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POLYSACCHARIDE USED IN CONTROLLED DRUG DELIVERY SYSTEM- A REVIEW

Gurjar P.N.*, Tare H.L., Dama G.Y., Thorave A.D.
SGMSPM's Sharadchandra Pawar College of Pharmacy, Otur, Pune, (M.S.), India

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For Correspondence:

Gujjar P.N.

Dept. of Pharmaceutics,

SGMSPM's Sharadchandra Pawar College of Pharmacy, Otur, Pune, (M.S.), India

E-mail:
gujjar_pravina@rediffmail.com

ABSTRACT

Polysaccharides are polymeric carbohydrate structures, formed of repeating units (either mono- or di-saccharides) joined together by glycosidic bonds. These structures are often linear, but may contain various degrees of branching. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. When all the monosaccharides in a polysaccharide are the same type the polysaccharide is called a *homopolysaccharide*, but when more than one type of monosaccharide is present they are called *heteropolysaccharides*. Polysaccharides have shown great value in drug delivery system one key reason is their hydrophilicity which makes them compatible with the aqueous environment in living thing. Thus, use of polysaccharides in formulation is one great choice for manufacturers instead of synthetic polymers.

Introduction

Polysaccharides consist of the repeat unit of monosaccharide or there derivatives, held together by glycoside bonds. It Contain about 20 monosaccharide units, thus high Molecular weight about more than 5000 units. Polysaccharides occur intracellular as large clusters or granules.They are usually tasteless (non Sugar) & Form Colloids with Water. Polysaccharides are linear as well as branched polymers. The occurrence of branches in Polysaccharides is due to the fact that glycosidase linkage can be formed at any one of the hydroxyl groups of monosaccharide.

1. Homopolysaccharides (Homoglycans):

They contain monosaccharides units of single type & may be represented by a general formuly (C6H10O5)n. They are named base on the nature of the monosaccharides unit. Thus glucans are polymer of glucose where as fructosans are polymer of Fructose **(A.C. Deb;2001).**

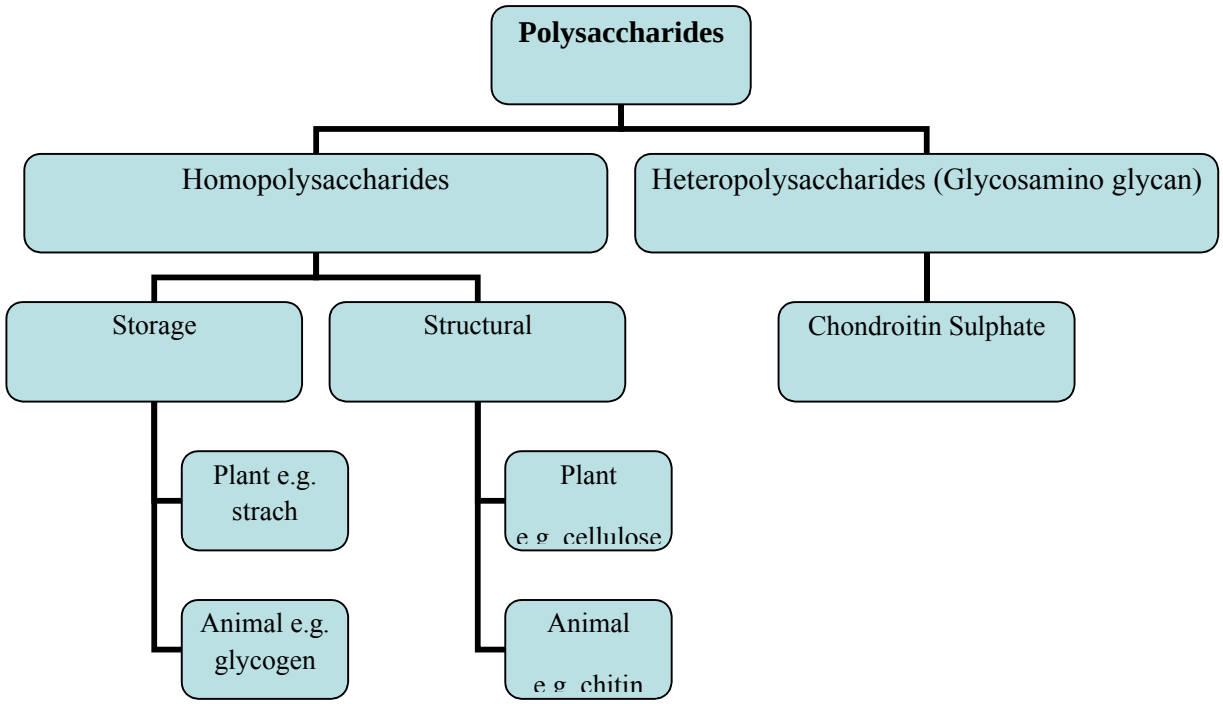
2. Heteropolysaccharides :

Heteropolysaccharides on hydrolysisyields a mixture of a few monosaccharides or their derivatives.e.g. Mucopolysaccharides

- **Advantages of Polysaccharides :**

1. Biodegradable
2. Non-toxic
3. Renewable Materials
4. Excellent biocompatibility

Classification



Controlled drug delivery system:

Controlled drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically for a specified period of time.The chief objective of most product should be controlled delivery to reduce dosing frequency to an extent that once daily dose is sufficient for therapeutic management through a uniform plasma concentration at steady-state.

Advantages

1. Improved patient convenience and compliance due to less frequent drug administration.

2. Reduction in fluctuation in stead-state level and therefore better control of disease condition

3. Increased safety margin of high potency drug due to better control of plasma level.

The controlled release dosage forms are so designed that they release the medicament over prolong period of time usually longer than typically dosing interval for a conventional formulation. The drug release rate that should should be so monitored that a steady plasma concentration is attained by reducing the ratio Cssmax/Cssmin while maintaining the drug level with in the therapeutic window.The rate controlled step in drug input should be determined not by the absorption rate but by rate of release from the formulation which ideally should be slower than rate of absorption.

The rate of drug release from such system should ideally be zero order or near zero order.

Oral Controlled Release System

It is most popular and successful used because of convenience and ease of administration.

This system classified by

1. Continuous release system.
2. Delayed transit and continuous release system.
3. Delayed release system.
4. Dissolution controlled release system.
5. Matrix (or monolith) dissolution controlled system.
6. Encapsulation/coating dissolution controlled system.
7. Diffusion controlled release system.
8. Matrix diffusion controlled system.
9. Reservoir Device.
10. Dissolution and diffusion controlled release system.
11. Ion-Exchange Resin drug complex.
12. PH independent formulation.
13. Osmotic pressure controlled system.
14. Osmotic pressure controlled system.-
15. Hydrodynamic presser controller system.
16. Mucoadhesive system.
17. Altered density system.
18. Size based system.
19. Intestinal release system.
20. Colonic release system.

The purpose of in-vivo bioavailability study on controlled release formulation is to determine,

1. The fraction of drug absorbed (should ideally be less then or equal to 80% of conventional release dosage form).
2. Occurrence of dose dumping.
3. Influence of food as well as circulation effect on drug absorption.
4. The time period for which please concentration stays within the therapeutic occupancy time.
5. C max/C min ratio at steady state.

Disadvantages

1. Decrease systemic availability in compare to immediate release dosage form due to incomplete release.
2. Poor in vivo and vitro correlation.
3. Higher cost of formulation.

Retrial of drug is difficult in case of toxicity, poisoning, or hypersensitivity reaction.

(Brahamkar. et al.1999)

Polysaccharides in Controlled Drugs Delivery System

Polysaccharides have shown great value in drug delivery systems one key reason is their hydrophilicity which makes them compatible with the aqueous environments in living things. Water-soluble polysaccharides, or hydrocolloids, have an additional extremely valuable feature – an ability to be cross linked in aqueous solution. Cross linking transforms the initial liquid of relatively low viscosity, depending on polymer concentration, to a highly swollen semisolid, or hydrogel, with elastic properties, or points in between with intermediate degrees of visco-elastic properties. Cross linking can occur under a variety of conditions depending on the particular polysaccharide. In a, the crosslinking of alginate was exploited for commercial, non-drug applications. crosslinkable hydrocolloids are used for delivery of ophthalmic drugs (such as glaucoma treatment).

Thus, a low-viscosity, aqueous solution of a drug and polysaccharide is topically administered to the eye where the liquid immediately forms a gel due to different pH and ionic strength conditions. The drug is thereby able to be administered accurately and diffuse from the semisolid hydrogel in a controlled fashion. A recent invention has improved delivery performance.

Some ionic polysaccharides can gel simply by a change in pH but often that involves a relatively large pH change which can be irritating to the eye. A milder gelation process is achieved by cross linking of certain polysaccharides, which contain cis-diol units, with borate ions. This cross linking occurs at near-neutral pH and only requires a change in pH of the initial drug/polysaccharide solution of 0.5 to 1.0 units.

Commercially available polysaccharides which possess the cis-diol unit and show the desired performance are galactomannans, polymers of mannose with galactose side groups. Examples of galactomannans, are guar gum, locust bean gum,

all produced naturally in plants and each possessing different ratios of galactose to mannose. Softer gel properties are obtained using partially substituted galactomannan derivatives, e.g., hydroxypropyl ethers. The mechanism of crosslinking involves formation of borate ester bonds between the polymer chains. The ester bonds of cis-diols are much more stable than single hydroxyl groups and afford effective crosslinks. The extent of crosslinking and thus the strength of the gel is controlled by the ratio of polysaccharide to borate. Control of gel strength is not as easily obtained with one-part, ionic polysaccharide gels. Overall, the borate-crosslinked-guar drug delivery system produces a clear viscous gel when applied to the eye from an initial low viscosity, pH-neutral liquid. Also, note that the same basic borate-guar chemistry is used on a MUCH larger scale in the oil drilling industry to create viscoelastic liquids to improve oil recoveries.

Polysaccharides are a classification of polymers that are widely used in drug delivery. They are polymers whose monomers are monosaccharides. Monosaccharides are the simplest form of carbohydrates, with a chemical formula of $(CH_2O)_n$. They are classified as ketones or aldehydes, depending on their structure and can be formed in chains or rings. The monosaccharides polymerize and are linked together through an oxygen atom to form polysaccharides.

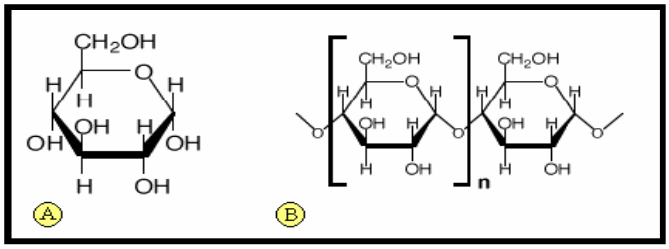


Figure : A) Typical monosaccharide B) Illustration of monosaccharide linkage

Polysaccharides are very useful in drug delivery applications because they have excellent biocompatibility and biodegradability. Polysaccharides are biocompatible because they are natural polymers. Due to their chemical makeup, they are biodegradable into simple digestible sugars in the human body. These characteristics make them very useful for drug delivery applications**.**

(Rotureau,et.al;2005).

Polysaccharide Structure

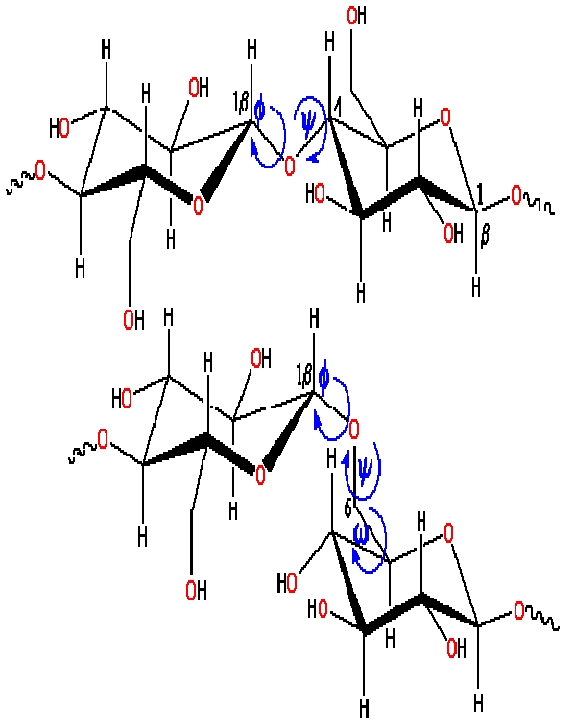
Polysaccharides have been proposed as the first biopolymers to have formed on Earth . They are classified on the basis of their main monosaccharide components and the sequences and linkages between them, as well as the anomeric configuration of linkages, the ring size (furanose or pyranose), the absolute configuration (D- or L-) and any other substituents present. Certain structural characteristics such as chain conformation and intermolecular associations will influence the physicochemical properties of polysaccharides. The most stable arrangement of atoms in a polysaccharide will be that which satisfies both the intra- and inter-molecular forces. Regular ordered polysaccharides, in general, are capable of assuming only a limited number of conformations due to severe steric restrictions on the freedom of rotation of sugar units about the interunit glycosidic bonds. There is also a clear

correlation between allowed conformations and linkage structure. The structural non-starch polysaccharides, such as cellulose and xylan, have preferred orientations that automatically support extended conformations. Storage polysaccharides such as the chains in amylopectin tend to adopt wide helical conformations. The degree of stiffness and regularity of polysaccharide chains is likely to affect the rate and extent of their fermentation. Pentose sugars such as arabinose and xylose can adopt one of two specific conformations, furanose rings (often formed by arabinose) that can oscillate and are more flexible, and pyranose rings (usually formed by xylose and glucose) which are less flexible. Cereal arabinoxylans are composed of β-linked xylan chains and are relatively stiff molecules with extended conformations. The flexibility of arabinoxylans is decreased with increasing arabinosylation, but the key parameter is likely to be the distribution of these side-chains along the backbone since this will have the most direct effect on conformation. Also, due to their extended conformation, arabinoxylans exhibit a very high viscosity in aqueous solution. Pectins, containing galacturonic acid residues, form more flexible extended conformations and also have regular "hairy" regions with pendant arabinogalactans. Carbohydrates, especially those containing large numbers of hydroxyl groups, are often thought of as being hydrophilic but they are also capable of generating apolar surfaces depending on the monomer ring conformation, the epimeric structure, and the stereochemistry of the glycosidic linkages. Apolarity has been shown for dextrin, α-(1⟶4)-linked glucans, while dextrans, α-(1⟶6) glucans, and cellulose, β-(1⟶4)-glucans, are much less hydrophobic (in solution) and unable to project an apolar surface. Hydrophobicity will also be affected by the degree of polysaccharide hydration, particularly the amount of intra-molecular hydrogen bonding. Hydrophobicity will affect their availability for fermentation in the gut, and their binding to bile acids.

Polysaccharides are more hydrophobic if they have a greater number of internal hydrogen bonds, and as their hydrophobicity increases there is less direct interaction with water. Carbohydrates contain hydroxyl (alcohol) groups that preferentially interact with two water molecules each if they are not interacting with other hydroxyl groups on the molecule. Interaction with hydroxyl groups on the same or neighboring residues will necessarily reduce the polysaccharide's hydration status. β-linkages to the 3- and 4- positions in mannose or glucose homopolymers allow strong inflexible inter-residue hydrogen bonding, so reducing polymer hydration, and giving rise to rigid inflexible structural polysaccharides whereas α-linkages to the 2-, 3- and 4- positions in mannose or glucose homopolymers give rise to greater aqueous hydration and more flexible linkages .

Sugar residues have a specific conformation, often the so-called $^4C_1$ chair conformation. This is illustrated on the right below where the ring oxygen is at the back, the 4-carbon is 'up' and the 1-carbon is 'down'. Conversely, furanose rings can oscillate and have a more flexible structure than pyranose rings, which means that they are less

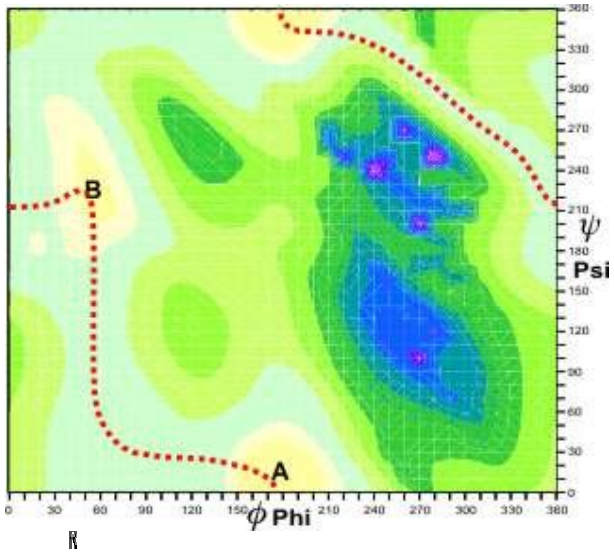
likely to have a fixed interaction with a molecule of water as energy will be lost in this process.

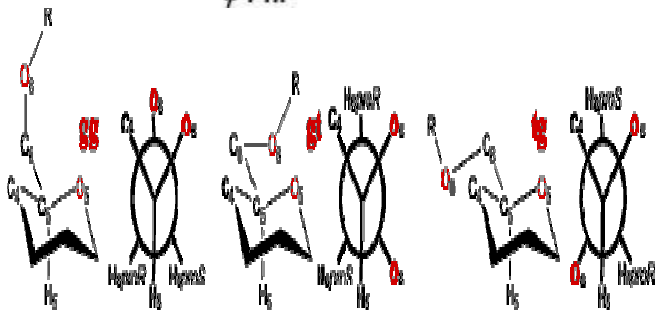


The flexibility of polysaccharide chains depends on the ease of rotation around the anomeric links

Rotation changes the energy of the structure and this can be visualized as a potential energy map (as shown for a β-(1⟶4)-xylan). In this case there are two main potential energy minima (at A and B) and the molecule can be seen to be rather flexible, with a low-energy route (shown in red) between them. Such differences in conformation can lead to effects on viscosity.

Polysaccharide linkage through the methyl hydroxyl group (for example, in α-(1⟶6) linked dextrans) are more flexible due to the extra degree of freedom in the link (ω). Such molecules often prefer trans conformations, around this bond, relative to one of the three other bonds neighboring the linking carbon atom (for example,





Examples Of Polysaccharides

1. Dextran

Dextran is a glucose polysaccharide. Structurally it consists of an α - D-1,6-glucan linked backbone with side chains forming off the oxygen atom bonded to carbon number three. The degree of branching is approximately 5% with each side chains typically 1-2 glucose units in length.

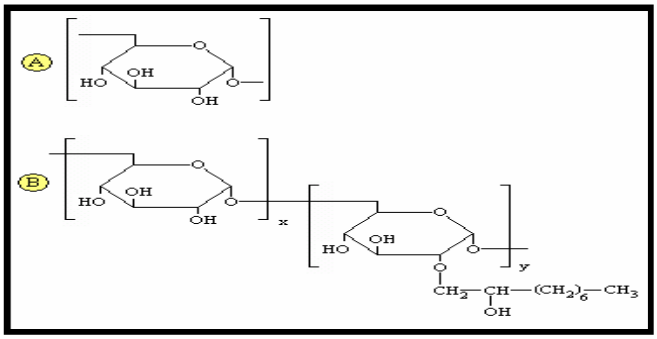


Figure : Chemical structure of A) Dextran monomer B) modified DexC6 polysaccharide

Dextran is produced in a laboratory by the bacterium Leuconostoc mesenteroids B512F during fermentation of sucrose from sugar beets (Amersham). The Dextran polymer is then divided into fractions based on average molecular weight. Dextran polymers range in molecular weight from 1,000-200,000 daltons. Typical fraction notation is the molecular weight divided by 1,000, therefore a Dextran fraction with an average molecular weight of 40,000 would be denoted Dextran 40.

Dextrans have several properties that make them good candidates for drug delivery applications. They are readily soluble in water and are neutrally charged. In a dry powder form they are stable for more than five years. However their most significant asset is their biocompatibility. Dextran may be ingested orally and is quickly digested as evidenced by an increase in blood sugar. It has been used intravenously as a blood plasma extender since the Korean War. Blood plasma extenders can help to temporarily keep trauma patients alive who have lost a lot of blood.

Dextran is also biodegradable and its byproducts are readily absorbed into the natural environment. Natural Dextran can be easily modified by substitution with functional groups to manipulate its physical properties. This study will focus on Dextran 40 reacted with epoxyoctane to form DexC6, or Dextran substituted with hexane chains, as shown in Figure 6. The hexane groups are soluble in organic materials, creating an amphiphilic polymer that gives DexC6 its surfactantproperties**. (Brannon Peppas,et.al;2005).**

2. Xyloglucan and Cellulose

Xyloglucan and cellulose have complementary abilities and work together to achieve something that neither could do alone. In naturally occurring wood, xyloglucan binds tightly and *specifically* to cellulose fibers. Cellulose fibers by themselves have very impressive strength, but an even greater composite strength is achieved when the fibers are "glued" together with xyloglucan. Xyloglucan's interaction with cellulose has also been employed in industrial applications to improve the performance of textiles and paper. Recently xyloglucan has been exploited as a vector or anchor to introduce new functional groups to cellulose surfaces and alter its properties under mild and environmentally friendly conditions.

Xyloglucan (XG) is widespread in nature in plants but is most commonly isolated from tamarind kernel powder (~60% XG) which is produced commercially on a large scale. XG actually is a group of polysaccharides defined generally as neutral, unbranched polymers of glucose with xylose pendent groups. This chemical structure is very similar to that of cellulose in that they both have the same poly(b-1,4-glucopyranose) backbone. The xylose substitution pattern along the backbone varys depending on the natural source. Certain other monosaccharides are also typically found attached to the xylose units. Whereas cellulose is highly crystalline and water-insoluble, XG is readily water soluble. However when XG is associated with cellulose as in wood, a strong complex results as evidenced by the fact that XG cannot be extracted from it with water. The complex can be broken however by extraction

with strong aqueous base. Cellulose acetate (CA) is produced by the synthetic derivatization of biologically produced cellulose. As such, it is one of the first synthetically modified, biobased polymers and has found wide utility. Acetylation of cellulose causes a dramatic change in properties. Cellulose is hydrophilic, highly crystalline, can't be melt processed due decomposition before its high melting point, and is poorly soluble in common solvents. CA is a hydrophobic, amorphous material that can be dissolved in common solvents or melted, especially after mixing with plasticizers, and can be readily processed into different forms for many applications. Its functional properties include: moisture resistance, optical clarity, high heat resistance (softening temperature), melt processability, high mechanical strength and toughness. Its combination of high clarity, isotropic transmittance (passes light equally in each direction), good moisture and temperature resistance, low cost, adhesion to high surface-energy polarizing films, and also its renewable raw material source make CA the material of choice.

Cellulose is the polymer produced in the greatest quantity in nature. It is primarily produced in plants, such as trees and cotton, but also in certain bacteria. Bacterially produced cellulose (BC) is an emerging industrial material with remarkable properties. BC is produced extracellularly by common, nonpathogenic bacteria in the form of a thick, highly hydrated, rubbery film, or pellicle. For many years it has been produced commercially in the form of a flavored gel food product, “nata de coco”. BC has properties that distinguish it from plant cellulose. It is produced without the lignin and hemicellulose that accompany plant cellulose and can be readily obtained in a very pure form by a simple washing process. The resulting BC is a highly hydrated (>99% water) network of highly crystalline, ribbonlike structures (60 nm wide) which are each composed of microfibrils (2x6 nm cross-section). The structure is very porous and readily permits the infusion of solutes and solids. This property of the native, hydrated material along with the desirable mechanical properties of the subsequently dried material have recently been exploited to produce unique composite materials.

Examples include composites containing metal particles for fuel cells, hydroxyapatite for orthopedic biomaterials, and carbon nanotubes for electrically conductive materials. Once dried, BC loses its ability to rehydrate to its initial high water content. The dried film has a very high surface area (200 times that of plant cellulose) and outstanding mechanical properties, such as stiffness which approachs that of aluminum (Young's modulus of 30 GPa, measured isotropically across surface of plane). Thus, its high purity, high crystallinity, and ultrafine network structure afford remarkable mechanical properties that have found applications in speaker diaphragm membranes, resin composites, , wound healing membranes, and artificial skin and blood vessels**.(Q.Zhou,et.al;2007).**

3. Alginate

Alginate is a versatile polysaccharide produced commercially from seaweed (20,000 tons/yr). It is primarily used as a thickener or gelling agent for aqueous mixtures (. It thus affects the flow

properties of a solution – its rheology. These properties are valuable in food preparations, pharmaceutical formulations, and specialized medical applications such as cell encapsulation. Alginate forms strong gels in the presence of divalent cations, particularly calcium, by way of ionic crosslinks between the polyanionic alginate chains. Thus, when an aqueous solution containing sodium alginate is mixed with a water-soluble calcium salt, the mixture forms a gel with elastic properties. Alginate's unique gelling properties result from its primary chemical structure. The polymer backbone is composed of two monosaccharide repeating units, mannuronate (M) and guluronate (G), which are isomers differing in configuration at the C-5 position. They can be present in different ratios and in different sized blocks of M or G units. The G-blocks form especially strong interchain crosslinks with calcium ions and are a large factor in the gel properties.

Alginate from different seaweed sources has different M/G ratios and different M and G block lengths and, as commonly occurs with natural products, has additional heterogeneity due to varying growth conditions. Recently, researchers have developed a method to synthesize alginate compositions in a controlled fashion with relatively long G-blocks at varying M/G ratios and an absence of M-blocks. These materials formed gels which showed markedly improved performance over native alginates.The structure of alginate was optimized to produce gel beads, or capsules, specifically for medical applications (cell encapsulation, tissue engineering) where strength, stability, and controlled, uniform porosity are important. It is very likely that lessons learned in this study will be valuable in alginate's industrial applications as well. Thus, a series of alginate compositions were synthesized with increasing lengths of G-blocks in a very controlled manner. Starting from a poly-mannuronate homopolymer obtained from a bacterial source, G-units were introduced by epimerization of every other M-unit using an enzyme catalyst. A second enzyme was then used to generate G-blocks by epimerization of more M units, the final content of the G-blocks depending on the reaction time. This series of alginates with increasing G-block lengths was tested and compared with two commercially available alginates with lower and more heterogeneous G-block content and at a relatively low and high M/G ratio. The new compositions affected calcium ion binding and backbone flexibility between crosslinks. Improvements in elasticity/gel strength, syneresis (shrinkage), capsule stability to treatment with saline solution, and permeability to small globular proteins were achieved relative to the two native alginate materials.

Alginate is a polysaccharide that is produced commercially from seaweed. Courtesy of its ionic substituents, alginate possesses very unique and useful properties. In particular, sodium alginate is water soluble while its calcium salts are water insoluble, although highly swelled. Conversion between the two salt forms is reversible by any ion exchange process. Aqueous sodium alginate solutions can be mixed with a second component (payload) and then gelled by introduction of

calcium ions. This material, either in wet form or after drying, can be utilized for specific purposes with respect to the payload and then at a later time, treated with a compound which exchanges sodium for calcium and the material becomes water soluble again, washed away, and releasing the payload. The calcium alginate composite is also porous and the payload may also be released by diffusion. Thus, calcium alginate can be used to immobilize and protect an active ingredient for storage and release it under subsequent use conditions. Similarly, calcium alginate can hold a component in an insoluble form while in use and then when no longer needed, it can be dissolved away. In these cases, alginate is a delivery agent with valuable material properties due to its polymeric character, such as mechanical strength and adhesion**.(A.W.J.Chan,et.al;2006)**

4. Xanthan gum

Xanthan gum is one of the most important polysaccharides in commercial use. It is used as a thickener, suspending agent, and emulsion stabilizer in food products, personal care formulations, and oil drilling. It is produced industrially by microbial fermentation and is classified as an extracellular polysaccharide as it is produced in the cell and then released into its surrounding aqueous medium. Its exceptional rheological properties include: high viscosity at low shear rates (when the liquid is barely moving), greatly reduced viscosity at high shear rates (when the liquid is stirred or poured – called shear thinning or pseudoplastic behavior), and low sensitivity to changes in pH and ionic strength. These properties are attributed to the high molecular weight chains which form rigid helical structures in aqueous solution. Shear thinning occurs as relatively weak, noncovalent intermolecular associations and chain entanglement are disrupted

Xanthan gum is produced commercially as a solid powder which must be redissolved in water prior to use in its various applications. Being a high molecular weight polymer, this is a relatively slow process and requires high-shear agitation to achieve homogeneous dispersion. Recently a new method has been developed which produces a form of xanthan gum that is rapidly and homogeneously dispersed in water. In this method a concentrated xanthan-water mixture is extruded in a twin screw extruder at 85 °C, followed by drying and grinding.

Interestingly, aqueous mixtures produced from the extruded xanthan exhibit a “particulate” behavior like a dispersion of crosslinked, highly swollen polyelectrolyte gel particles, in contrast to the molecular solution behavior of non-extruded xanthan. The aqueous dispersions reached their maximum viscosity in less than one minute of mixing (0.75% concentration) which was much faster than nonextruded xanthan. The extruded xanthan also produced a higher final viscosity than the nonextruded material at concentrations greater than 0.1% at various shear rates in pure water. At low shear rates the aqueous mixtures of extruded xanthan did not show the viscosity plateau typical of polymer solutions, but rather was consistent with a dispersion of swollen particles. The viscosity of extruded xanthan dispersions also showed a high sensitivity to salt concentrations

unlike the non-extruded material. The particulate structure reverted to a disordered coil structure, in common with that of the nonextruded xanthan, after heating to a temperature above its order/disorder transition.

Generation of the unique network structure under the high shear and high concentration conditions during extrusion was postulated as a sequence of disruption of the initial ordered structure, followed by realignment of the polymer molecules and formation of new intermolecular helical junction zones amid unordered amorphous regions. As the polymer emerges from the extruder and cools, a kinetically stable network structure is obtained. Upon heating in water, this structure reverts to the random coil of non-extruded xanthan and ultimately, after cooling, to the helical molecular solution of non-extruded xanthan. In summary, an intriguing and potentially very useful form of xanthan gum is prepared by extrusion processing which rapidly forms aqueous dispersions that possess unique rheological properties. Its properties can be returned to that of non-extruded xanthan by a heating/cooling cycle**. (N.M.Sereno,et.al;2007).**

Synthesis of polysaccharide derivatives for production of hydrogels with controlled morphology for drug delivery

Polysaccharides such as chitosan and alginate have been used for drug delivery applications due to its unique properties of biodegradability and non toxicity. Alginate (ALG) and Chitosan (CHI) can form ionic hydrogels by polyelectrolyte complexation(PEC) and have been used for drug delivery systems. However, chitosan is only soluble in acid media, restricting the drugs used in their systems once low pH may biodeactivate for example, proteins.

To overcome this limitation water-soluble chitosan derivatives have been synthesized. In this work, microspheres of carboxymethylchitosan (NOCC) hydrogels were prepared for peptides delivery. A set of experiments were carried out using ALG, CHI and/or NOCC and the composition effect on the hydrogels properties was evaluated. The reactions conditions were favorable to produce hydrogels with acceptable size and swelling behavior.

The NOCC-ALG hydrogels presented spherical morphology, similar size and lower swelling degree than CHI-ALG hydrogels. The former presented satisfactory balance of properties to be used in peptide and protein delivery systems. Keywords: hydrogels, chitosan, carboxymethylchitosan, synthesis, microspheres. Controlled drug delivery systems have been used to overcome the shortcomings of conventional drug formulations. These systems may have the ability to maintain therapeutic concentrations at a target site, reducing the chance for toxicity and collateral damage, enhancing the drug efficiency. Hydrogels of different polymers have been investigated over the last decade, and hydrogels consisting of natural polymers have been prepared due to the promissory properties of biodegradability and biocompatibility.

Alginate is a polyanionic copolymer of mannuronic and guluronic sugar residues and has been widely used in biomedical applications .

Chitosan, 2-amino 2-deoxy □-D glucan, is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells. Both polymers are biodegradable, and no toxic when administered orally, propitious for drug delivery Chitosan (CHI) and alginate (ALG) are both polyelectrolyte polymers and can produce hydrogels by ionic complexation (PEC). The PEC process requires an ionic pair of polymers, and is generally performed in aqueous solution, favoring biocompatibility and avoiding purification before administration.

However, chitosan it's only soluble in acid solution, limiting its use for peptide and protein delivery, which are readily degraded in low pH. So, various studies were conducted to make water-soluble derivatives of chitosan by chemical modification techniques. Carboxymethyl chitosan (NOCC) is a non toxic chitosan derivative which has carboxymethyl groups in some of both amino and primary hydroxyl of the glucosamine units of the chitosan structure. NOCC is an amphoteric polyelectrolyte containing both cationic and anionic fixed charges. The aim of this work was to obtain hydrogels with controlled morphology from ionically crosslinked ALG-CHI or ALG-NOCC to be used in controlled release system for peptides delivery. Initially, the chitosan derivative (NOCC) was synthesized and characterized. Afterwards, the hydrogels were prepared by polyelectrolyte complexation (PEC) at different alginate content and the pH, and their morphology and swelling degree were evaluated at different pHs.

METHODS

Preparation of ALG-CHI and ALG-NOCC hydrogels

A 2% sodium alginate aqueous solution was prepared and diluted with 0.9% NaCl solution having a final concentration of 0.2% (w/v). A 0.2% (w/v) Chitosan and 0.2% NOCC solutions in acetic buffer were also prepared. The alginate solution was extruded dropwise into a 2000 mM CaCl2 solution, in order to obtain Alginate:CaCl2 with molar ratio of 10:1. After stirring for during 30 min, the chitosan and/or NOCC solution was added dropwise into the alginate solution, at different alginate/chitosan volume ratio, producing the final product. The hydrogels were rinsed with distilled water followed by centrifugation at 3500 rpm for 20 min three times. The reaction yield was determined by weight difference of a pre-weighted flask having the wet beads before and after being in a stove at 50°C until dryness**. (Hoffman,et.al;2002).**

Improvement of simple cultivation conditions for polysaccharide synthesis by *Haemophilus influenzae* type b

H. influenza type b is a Gram-negative bacteria with a capsular polysaccharide of repeating units of polyribosylribitol phosphate (CPS-b) and it is the most important factor for its virulence [1, 2]. This Bacteria is an important cause of meningitis and others severe infections in children under five years old.

The purified CPS-b conjugated to a protein is effective vaccine for children less than five years old [3].This conjugated vaccine is expensive because there are many steps involved in the

production processes.The CPS-b has to be produced and purified; the protein (tetanus toxin or diphtheria toxin) have to be produced purified and inactivated; the CPS-b and the protein is chemically bounded and the new product has to be purified from the reagents. Improvements in any of these steps would contribute to reduce the total cost of production.

The medium culture for H. influenzae type b developed by Carty [4] is composed of soy peptone, yeast extract with a culture conditions of 0.25 volume of air per volume of medium (VVM), agitation 200 rpm, without pH control and overlay and sparged aeration.

Communicating Current Research and Educational Topics and Trends in Applied Microbiology and NAD to the medium and 100 rpm agitation without pH control [5, 6]. Recently, Merrit et al. used a medium with casamino acids, yeast extract, NAD and hemin, the pH was controlled at 7.5 and dissolved oxygen tension set at 50% (DOT 50%) with a production of 490 mg/L of polysaccharide.

The purpose of this study is the improvement of the polysaccharide production carried out in three different cultivation conditions and the best process could be used to replace the current one in order to reduce the final cost of vaccine production. This improvement was established with simple methodology like control of dissolved oxygen tension in conjunction with ph control.

Methods

Medium Composition and Preparation Medium

The cultivation medium was made according to Takagi et al [8] with modification in the hemin and NAD concentrations: 10.0 g of soy peptone (Difco, Detroit, MI), 5.0 g of dialized yeast extract (Merck), 2.5 g of K2HPO4 (Merck), 13.1 g of Na2HPO4 (Sigma, St. Louis, MO), 3.3 g of NaH2PO (Sigma), 5.0 g of glucose (Merck), 30 mg of hemin chloride (Sigma), and 15 mg of nicotinamide adenine dinucleotide (NAD) (Sigma) in a final volume of 1 L of distilled water. The pH was adjusted to 7.2 with 5.0 M NaOH. The medium was sterilized by filtration in a Millipore system with a 0.22-µm membrane previously autoclaved at 120°C for 15 min and aseptically transferred to the fermentation vessel. UCONLB 652 was used as antifoam. The bioreactor BioFlo 2000 (New Brunswick Scientific Co.) has temperature control (heater/cooler), pH electrode, oxygen probe for measurement of DOT, five peristaltic pumps to add fresh medium, acid or alkali for pH control, anti foam etc,. The bioreactor keeps the temperature, DOT and pH in the elected set values automatically. The DOT probe keeps the desired value by changing the stirring speed, the pH electrode switch-on switch-off the acid/alkali pumps to keep the pH.

Cultivations

The experiments were conducted in a Bioflo 2000 with a 13-L nominal volume, under the following conditions: stirring speed of 100–600 rpm, initial medium volume of 7.4 L, temperature of 37°C and 0.25 VVM of air.

The experiments were conducted as follows: (a) overlay aeration without pH control; (b) air sparged aeration with DOT controlled at 30% of air saturation, without pH control; (c) same as (b) with pH controlled at 7.2. A pulse of glucose to restore the initial concentration of 5g/L was added twice during the batch cultivations when glucose was near depletion.

Biomass concentration

Optical density of the culture was measured at 540 nm using a Ultrospec 100 spectrophotometer (GE Healthcare). Dry cell weight (DCW) was determined in 10-mL samples collected in preweighed tubes. After centrifugation at 3220*g* and 4°C for 60 min, the pellet was resuspended in 10 mL 0.15 M NaCl and centrifuged again. The centrifuge tube containing the cells was dried at 60°C to achieve constant weight 603. Communicating Current Research and Educational Topics and Trends in Applied Microbiology

Glucose concentration

Glucose concentration was determined in cell-free samples by the glucose oxidase method. The consumed glucose was calculated by the difference between total input and residual glucose present in every sample-collecting time.

CPS-b concentration

Samples of 10mL were withdrawn from bioreactor and centrifuged at 3220 × *g*, 4◦C for 60min. The supernatant were submitted to dialysis (membrane cut-off 12 000–14 000) against distilled water for 24 h in order to eliminate low molecular weight components from the medium. The polysaccharide concentration was determined using modified Bial method, using ribose (Sigma) as standard [10]. The concentration of CPS-b, polyribosylribitol phosphate, was estimated using a conversion factor in which 1 mg of ribose corresponded to 2.55 mg of polyribosylribitol phosphate. The value for the conversion factor was based on the polyribosylribitol phosphate structural formula.

Organic acids concentrations

The metabolic acids produced by *H. influenzae* type b were measured by high-pressure liquid chromatography (HPLC; Shimadzu Co.) with aminex HPX-87H *(*300mm × 7.8mm; Bio-Rad) type column, UV detector (210 nm) and an integrator program (class VP, version 6.2; Shimadzu Co.). An aliquot of 20 µl of cell-free supernatant, collected during cultivation, was diluted with 0.1 M H2SO4 (1:5), filtered in Millex 0.22 µm and injected into the column at 35 ◦C. A 50 mM H2SO4 solution was used as mobile phase with a flow rate of 0.6 ml/min. Organic acids standard was used to identify and quantified the peak (Bio-Rad Lab. CA, USA No. 125-0586).

Kinetic parameters

The kinetic parameters were estimated based on the results obtained from biomass formation, glucose consumption, cell-free CPS-b and organic acids production in shake flask experiments and batch cultivations. Maximum specific growth rate (µmax), calculated as slope of a plot of time versus Ln of biomass concentration. Yield coefficients YP/S and YX/S, are the conversion of substrate to products, amount of product (CPS-b) or cells obtained per gram of consumed glucose (S,

substrate), calculated as slope of plot of consumed glucose to product or cells.

Specific production is amount of product per gram of cells and the productivity amount of product or cells per volume and per time (P/L/h). All parameters were estimated at the end of the growth, when the maximum CPS-b concentration had been reached. The datas collected from the two independent fermentation batches were statistically processed at the 95% confidence interval**.(C.W.Norden,et.al;1982).**

Conclusion

Polysaccharide have shown great value in drug delivery system one key reason is their hydrophilicity which makes them compatible with the aqueous environment in living thing. Water soluble polysaccharide or hydrocolloids have an additional extremely valuable feature an ability to cross linked in aqueous solution. Cross linking can occur under variety of condition depending on particular polysaccharide. Polysaccharide is naturally occurring substance. It has greater advantages over synthetic material like non toxicity, biodegradability, excellent biocompatibility etc. Due to natural source, it is sufficiently available in nature. Thus, use of polysaccharide in formulation is one more choice for manufacturer instead of synthetic polymer. Naturally occurring polysaccharide reduces the cost of production.

References

- A.J. Shuhendler, R. Cheung, J. Manias, A. Connor, A.M. Rauth, X.Y. Wu (2009) A novel doxorubicin-mitomycin C co-encapsulated nanoparticle formulation exhibits anti-cancer synergy in multidrug resistant human breast cancer cells, *Breast Cancer Res. Treatment*, in press.
- Amersham Biosciences. "Dextran Data File." 9 Feb. 2006 http://www.dextran.nu/media/18-1153-41AA.pdf.
- Brannon-Peppas, L. "Polymers in Controlled Drug Delivery." Medical Plastics and Biomaterials Magazine Nov (1997): 34. 30 Jan. 2006.
- Drug Release from Biodegrading Microspheres, J. Pharm Sci 1997; 86(12): 1464
- G. Ashwell in Methods of Enzymology, Vol. III, edited by S.P. Colwick and N.O. Kaplan. Academic Press,New York, USA, p 87–90, (1957).
- H.L. Wong, X.Y. Wu, R. Bendayan (2009) Multidrug resistance in solid tumors and its reversal, in: Pharmaceutical Aspects of Cancer Chemotherapy (on invitation), in press.
- http://www.devicelink.com/mpb/archive/97/11/003.html.
- http://www.touchbriefings.com/pdf/17/ACF9CFB.pdf
- J. Liu, D. Meisner, E. Kwong, X.Y. Wu, M.R. Johnston (2009) Trans-lymphatic delivery of paclitaxel by intrapleural placement of gelatin sponge impregnated with PLGA-paclitaxel microspheres effectively controls lymphatic

metastasis in an orthotopic lung cancer model, *Cancer Research*, in press.

- J. Merrit, G. Allard, L. O'Toole, R. Swartz and P. Licari. Journal of Biotechnology 81, 189, (2000).
- M. Takagi, J. Cabrera-Crespo, T.C. Zangirolami, I. Raw and M.M. Tanizaki. Journal of Chemical Technology and Biotechnology 81, 182, (2006)

- N.M. Sereno,S.E. Hill and J.Mitchell, Carbohydrate research 2007.(343,1333,1342)
- Optimization,Applications. New York,NY: CRC Press: 1992

- Q.Zhou,M.W. Rutland, TT. Teeri and H. Brumer Cellulose 2007(14,625,641)

- Rotureau, E., M. Leonard, E. Dellacherie, and A. Durand. "Amphiphilic derivitives of dextran: Adsorption at air/water and oil/water interfaces." Journal of Colloid and Tnterface Science 279 (2004): 68-77.

- Rouzes, C., A. Durand, M. Leonard, and E. Dellacherie. "Surface Activity and Emulsification Properties of Hydrophobically Modified Dextrans." Journal of Colloid and Interface Science 253 (2002): 217-223.

- Rouzes, C., M. Leonard, A. Durand, and E. Dellacherie. "Influence of polymeric surfactants on the properties of drug-loaded PLA nanospheres." Colloids and Surfaces B: Biointerfaces 32 (2003): 125-135.

- Stevens, J., Mims, P., and Coles, N. Lipid Emulsions as Drug Delivery Systems. Fresenius Kabi Clayton L.P. 2003. 23 Feb. 2006.

- Surfactants Derived from Dextran: A Study of Their Aqueous Solution Behavior." Macromolecular Chemistry and Physics 206 (2005): 2038-2046. Wikipedia. "Micelle." 16 Feb.2006 <http://en.wikipedia.org/wiki/Micelle>.63