

POLYSACCHARIDE NANOPARTICLES: PREPARATION AND THEIR POTENTIAL APPLICATION AS DRUG DELIVERY SYSTEMS

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ABSTRACT

There is an increasing interest in using nanoparticles, and in particular polysaccharide-based ones as carriers for the delivery of chemotherapeutic agents. They are also being investigated for enhancing their blood circulation time thereby resulting in increased therapeutic efficiency. Natural Polysaccharides as drug delivery systems, have received the most attention, owing to their benefits, which include their biodegradability, upgradability, biocompatibility, multiple reacting groups and low cost. This results in polysaccharides being seen as the materials with the highest promise in preparation of nanometric carriers. In this review, polysaccharides-based nanoparticles and their connections with drugs were analyzed. The different methods that are adopted to prepare polysaccharides-based nanoparticles were enumerated and finally with a discussion on the potential for these nanoparticles in controlled drug delivery and biomedical imaging.

KEYWORDS: Polysaccharides, Nanoparticles, Chitosan, Drug Delivery System

INTRODUCTION

The major problem in treatment of many diseases is the delivering of therapeutic compound to the target site. A conventional application of drugs is characterized by, poor biodistribution, lack of selectivity and limited effectiveness (Nevozhay et al. 2007). These drawbacks can be overcome by controlling drug delivery. In controlled drug delivery systems (DDS) the drug is carried to the place of action; thus, its impact on vital tissues and undesirable side effects can be minimized. Moreover, DDS safeguard the drug from clearance or rapid degradation and increases drug concentration in target tissues; hence, minimum doses of drug are required (Nevozhay et al. 2007). This modern form of therapy is especially important when there is a discrepancy between a dose and concentration of a drug and its therapeutic results or toxic effects. By attaching drugs to individually designed carriers cell-specific targeting can be achieved. It has been reported recently that nanoparticles (structures smaller than 100 nm in at least one dimension) have a great potential as drug carriers. Because of their small sizes, the nanostructures display unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that formulate them a desirable material for biomedical applications.

NANOCARRIERS AS DRUG DELIVERY SYSTEM

As per NNI (National Nanotechnology Initiative) definition, nanoparticles are structures of sizes ranging from 1 to 100 nm in minimum one dimension. However, particles that are up to several hundred nanometers in size also commonly prefixed as “nano”. Nanocarriers with standardized physicochemical and biological properties are taken up by cells more obviously than larger molecules, so they can be successfully used as delivery tools for the available bioactive

compounds (Suri et al. 2007). Liposomes, solid lipids nanoparticles, silicon or carbon materials, polymers, dendrimers, and magnetic nanoparticles are the examples of nanocarriers that have been tested as drug delivery systems.

Nanoparticle drug delivery systems have wide range of advantages : (1) Because of their small size, they can easily pass through the smallest capillary vessels and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged; (2) they can penetrate cells and tissue gaps to arrive at target organs such as liver, spleen, lung, spinal cord and lymph; (3) they could show controlled release properties due to the biodegradability, pH, ion and/or temperature sensibility of materials; (4) they can improve the utility of drugs and reduce toxic side effects; etc.

The way of conjugating the drug to the nanocarrier and the strategy of its targeting is very important for a targeted therapy. A drug may be adsorbed or covalently attached to the nanocarrier's surface or else it can be encapsulated into it. When compared to other ways of attaching, covalent linking has the advantage, as it enables to control the number of drug molecules connected to the nanocarrier, i.e., an accurate control of the amount of therapeutic compound delivered. Cell-specific targeting with nanocarriers may be accomplished by using active or passive mechanisms.

Once the drug-nanocarrier conjugates reach the diseased tissues, the therapeutic agents are released. A controlled release of drugs from nanocarriers can be achieved through changes in physiological environment such as temperature, pH, osmolarity, or *via* an enzymatic activity.

POLYSACCHARIDES AS A SOURCE OF NANOPARTICLES

Nanocarriers used for medical applications have to be biocompatible (able to integrate with a biological system without eliciting immune response or any negative effects) and nontoxic (harmless to a given biological system). Many polymeric materials which are biocompatible and biodegradable are used in preparing nanoparticles for drug delivery, which include poly (lactic acid), poly (glycolic acid), polycaprolactone, polysaccharides (particularly chitosan), poly (acrylic acid) family proteins or polypeptides (such as gelatine). Among these systems, the role of natural polysaccharides in developing prepared nanoparticles has significantly increased (Zhang et al. 2011; Yang et al. 2008a; Aumelas et al. 2007; Leonard et al. 2003).

Polysaccharides are long carbohydrate molecules of repeated monosaccharide units joined together by glucosidic bonds and are often one of the main structural element of plant and animals exoskeleton (cellulose, carrageenan, chitin, chitosan, etc.) or have an important role in plant energy storage (starch, paramylon, etc; Aminabhavi et al, 1990). In nature, polysaccharides are available from various resources like plant (eg. Pectin, guar gum), animal (chitosan, chondrotin) algal (eg. Alginate) and microbial origin (eg. Dextran, xantha gum) (Sinha and Kumria, 2001).The majority of natural polysaccharides present are hydrophilic groups such as carboxyl, hydroxyl and amino groups, which endow their solubility in water and the formation of non-covalent bonds with biological tissues and mucosal membranes (Liu et al. 2008). This way, the hydrophilic properties of most of the polysaccharide nanoparticles provide bioadhesion and mucoadhesion characteristics to these biomaterials, as well as giving the possibility of chemical modification of the macromolecules to bind drugs or targeting agents. The hydrophilic nanoparticles also possess the enormous advantage of extended circulation in blood, which increases the probability of passive targeting of the nanoparticles into the tumour tissues (Mitra et al. 2001).

The most profitable use of polysaccharides as natural biomaterials is their availability in nature and low cost in

processing, which makes them quite accessible materials to be used as drug carriers. Moreover, they are highly stable, safe, non-toxic, biodegradable and hydrophilic (Liu et al. 2008). Thus, they have a large variety of composition and properties that cannot be replicated in a chemical laboratory, and the ease of their production makes various polysaccharides cheaper than synthetic polymers (Coviello et al. 2007). Therefore, the use of polysaccharides as biomaterials is quite promising in terms of biomedical, environmental and food-related fields and even pharmaceutical applications (Lemarchand et al. 2005; Rinaudo 2008; Park et al. 2010).

Recently, many studies have been using polysaccharides and their derivatives for their potential application as nanoparticles drug delivery systems. The most commonly used ones are chitosan, alginate, hyaluronic acid, pullulan, pectin, cellulose, dextran, and guar gum (Mizrahy et al. 2012; Luximon 2011; Boddohi et al. 2009; Liu et al. 2008; Sarvanakumar et al. 2012). A brief description of some of the characteristics and techniques used to prepare polysaccharide-based nanoparticles are discussed subsequently.

Characteristics of Some of The Polysaccharides

An enormous number of polysaccharides have been in use as drug delivery systems. The characteristic features and applications in various fields of some commonly used polymers are discussed below. This is done emphasizing their role in preparation of drug delivery systems.

Chitosan

Chitosan has appeared the most promising biomaterial for the development of ideal hydrophilic drug vehicles for the controlled drug delivery and therefore has been rigorously investigated over the last two decades (Felt et al. 1998; Janes et al. 2001; Mitra et al. 2001). Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Fig.1). The average molecular weight of industrially produced chitosan is between 3.8 - 20 kDa. Chitosan is positively charged at neutral and alkaline pH. It is a weak base and it is insoluble in water and organic solvents. However, it is soluble in diluted aqueous acidic solution (pH <6.5), which can convert the glucosamine units into a soluble form with protonated amine groups (Sinha et al. 2004). The solubility of chitosan in water can be increased by removing one or two hydrogen atoms from the amino groups of chitosan, and introducing some hydrophilic segments (Srinophakun and Boonmee 2011).

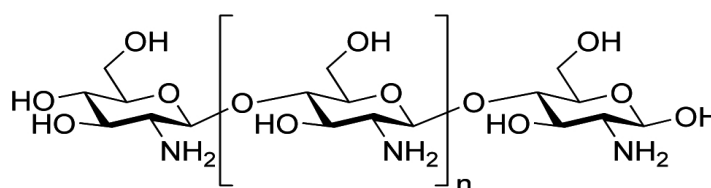


Figure 1: The Chemical Structure of Chitosan

The various biological characteristics of chitosan such as low or non toxicity, biocompatibility, biodegradability, low antimicrobial and immunogenic properties, provide its potential for various applications (Guerrero et al. 2010). Its rare positive charge converts chitosan into a special polysaccharide, since it provides strong electrostatic interaction with negatively charged mucosal surfaces and macromolecules such as DNA and RNA (Fang et al. 2001; Morille et al. 2008), which is an attractive feature for the treatment of solid tumours (Li et al. 2009). The polyelectrolyte nature of chitosan, can

be used as an absorbent of heavy metal ions and textile industry effluents from waste waters. It has been also used as template for the preparation of mesoporous metal oxides spheres (Braga et al., 2009). It is also reported to find uses as an antimicrobial compound, as a drug in the treatment of hyperbilirubinaemia and hypercholesterolaemia and, also, it has been prepared and evaluated for its antitumour activity carrying several antineoplastic agents (Blanco et al. 2000).

Chitosan has attracted attention as a matrix for controlled release in the field of nanomedicine, due to its reactive functionalities, polycationic nature, easy degradation by enzymes and non-toxic degradation products. From long, various natural and synthetic polymers have been used for the preparation of drug-loaded microparticles; out of those chitosan found to be very promising and has been extensively investigated (Davidenko et al. 2009; Muzzarelli and Muzzarelli, 2005). Because of its bioadhesive properties, chitosan has received significant attention as carrier in novel bioadhesive drug delivery systems which increase the residence time of the drugs at the site of absorption and thereby increase the drug bioavailability (Varum et al. 2008). Hence, some drugs administered via nasal (Learoyd et al. 2008) or gastrointestinal routes have improved their treatment efficacy when they are included into chitosan-based systems (Guerrero et al. 2010).

Considering the various advantages of chitosan, it is found to be a promising matrix for the controlled release of pharmaceutical agents. Both *in vitro* and *in vivo* experiments have clearly demonstrated chitosan as an ideal carrier for a variety of drugs whose effectiveness is increased when they are admitted into these systems.

Dextran

It is a polysaccharide consisting of many glucose molecules composed of chains of different lengths. It has a significant amount of α 1-6 glucosidic linkages in its main chain (Fig.2) and a variable number of α (1-2), α (1-3) and α (1-4) branched linkages (Misaki et al. 1980). The average molecular weight of dextran is as high as $10^7 - 10^8$ Da (Heinze et al. 2006); but, can be reduced by acidic hydrolysis to obtain molecular weight fractions that are of specific interest. Dextran is water soluble, neutral, biodegradable and biocompatible. Its features may vary depending on the molecular weight as well as the distribution, type of branches and the degree of branching. It is synthesized by a wide variety of bacterial strains.

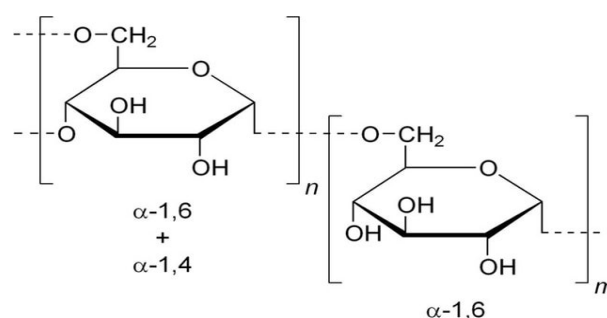


Figure 2: The Chemical Structure of Dextran

Dextran has a broad range of applications in varied areas like, clinical, chemical, food and pharmaceutical industry. It is used as an emulsifier, stabilizer, adjuvant, stabilizer, thickener in jam and ice cream and mainly as a drug (as blood plasma volume expander). By using matrix of cross-linked dextran gel layer, proteins can be separated and purified by size exclusion chromatography. Dextran and its derivatives (which are produced by structural modifications) are used for the preparation of modified drug delivery systems (Aumelas et al. 2007; Coviello et al. 2007; Chen et al. 2003). In the field of nanomedicine, because of its biocompatibility, good availability and biodegradability, it is not only used as a nanoparticulate carrier system; but, also engaged to encapsulate these systems (Gavory et al. 2011).

Pullulun

Pullulun is a naturally occurring fungal polysaccharide produced by fermentation of liquefied corn starch by *Aureobasidium pullulans*, a ubiquitous yeast-like fungus. It has a linear structure consisting of predominantly repeating maltriose units, which are made up of three α -1-4 linked glucose molecules (Fig.3; Wallefels et al. 1965; Catley 1971; Carolan et al. 1983), linked by α -1-6-glucosidic bonds. The M.W of pullulun ranges from thousands to 2000 kDa depending on the growth conditions, (Rekha and Chandra 2007). It is soluble in water but insoluble in organic solvents. Aqueous solutions of pullulun are viscous but do not form gels. It forms transparent, water-soluble, odourless, flavourless, fat resistant, anti-static films.

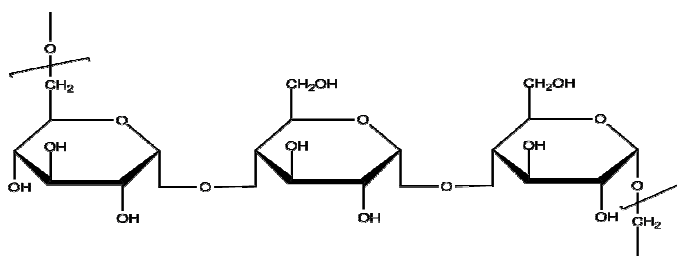


Figure 3: The Chemical Structure of Pullulun

As an edible, bland and tasteless polymer, the main use of pullulan is in the manufacture of edible films that are used in various breath freshener or oral hygiene products such as Listerine Cool Mint PocketPaks. In pharmaceuticals, it is used as a coating agent; in manufacturing and electronics it is used because of its film- and fiber-forming properties. It is used as a thickener or as a carrier in the production of capsules for dietary supplements as a replacement to gelatine. It is also used in the production of various jams and jellies, confectionery and some fruit and meat products. It is commonly used as a foaming agent in milk-based desserts and as a texturing agent in chewing gums (Sugimoto 1978; Wiley et al. 1993; Gibbs and Seviour 1996; Madi et al. 1997; Lazaridou et al. 2002).

Because of its non-carcinogenic, non-toxic, non-immunogenic and hemocompatible properties (Coviello et al. 2007), the FDA has approved it for various biomedical applications such as drug and gene delivery (Rekha and Chandra 2007), wound healing (Bae et al. 2011), and tissue engineering (Thebaud et al. 2007). It has been reported that pullulan hydrogels are used as drug delivery systems, particularly in the form of micro and nanogels. Interest in using pullulan nanogels has increased over the last decade due to its related potential applications in the development and implementation of new environmentally responsive or smart materials, artificial muscles, biomimetics, biosensors, drug delivery systems, and chemical separations (Coviello et al. 2007).

To prepare pullulan nanostructures which act as carriers of different drugs, its backbone structure is modified with hydrophobic molecules, results in a molecule of hydrophobized pullulan that self-assembles in water solutions.

Hyaluronic Acid

Hyaluronic acid (HA) is a carbohydrate, more precisely a mucopolysaccharide, present naturally in all living organisms. It is composed of repeating disaccharide units of D-glucuronic acid and N-acetyl D-glucosamine linked by β (1-3) and β (1-4) glycosidic bonds (Fig.4). HA can be modified in many ways to alter the properties of the resulting materials, including modifications leading to hydrophobicity and biological activity (Burdick and Prestwich 2011). HA has a

molecular weight which can reach as high as 10^7 Da. In several organisms, generally HA is linked to various biopolymers and this requires several separation procedures such as protease digestion, HA ion-pair precipitation, membrane ultra filtration, HA non-solvent precipitation and/or lyophilisation (Mendichi and Soltes 2002) to obtain the pure compound. These methods generate HA with a molecular weight of several thousands to about 2.5 MDa. Nonetheless, some microorganisms such as *Streptococcus zooepidemicus* and *S. Equi* can produce HA with a molar mass in the range of several MDa.

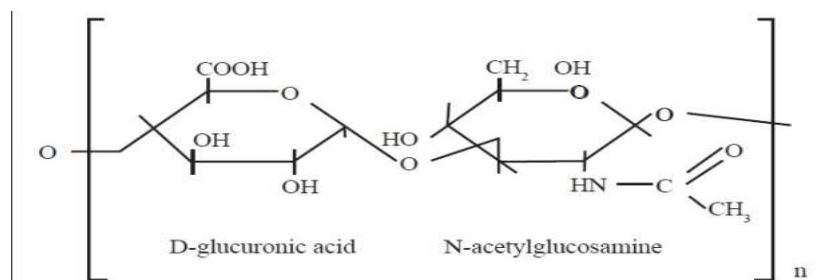


Figure 4: The Chemical Structure of Hyaluronic Acid

The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, including the supplementation of joint fluid in arthritis (Neo et al. 1997; Barbucci et al. 2002; Uthman et al. 2003; Medina et al. 2006), to facilitate the healing and regeneration of surgical wounds, and as a surgical aid in eye surgery

Hyaluronan as other glycosaminoglycans, serve as a targeting vehicle for the delivery of chemotherapeutic agents to cancerous tissues, as many tumours over-express the hyaluronan CD44 and RHAMM receptors (Yip et al. 2006). Recently, HA has been studied as a drug delivery agent for various administration routes, including nasal, parenteral, ophthalmic, pulmonary and topical (Brown and Jones 2005). As a drug delivery carrier, HA has several advantages including the negligible non-specific interaction with serum components due to its polyanionic characteristics (Ito et al. 2006) and the highly efficient targeted specific delivery to the liver tissues with HA receptors (Zhou et al. 2003).

In tissue engineering and regenerative medicine, HA has become been recognised as an important building block for the creation of new biomaterials (Allison and Grande-Allen 2006; Prestwich 2008). Furthermore, it has been shown that HA binds to cells and effectively promotes new bone formation. As there are various applications of HA, it has been used as a promising biomaterial in diverse fields of biomedicine.

Alginate

Alginate is a naturally occurring anionic and hydrophilic polysaccharide. It is one of the most abundant biosynthesized materials (Narayanan et al., 2012; Braerk et al. 1989), and is derived primarily from brown seaweed and bacteria. Alginate contains blocks of (1–4)-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) monomers (Fig.5). It has a variable molecular weight, depending on the degree of depolymerisation caused by its extraction and the enzymatic control during its production. Industrially produced alginates have a molecular weight ranging from 400-500 kDa, with average M.W of 200 kDa (Rehm 2009).

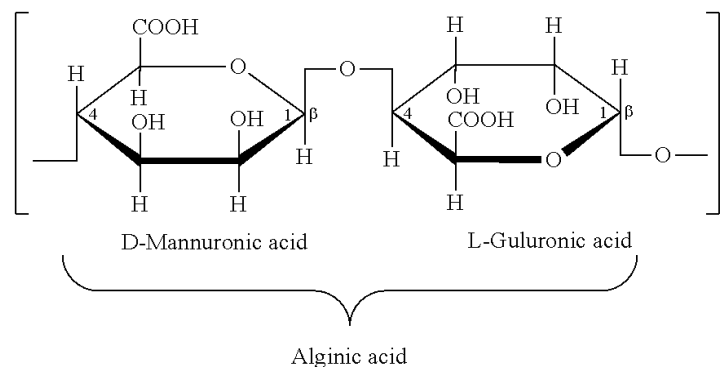


Figure 5: The Chemical Structure of Alginate

Alginate is of specific interest for a wide range of applications as a biomaterial and especially as a supporting matrix or delivery system for tissue repair and regeneration. As it is a biopolymer and a polyelectrolyte which is biocompatible, non-immunogenic, non-toxic, and biodegradable with chelating ability, it has been used in a wide range of biomedical applications. Due to its abundance, low price and non-toxicity, alginate has been extensively used in different industries. For example, it has been used as food additive and thickener in salad dressings and ice-creams in the alimentary industry (Nair and Laurencin 2007). Moreover, the biocompatibility behaviour and the high functionality make alginate a favourable biopolymer material for its use in biomedical applications, such as immobilization of cells (Lan and Starly 2011), controlled drug release devices (Pandey and Ahmad 2011) and as scaffolds in tissue engineering (Barbosa et al. 2005).

Alginate exhibits a pH-dependent anionic nature and has the ability to interact with cationic polyelectrolytes and proteoglycans. Therefore, by simple electrostatic interactions, delivery systems for cationic drugs and molecules can be obtained. Depending on the site of implantation, the biomaterials are subjected to various pH environments, which affect the degradation and mechanical properties as well as the swelling behaviour of the biomaterials. Alginate plays a crucial role in the long term stability and performance of alginate-based biomaterials *in vitro*. Being a natural polymer, it is compatible with a wide variety of substances, and does not require multiple and complex drug-encapsulation process. Moreover, it is mucoadhesive and biodegradable and, consequently, it can be used in the preparation of controlled drug-delivery systems achieving an enhanced drug bioavailability (Pandey and Ahmad, 2011). Therefore, the biocompatibility, availability and versatility of this polysaccharide make it an important and hopeful tool in the field of nanomedicine, especially in the preparation of nanoparticulate drug delivery systems.

Pectin

Pectins are a complex family of heteropolysaccharides that constitute a large proportion of the primary cell walls of dicotyledons and play important roles in growth, development and senescence (van Buren 1991; Tombs and Harding 1998; Ridley et. al. 2001; Willats et. al. 2001). The chemical structure of this natural polymer has large amounts of poly (D-galacturonic acid) bonded via α -(1 \rightarrow 4) glycosidic linkage (Fig. 6).

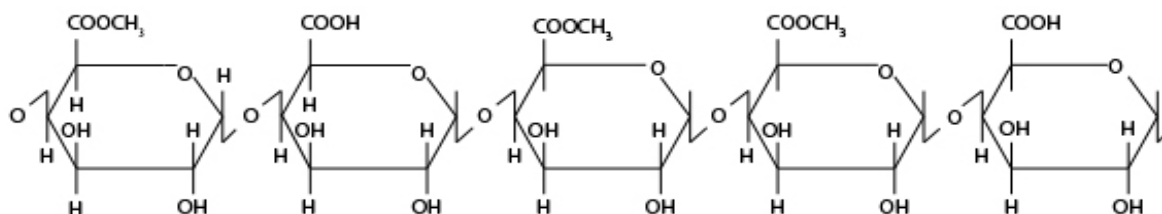


Figure.6: The Chemical Structure of Pectin

Pectins have been used earlier as gelling and thickening agents for a large number of years in food industry; but, recently there has been interest in using pectin gels for pharmaceutical applications (Liu, et al 2003). Interesting uses of pectin in biomedical applications include the specific delivery of unique amino acid sequences, wound healing substances, anti-inflammatory agents and anti-coagulants to specific tissue sites. Moreover, in the physiological environment of stomach and small intestine, pectin remains intact, but the bacterial inhabitants of human colon degrades pectin by secreting pectinases. Because of its controlled drug delivery property (Sungthongjeen et al. 2004; Lui et al. 2003; Lui et al. 2007), long standing reputation of being non-toxic (GRAS – generally regarded as safe) (Lui et al. 2003; Lui et al. 2007; Watts and Smith 2009), relatively low production costs (Sungthongjeen et al. 2004) and high availability (Beneke et al. 2009) pectin could be used as a delivery vehicle to assist protein and polypeptide drugs from mouth to the colon (Sinha and Kumria 2001) orally, nasally and vaginally (Peppia et al. 2000; Sinha and Kumria 2001; Lui et al. 2003; Nafee et al. 2004; Valenta 2005; Lui et al. 2007; Chelladurai et al. 2008; Thirawong et al. 2008), which are generally well accepted by patients (Lui et al. 2003; Lui et al. 2007; Yadav et al. 2009).

As pectin is not able to safeguard its drug delivery while passing through stomach and small intestine due to its high water solubility (Sinha and Kumria 2001), research is focused on developing water-resistant pectin derivatives. For this purpose, calcium salts which binds non-covalently with the carbohydrate chains of pectin were found out, which can reduce the solubility and are stable in low pH solution while resisting extensive hydration *in vivo* in the gastrointestinal tract. Hence, calcium pectinate is a prospective candidate as a drug carrier for colon-specific delivery in different formulations such as gels or droplets, films, microspheres etc. (Liu et al. 2003). Pectin in combining with natural polymers or synthetic polymers, various useful novel formulations have been obtained. The combination of pectin and a second polymer into a composite may alter degree of swelling and change mechanical properties (Liu et al. 2003), improving in the most cases the stability of the drug and controlling the drug release. It has been combined with 4-aminothiophenol (Perera et al., 2010), hyaluronic acid (Pliszczak et al. 2011) chitosan (Fernandez-Hervas and Fell 1998), or poly (lactide-co-glycolide) (Liu et al. 2004), showing good results as controlled drug release devices.

Gum Arabic

It is a complex heteropolysaccharide derived from exudates of *Acacia senegal* and *Acacia seyal* trees. The carbohydrate moiety is made up of D-galactose (~40% of residues), L-arabinaose (~24%), L-rhamnose (~13%) and two uronic acids, responsible for the polyanionic character of the gum, the D-glucuronic acid (~21%) and 4-O-methyl-D-glucuronic acid (2%) (Fig.7). Due to the presence of flexible structure that allows molecules to be easily deformed at interfaces (Jayme et al. 1999; Fauconnier et al. 2000), acacia gum is mainly used as an emulsifier/stabiliser. Gum arabic presents high water solubility, low viscosity in aqueous solutions and good emulsifying abilities, due to the existent protein fraction (Gabas et al. 2007; Kurozawa et al. 2009).

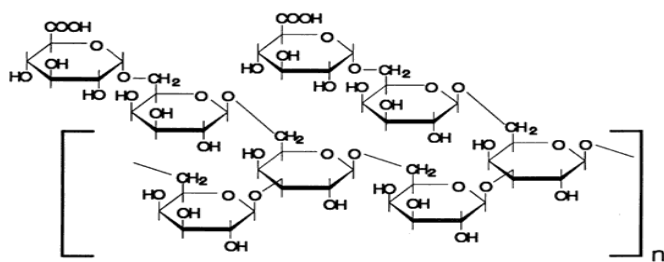


Figure 7: The Chemical Structure of Gum Arabic

Together maltodextrin and gum arabic based nanoparticles are being used as catechin delivery systems (Gomes et al. 2010; Peres et al. 2010). To enhance the protein delivery by gum Arabic based nanoparticles, interaction between gum arabic and chitosan is starting to be exploited (Avadi et al., 2010; Coelho et al. 2011). Very little has been known about the use of gum Arabic based nanoparticles and drug delivery system, it has to be exploited further.

Preparation of Polysaccharide Based Nanoparticles

Many studies have demonstrated that nanoparticles have a number of advantages over microparticles (Panyam and Labhasetwar 2003). Alonso and co-workers (2001) and Prabakaran and Mano (2005) have written excellent reviews that focus on the preparation and application of chitosan nanoparticle carriers. Polysaccharide-based nanoparticles greatly enrich the versatility of nanoparticle carriers in terms of category and function. Polysaccharide-based nanoparticles are basically prepared by four different mechanisms depending on their structural characteristics; namely,

- Covalent crosslinking
- Ionic cross linking
- Polyelectrolyte complexation and
- Self assembly of hydrophobically modified polysaccharides.

The select of method depends on a number of factors, such as, particle size, particle size distribution, area of application and etc. Particle size is the most important characteristics of nanoparticles.

Covalently Crosslinked Polysaccharide Nanoparticles

Preparation of polysaccharide nanoparticles by covalent cross linking is the earliest method that was adopted. Among various polysaccharides, chitosan is the initial one to be used for nanoparticle preparation. Initially, chitosan-based polysaccharides were cross-linked using glutaraldehyde, a common cross linker (Zhi et al. 2005; Liu et al. 2007). However, because of the cellular toxicity of glutaraldehyde, its use in drug delivery was limited. Hence the use of a biocompatible cross linking agent is desirable. . Presently, various water-soluble condensation agents such as carondiamide, natural di- and tricarboxylic acids, including succinic acid, tartaric acid, malic acid, citric acid, etc. are being used as intermolecular crosslinkers for chitosan nanoparticles (Bodnar et al. 2005). Hence, biodegradable chitosan nanoparticles were obtained by performing the condensation reaction between carboxylic groups of natural acids and the pendent amino groups of chitosan. This method allows the formation of polycations, polyanions, and polyampholyte nanoparticles. The prepared nanoparticles were stable in aqueous media at low pH, neutral, and mild alkaline conditions. Depending on the pH in the swollen state, the average size of the particles ranged between 270–370 nm.

Ionic Crosslinked Polysaccharide Nanoparticles

Ionic cross linking has more advantages than covalent cross linking as the procedure is simple and the preparation conditions are mild. For charged polysaccharides, low MW polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. To date, the most widely used polyanion cross-linker is tripolyphosphate (TPP) as it is non-toxic and has multivalent cations. TPP cross linked chitosan nanoparticles were first reported in 1997 by Alonso et al (Calvo et al. 1997). TPP can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counter ions of TPP (Jain et al. 2008). From then on, TPP-chitosan nanoparticles have been widely used to deliver various drugs and macromolecules (Lu et al. 2006; Zhang et al. 2004; Anan et al. 2005; Vila et al. 2004; Aktas et al. 2005; Qi et al. 2005; Sun et al. 2007; Tsai et al. 2008; Zhang et al. 2008; Gan et al. 2007; Maestrelli et al. 2006).

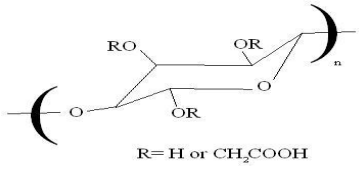
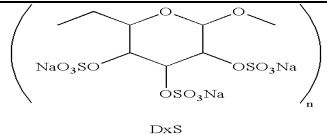
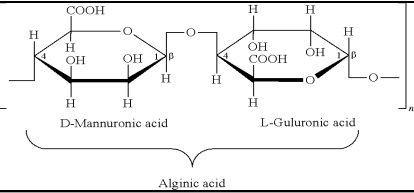
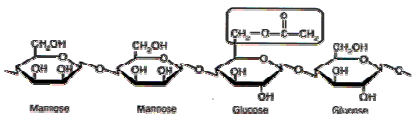
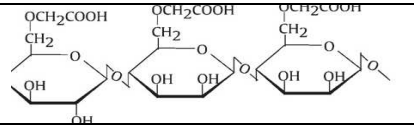
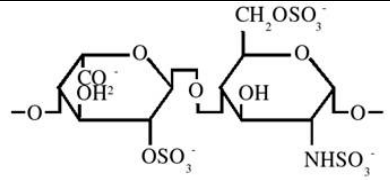
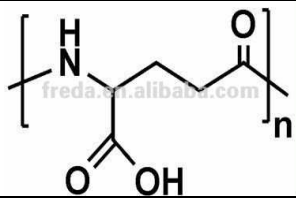
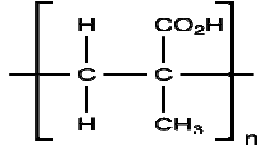
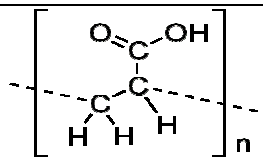
Recently apart from TPP, water soluble chitosan derivatives were also ionically cross linked to prepare nanoparticles. A water soluble chitosan derivative, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride was synthesized by Xu et al in 2003 by the reaction between glycidyl-trimethyl-ammonium chloride and chitosan. Based on ionic gelation process of the derivative and TPP, nanoparticles of 110–180 nm in size were obtained. In addition, Amidi et al. (2006) prepared N-trimethyl chitosan nanoparticles by ionic crosslinking of N-trimethyl chitosan with TPP and their potential as a carrier system for the nasal delivery of proteins, ovalbumin were evaluated. It is found that the nanoparticles had an average size of about 350 nm and a positive zeta potential. They showed a loading efficiency up to 95% and a loading capacity up to 50% (w/w). The integrity of the entrapped ovalbumin was preserved. More recently, calcium-cross linked negatively charged polysaccharide nanoparticles have found efficacy as drug carrier. As some polysaccharides bear carboxylic groups on molecular chains, they can be cross linked by bivalent calcium ion to form nanoparticles. By using water-in-oil reverse microemulsion method You et al. (2004) prepared Ca-cross linked alginate nanoparticles. To examine the potency of the nanoparticles for gene delivery, green fluorescent protein-encoding plasmids were encapsulated in the nanoparticles to investigate the degree of endocytosis by NIH 3T3 cells and ensuing transfection rate. Results showed that Ca-alginate nanoparticles with an average size around 80 nm in diameter were very efficient gene carriers. Zahoor et al (2005) also prepared ca-alginate nanoparticles (235.5 nm in size) by ion-induced gelification. It is found that the drug encapsulation efficiency in these nanoparticles were 70-90 % for isoniazid, pyrazinamide and 80-90% for rifampicin. The bioavailabilities of encapsulated drugs were found to be relatively higher when compared with oral free drugs. It was observed that all the drugs which are encapsulated were found to be present in the organs (liver, lungs and spleen) for about 15 days post nebulisation, while free drugs stayed up to 1 day. Therefore, these inhalable nanoparticles could serve as carriers for controlled release of drugs. Kim et al. (2006) encapsulated retinol into chitosan nanoparticles by ion complex due to the electrostatic interaction between amine group of chitosan and hydroxyl group of retinol and reconstituted it into aqueous solution for pharmaceutical and cosmetic applications. By encapsulation it is found that the solubility of retinol is able to increase by more than 1600- fold.

Polysaccharide Based Nanoparticles By Polyelectrolyte Complexation (PEC)

Polyelectrolyte polysaccharides can form PEC with oppositely charged polymers by intermolecular electrostatic interaction. Although theoretically any polyelectrolyte could interact with polysaccharide to form PEC nanoparticles, in practice these polyelectrolytes are restricted to biocompatible and water-soluble polymers in view of safety purpose.

Chitosan is the only natural polycationic polysaccharide that fulfills all the needs; however, there are many negative polymers (Table 1) which complex with chitosan to form PEC nanoparticles, peptides, polyacrylic acid family etc.

Table.1: Negative Polymers Complexed with Chitosan and Their Chemical Structures

S.No.	Polymer	Structure
Negative Polysaccharides Complexed with Chitosan		
1	Carboxymethyl cellulose	
2	Dextran sulfate	
3	Alginate	
Negative Peptides Complexed with Chitosan		
4	Glucomanan	
5	Carboxymethyl konjac	
6	Heparin	
Polyacrylic Acid Family Complexed with Chitosan		
7	Poly-γ-glutamic acid	
8	Polymethylacrylic acid	
9	Poly acrylic acid	

Negative Polysaccharides Complexed With Chitosan-Based Nanoparticles

Carboxymethyl cellulose can complex with chitosan and form stable cationic nanoparticles. Cui et al (2001) coated the plasmid DNA on pre-formed cationic chitosan/ carboxymethylcellulose nanoparticles. These chitosan-based nanoparticles containing plasmid DNA resulted in both detectable and quantifiable levels of luciferase expression in mouse skin after 24 h topical application, and significant antigen-specific IgG titre expressed β -galactosidase at 28 days. Tiyaboonchai et al. (2007) developed a nanoparticulate delivery system for amphotericin B with chitosan and dextran sulphate together with zinc sulphate as a cross linking and hardening agent. The nanoparticles possessed a mean particle size of 600–800 nm with a polydispersity index of 0.2, indicating a narrow size distribution. Insulin loaded nanoparticles was prepared by Sarmiento et al. (2006) using ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan. They also studied the structural integrity of insulin after being entrapped into chitosan/alginate nanoparticles (Sarmiento et al. 2007). Their results clearly showed that no significant conformational changes of insulin occurred in terms of α -helix and β -sheet content. Alonso-Sande et al. (2006) prepared nanoparticles using two different types of glucomannan (non-phosphorylated and phosphorylated) and two different approaches. The interaction of chitosan and glucomannan in these procedures involved the presence or absence of sodium tripolyphosphate, which acted as an ionic cross-linking agent for chitosan. The nanoparticles showed a great capacity for the association of insulin and the immunomodulatory protein P1, reaching association efficiency values as high as 89%.

Negative Peptides Complexed with Chitosan Based Nanoparticles

By using ionic-gelation method, Lin et al. (2005) prepared poly- γ -glutamic acid/chitosan nanoparticles system. These nanoparticles showed enhanced intestinal paracellular transport in Caco-2 cell monolayers *in vitro*. These nanoparticles which have chitosan dominated on the surface could effectively reduce the transepithelial electrical resistance of Caco-2 cell monolayers and opened the tight junctions between Caco-2 cells; thus, allowing transport of the nanoparticles *via* the paracellular pathways. When compared to chitosan/DNA, chitosan/ poly- γ -glutamic acid/DNA improved their penetration depth into the mouse skin and enhanced gene expression. These studies clearly showed that chitosan/poly- γ -glutamic acid/ DNA were more compact in their internal structures and had a greater density than their chitosan/DNA counterparts, thus they can penetrate into the skin barrier (Lee et al. 2008) more easily.

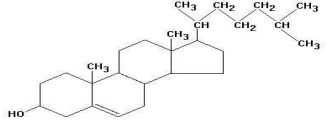
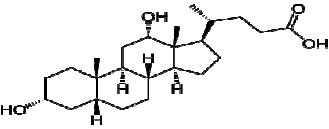
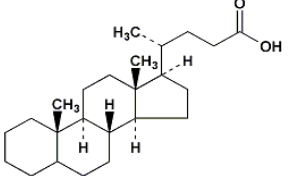
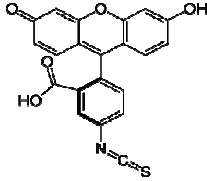
Polyacrylic Acid Family Complexed with Chitosan Based Nanoparticles

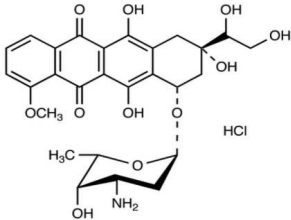
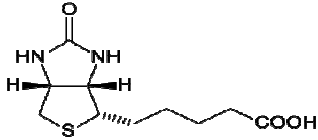
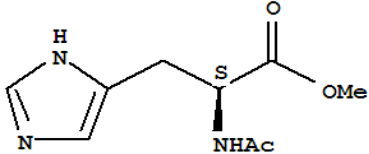
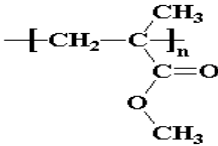
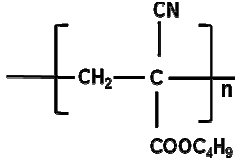
pH-sensitive polymethacrylic acid/ chitosan/polyethylene glycol nanoparticles were produced under mild aqueous conditions (Sajeesh et al. 2006). The procedure involves free radical polymerisation of methacrylic acid in the presence of chitosan and polyethylene glycol using a water-soluble initiator. Nanoparticles were obtained instantaneously without adding any organic solvents or surfactants/steric stabilizers. Model proteins like insulin and bovine serum albumin were added into the nanoparticles by diffusion filling method and at pH 1.2 and 7.4, there *in vitro* release characteristics were evaluated. The nanoparticles exhibited good protein encapsulation efficiency and pH responsive release profile under *in vitro* conditions. Chen et al. (2005) developed chitosan/poly (acrylic acid), When polyanionpoly(acrylic acid)was dropped into polycation chitosan solution. It was reported that plasmid DNA was encapsulated very well in these nanoparticles, giving them great potential in gene delivery.

Polysaccharides-Based Nanoparticles through Self-Assembly Method

There are several reports that have been carried out to understand the synthesis and potential applications of polysaccharide based self-assembled nanoparticles as drug delivery systems. When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are formed. When these copolymers exposed to aqueous environment, they spontaneously form micelles or micelle-like aggregates by intra- or intermolecular associations between hydrophobic moieties, particularly to minimize the interfacial free energy. Depending on the hydrophilic/hydrophobic constituents, these polymeric micelles display unique characteristics, like thermodynamic stability, unusual rheology feature, small hydrodynamic radius (less than microsize) with core-shell structure etc (Table.2). Polymeric micelles have been recognized as a promising and potential drug carrier as they have a hydrophobic domain, surrounded by a hydrophilic outer shell, which serves as a preservative for various hydrophobic drugs (Letchford and Burt 2007).

Table.2: Hydrophobic Molecules and Their Chemical Structures That are Used to Modify Polysaccharides

Polysaccharides	Hydrophobic Molecules	Chemical Structure of Hydrophobic Molecules
Chitosan	Poly (ethylene)glycol	$HO-CH_2CH_2-O-(CH_2CH_2O)_n-CH_3$
β -Cyclodextrin	Hexanoic acid Decanoic acid	$CH_3(CH_2)_4COOH$ $CH_3(CH_2)_8COOH$
Chitosan, Amylose	Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
Chitosan	Linolenic acid	$CH_3(CH_2CH=CH)_3(CH_2)_7COOH$
Chitosan	Palmitic acid	$CH_3(CH_2)_{14}COOH$
Chitosan	Stearic acid	$CH_3(CH_2)_{16}COOH$
Chitosan	Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Dextran, chitosan	Poly(ϵ -caprolactone)	$-[O-(CH_2)_5-CO]_n-$
Heparin, Hyaluronic acid	Pluronic	$-(CH_2CH_2O)_n-(CH_2CH(CH_3)O)_m-$
Pullulan	Hexadecanol	$CH_3(CH_2)_{15}OH$
Chitosan, Carboxymethyl chitosan, Pullulan	Cholesterol	
Chitosan, heparin, Glycol chitosan	Deoxycholic acid	
Glycol chitosan	5 β -Cholanic acid	
Glycol chitosan	Fluorescein isothiocyanate (FITC)	

Glycol chitosan	Doxorubicin	
Pullulan	Vitamin H	
Glycol chitosan	N-Acetyl histidine	
Heparin, Dextran	Poly(methyl methacrylate)	
Chitosan, Dextran, Dextran sulfate, Thiolated chitosan, Heparin, Hyaluronic acid, Pectin	Poly(isobutyl Cyanoacrylate)	

MEDICAL APPLICATIONS OF POLYSACCHARIDE-BASED NANOPARTICLES

Polysaccharide-based nanoparticles have received the most promising drug delivery systems because of their unique properties. They are characterized as particulate dispersions or solid particles with a size ranging from 10-1000 nm with various morphologies, like nanocapsules, nanospheres, nanoliposomes, nanodrugs, nanomicelles etc. In this system, the drug is dissolved, encapsulated, entrapped or attached to the nanoparticle matrix (Kommareddy et al. 2005; Lee and Kim 2005). Polysaccharide-based nanoparticles drug delivery system has wide advantages, such as efficient drug protection against enzymatic and chemical degradation, ability to create a controlled release to a specific tissue, high drug encapsulation efficiency, cell internalization as well as ability to reverse the multidrug resistance of tumour cells (Soma et al. 1999). Starch-based nanoparticles have received a significant amount of attention because of their biocompatibility, good hydrophobicity and biodegradability. Hydrophobic-grafted and cross-linked starch nanoparticles were used for drug delivery and Indomethacin was incorporated as the model drug (Abraham and Simi 2007). Hydrophilic amylopectin was modified by grafting hydrophobic poly (lactic acid) chains (PLA) for the fabrication of polymeric micelles for drug delivery. When these spherical nano-aggregates were used as the drug carrier, it was found that they had a good loading capacity and *in vitro* release properties for hydrophobic indomethacin drug (Brecher et al. 1997; Dufresne et al. 2006).

Nanoparticles of poly (DL-lactide-co-glycolide)-grafted dextran were synthesized for use as oral drug carrier. These nanoparticles have particle size ranging from 50-300nm and were able to form nanoparticles in water by self-aggregating process. Super paramagnetic chitosan-dextran sulfate hydrogels as drug carriers was synthesized. The 5-

aminosalicylic acid was incorporated as model drug molecule (Saboktakin et al. 2010). To overcome the pharmacokinetic problems and to obtain the full benefits of the drug Anitha et al. 2011, prepared dextran sulphate–chitosan nanoparticles. Self-assembled hydrogel nanoparticles composed of dextran and poly (ethylene glycol) was synthesized and prepared nanoparticles used for drug carrier with hydrophobic model drug in vitro (Kim et al. 2000).

Hydrophobized pullulan Specifically, cholesterol pullulan and a copolymer of N- isopropylacrylamide and N-[4-(1-pyrenyl)butyl]-N-noctadecylacrylamide via their hydrophobic moieties, as well as hexadecyl group-bearing pullulan self-assembly nanoparticles (Akiyoshi et al., 1998; Akiyoshi et al. 1993; Jung et al. 2004) has been used as drug delivery systems. These hydrophobized pullulan self-associate to form colloiddally stable nanoparticles with inner hydrophobic core. This hydrophobic core can encapsulate only hydrophobic substances like insoluble drugs and proteins (Gupta and Gupta 2004). However, amphiphilic polysaccharides composed of pullulan and poly (DL-lactide-coglycolide) (PLGA) were synthesized to give amphiphilic and biodegradable novel drug carriers. For the controlled release of drugs, PLGA is commonly used because of its biodegradability (Jeong et al. 2006). *In vivo* studies showed that hydrophobically modified glycol chitosan (HGC) nanoparticles found to be potential as carriers for anticancer peptides and anticancer drugs because of their biocompatible nature (Kwon et al. 2003; Yoo et al. 2005). Modified chitosan derivatives, are emerging as novel carriers of drugs because of their solubility and biocompatibility in vivo (Sinha et al. 2004; Jiang et al. 2006; Chen et al. 2003b). Nanoparticles of carboxymethyl chitosan (CM-chitosan) as carriers for the anticancer drug were prepared by gelification with calcium ions with Doxorubicin (DOX) chosen as a model drug.

CONCLUSIONS AND FUTURE PERSPECTIVES

The literature enumerated in this review showed that a lot of attention has been aimed in the combination of polysaccharide-based polymers with inorganic nanoparticles, so as to profit from the advantages of both organic and inorganic components. The literature above clearly depicted the significant use of polysaccharide based nanoparticles, because of their availability in natural source, renewability, biocompatibility, biodegradability, low cost and non-toxic nature. Hence, formulations of such bionanocomposites can perform outstanding characteristics, like optical, antimicrobial functionalities, surface coverage, size particles, enzyme degradability, colloidal stability, and their derivatives for various biotechnological and biomedical applications were explained. The important step of this kind of material depends strongly on the earlier steps of their production and their modification steps which emphasize the correlation of preparative strategies that rely on their final applications. Until now, these nanoparticles are mostly investigated in terms of their physicochemical properties, *in vitro* toxicity, drug-loading ability and comparatively simple in vivo tests. However, the more critical issues, such as the specific interaction of these nanoparticles with human organs, tissues, cells, or biomolecules, their effect on human's metabolism brought by the nanoparticles, and the wider application of these nanoparticles for drug delivery, etc. needs to be focused on in the near future. Furthermore, attempts in finding new methods for the earlier diagnosis of diseases and more efficient therapies to synthesize the new generation of multifunctional nanostructured materials based on polysaccharides, modified polysaccharides and polysaccharide-based dendrimers is very fast emerging. Hence, in near future, more polysaccharide-based nanoparticles emerge, which greatly enriches the versatility of nanoparticle carriers agents in terms of category and function.

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