme. The permeation of the inner materials is obviously dependent on their molecular masses, molecular structures and electrical charges. Indeed, an exclusion limit for the

diffusion out of the membrane has been experimentally found. Furthermore, it was evidenced that the loaded enzyme recovered its catalytic activity after the drying and selling steps, indicating that this formulation can be repeatedly used. Hence, it is a potentially powerful tool for applications in the field of biotechnology. It should be pointed out that both polymers are biodegradable and biocompatible, thus, the enzyme-encapsulating Gellan-chitosan capsules can be implanted directly, eliminating the need for surgical removal because of their bio-resorbability. Finally it is important to underline that the enzyme encapsulating procedure does not need any chemical modification and can be conveniently performed in aqueous solution.

Formation of Gellan beads has also recently experimented to evaluate the effect of various divalent cations on the encapsulation efficiency using a constant concentration of polymer and ionotropic medium. [78] It is well known that the gelling mechanism of gellan can be induced by cations and is temperature-dependent. In aqueous solution the gelation of gellan is accompanied by a two-step process which involves formation of double helices from random coil chains (coil-to-helix transition) and an aggregation of pairs of double helices. The coil-helix transition is greatly affected by the electrostatic interaction with the cations present in the solution. Gellan forms gels in the presence of mono (Na^+ and K^+) and divalent (Ca^{2+} and Mg^{2+}) cations but its affinity for the latter is much stronger than for the former. Preparing hard gelled beads with different cations has a significant effect on the aqueous solubility of the drug. Furthermore, drug loading increases as the atomic number of the divalent ion increases, thus suggesting that the electro-positivity of cations plays an important role in the ionotropic gelation of gellan. Although the drug loading efficiency was much higher in the presence of transition elements compared to alkaline earth metal ions, the beads prepared with Ca^{2+} were the best in terms of quality and mechanical strength.

Gellan has also been tested for oral drug delivery. [79] The formulation adopted was a Gellan solution containing calcium chloride (as the source of calcium ions) and sodium citrate, which complexes the free calcium ions and only releases them in the highly acidic environment of the stomach. The formulation remained in its liquid form until it reached the stomach where the gelation occurred after a few minutes and lasted for several hours. Plasma drug levels, tested after oral administration of Gellan solutions, showed that a sustained release was achieved, along with a higher bioavailability compared to that of a sustained release commercial solution product.

In terms of oral sustained delivery, Gellan has been compared to sodium alginate which, as previously reported, is also capable of giving *in situ* gelation in the acidic environment of the stomach. The *in vivo* release curves from the gels had a profile similar to that of a commercial suspension. The sustained release effect of the gel formulation was a consequence of the resistance of the gel structure to the diffusion of the drug whereas that of the suspension arose from the reservoir effect of the suspension particles as they slowly dissolved in the intestine. The Gellan gels were detected *in vivo* for a longer period of time if compared to those formed with alginate, indicating the formation of a mechanically stronger gel. There is an obvious advantage of using polymer solutions because such formulations are homogeneous liquids and do not have the problems that may be associated with the formulation and oral administration of suspensions.

Beads prepared with Gellan have also been studied in order to delay the delivery of loaded substances employed for weed control in agriculture (e.g. metribuzin) as well as model molecules for drug delivery (theophylline and benzamide)[80] Several formulations, containing different amounts of surfactants and/or oil, were tested. The beads were prepared by adding

calcium ions to homogeneous slurries of the various components. The presence of the oil in the formulations reduced the penetration rate of water into the beads leading to a decreased delivery rate. Similar beads, prepared with alginate, showed a slightly faster release than those obtained with Gellan. Hence, confirming previous researches, the strength of the gellan gels was higher than that of alginate, though a quantitative estimation was not given. It was also pointed out that, when the hydrophilicity of the model molecule was changed using a molecule more water soluble, a remarkable increase of the delivery rate was obtained. These results clearly indicate that, the swelling of the matrix together with the composition of the beads played a key role in the delivery process.

A recent study reports the preparation of microspheres obtained by the emulsion cross-linking method of gellan and poly (vinyl alcohol) in the presence of different amounts of glutaraldehyde as a cross-linking agent and of an antihypertensive drug.[81] The use of such IPN improved the mechanical strength obtained when only Gellan was used. The new microspheres were spherical, with smooth surfaces and with a narrow unimodal size distribution. By increasing the cross-link density, microspheres with smaller size were obtained due to the formation of a more rigid network. An increase in the amount of Gellan in turn increased the size of microspheres with the formation of a more crystalline matrix. Similarly the drug-loaded microspheres also showed a crystalline dispersion of the drug into the polymer matrix. In comparison to microspheres prepared with only Gellan, the new IPN microspheres showed a higher tensile strength. The *in vitro* studies evidenced that the drug release rates were higher for microspheres with a lower amount of Gellan, while the different diffusion media produced differences related to the solubility of the drug in the two environments [82]

8. Curdlan

Curdlan, an insoluble microbial exopolymer is composed almost exclusively of -(1,3)-glycosidic linkages. One of the unique features of Curdlan is that aqueous suspensions can be thermally induced to produce high-set gels, which will not return to the liquid state upon reheating and this has attracted the attention of the food industry. In addition to this, Curdlan offers many health benefits, as the betaglucan family is well known among the scientific community to have immune stimulatory effects. Curdlan was discovered in 1966 by Professor Harada and coworkers, and given its name because of its ability to ™curdle∫ when heated. At this time, Harada and his colleagues were working on the identification of organisms capable of utilizing petrochemical materials, and isolated Alcaligenes faecalis var. myxogenes 10C3 from soil. This organism was found to be capable of growing on a medium containing 10% ethylene glycol as the sole carbon source and also produced a new -(1,3)-glucan that contained about 10% succinic acid, and which was named succinoglucan. They were also able to derive a spontaneous mutant that mainly produced a water-insoluble neutral polysaccharide, (1,3)-glucan, and which did not contain succinoglucan.[83] Scientists at Takeda Chemical Industries Ltd. (Osaka, Japan) have played a pioneering role in both the research and development of Curdlan. Thus, as early as 1989, curdlan was approved and commercialized for food usage in Korea, Taiwan, and Japan. Upon obtaining approval in December 1996, PureglucanTM □} the trade name of Curdlan □} was launched in the US market as a formulation aid, processing aid, stabilizer, and thickener or texture modifier for food use .No evidence of any toxicity nor carcinogenicity of PureglucanTM has been observed.

8.1 Structure

Curdlan is composed of a minimum carbohydrate concentration of 90% and a maximum water content of 10%. Curdlan is a neutral polysaccharide consisting of D-glucose with β-1,3

glycosidic linkages, which means there are repeating glucose subunits joined by a beta linkage between the first and third carbon of the glucose ring. The 1,3 β-D-linkage is similarly seen in other hydrocolloids such as Carrageenan, agarose, and Gellan gum, however Curdlan does not possess any acidic components. The D-glucose that makes up Curdlan is similar to cellulose however, the linkages differ.[83]

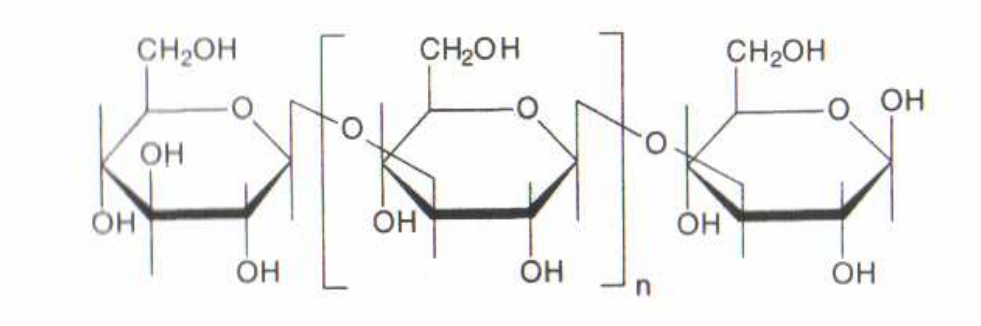


Figure 4: The Molecular Structure of Curdlan

Curdlan, in its solid state, may exist in a triple helix shown by 13C NMR analysis (Nishinari 2000). It is in granular form similar to that of a donut shape. Curdlan is not water soluble although it can be dissolved in aqueous NaOH because of the ionization of hydrogen bonds. Upon introduction to aqueous NaOH, the bonds are broken due to swelling and the granular structure of Curdlan is lost.[84]

8.2 Properties

Curdlan gum is not soluble in water, alcohol, and most organic solvents, however it is soluble in alkaline solutions such as sodium hydroxide and trisodium phosphate. Ogawa et al studied Curdlan conformation in various concentrated alkaline solutions. At low concentrations of sodium hydroxide (below 0.19 N NaOH), Curdlan has an ordered (helical) conformation, however a significant change is seen once the sodium hydroxide concentration is increased to a concentration between 0.19 and0.24 N NaOH. At a concentration higher than 0.2 N NaOH, Curdlan is fully soluble and has a random structure. However, once this solution is neutralized, the soluble turns to an order state which consists of single and triple helices. Curdlan is a linear homopolymer chain, but forms complex tertiary structures believed to be caused by intramolecular and intermolecular hydrogen bonding. It is these hydrogen bonds that may confirm why Curdlan is not soluble in water. It acts somewhat similar to cellulose. Although Curdlan is not soluble in water, an aqueous solution containing curdlan can form a gel depending on the heating temperature.[85]

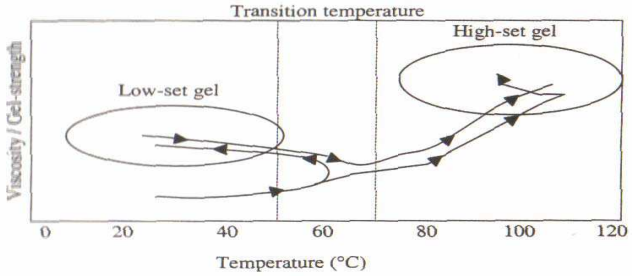


Figure 5: Effect of temperature on viscosity and gel strength (adapted from Nakao 1997)

Two types of Curdlan gels can be formed: a low-set gel and a high-set gel. The low-set gel, which is thermo-reversible, can be created one of three ways: addition of the cations Ca^{++} or Mg^{++}, neutralization of an aqueous alkaline solution of Curdlan, or by heating a Curdlan solution between 55 and 60ºC and then cooling it below 40ºC. A low-set Curdlan gel exhibits properties similar to those of Carrageenan and agar-agar. The Curdlan molecules swell around 55ºC which results in partial rupture of intramolecular hydrogen bonding [86]. Therefore upon cooling, new hydrogen bonds cross-link Curdlan micelles, which are occupied by molecules of a single-helix. If the aqueous solution is heated to temperatures exceeding 80ºC a high set gel will result, which is also identified as a thermo-irreversible gel. The high-set Curdlan gel is a much stronger and more resilient gel then the low-set gel. The texture of the high-set gel is described as being between a soft, elastic gel as seen with gelatin and a hard brittle gel seen with agar-agar. It is believed the molecular mechanism for a high-set gel results from cross-linking between Curdlan micelles with hydrophobic interactions. [87] The Curdlan micelles are occupied by molecules of multiple-chain helix or triple stranded helix. The high set gel is stable at low temperatures, such as freezing as well as at high temperatures, as seen in processing conditions such as retorting. It is also resistant to enzymatic and acidic hydrolyses It is possible to change a low set gel into a high-set gel by increasing the temperature to 80ºC. Either type of Curdlan gel does not add any color, smell, or taste to products, however, can make a profound difference when added in small amounts. A general property of Curdlan gel is as the concentration of Curdlan is increased, the gel strength is also increased. For higher strength gels, a higher concentration of Curdlan is needed, however, if a low strength gel is needed; only a minimal amount of Curdlan is needed. Gel strength is not only dependent on the concentration of Curdlan, but on the heating temperature and time as well. Gel strength increases with increasing temperature as well as heating time. In addition to Curdlan concentration, heating time and temperature, gel strength is stable over the pH range of 2-10.[88] There are no significant effects on gel strength with addition of inorganic salts.

8.3 Pharmaceutical Applications

Curdlan, which is not soluble in water, can be made into a gel if heat is applied. The distinguishing characteristic of Curdlan is that no other conditions need to be met to induce gel formation, such as pH, sugar concentration, and cation presence. Before heating though, the curdlan needs to be properly dispersed in water. Usually a high speed mixer or homogenizer would be sufficient. There are many potential uses for Curdlan gel as it is a tasteless, odorless, and colorless gel. The gel is able to withstand the temperature extremes in the case of freezing and retorting. The safety of Curdlan has been assessed in animal studies and *in vitro* tests and it is approved in food use in Korea, Taiwan and Japan as an inert dietary fibre. It is registered in the USA as a food additive. The rats were feeded with Curdlan, the faecal mass increased significantly as the amount of lactic acid in the faecal content. The significant increase in the mass of the caesium was accompanied by a decrease in faecal mass. The transit time of the gastrointestinal tract was extended by Curdlan supplementation. Significant decrease was observed in the total hepatic cholesterol and low values were measured in the proportion with secondary bile acids. All those parameters revealed that Curdlan is easily degraded and fermented by intestinal bacteria in the caesium and lowers cholesterol concentration in the liver.[89] But still bacterial degradation studies of Curdlan as a polysaccharide for colon-specific drug delivery yet to be reported. Masumi Sato, et al has found that the dialysis of Curdlan dissolved in alkaline solution into aqueous solutions of metal salts yielded multifold gel structures. Aqueous sodium chloride and potassium chloride as well as pure water induced isotropic gels. Aqueous calcium salts induced liquid crystalline gel with refractive index gradient/amorphous gel alternative structure. Aqueous salts of trivalent aluminum and ferric cations induced a rigid liquid crystalline gel, which shrank above a threshold concentration of

each salt. On the other hand, Lies gang ring-like pattern was observed with aqueous solutions of mixed salts of calcium chloride and magnesium chloride.[90]

Kanke, M, et, al was studied in vitro of sustained release suppositories To prepare the suppositories, indomethacin, prednisolone or salbutamol sulfate was mixed with Curdlan gel. Preparation conditions, including heating time and Curdlan concentrations of 5 and 10%, had little effect on the drug release. The tonicity (hypotonic or isotonic) of the media for the suppository preparation and for in vitro drug release study also had little effect on drug release. However, the heating temperature during gel preparation, the drug amount in the suppository and the type of release media did affect drug release. It was found that drug release was sustained and diffusion-controlled in the three drugs. And finally, Curdlan can be applicable for use in a sustained release suppository.[91]

KunNa,et,al was prepared self-assembled Hydrogels nanoparticles were synthesized from carboxymethylated (CM)-Curdlan, substituted with a sulfonylurea (SU) as a hydrophobic moiety for self-assembly. The degree of SU substitution was 2.4, 5.6, or 7.2 SU groups per hundred anhydroglucose units of Curdlan. The loading and release of all-*trans* retinoic acid (ATRA) was studied. The ATRA loading efficiencies and loading contents of CM-curdlan/SU nanoparticles increased as the degree of SU substitution increased. The ATRA release rate was controlled by the degree of substitution and drug-loading. For specific interaction with a hepatic carcinoma cell line (HepG2), CM-Curdlan was additionally conjugated with lacto-bionic acid (LBA; galactose moiety). HepG2 was strongly laminated by legend–receptor interactions with fluorescence-labeled LBA/CM-Curdlan/SU hydrogel nanoparticles. The luminescence was not observed for other control cases. It is concluded that LBA/CM-Curdlan/SU hydrogel nanoparticles are a useful drug carrier for the treatment of liver cancer, because of the potential immunological enhancement activities of CM-Curdlan in the body, the legend–receptor mediated specific interactions, and the controlled release of the anti-cancer drug.[92]

Y.M. Lo, K.L. Robbins studied the diffusion properties of Curdlan gels were characterized against its viscoelastic behavior. With increased junction zones involved in rigid crosslink's in higher concentration gels, the three-dimensional structure of Curdlan is characteristic. As the gel concentration increases, the intermolecular cross linking and apparent viscosity increase, reducing mobility of the polymer chains with slower release rates. The scaling laws confirmed that Curdlan gel network structure corresponds to that of polymer chains in a good solvent at the semi-dilute regime. Diffusion of the entrapped agent in 6% gels resembled pseudo-controlled release behavior, and a further increase in gel concentration might produce microspheres that support a sustained release. The network of higher concentration curdlan gels exhibits a greater degree of tortuosity, providing more resistance to solvent and solute transport. The intermolecular cross-linking and apparent viscosity increase with increasing gel concentration, resulting in reduced mobility of the polymer chains and slower release rates. With the entrapped agent distributed homogeneously throughout the non-eroding Curdlan microspheres, 2 significant diffusion stages were observed. By modeling the interaction of 2 dimensionless groups—the concentration number and the diffusion number the fractional release of dye from the microspheres was characterized. Whereas the pseudo-controlled release behavior in 6% gels was distinctive, a sustained release of the entrapped agent might be reached with further increase in gel concentration.[93-94]

Conclusion

Exopolysaccharides Hydrogels like Scleroglucan, Gellan, Pullulan and Curdlan as promising biomaterials in various modified drug delivery systems. These Hydrogels that have been obtained by means of different cross linking agents are suitable for release modulation from various dosage forms and their characterization, in terms of water uptake, diffusion studies rheological behaviors could evidence to use in sustained release and environmental-controlled delivery. Therefore Scleroglucan, Gellan Curdlan and Pullulan are Exopolysaccharides Hydrogels as an interesting challenge for future researches to be further investigated since these already showed interesting and in some cases very peculiar properties indicating the wide potentiality in several pharmaceutical technologies

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