

# Polysaccharide-Based Nanoparticles for Controlled Release Formulations

A. Martínez<sup>1</sup>, A. Fernández<sup>2</sup>, E. Pérez,  
M. Benito<sup>3</sup>, J.M. Teijón<sup>2</sup> and M.D. Blanco<sup>2</sup>

<sup>1</sup>*Departamento de Farmacología, Facultad de Farmacia,  
Universidad Complutense de Madrid,*

<sup>2</sup>*Departamento de Bioquímica y Biología Molecular, Facultad de Medicina,  
Universidad Complutense de Madrid,*

<sup>3</sup>*Centro Universitario San Rafael-Nebrija. Ciencias de la Salud, Madrid  
Spain*

## 1. Introduction

Nanoscience is the science of the phenomena peculiar to matter on the scale from 1 to several hundred nanometers ( $10^{-9}$  m). Some unique features of matter emerge when features are on the nanoscale, and the appreciation of these new properties opens new opportunities. Ignored in the past decades due to the lack of technology, these new emerging opportunities offered by nanoscience have been one of the most important areas of researching from the middle of the twentieth century to nowadays (Tibbals, 2010).

New opportunities have been realized in a wide variety of areas of technology, ranging from intelligent nanoscale materials, faster electronics or nanomotors, to medicine and biology, where first nanotechnology applications have demonstrated an enormous potential.

While medical nanotechnology was improving a wide range of medical resources and practice, the concept of nanomedicine was taking shape. Nanomedicine has recently been referred by the National Institutes of Health as the applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems (Moghimi et al., 2005). Although this term has been defined in the literature in many ways, nanomedicine means essentially applying nanotechnology to medicine.

In contrast with other therapies, nanomedicine attempts to use sophisticated approaches to either kill specific cells or repair them one cell at a time. This approach also offers new possibilities towards the development of personalized medicine (Gurwitz & Livshits, 2006). Because nanomedicine inherits its focus on certain diseases which are currently being investigated, its primary aims have been towards non-infectious diseases, especially cancer, and on degenerative diseases in order to characterize them in the increasingly sedentary and aging populations of the wealthiest countries that lead in medical research (Tibbals, 2010).

One of the most important and hopeful tools employed in nanomedicine for medical applications are nanoparticles. Nanoparticles are solid, colloidal particles consisting of

macromolecular substances that vary in size from 10 nm to 1000 nm. However, particles >200 nm are not heavily pursued and nanomedicine often refers to devices <200 nm (i.e., width of microcapillaries). Depending on the method of preparation nanoparticles, nanospheres, or nanocapsules can be constructed to possess different properties and release characteristics for the best delivery or encapsulation of the therapeutic agent (Barratt, 2000).

One advantage of nanovectors – nanoparticles is their ability to overcome various biological barriers and to localize into the target tissue. The nanovectors currently used and investigated can be classified into three main groups or “generations” (Sakamoto et al., 2007). The first generation comprises a passive delivery system that localizes into the target site. In case of a tumour, the system reaches the tumour through the fenestrations in the adjacent neovasculature, and is normally decorated by a “stealth” layer in order to avoid their uptake by phagocytic blood cells, thus substantially prolonging their circulation time (Romberg et al., 2008). The unique mechanism of driving systems to the tumour site is the size of particles, not specific recognition of the tumour or neovascular targets. As a case in point, particles based on albumin-paclitaxel have been recently approved by FDA for their use in metastatic breast cancer (Kratz, 2008). The second generation of nanosystems includes additional functionalities that allow for molecular recognition of the target tissue or for active or triggered release of the payload at the disease site. These include ligands, aptamers and small peptides that bind to specific target-cell surface markers or surface markers expressed in the disease microenvironment (Kang et al., 2008). Responsive systems, such as pH-sensitive polymers, are included in this category. Although the representatives of the second generation have not yet been approved by the FDA, there are numerous ongoing clinical trials involving targeted nanovectors, particularly in cancer applications. Finally, the third generation nanovectors are focused to successfully overcome the natural barriers that the vector needs to bypass to efficiently deliver the drug to the target site. This goal will only be reached by a “multistage” approach, and such a system has been recently reported (Tasciotti et al., 2008).

Polymeric nanoparticles made from natural and synthetic polymers have received the majority of attention due to their stability and ease of surface modification. Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible at least and biodegradable best. Among natural polymers, proteins or polysaccharides tend to be internalized and degraded rapidly, thus enabling a moderate intracellular release of the drug or gene (Sinha & Trehan, 2003). Polysaccharides have been especially used in the preparation of drug delivery systems.

Polysaccharides are the polymers of monosaccharides. In nature, polysaccharides have various resources from algal origin (e.g. alginate), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum), and animal origin (chitosan, chondroitin) (Sinha & Kumria, 2001). They offer a wide diversity in structure and properties due to their wide range of molecular weight and chemical composition.

Due to the presence of various reactive groups in their structure, polysaccharides can be easily modified chemically and biochemically. Moreover, the presence of hydrophilic groups in their structure, such as hydroxyl, carboxyl and amino groups, enhance bioadhesion with biological tissues, like epithelia and mucous membranes, forming non-covalent bonds, which is an useful strategy to improve bioavailability of drugs included in drug delivery systems (Lee et al., 2000).

One of the main advantages of polysaccharides as natural biomaterials is their availability in natural resources and low cost in their processing, which make them very accessible materials to be used as drug carriers. Furthermore, polysaccharides are highly stable, safe, non-toxic, hydrophilic and biodegradable (Liu et al., 2008). Thus, they have a large variety of composition and properties that cannot be easily mimicked in a chemical laboratory, and the ease of their production makes numerous polysaccharides cheaper than synthetic polymers (Coviello et al., 2007). Therefore, polysaccharides have a promising future as biomaterials.

In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application as nanoparticle drug delivery systems. The number of polysaccharides that have been investigated for the preparation of nanoparticles suitable as delivery systems is extremely large. As a result, attention has been focused on the latest studies and exploitations related to such systems, including some of the most used polysaccharides, a brief description of their structural features and some of the techniques carried out to prepare polysaccharide-based nanoparticles.

## 2. Structural features and characteristics of polysaccharides

As the number of polysaccharides used in the preparation of drug delivery systems is very large, some of the most commonly used polymers have been collected, describing their chemical structure, chemical features, and highlighting their applications in different fields, especially, in the preparation of drug delivery systems.

### 2.1 Alginate

Alginate is a well known polysaccharide obtained from natural sources, such as its extraction from cell walls and intercellular spaces of marine brown algae, and its production by bacteria. It can be characterized as an anionic copolymer whose chemical structure is based on a backbone of (1-4) linked  $\beta$ -D-mannuronic acid (M units) and  $\alpha$ -L-guluronic acid (G units) (Fig. 1) of widely varying composition and sequence depending on the source of the alginate, resulting in an irregular blockwise pattern of GG, MG and MM blocks. Alginate has a variable molecular weight, depending on the enzymatic control during its production and the degree of depolymerization caused by its extraction. Typically, commercial alginates have an average molecular weight of approximately 200,000 Da, but alginates with values as high as 400,000-500,000 Da are also available (Rehm, 2009).

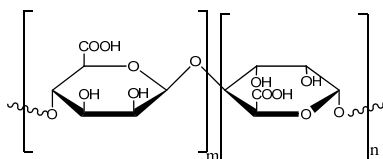


Fig. 1. Chemical structure of alginate

The physico-chemical properties of alginate have been found to be highly affected by the M/G ratio as well as by the structure of the alternating zones, which can be controlled by enzymatic pathways (Coviello et al., 2007). The alginate composition influence on the flexibility of the polysaccharide chain was first reported by Smidsrod (1973), who described

that the extension of the alginate chain was dependent on its composition, with the intrinsic flexibility of the blocks decreasing in the order MG>MM>GG. M block segments show linear and flexible conformation because of the  $\beta$  (1 $\rightarrow$ 4) linkages. Besides, the guluronic acid gives rise to  $\alpha$  (1 $\rightarrow$ 4) linkages, which serves to introduce a steric hindrance around the carboxylic groups, and provide folded and rigid structural conformations that are responsible for a pronounced stiffness of the molecular chains (Yang et al., 2011).

Alginate is a biopolymer and a polyelectrolyte considered to be biocompatible, non-immunogenic, non-toxic and biodegradable, and the composition of the polymer has been reported to affect to its applications. Alginate with high content of guluronic acid block can produce, in the form of calcium salts, cross-links stabilizing the structure of the polymer in a rigid gel form. This properly enables alginate solutions to be processed into the form of films, beads and sponges (Sujata, 2002). However, high mannuronic acid alginate capsules are interesting for cell transplantation and for biohybrid organs, because of their less viscosity. In the case of cellular response, some research groups found immunostimulatory activity caused by those alginates with high mannuronic acid content, and immunosuppressive activity caused by alginates with high guluronic acid content. It was concluded that mannuronic acid oligomers would provoke cytokine release by macrophages by a receptor-mediated mechanism, whereas guluronic oligomers should inhibit this reaction (Orive et al., 2002).

Compositional modifications of natural alginates can be obtained by several mannuronan C-5 epimerases produced by alginate-producing bacteria, such as *A. vinelandii*. Recently, the combination of different epimerases has been used as a fundamental tool in order to create specific engineered alginates with any desired block length and composition (Rehm, 2009). Moreover, alginate has a large number of free hydroxyl and carboxyl groups distributed along the backbone, which are highly reactive and turn it into an ideal candidate for being appropriately modified by chemical functionalization. Thus, properties such as solubility, hydrophobicity and physicochemical and biological characteristics may be modified, having proved alginate derivatives to have a lot of potential applications. These chemical modifications of alginate have been achieved using techniques such as oxidation, sulfation, esterification, amidation, or grafting methods (Yang et al., 2011).

Due to its abundance, low price and non-toxicity, alginate has been extensively used in different industries. For instance, it has been used as food additive and thickener in salad dressings and ice-creams in the alimentary industry (Nair & Laurencin, 2007). Moreover, the biocompatibility behavior and the high functionality make alginate a favorable biopolymer material for its use in biomedical applications, such as scaffolds in tissue engineering (Barbosa et al., 2005), immobilization of cells (Lan & Starly, 2011), and controlled drug release devices (Pandey & Ahmad, 2011).

In case of its applications in nanomedicine, alginate has also been extensively investigated as a drug delivery device in which the rate of drug release can be modified by varying the drug polymer interaction, as well as by chemical immobilization of the drug in the polymer backbone using the reactive carboxylate groups (Nair & Laurencin, 2007). Apart from its easy functionalization due to its reactive structure, there are many advantages and favorable properties of alginate for its use in drug delivery. It is a natural polymer compatible with a wide variety of substances, which does not need 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 & 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.

## 2.2 Chitosan

Chitosan is a linear polysaccharide composed by units of glucosamine and N -acetyl-glucosamine linked by (1 → 4)  $\beta$ -glycosidic bonds (Fig. 2). It is a hydrophilic biopolymer obtained industrially by hydrolysing the aminoacetyl groups of chitin – which is the main component of the shells of crab, shrimp and krill – by an alkaline deacetylation treatment (Muzzarelli & Muzzarelli, 2005).

The degree of deacetylation (%DD) can be determined by NMR spectroscopy, and generally the %DD in commercial chitosan is in the range 60–100%. On average, the molecular weight of commercially produced chitosan is between 3,800 to 20,000 Da. A commonly used method for the synthesis of chitosan is the deacetylation of chitin, using sodium hydroxide in excess as a reagent and water as a solvent. This reaction pathway, when allowed to go to completion (complete deacetylation), yields up to 98% product (Yuan, 2007). So, once deacetylation happens, chitosan is consisting primarily of repeating units of  $\beta$ -(1,4)-2-amino-deoxy-D-glucose (D-glucosamine).

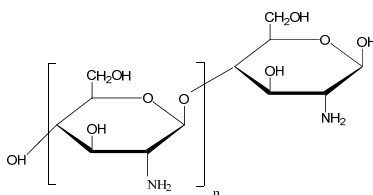


Fig. 2. Chemical structure of chitosan.

This biopolymer is accepted as a biodegradable and non toxic polymer. Despite its biocompatibility, the applications of chitosan are limited due to its insolubility above pH 6. Chitosan 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). It is possible to increase the solubility of chitosan in water removing one or two hydrogen atoms from the amino groups of chitosan, and introducing some hydrophilic segments (Srinophakun & Boonmee, 2011).

The non-toxic, biodegradable and biocompatible properties of chitosan provide potential for many applications (Guerrero et al., 2010). Due to its polyelectrolyte nature, chitosan can be used as absorbent for treatment of textile industry effluents as well as for heavy metal ions uptaking from wastewater. It has been also used as template for the preparation of mesoporous metal oxides spheres (Braga et al., 2009). However, it has been more frequently proposed for applications in pharmaceutical and biomedical fields due to its

biocompatibility and biodegradability. It has been assayed as biomaterial for wound healing and prosthetic material, since it can be biodegraded by enzyme action (Bernardo et al., 2003). Also it is reported to find applications 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).

In the field of nanomedicine, chitosan has attracted attention as a matrix for controlled release due to its reactive functionalities, polycationic character, easily degradation by enzymes and non-toxic degradation products. Over the years, a variety of natural and synthetic polymers have been explored for the preparation of drug-loaded microparticles and chitosan has been extensively investigated (Davidenko et al., 2009; Muzzarelli & Muzzarelli, 2005). Because of its bioadhesive properties, chitosan has received substantial attention as carrier in novel bioadhesive drug delivery systems which prolong the residence time of the drugs at the site of absorption and increase the drug bioavailability (Varum et al., 2008). Thus, 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).

Taking all into account, chitosan appears to be a promising matrix for the controlled release of pharmaceutical agents. Experimental *in vitro* and *in vivo* results show chitosan as an ideal carrier for a wide variety of drugs whose efficacy is increased when they are included into these systems.

### 2.3 Hyaluronic acid

Hyaluronic acid (HA) (also called sodium hyaluronate or hyaluronan) is a polysaccharide with a structure composed of repeating disaccharide units of D-glucuronic acid and N-acetyl D-glucosamine linked by  $\beta$  (1-3) and  $\beta$  (1-4) glycosidic bonds (Fig. 3) (Cafaggi et al., 2011). HA can be modified in many ways to alter the properties of the resulting materials, including modifications leading to hydrophobicity and biological activity. There are three functional groups that can be chemically modified: the glucuronic acid carboxylic acid, the primary and secondary hydroxyl groups, and the N-acetyl group (Burdick & Prestwich, 2011). HA has a molecular weight that can reach as high as  $10^7$  Da.

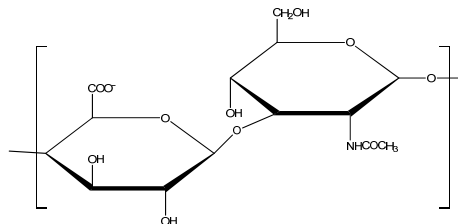


Fig. 3. Chemical structure of disaccharide repeating unit of hyaluronic acid.

It belongs to a group of substances known as glycosaminoglycans. It is the most simple among them, the only one not covalently associated with a core protein, and the only one which is non-sulfated (Kogan et al., 2007). Since hyaluronan is a physiological substance, it

is widely distributed in the extracellular matrix of vertebrate tissues. It is mainly synthesized in vertebrate organisms as an essential functional component due to its viscoelastic and rheological properties. It is a major and important component of cartilage, skin and synovial fluid.

HA is usually linked to other biopolymers in the organism, and several separation procedures have to be applied in order to obtain the pure compound, such as protease digestion, HA ion-pair precipitation, membrane ultrafiltration, HA non-solvent precipitation and/or lyophilisation (Mendichi & Soltes, 2002). With these methods HA from several hundred thousand Da up to 2.5 MDa can be obtained. However some microorganisms secreted HA with a molar mass in the range of several MDa, such as attenuated strains of *Streptococcus zooepidemicus* and *S. Equi. Bacillus subtilis* has been recently genetically modified to culture a proprietary formula to yield hyaluronans (Mendichi & Soltes, 2002).

It is a biodegradable, bioactive, non immunogenic, non cytotoxic and negatively charged polysaccharide (Oh et al., 2010) that has been associated with several cellular processes, including angiogenesis and the regulation of inflammation (Leach & Schmidt, 2005).

Among its applications, it is widely used as a coating for the surface modification of various biomaterials used for prosthetic cartilage, vascular graft, guided nerve regeneration and drug delivery (Li et al., 2006).

Like other glycosaminoglycans, hyaluronan can 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). 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).

More recently, HA has become recognized as an important building block for the creation of new biomaterials with utility in tissue engineering and regenerative medicine (Allison & Grande-Allen, 2006; Prestwich, 2008). Moreover, it has been shown that HA binds to cells and effectively promotes new bone formation. Balazs classified the biomedical applications of the HA and its derivatives in areas as vicosurgery, viscoaugmentation, viscoseparation, viscosupplementation, viscoprotection (Balazs, 2004).

So, in this way, there is a wide number of usages of HA in medicine and cosmetics, such as ophthalmology, orthopaedic surgery and rheumatology, otolaryngology, wound healing, pharmacology and drug delivery (Kogan et al., 2007), which shows HA as a successful biomaterial used in different fields of biomedicine.

## 2.4 Dextran

Dextran is a polysaccharide made of many glucose molecules composed of chains of varying lengths. It has a substantial number of  $\alpha$  (1 $\rightarrow$ 6) glucosidic linkages in its main chain (Fig. 4), and a variable amount of  $\alpha$  (1 $\rightarrow$ 2),  $\alpha$  (1 $\rightarrow$ 3) and  $\alpha$  (1 $\rightarrow$ 4) branched linkages (Misaki et al., 1980). The degree and type of branching will be determined by the bacterial strain that synthesizes it. Its average molecular weight is as high as  $10^7 - 10^8$  Da (Heinze et al., 2006) but can be reduce by acidic hydrolysis obtaining molecular weight fractions that also can interest.

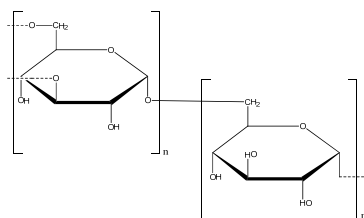


Fig. 4. Chemical structure of dextran with  $\alpha$  (1 $\rightarrow$ 3) branched linkage.

The natural structure of dextran can be modified by reacting different molecules (such hydrophobic molecules) with its different hydroxyl groups (Lemarchand et al., 2003b). Many amphiphilic dextran derivatives have been obtained by varying the nature of the reacting molecules (aromatic rings, aliphatic or cyclic hydrocarbons) and the number of grafted, that is the number of hydrophobic groups per 100 glucopyranose units or the degree of substitution (Rotureau et al., 2004).

Dextran is neutral, water soluble, biocompatible and biodegradable. Its features may vary depending on the molecular mass as well as the distribution, type of branches and the degree of branching, which depend on the bacterial synthesis or post-synthesis reactions to form derivatives.

Dextran is synthesized by a wide variety of bacterial strains. *Leuconostoc mesenteroides* produces dextran from sucrose and *Gluconobacter oxydans* produces dextran from maltodextrin. *Streptococcus mutans* also produces dextran from sucrose (Heinze et al., 2006). It can be also obtained enzymatically using cell-free culture supernatant (Wang et al., 2011). Apart from these methods, dextran can be also produced by chemical synthesis, developing a cationic ring opening polymerisation of levoglucosan (Heinze et al., 2006).

It has wide applications in different areas such as pharmaceutical, chemical, clinical, and food industry. Dextran is used as a drug (as blood plasma volume expander), adjuvant, emulsifier, carrier, stabilizer and thickener of jam and ice cream. Also it is widely used for the separation and purification of proteins (Naessens et al., 2005) based on size exclusion chromatography with a matrix of cross-linked dextran gel layer. Its derivatives also have multiple applications depending on the characteristics that structural modifications give them.

Both dextran and its derivatives have potential application for the preparation of modified drug delivery (Aumelas et al., 2007; Coviello et al., 2007; Chen et al., 2003). Not only has this polysaccharide been used to prepare nanoparticulate systems as a carrier, but also it has been employed to cover these systems (Gavory et al., 2011).

It seems that dextran is a very useful tool in the field of nanomedicine, showing also good availability, biocompatibility and biodegradability, being selected by a lot of researchers as biomaterial in the preparation of nanosystems.

## 2.5 Other polysaccharides

### 2.5.1 Pullulan

Pullulan is a linear bacterial homopolysaccharide produced from starch by the fungus *Aureobasidium pullulans*. The backbone is formed by glycosidic linkages between  $\alpha$ -(1 $\rightarrow$ 6) D-



glucopyranose and  $\alpha$ -(1 $\rightarrow$ 4) D-glucopyranose units in a 1:2 ratio (Fig. 5). The molecular weight of pullulan range from thousands to 2,000,000 Da depending on the growth conditions (Rekha & Chandra, 2007).

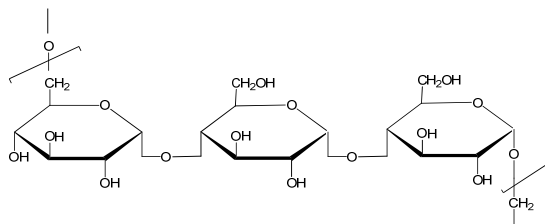


Fig. 5. Chemical structure of pullulan.

The backbone structure of pullulan tends to behave as a random expanded flexible coil in aqueous solution with modelling studies suggesting that this flexibility is imparted by the  $\alpha$ -(1 $\rightarrow$ 6) linkage. This could be the reason why pullulan is biodegradable and has high adhesion, structural flexibility and solubility (Leathers, 2003). Pullulan can also be easily derivatized in order to impart new physico-chemical properties, e.g. to increase the solubility in organic solvents or to introduce reactive groups.

This polysaccharide has numerous uses: in foods and beverages as a filler; as an edible, mostly tasteless polymer, the chief commercial use of pullulan is in the manufacture of edible films that are used in various breath freshener or oral hygiene products; in pharmaceuticals as a coating agent; in manufacturing and electronics it is used because of its film- and fiber-forming properties. It is worth noting that pullulan films, formed by drying pullulan solutions, are clear and highly oxygen-impermeable and have excellent mechanical properties.

Due to it is hemocompatible, non-immunogenic, non-carcinogenic, FDA approved it for a variety of applications (Coviello et al., 2007). Recently, pullulan has been investigated for being used in various biomedical applications such as drug and gene delivery (Rekha & Chandra, 2007), tissue engineering (Thebaud et al., 2007), and wound healing (Bae et al., 2011).

Numerous papers deal with pullulan hydrogels as drug delivery systems, particularly in the form of micro and nanogels. Despite pullulan is not a natural gelling polysaccharide, an appropriate chemical derivatization of its backbone can actually lead to a polymeric system capable of forming hydrogels. The study of nanogels has been intensified over the last decade due to related potential applications in the development and implementation of new environmentally responsive or smart materials, biomimetics, biosensors, artificial muscles, drug delivery systems and chemical separations (Coviello et al., 2007).

In order to obtain nanostructures that may act as carriers of different drugs, the backbone structure of pullulan is modified with hydrophobic molecules, resulting in a molecule of hydrophobized pullulan that self-assembles in water solutions. Cholesterol, hexadecanol or vitamin H are some molecules that are attached to the structure of pullulan in order to obtain micelles in water solution (Liu et al., 2008).

### 2.5.2 Guar gum

Guar gum is a water soluble polysaccharide extracted from the seeds of *Cyamopsis tetragonoloba*, which belongs to Leguminosae family. Also called guaran, it is a non-ionic natural polysaccharide derived from the ground endosperm of guar beans. Its backbone consists of linear chains of (1 → 4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1 → 6) linkages (Fig. 6), forming short side-branches (Sarmah et al., 2011).

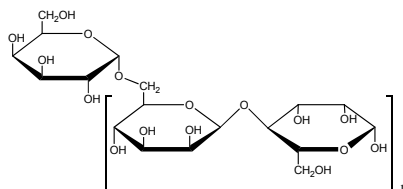


Fig. 6. Chemical structure of guar gum.

Guar gum hydrates in cold water to form a highly viscous solution in which the single polysaccharide chains interact with each other in a complex way (Barbucci et al., 2008). Its nine hydroxyl groups are available for the formation of hydrogen bonds with other molecules, but it remains neutrally charged due to the absence of dissociable functional groups. Extreme pH and high temperature conditions (e.g. pH 3 at 50°C) degrade its structure (Tirafferri et al., 2008). It remains stable in solution over pH range 5-7. Strong acids cause hydrolysis and loss of viscosity, and alkalis in strong concentration also tend to reduce viscosity. It is insoluble in most hydrocarbon solvents.

As the guar gum polymer is a low-cost, easily available and non-toxic polysaccharide, it is widely applied in many industrial fields. Thanks to its property of producing highly viscous aqueous solutions, it is commonly used as a thickening agent in cosmetics and in sauces, salad dressings and ice creams in the food industry (Barbucci et al., 2008). In pharmaceuticals, guar gum is used in solid dosage forms as a binder and disintegrant, and it has also been used as hydrophilic matrix, for designing oral controlled release dosage forms (Sarmah et al., 2011). Guar gum has been extensively used for colon delivery due to its drug release retarding property and susceptibility to microbial degradation in the large intestine (Soumya et al., 2010).

Not only the native guar-gum is used, but also chemically modified products can be used with the objective of changing its intrinsic characteristics of solubility, viscosity and rheological behaviour. For instance, hydrossilalchyl derivatives, which are often used for the formulation of cements and plasters, or carboxymethyl derivatives, which are employed as thickening agents.

In case of biomedical fields or pharmaceutical fields, such as 3D scaffolds for cell culture, fillers for tissue engineering and carriers for drugs, the physically cross-linked product is obtained through a spacer arm between the polymer chains and allows the obtainment of an insoluble compound in a wide range of pH with a good mechanical stability (Barbucci et al., 2008).

Little information is available in the literature for the possibility of using guar gum based nanosized materials as drug carriers due to its solubility in water, what makes difficult to

use it as adsorbent in aqueous conditions. Some researchers have incorporated to its structure some compounds like silica, in order to obtain insoluble compounds which could act as adsorbents in aqueous media (Singh et al., 2009). Moreover, guar gum-based nanosystems have been prepared by nanoprecipitation and cross-linking methods (Soumya et al., 2010). A different application of this polysaccharide has been found as stabilizer of nanosuspensions, where the presence of guar gum during the synthesis process allows the achievement of a better stability of the nanoparticles (Tirafferri et al., 2008).

### 2.5.3 Pectin

Pectin is a structural polysaccharide obtained from the cell wall of all plants, where is implicated in cell adhesion. This natural polymer has a heterogeneous chemical structure based on large amounts of poly (D-galacturonic acid) bonded via  $\alpha$  (1  $\rightarrow$  4) glycosidic linkage (Fig. 7). Pectin has a few hundred to about one thousand building blocks per molecule, corresponding to an average molecular weight of about 50,000 to about 180,000 Da (Sinha & Kumria, 2001). The carboxyl groups are partially in the methyl ester form with different degree of esterification (DE) and amidation (DA), which determine the content of carboxylic acid in pectin chains.

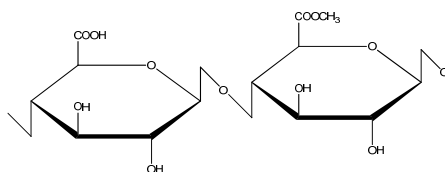


Fig. 7. Chemical structure of pectin

In the beginning, applications of pectin concentrated in food industry, as gelling or thickening agent, but lately it started being also used as an excipient for pharmaceutical purposes (Liu et al., 2003). Nowadays, some of the uses of pectin in biomedical applications include the facilitation of the delivery of specific sequences of amino acids, anti-inflammatory agents, anti-coagulants, and wound healing substances to tissue sites. Also, pectin remains intact in the physiological environment of the stomach and the small intestine, but is degraded by pectinases, which are secreted by the bacteria inhabitants of the human colon. Due to these properties it is highly possible that pectin could function as a delivery vehicle to escort protein and polypeptide drugs from the mouth to the colon (Sinha & Kumria, 2001). To be used as such, pectin based composites can be formed into membranes, microspheres, scaffolds, or injectable gels (Liu et al., 2004).

The most attractive property of pectin for industrial applications is its gelling activity. Parameters such as type and concentration of pectin (DE, DA), modification of hydroxyl groups, pH, temperature and the presence of cations, determine the gel process. For example, a high DE of pectin provides the gel formation, increasing the amounts of hydrophobic areas and reducing the solubility of pectin. In contrast, when the DE is less than 50%, pectin is highly water soluble and gel formation is only at extremely low pH solution or in the presence of divalent cations, which cross-link the galacturonic acids of the main polymer chains (Liu et al., 2003). Also, it is possible to reduce the hydrophilic property with an increasing tendency to form gels by the introduction of amide groups in low DE pectin.

With regard to its use in the preparation of drug delivery systems, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine due to its high water-solubility (Sinha & Kumria, 2001). Consequently, most of the researching groups focused on looking for water resistant pectin derivatives, which were also enzymatically degradable. For this purpose, calcium salts binding by non-covalent associations with the carbohydrate chains of pectin were investigated, which can reduce the solubility and are stable in low pH solution while resisting extensive hydration *in vivo* in the gastrointestinal tract. Thus, calcium pectinate is a potential candidate as a drug carrier for colon-specific delivery in different formulations such as microspheres, films, gels or droplets (Liu et al., 2003). Another derivative of pectin, amidated pectin cross-linked with calcium, was considered for colonic delivery, with retarding drug release and because of its biodegradability, higher tolerance to pH variations and fluctuations in calcium levels (Sinha & Kumria, 2001).

In addition, combinations of pectin with other polymers, either naturally occurring or synthetic, have been developed in order to obtain useful novel formulations. 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. As a case in point, pectin has been combined with 4-aminothiophenol (Perera et al., 2010), chitosan (Fernandez-Hervas & Fell, 1998), hyaluronic acid (Pliszczak et al., 2011) or poly (lactide-co-glycolide) (Liu et al., 2004), showing good results as controlled drug release devices.

### **3. Preparation methods of polysaccharide-based nanoparticles**

As for polysaccharide-based nanoparticles, it can be seen in the literature wide research carried out focusing on the preparation and application of these systems, which enhances their importance and versatility in terms of category and function.

According to the literature and the structural features of the employed polysaccharides, five mechanisms can be mainly applied in order to obtain nanoparticles, namely gelation of emulsion droplets, covalent cross-linking, ionic cross-linking, self-assembling and nanoprecipitation.

#### **3.1 Formation of nanoparticles from an emulsion: nanoparticles obtained by gelation of emulsion droplets**

Different methods to prepare emulsified systems have been significantly developed. All of them require two immiscible phases and the presence of a surface active agent, whose nature has been already evolved, replacing the commonly used pluronic or span by new amphiphilic copolymers (Qiu & Bae, 2006). These methods are two-step processes, where the first step consists of the preparation of an emulsified system while nanoparticles are formed during the second step. Generally, the principle of the second step gives its name to the method (Vauthier & Bouchemal, 2009).

Nanoparticles can be obtained from an emulsion method by gelation of the emulsion droplets where the polymer is dissolved, and that have been formed in the first step of the emulsion procedure. Polysaccharides show good gelling properties as well as good

solubility in water, which make them ideal candidates to be used for preparing nanoparticles by this method (Vauthier & Couvreur, 2000). Different mechanisms of gelation can be applied depending on the gelling properties of the polymer. Changes in temperature of the emulsion system or gelation induced by covalent or ionic cross-linking are some of these mechanisms which induce the gelation of the pre-formed droplets and allow nanoparticles to be obtained.

Alginate and pectin particles have been obtained by using a modified emulsification/internal gelation method (Opanasopit et al., 2008). The preparation of two different emulsions is required: one containing the gelling polymer in the dispersed phase and the other containing the gelling agent (usually counter-ions) or the pH controlling agent in the dispersed phase. Both emulsions are mixed together under strong stirring conditions in order to achieve collisions between droplets, which are necessary to promote the gelation of the polymer and, consequently, the formation of nanoparticles (Fig. 8). In case of alginate, the size range of particles is greatly dependent on the order of addition of counter-ion to the alginate solution. Some studies show that the addition of a polyelectrolyte complexation step in this procedure shows some benefits in order to obtain a better control of size distribution. Dextran or chitosan can be used as complexing agents in the *in situ* gelation of the droplets obtained in the previous nanoemulsion (Reis et al., 2007). The resulting nanoparticles range in size from 267 nm to 2.76  $\mu\text{m}$ .

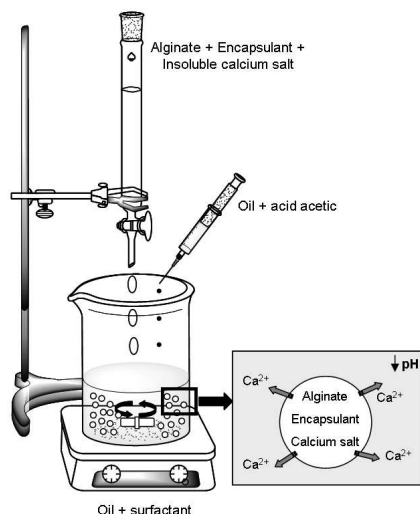


Fig. 8. Schematic representation of the emulsification-internal gelation technique using alginate.

Chitosan particles have been formed from an emulsified system by emulsion cross-linking method or by emulsion-droplet coalescence method. In the first method, the reactive functional amine group of chitosan reacts with aldehyde groups of the cross-linking agent, which usually is glutaraldehyde. A water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. Aqueous droplets are stabilized using a suitable surfactant. The stable emulsion is cross-linked by glutaraldehyde to harden the

droplets. Microspheres are filtered and washed repeatedly with n-hexane, followed by alcohol and then dried (Akbuga & Durmaz, 1994). Particle size can be determined by controlling the size of aqueous droplets, but it is usually ranged in a micrometric scale (Kumbar & Aminabhavi, 2003).

Chitosan nanoparticles with a mean size of 400 nm can be obtained by emulsion-droplet coalescence method. This method, introduced by Tokumitsu and coworkers (Tokumitsu et al., 1999), utilizes the principles of both emulsion cross-linking and precipitation. A stable emulsion containing aqueous solution of chitosan and the encapsulant drug is produced in liquid paraffin. Another emulsion containing NaOH aqueous solution is produced in the same manner and is finally mixed with the other under high speed stirring. Droplets of each emulsion would collide at random and coalesce, precipitating chitosan droplets to give small solid particles.

With a polymer like agarose, gel beads can be formed by cooling down the temperature of the solution which is prepared at high temperature. Thermal gelation results from the formation of helicoidal structures responsible for a three-dimensional network in which large amounts of water can be entrapped. The hydrogel, being hydrophilic, inert, and biocompatible, forms a suitable matrix for macromolecules that can be entrapped in the gel during formation (Vauthier & Couvreur, 2000). Agarose nanoparticles are produced using an emulsion-based technology which requires the preparation of an agarose solution in corn oil emulsion at 408°C. Macromolecules to be encapsulated are initially added to the agarose solution. The small size of the dispersed aqueous nanodroplets is achieved by homogenization. Gelation of agarose is then induced by diluting the emulsion with cold corn oil under agitation at 58°C. The liquid nanodroplets then gel to macromolecule-containing agarose hydrogel nanoparticles (Wang & Wu, 1997). The mean average size of the obtained nanoparticles is 504 nm.

### **3.2 Polysaccharide-based nanoparticles with covalent cross-links**

Among various polysaccharides, chitosan is the early one to be used to prepare nanoparticles based in covalent cross-links. Glutaraldehyde has been usually used as a cross-linker to obtain nanoparticles by emulsion cross-linking method (previously described), but its cytotoxicity limits its utility in the field of drug delivery systems. However, some chitosan nanoparticles are still being produced using glutaraldehyde as cross-linker agent (Zhi et al., 2005).

To overcome the problems of toxicity that are presented by glutaraldehyde, some biocompatible cross-linkers, such as natural di- and tricarboxylic acids, including succinic acid, malic acid, tartaric acid and citric acid, are used for intermolecular cross-linking of chitosan nanoparticles (Bodnar et al., 2005). By this method, the pendant amino groups of chitosan react in aqueous media with carboxylic groups of natural acids which were previously activated by a water-soluble carbodiimide, obtaining polycations, polyanions, and polyampholyte nanoparticles with an average size in the range of 270–370 nm depending on the pH.

Hyaluronic acid is another polysaccharide used to prepare nanoparticles by using a carbodiimide method. The preparation of nano-sized particulate systems based on hyaluronic acid takes place by covalently cross-linking via carboxyl groups of the

hyaluronic acid chain with a diamine in aqueous media at room temperature. Bodnar and coworkers have obtained spherical nanoparticles whose size varies less than 130 nm (Bodnár et al., 2009).

Recently, nanoparticles based on thiolated alginate and modified albumin have been synthesized and stabilized by the formation of disulphide bonds between both polymers (Martínez et al., 2011). In this case, the covalent interaction is established between the sulfhydryl groups of the albumin, obtained after a reduction process of the protein, and the sulfhydryl groups of the L-cysteine which has been attached to the polysaccharide structure using a carbodiimide reaction. Nanoparticles with a size range of 42-388 nm are obtained by this coacervation method based on a pH change that induces the disulphide bond formation between both structures (Fig. 9).

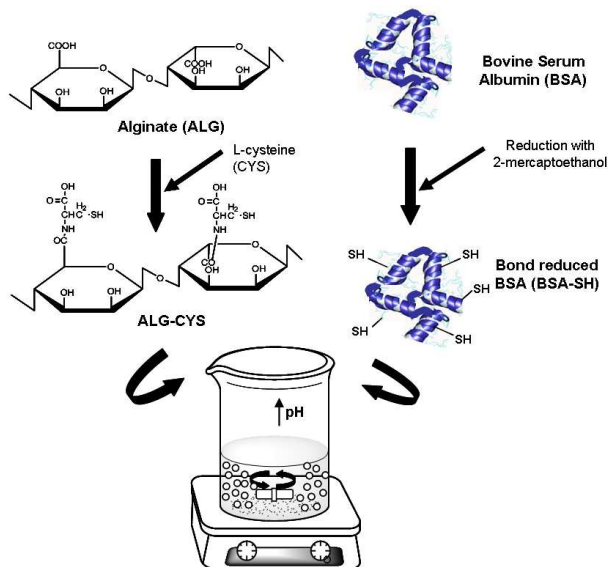


Fig. 9. Schematic representation of preparation method of nanoparticles based on thiolated alginate and modified albumin, stabilized by disulphide bond formation.

### 3.3 Polysaccharide-based nanoparticles with ionic cross-links

Ionic gelation procedure to obtain nanoparticles is included among the few organic solvent free methods, as nanoparticles are totally synthesized in aqueous media. Compared with covalent cross-linking, this method shows more advantages, such as simple procedures and mild preparation. Nanoparticles can be obtained from aqueous solutions of charged polysaccharides which gel in the presence of small ions of opposite charges. Thus, polyanions and polycations could act as cross-linkers with polycationic and polyanionic polysaccharides, respectively. Very dilute solutions of the polysaccharide are used to perform the gelation process, in which the chains of the polymer reacting with the gelling agent are forming small clusters. These clusters are stabilized by forming complex with opposite charged electrolytes (Vauthier & Bouchemal, 2009).

The cationic nature of chitosan when it is dissolved in an acidic aqueous solution (pH 4-6) can be exploited to form nanoparticles by adding small amounts of tri-polyphosphate (TPP) included in an alkaline phase (pH 7-9), upon mixing of the two phases through inter and intra molecular linkages are created between TPP phosphates and chitosan amino groups (Janes et al., 2001).

Depending on the pH and the ionic strength of the dispersing medium, these nanoparticles are capable of swelling and shrinking, which is used to trigger the release of a drug encapsulated in the nanoparticles upon the action of a pH or an ion concentration variation stimulus. For instance, KCl can be added to the dispersing medium to vary the ionic strength and cause the nanoparticle swelling; or glucosamin groups of chitosan can be deprotonated by raising the pH from acid to basic values causing a shrinking on the gel because the intramolecular electric repulsions inside the particle mesh are reduced. The average size of the obtained chitosan nanoparticles ranges between 20 and 400 nm (Pan et al., 2002).

Some water-soluble chitosan derivatives, like N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride or N-trimethyl chitosan, have been also ionically cross-linked to prepare nanoparticles. The average size of the obtained systems is between 110 and 350 nm (Amidi et al., 2006).

A slightly modified ionotropic gelation technique was used by de la Fuente and coworkers in order to obtain nanoparticles based on chitosan and hyaluronic acid using TPP as ionic cross-linker. Their results show that hyaluronic acid/chitosan nanoparticles have a small size in the range of 110-230 nm (de la Fuente et al., 2008).

Not only TPP is used as a cross-linker to obtain chitosan nanoparticles by ionic gelation method. Some researchers as Kim and co-workers have obtained chitosan-based nanoparticles by this method using the encapsulated drug itself as a cross-linker, establishing electrostatic interactions between amine group of chitosan and hydroxyl group of the drug (Kim et al., 2006).

Among negatively charged polysaccharides, alginate is one of the most used to obtain nanoparticles by ionic gelation. In this case, carboxylic groups on molecular chains of alginate structure can be cross-linked by bivalent calcium ions to form nanoparticles. Then, clusters formed in the pre-gel phase can be stabilized with polycations like polylysine and chitosan (De & Robinson, 2003). Polylysine can form polyelectrolyte complexes with alginate without the previous formation of the pre-gel phase with calcium, but more compact nanoparticles are obtained when this previous step is carried out. The size of nanoparticles using polylysine as stabilizer depends, not only on the concentration of alginate, but also on the molecular weight. In fact, an optimal mass balance between sodium alginate:  $\text{CaCl}_2$ : cationic polymer (poly-Llysine or chitosan) has been found to obtain particles with nanometric size (Vauthier & Bouchemal, 2009). Nanoparticles with an average size ranged between 194 nm and 1.6  $\mu\text{m}$  can be obtained by this method (Ahmad et al., 2006; Azizi et al., 2010).

In addition, the interaction of alginate with divalent calcium ions was used to obtain nanoparticles from water-in-oil microemulsions, as it was previously described (Reis et al., 2007).



### 3.4 Methods based on self assembling macromolecules

#### 3.4.1 Polysaccharide based nanoparticles by polyelectrolyte complexation (PEC)

Polyelectrolyte complexes (PECs) are formed by the interaction between oppositely charged polymers by intramolecular electrostatic interactions. PECs are very interesting materials for different applications because some of their properties, like swelling or permeability, can be easily modified by external stimuli, such as the pH of the medium.

Positively or negatively charged nanoparticles with a core/shell structure can be obtained according to the nature of the polyelectrolyte used in excess. The hydrophobic core is composed by the complexed segments whereas the excess of component not incorporated in the polyelectrolyte complex is segregated in the outer shell ensuring the colloidal stabilization of the nanoparticles against coagulation and conferring the charge of the nanoparticle surface. This charge could affect to the interaction between cells and nanoparticles. Moreover, molecular weight of the two polyelectrolytes influences the size of the nanoparticles (Vauthier & Bouchemal, 2009).

Although any polyelectrolyte could interact with polysaccharides in order to obtain PEC nanoparticles, only water-soluble and biocompatible polymers are used as polyelectrolytes with this goal. Among the existing polyanionic and polycationic polysaccharides to form PEC nanoparticles, chitosan is widely used because it satisfies the needs of safety and solubility. It can be seen in the literature that much research has been carried out on PECs with chitosan as polycation and different negative polymers, such as negative polysaccharides, poly(acrylic acid) (PAA) or nucleic acids.

There is a wide variety of negative polysaccharides that can be attached to chitosan to form PEC nanoparticles. Carboxymethyl cellulose (Cui & Mumper, 2001), dextran-sulfate (Drogoz et al., 2007), alginate (Sarmiento et al., 2006) and glucomannan (Alonso-Sande et al., 2006) are just a few examples of PEC combinations that allow the preparation of nanoparticles whose size ranges between 100 and 800 nm.

The formation of the complex between chitosan and poly(acrylic acid) (PAA) has been widely studied. The influence of molecular weight of chitosan and PAA, the ratio of the initial polyelectrolyte concentrations, dropping temperature, pH of the initial solutions and the purification process on the size, stability and morphology of the nanoparticles has been studied by different authors (Chen et al., 2005; Davidenko et al., 2009).

Nucleic acids can also be combined with chitosan to obtain nanospheres. In this case, the drug being the nucleic acid is incorporated in the nanocarrier as part of its structure (de Martimprey et al., 2009). The N/P ratio, which is defined as the ratio number of amine groups of the polycation (N) divided by the number of phosphate groups of the nucleic acid (P), has to be taken into account to obtain the desired size of nanospheres.

Apart from chitosan, polyelectrolyte complexes with nanometric size can be formed using alginate, a negatively charged polysaccharide, combined with polylysine, a positively charged peptide (George & Abraham, 2006). Although both structures could interact without the previous formation of the alginate pre-gel phase with calcium, more compact nanoparticles are obtained when this previous step is carried out. Nanoparticles with a mean size of 250–850 nm are obtained using very well defined concentrations of both electrolytes.

### 3.4.2 Nanoparticles obtained from self-assembling of hydrophobically modified polysaccharides

Amphiphilic copolymers are synthesized when hydrophobic segments are added to chains of hydrophilic polymers. In aqueous solutions, amphiphilic molecules orientate themselves in order to achieve a state of minimum free energy and the hydrophobic blocks are removed from the aqueous environment. Consequently, polymeric micelles with core/shell structure are formed. Thanks to their hydrophobic domain, surrounded by a hydrophilic outer shell, they can serve as reservoir for various hydrophobic drugs (Letchford & Burt, 2007).

The synthesis and application of polysaccharide-based-self-aggregate nanoparticles as drug delivery systems have been recently investigated. There are various hydrophobic molecules that can be attached to polysaccharides in order to obtain these kind of systems, such as poly(ethylene glycol) derivatives, long chain fatty acids, poly( $\epsilon$ -caprolactone), pluronic copolymers, cholesterol and poly(isobutylcyanoacrylate) (PIBCA).

Poly(ethylene glycol) (PEG) has been often used in pharmaceutical and biomedical fields as soluble polymeric modifier in organic synthesis, and as a pharmacological polymer with high hydrophilicity, biocompatibility and biodegradability. PEG and its derivatives can be attached to the polysaccharide structure to form micelles directly in an aqueous medium by adjusting the hydrophobicity/hydrophilicity of the polysaccharide chain. Chitosan has been grafted with different molecules of poly(ethylene glycol), obtaining nanoparticles with an average size ranged between 80-260 nm (Yang et al., 2008; Yoksan et al., 2004).

Some long-chain fatty acids like hexanoic acid, linoleic acid, linolenic acid, palmitic acid or stearic acid have been used for modifying polysaccharides and obtaining polymeric micelles. Nanoparticles based on linoleic acid-chitosan have been obtained through a carbodiimide-mediated reaction, and their size ranged between 200-600 nm. (Chen et al., 2003). Hu and coworkers employed a similar methodology in order to obtain stearic acid-chitosan nanoparticles. To increase the stability of the micelle *in vivo* and controlled drug release, the shells of micelles were cross-linked by glutaraldehyde (Hu et al., 2006). Dextran has been also employed to obtain nanoparticles by coupling lipoic acid to the structure of dextran and forming nanoparticles in water, whose size varied from 145 to 221 nm (Li et al., 2009).

Poly( $\epsilon$ -caprolactone) (PCL) is a well known biodegradable polyester, frequently used as implantable carrier for drug delivery systems, and a promising molecule which allows the formation of nanometric micelles when it is attached with a polysaccharide structure. The combination of the hydroxyl groups of dextran with the carboxylic function present on preformed PCL monocarboxylic acid results in the formation of nanoparticles of less than 200 nm (Lemarchand et al., 2003a).

Hyaluronic acid and pluronics form the polymeric shell of nanoparticles obtained by Han and coworkers (Han et al., 2005). Pluronic self-assemble to form a spherical micellar structure above the lower critical solution temperature by hydrophobic interaction of the poly(propylene oxide) middle block in the structure. Depending on the composition and molecular weight, they show at high concentration a sol-gel transition behaviour when raising the temperature above the lower critical solution temperature, which means a swelling or de-swelling behaviour temperature-dependent (Liu et al., 2003).

Among different cyclic hydrophobic molecules, cholesterol is one of the most used to give hydrophobic character to polysaccharide structures and form self-assembly nanoparticles in aqueous solution. Cholesterol attached to pullulans of different molecular weights, or to chitosan, allow to obtain nanoparticles of very small sizes: 20-30 nm in case of cholesterol-pullulans based nanoparticles (Akiyoshi et al., 1998) and 417 nm in case of cholesterol-chitosan systems (Wang et al., 2007). Apart from cholesterol, 5 $\beta$ -Cholanic acid is a non-toxic bile acid present in humans that can be chosen as the hydrophobic moiety to be attached to hyaluronic acid in order to form amphiphilic conjugates. The mean diameters of the obtained nanoparticles are in the range of 237–424 nm (Choi et al., 2010).

Poly(isobutylcyanoacrylate) (PIBCA), which belongs to polyacrylate family, can form amphiphilic copolymers in combination with polysaccharide structures due to the hydrophobic nature of this molecule that shows carboxylic esters on its structure. Among the different existing combinations of PIBCA with polysaccharides, chitosan and dextran are good examples of hydrophilic molecules that have been used to obtain nanometric micelles through an emulsion polymerization of IBCA in the presence of polysaccharides. In case of chitosan, the obtained micelles show very small sizes, lower than 35 nm (Bertholon et al., 2006).

### 3.5 Nanoprecipitation

Nanoprecipitation is one of the most used methods to produce nanoparticles owing to the reproducibility, the simplicity and the economy of the technique. Systems of three components are needed to perform nanoprecipitation process: the polymer, the polymer solvent and the non-solvent of the polymer. To produce nanoparticles, the polymer solution is mixed with the non-solvent. Nanoparticles are instantaneously formed during the fast diffusion of the polymer solution in the non-solvent. This technique has been up to date mainly applied for poly(lactic acid), poly(lactic-*co*-glycolic acid) (Lassalle & Ferreira, 2007) as well as for polysaccharide derivatives (Hornig & Heinze, 2008). Chitosan and amphiphilic cyclodextrins are some examples of polysaccharide derivatives employed to obtain nanoparticles by this method.

In case of chitosan, sodium sulfate is commonly used as a precipitating agent to form chitosan particles. When sodium sulfate is slowly added into a solution of chitosan and polysorbate 80 under both stirring and ultrasonication, desolvated chitosan in a particulate form is obtained. The precipitated particles are at micro/nano interface ( $900 \pm 200$  nm) (Berthold et al., 1996).

Skiba and coworkers synthesised nanoparticles based on amphiphilic cyclodextrines (Skiba et al., 1996). Nanospheres are prepared by progressive dispersion of an organic solution of modified  $\beta$ -cyclodextrin in an aqueous phase with or without surfactant. Various physicochemical parameters were studied such as the effect of the chain length of acyl groups and type of surfactant on the size and physicochemical properties and stability of the nanospheres. Systems with a mean diameter varying between 90 and 150 nm were obtained by this method.

## 4. Application as drug delivery systems

In the design of drug carriers, issues of safety, toxicity and availability have to be taken into account, and the application of polysaccharides simplifies some of these issues. Thus,

polysaccharides are emerging as new promising materials to be applied in the design of drug delivery systems. Some examples of drug delivery systems that have been prepared during the last years using polysaccharides in their composition have been collected below (Table 1).

#### 4.1 Peptides and proteins included in polysaccharide-based delivery systems

Some of the most significant advances in biotechnology in recent years are related with the discovery of some therapeutic and antigenic peptides and proteins (Vila et al., 2002). Despite these great advances, some problems like low stability, short biological half-life and the need to cross biological barriers limit the use and the *in vivo* application of the most of biologically derived drugs. The inclusion of the biologically derived drug into drug delivery systems based on polysaccharides have shown good results and some of the problems of systemic administration can be overcome.

**Insulin** is one of the most widely used therapeutic peptides. It is a 5.8 kDa protein used exogenously to treat insulin-dependent diabetes mellitus when normal pancreatic production is insufficient. Orally administration of insulin normally shows low bioavailability due to acidic gastric pH, the enzymatic barrier of the intestinal tract and the physical barrier made up of the intestinal epithelium. The inclusion of insulin into nano- and microparticulate systems potentially provides gastric protection, controlled release and enhanced absorption by mucosal adhesion and nanoparticle direct uptake (Tiyaboonchai et al., 2003). There are various studies in which insulin has been successfully included into polysaccharide-based nanoparticles prepared by different methods, that have shown good results in loading efficacy of the drug, good release control and, in some of them, *in vivo* efficacy of the systems.

Nanoemulsion/*in situ* triggered gelation is one of the methods employed to prepare insulin-loaded nanoparticles. Pinto Reis and co-workers have designed nanoparticles based on alginate and alginate coated with chitosan that have been loaded with insulin obtaining an encapsulation efficiency between 76-93% due to the interaction of amino groups of insulin with carboxylic groups of alginate. They observed that insulin was strongly retained into both systems at low pH, but when the release medium was changed by one at near neutral pH, up to 40-70% of the insulin was released almost immediately. Alginate-chitosan nanospheres demonstrated the best controlled insulin profile release in simulated intestinal conditions (Reis et al., 2008). The same method has been used to prepare insulin-loaded nanoparticles based on alginate-dextran, achieving an encapsulation efficiency of 82.5%. At low gastric pH, insulin was fully retained likely due to alginate polymer forming a compact acid-gel structure reducing permeability and potentially stabilizing insulin from acid attack. Up to 89% of the insulin was released almost immediately after changing the medium to near neutral pH, and full release was observed after 1h. Nanoencapsulated insulin was bioactive, which was demonstrated through both *in vivo* and *in vitro* bioassays (Reis et al., 2007).

Insulin-loaded nanoparticles have been prepared by ionic cross-link methods using chitosan as the selected polysaccharide and TPP anions as cross-linker agent. Pan and co-workers used this method in order to prepare chitosan nanoparticles which enhanced the intestinal absorption of insulin *in vivo* (Pan et al., 2002). The association between the drug and the

system was up to 80%, and the drug release was very dependent on the pH of the release medium. *In vivo* studies revealed that insulin was released in its active form, and the dosages of insulin loaded into chitosan nanoparticles were found orally effective, prolonging the hypoglycemia over 15 h.

Group of drugs	Entrapped Drug	Method of synthesis	Polysaccharide	NP size (nm)	References
Peptides and proteins	Insulin	Emulsification / ionic gelation	Alginate	564	Reis et al., 2008; Reis et al., 2007
			Alginate-chitosan	1280	
			Alginate-dextran	267-2760	
		Ionic cross-link	Chitosan	300-400	Pan et al., 2002
				250-400	
		Polyelectrolyte complexation (PEC)	Chitosan-dextran	423-850	Sarmiento et al., 2006
	Chitosan-alginate				
	Self-assembling	Pullulan-cholesterol	20-30	Akiyoshi et al., 1998	
					Chitosan-glucomannan
Growth factors	Ionic cross-link	Chitosan	301-424	Cetin et al., 2007	
Antisenses	Ionic cross-link	Alginate-chitosan	196-430	Azizi et al., 2010	
Anticancer drugs	Tamoxifen	Covalent cross-link	Alginate-albumin	42-388	Martínez et al., 2011
		Emulsification	Guar gum	200-300	Sarmah et al., 2009
	Mitoxantrone	Ionic cross-link	Chitosan	75	Lu et al., 2006
	Methotrexate	Self-assembling	PEG-chitosan	262	Yang et al., 2008
	Doxorubicin	Emulsification	Oleoyl-chitosan	255	Zhang et al., 2007
	Paclitaxel	Self-assembling	Stearic acid-chitosan	28-175	Hu et al, 2006
	Epirubicin	Self-assembling	Chitosan-cholesterol	417	Wang et al., 2007
Nucleic acids and genetic material	Nucleic acids	Ionic cross-link	Chitosan	200-700	Katas & Alpar, 2006
		Polyelectrolyte complexation (PEC)	Chitosan-alginate	323-1600	Douglas & Tabrizian, 2005
			Chitosan-CMC	180-200	Cui & Mumper, 2001

Group of drugs	Entrapped Drug	Method of synthesis	Polysaccharide	NP size (nm)	References
			Chitosan-nucleic acids	129-450	de Martimprey et al., 2009
	Antisenses	Polyelectrolyte complexation (PEC)	Alginate-poly-L-lysine	28.000-10.000	González Ferreiro et al., 2002
	Genes	Emulsification / ionic gelation	Alginate	80	You & Pen, 2005
<b>Other drugs</b>	Furosemide	Emulsification / ionic gelation	Chitosan	30-150	Zhi et al., 2005
	Cyclosporine A	Ionic cross-link	Chitosan	250-400	De la Fuente et al., 2010
	Rertinol	Nanoprecipitation	Chitosan	50-200	Kim et al., 2006
	Antitubercular drugs	Ionic gelation	Alginate	236	Ahmad et al., 2006
	Anfortericin B	Polyelectrolyte complexation (PEC)	Chitosan-dextran sulfate	600-800	Tiyaboonchai & Limpeanchob, 2007

Table 1. Some groups of drugs included in polysaccharide-based nanoparticles.

Ionotropic complexation (PEC) between chitosan and different polyanions (alginate, dextran sulfate and glucomannan) has been described in different works as another method that allowed the preparation of insulin-loaded nanoparticles. Insulin association efficiency from 63 to 94% and loading capacity from 5 to 13% were obtained with chitosan/alginate and chitosan/dextran nanoparticles, providing dextran sulfate combination the highest insulin association efficiency. It might be seen that insulin release occurred very rapidly from both systems, but a significant increment of insulin retention when using dextran sulfate in the formulation compared with alginate was observed in simulated gastric conditions (Sarmiento et al., 2006). Alonso-Sande and co-workers prepared insulin-loaded chitosan/glucomannan nanoparticles reaching association efficiency values of 89%. They analyzed the influence of the pH release medium and the TPP presence on the release rate, obtaining more restricted diffusion of the peptide through a highly cross-linked system with TPP and at high pH of the release medium (Alonso-Sande et al., 2006).

Finally, nanoparticles based on self-assembling methods were also obtained and loaded with insulin. In this case, insulin was easily complexed with the hydrogel nanoparticle of hydrophobized cholesterol-bearing pullulan in water. The presence of different concentrations of bovine serum albumin (BSA) in the release medium was analyzed, and approximately 15% of insulin still remained in the complex, even at concentrations of BSA comparable to its physiological value in the blood. The physiological activity of complexed insulin was preserved *in vivo* after i.v. injection, showing an excellent behaviour of the complex as a possible protein drug carrier (Akiyoshi et al., 1998).

**Basic fibroblast growth factor (bFGF)** is a protein with molecular mass of 18 kDa that is a potent mitogen which regulates angiogenesis during growth and development. This growth factor stimulates the proliferation of a wide variety of cells, including mesenchymal, neuroectodermal, and endothelial cells, and it is effective on protecting neurons from oxidative stress processes (Li et al., 2006). However, bFGF has minimal pharmacological effects in the central nervous system (CNS) because of the presence of bloodbrain barrier that reduces its transport to CNS. In order to enhance the amount of bFGF that could reach the CNS, Cetin and co-workers have developed bFGF-loaded chitosan nanoparticles according to a ionotropic gelation process, using TPP as cross-linker agent. Nanoparticles displayed a low bFGF-association efficiency (27.4%) leading to final bFGF-loading values as low as 0.021%, but since bFGF exerts its biological activity in the concentration range of 0.1 to 10.0 ng/mL, they concluded that the bFGF loading was acceptable. *In vitro* release studies showed that around 30% of the loaded protein was immediately released into PBS and that the highest extent of release (68%) was observed at 24h. Moreover, their results showed that integrity of the encapsulated bFGF was not affected by the entrapment procedure and release conditions (Cetin et al., 2007).

**Epidermal growth factor receptor (EGFR) antisense (AS)** is used to reduce the expression of EGFR, a receptor tyrosine kinase proto-oncogene that plays a central role in the initiation and development of several human malignancies, notably of the breast, brain, and lung. The problem is that the macromolecular drug can degrade during preparation of the nanoparticles, during storage, and also during *in vitro* or *in vivo* release. For this reason, the ability of nanoparticles to protect the antisense molecules from degradation in aqueous medium is necessary to be investigated. Azizi and co-workers prepared EGFR-AS-loaded chitosan/alginate nanoparticles with different composition ratios, obtaining that nanoparticles can release antisense over 45-50 hours and 67-96% loading efficacy. Thus, they concluded that the nanoparticles could retain and stabilize the content of antisense in their hydrogel matrix (Azizi et al., 2010).

## 4.2 Anticancer drugs included in polysaccharide-based delivery systems

One of the major problems facing cancer chemotherapy is the achievement of the required concentration of the drug at the tumour site for a desired period of time, since tumours usually present resistances to treatment, and high dosages are frequently toxic. Thus, one of the main goals of nanomedicine is to develop safe and effective drug carriers that are systemically applied but will selectively deliver cytotoxic drugs to tumour cells without harming normal cells (Gullotti & Yeo, 2009). Among the available potential drug carrier systems in this size range, polysaccharide-based nanoparticles play an important role and their use with some anticancer drugs show promising results.

**Tamoxifen** has been successfully used since 1970s in treatment of hormone dependent breast cancer (Ameller et al., 2004). However, tamoxifen shows low water solubility, which limits the administration of this drug only to the oral route. Furthermore, following a long-term therapy, tamoxifen has some side effects, such as endometrial cancer and development of drug resistance. To overcome the undesirable side effects of tamoxifen, and to increase the concentration at the tumour site, tamoxifen could be entrapped into polymeric nanoparticles, which may provide better means of delivery in terms of enhanced uptake by the tumour and increased local concentration of the drug at the receptor site. Tamoxifen-

loaded nanoparticles were prepared by Sarmah and co-workers based on guar gum, which is commonly used for colon specific drug delivery in the pharmaceutical industry. Nanoparticles were obtained by o/w emulsification and in situ polymer cross-linking, using dichloromethane as the best solvent of the drug and glutaraldehyde as cross-linker agent during the process. An efficiency loading of 15% was obtained when dichloromethane was used as selected solvent (Sarmah et al., 2009). Recently, nanoparticles based on thiolated alginate (ALG-CYS) and disulfide bond reduced albumin (BSA-SH) have been synthesized by coacervation method and stabilized by disulfide bond formation between both polymers. *In vitro* studies revealed that total release of the drug was not achieved in any case; only the 23–61% of the drug was released. Maximum release took place between 7 and 75 h. According to the results, it was concluded that the presence of alginate in the nanoparticle composition allowed the modulation of the amount of released TMX (Martínez et al., 2011).

**Mitoxantrone** is often used to treat breast cancer clinically, but the prolonged treatment with this drug results in some side-effects, such as heart toxicity and myelosuppression, which are often a problem. Mitoxantrone is positively charged and it can be absorbed by negatively charged polysaccharides, such as chitosan. Nanospheres can be obtained by ion gelation method using sodium TPP as gelation agent, and obtaining an encapsulation efficacy of 98%. Tests for *in vitro* release in physiological saline or physiological saline containing 0.5% (w/v) ascorbic acid by a dialysis bag showed sustained release and little burst effect (Lu et al., 2006).

**Methotrexate (MTX)** is a folate antimetabolite and has been used in the treatment of various malignancies, including childhood acute lymphocytic leukemia, osteosarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, head and neck cancer, lung cancer, and breast cancer. However, it may cause some adverse effects such as bone marrow suppression, acute and chronic hepatotoxicity, interstitial pneumonitis and chronic interstitial obstructive pulmonary disease. Therefore, in order to reduce its toxic and side effects and to improve specificity and selectivity, it is a good candidate to be included in drug delivery systems. Yang and co-workers have designed methoxy poly(ethylene glycol)-grafted-chitosan (mPEG-g-CS) self-aggregated nanoparticles being used as a carrier of MTX. Depending on the formulation, loading efficiency varied from 21 to 95%. *In vitro* release studies showed that the MTX appeared to be released in a biphasic way, which characterized by an initial release or rapid release period followed by a step of slower release. A fast release was observed in 4 h, in which 40% of the drug was released from nanoparticles. After this initial effect, MTX was released in a continuous way for up to 48 h, reaching percentage of cumulative release close to 60%. Therefore, the self-aggregated nanoparticles delayed the drug release in the release process when these results were compared with the release of the free drug (Yang et al., 2008).

**Doxorubicin (DOX)** is a member of the anthracycline ring antibiotics, with a broad spectrum of antitumor activity, including a variety of human and animal solid tumors. Despite advances of this antitumor agent, it is hydrophobic and possesses inevitable, serious side effects such as nonspecific toxicity that limit the dose and use of the drug. Therefore, a lot of studies have been carried out in order to entrap this drug into different drug carriers. DOX has been successfully entrapped into oleoyl-chitosan nanoparticles prepared by o/w emulsification method. Nanoparticles had a high encapsulation efficiency of 53% in loading doxorubicin, being drug incorporation into nanoparticles generally limited by the large



surface area of the latter, as well as by the solubility of the drug in water. The drug was completely released from nanoparticles in the buffer medium of  $\text{Na}_2\text{HPO}_4$ - citric acid (pH 3.8), whereas in PBS (pH 7.4) 65% of doxorubicin was released after 6 hours, followed by a sustained release until 72 hours. Approximately 72% of DOX was released for 3 days, showing the potential of the nanoparticles as a sustained drug delivery system, suggesting that the nanoparticles might act as a barrier against the release of entrapped DOX. This result indicated that the nanoparticles contributed to an extended circulation of DOX and thus an improvement in therapeutic efficacy (Zhang et al., 2007).

**Paclitaxel** is an anticancer drug which is used in different types of malignancies, such as ovarian and breast cancer. Despite its multiple applications, it has important problems of solubility in water and, consequently, its administration is carried out including an oily component (Cremophor) and dehydrated alcohol within its formulation. Thus, relevant side effects such as hypersensitivity, hypotension or breast pain are frequently observed after its intravenous administration. In the last years, a lot of studies have included this drug as good candidate to be included in drug delivery systems to overcome its administration problems. Hu and coworkers have obtained paclitaxel-loaded nanoparticles by self-cross-linking stearic-grafted chitosan and using glutaraldehyde as cross-linker in order to stabilize the systems. These formulations showed high encapsulation efficiency which ranged from 95% to 99%. When the surfaces of micelles were cross-linked by glutaraldehyde, the burst release of the micelles at earlier stage was highly improved and the drug release time was prolonged. By controlling the amino substitution of stearic-grafted chitosan and the cross-link degree, the prolonged and controlled release could be achieved (Hu et al., 2006).

As an anthracycline anticancer agent, **epirubicin** has a wide range of antitumor activity and is used to treat various carcinomas. However, this therapy may cause some serious side effects such as allergic reactions, cardiotoxicity and blood problems. Therefore, nanoparticles being used as a carrier of epirubicin are hoped to sustain its release, prolong its circulation time, enhance its therapeutic index and decrease its toxic effects. Cholesterol-modified nanoparticles have been prepared by self-assembling method by Wang and coworkers (Wang et al., 2007). They synthesized cholesterol-modified chitosan conjugate with succinyl linkages, investigated its self-aggregation behaviour, and prepared self-aggregated nanoparticles by sonication in aqueous media. Nanoparticles loading efficiency varied from 25 to 71% depending on the weight ratio of epirubicin and the nanoparticles and exhibited the release profile relating to the pH of the release media. When the pH of the release media increased, an evident decrease of drug release rate was observed owing to the solubility of epirubicin is greatly influenced by the pH of the aqueous solution, and self-aggregated nanoparticles were also pH sensitive because of the presence of many amino groups in their molecules. Thus, the drug release from self-aggregated nanoparticles was very slow in PBS (pH 7.4) and the total release amount was about 25% in 48 h, which suggested self-aggregated nanoparticles had a potential as a sustained-release carrier of epirubicin.

### 4.3 Nucleic acids and genetic material included in polysaccharide-based delivery systems

**Small interfering RNAs** (siRNAs) have proven to be versatile agents for controlling gene expression in mammalian cells. They have been employed as a novel tool since they can block the expression of genes, such as those expressed in infectious diseases and cancers.

However, siRNA suffers particular problems including poor cellular uptake, rapid degradation as well as limited blood stability. Therefore, effective systems which can protect and transport siRNA to the cytoplasm of the targeted cells are needed to exploit the promising potential applications offered by successful delivery of siRNA. Chitosan has been used to prepare nanoparticles based on modified ionic gelation with TPP as cross-linker agent, and they have been applied as possible vector that should be able to be taken up by the cells and escape the endosomal vesicle to avoid lysosomal degradation. *In vitro* study revealed the transfection efficiency of siRNA depends on the method of siRNA association to the chitosan, and entrapping siRNA using ionic gelation has shown to yield a better biological effect than simple complexation or siRNA adsorption onto the chitosan nanoparticles (Katas & Alpar, 2006).

Ionotropic complexation (PEC) between chitosan and different polyanions has been used as an useful method of preparation of nanoparticles in order to include nucleic acids, since chitosan-DNA nanoparticles demonstrated low transfection efficiencies and the incorporation of secondary polymers improved the characteristics of these systems (Kaul & Amiji, 2002). Among the chitosan-polyanion complexes investigated, the combination of chitosan and alginate is considered to be among the most interesting for delivery systems. The method used to prepare the nanoparticles is a two-step method where the first step is the formation of a calcium-alginate pre-gel. Various concentrations of chitosan solutions were then added with continuous stirring. High loading efficacy was obtained (26-60%), while maximum mass loading was 60  $\mu\text{g}$  DNA/mg nanoparticles and 6% and 3.5% of the adsorbed DNA was released. Therefore, the high encapsulation of DNA from alginate-chitosan nanoparticles is encouraging for application in the field of gene therapy (Douglas & Tabrizian, 2005). The complexation between chitosan and carboxymethylcellulose (CMC) is another PEC combination employed in the inclusion of nucleic acid into delivery systems. Cui and Mumper coated the surface of this pre-formed nanoparticles with plasmid-DNA, obtaining a final plasmid DNA concentration of up to 400 mg/ml. Chitosan/CMC based nanoparticles containing plasmid DNA were applied topically to the skin of shaved mice and resulted in detectable and quantifiable levels of luciferase expression in skin after 24 h (Cui & Mumper, 2001). Nucleic acids can be also combined with chitosan in order to obtain nanospheres, being part of the structure of the system at the same time they are the encapsulant of the nanospheres. Chitosan showed a very high protection efficiency for siRNA. Polyplexes prepared with this polysaccharide are able to protect a siRNA for 7 h and an efficient transfection of the cells may be obtained after the intranasal administration of siRNA/chitosan polyplexes (de Martimprey et al., 2009).

**Antisense oligonucleotides** are therapeutic agents known to selectively modulate gene expression. The development of non-parenteral dosage forms for these compounds is desirable. However, the high molecular weight, the hydrophilicity and multiple negative charges result in a poor absorption of antisense oligonucleotides. Moreover, the oral administration faces additional problems such as degradation in the acidic gastric environment, enzymatic metabolism in the lumen and at the gastrointestinal epithelium and first-pass hepatic clearance. To achieve a successful non-parenteral delivery of antisense therapeutics, it is necessary to solve the specific problems of the oral administration route, together with general concerns of correct time-space targeting, improved cellular uptake and nuclear localization to exert gene transfection (Akhtar et al., 2000). Using a ionotropic

gelation method, microparticles made of alginate cross-linked with calcium ions and poly-L-lysine have been reported to effectively act as transfecting agents. Encapsulation efficacies of 32-95% depending on the composition of the formulation have been obtained and the *in vitro* release behaviour depended mostly on the medium composition. The importance of competitive anions and ionic strength on the mechanism of dissociation of the oligonucleotide from the polymeric matrix was observed. Thus, the presence of phosphate anions preferably displaced alginate from the structure, resulting in the release of complexed oligonucleotide. Rat *in vivo* studies showed promising oligonucleotide bioavailability for microparticles after intrajejunal administration in the presence of a mixture of permeation enhancers to achieve a successful intestinal application (Gonzalez Ferreira et al., 2002).

Natural biopolymers are also widely used in the field of **gene delivery**. In fact, alginate nanoparticles have been prepared using w/o microemulsion as a template followed by calcium cross-linking of guluronic acid units of alginate polymer. Ca-alginate nanoparticles were loaded with GFP-encoding plasmids in order to study their potency as carriers for gene delivery. The degree of endocytosis by NIH 3T3 cells and ensuing transfection rate were investigated. Results showed that Ca-alginate nanoparticles were very efficient gene carriers (You & Peng, 2004).

#### 4.4 Other drugs included in polysaccharide-based delivery systems

**Furosemide** is a loop diuretic used in the treatment of congestive heart failure and edema, and it has been recently incorporated into chitosan nanoparticles (Zhi et al., 2005). Since the chitosan molecule has strong interaction with the organic compounds, it can be applied to adsorb diuretics from the water samples. Zhi and coworkers prepared chitosan nanoparticles by a nanoemulsion system, in which the chitosan nanoparticles were prepared by adding NaOH solution or glutaraldehyde as the solidification solution. Size was smaller in case of using NaOH as cross-linker agent (30 - 150 nm). The adsorptive efficiency of furosemide on the nanoparticle was 51.9%, and the presence of less cross-linking agent could be in favour of the adsorption ability. The furosemide adsorption capacity on the chitosan nanoparticles was affected not only by the -NH<sub>2</sub> content, but also by the molecular weight of chitosan.

Ophthalmic drug delivery, probably more than any other route of administration, may benefit from the characteristics of nanotechnology-based drug delivery systems, mainly because of their capacity to protect the encapsulated molecule while facilitating its transport to the different compartments of the eye (Raju & Goldberg, 2008). For this purpose, the common use of chitosan is justified by its mucoadhesive and penetration enhancing properties, as well as by its good biocompatibility with the ocular structures. **Cyclosporine A (CyA)** is an immunosuppressant drug widely used in post-allogeneic organ transplant to reduce the activity of the immune system, and therefore the risk of organ rejection. The entrapment of the hydrophobic polypeptide CyA was achieved by ionic gelation technique using TPP and a previous dissolution of the peptide in an acetonitrile: water mixture, and a further nanoprecipitation into the nanoparticles in the form of small nanocrystals (de la Fuente et al., 2010). Entrapment efficiencies were reported to be as high as 73.4%. The *in vivo* evaluation of this new prototype, in rabbits, evidenced the capacity of these nanoparticles providing a selective and prolonged delivery of CyA to the cornea and conjunctiva. More

importantly, it was observed that CyA-loaded chitosan nanoparticles provided therapeutic levels of CyA in the conjunctiva and the cornea for up to 24 and 48 h postadministration, respectively, while reducing the access of CyA to the blood circulation. This positive behavior of chitosan nanoparticles was attributed to their improved interaction with the corneal and conjunctival and the prolonged delivery of the CyA molecules associated to them (de la Fuente et al., 2010).

**Retinol** and its derivatives are extensively used in the pharmaceutical and cosmetic area. Especially, retinoids are recognized as being important for modern therapy of dermatological treatment of wrinkled skin. The stability and solubility problems of these compounds make them ideal candidates to be included into carriers. Nanoparticles are reported to be useful formulation to solve the poor aqueous solubility of retinoids and are able to use it by intravenous injection. Chitosan has been used by Kim and coworkers in order to obtain retinol-encapsulated chitosan nanoparticles for application of cosmetic and pharmaceutical applications (Kim et al., 2006). Retinol-encapsulated nanoparticles were completely reconstituted into aqueous solution as same as original aqueous solution.

The requirement of multidrug administration daily or several times a week for at least 6 months is the main cause of the failure of antitubercular chemotherapy. The dose-dependent side effects with high incidence and the need of daily-dosing results in discontinuation of medication, relapse of symptoms and an alarming increase in the prevalence of multidrug-resistant strains (Pandey & Khuller, 2005). Consequently, reductions in dose and dosing frequency are the major goals of tuberculosis research and the application of drug delivery systems constitutes an important therapeutic strategy. **Isoniazid, pyrazinamide and rifampicin**, which are important antitubercular drugs, were encapsulated into alginate nanoparticles prepared by cation-induced gelification, reaching drug encapsulation efficiencies of 70–90% for isoniazid and pyrazinamide and 80–90% for rifampicin (Ahmad et al., 2006). When alginate nanoparticles were administered through nebulisation, the drugs were detected in plasma from 3 h onwards. Encapsulated drugs were observed up to 14, 10 and 14 days, respectively, in contrast with free drugs that were cleared from the circulation within 12–24 h. The levels of drugs in various organs remained above the minimum inhibitory concentration at both doses for equal periods, demonstrating their equiefficiency. Alginate nanoparticles hold great potential in reducing dosing frequency of antitubercular drugs.

**Amphotericin B (AmB)** is a polyene macrolide antifungal agent and the drug of choice for systemic fungal infection. Unfortunately, it is poorly absorbed from the gastrointestinal tract due to its low aqueous solubility and it must be given parenterally to treat systemic fungal infections. Currently, two types of drug formulations for AmB are available. The first one is a micellar solution of AmB and the second one are lipid-based nanoparticulate formulations. Despite these formulations reduce nephrotoxicity of the treatment, they are still quite expensive. Thus, much effort has been spent to develop cheaper delivery systems with reduced amphotericin B toxicity. Polysaccharide based nanoparticle delivery systems are one approach that has been investigated by Tiyaboonchai and coworkers (Tiyaboonchai & Limpeanchob, 2007). They prepared AmB-chitosan-dextrane sulfate nanoparticles by polyelectrolyte complexation at room temperature, showing an association efficiency of 50–65%. They observed a fast release characteristic of AmB independent of the processing conditions, with most of AmB released from particles within 5 min. This suggested that

AmB exhibited only moderate interaction with the weakly cross-linked polymers of the nanoparticles. A reduction of nephrotoxicity was observed in an *in vivo* renal toxicity study.

## 5. Conclusion

As pointed out throughout this review, polysaccharides show variability and versatility, due to their complex structure, which is difficult to be reproduced with synthetic polymers. Thus, native polysaccharides and their derivatives are emerging in the last years as one of the most used biomaterials in the field of nanomedicine, especially being chosen by a lot of researchers as carriers to be used in the preparation of nanoparticulate drug delivery systems.

A wide variety of preparation methods of nanoparticles has been developed, and three aspects have marked the evolution of these methods: need for less toxic agents, simplification of the procedures and optimization to improve yield and entrapment efficiency. Now it is possible to choose the best method of preparation and the best suitable polymer to achieve an efficient encapsulation of the drug, taking into account the drug features in this selection.

As reviewed above, so many nanoparticle drug delivery systems have been prepared using various polysaccharides as carriers combined with different groups of drugs, and they have been investigated in terms of physicochemical features, drug-loading efficiency, *in vitro* toxicity and comparative *in vivo* test. Deeper studies, such as evaluation of interaction between cells, tissues and organs, as well as how the administration of these systems can affect to the metabolism, need to be carried out. In fact, more and more nanoparticle systems are emerging nowadays, and these necessary studies will be focused on in the near future, completing the evaluation of these hopeful polysaccharide-based systems.

## 6. Acknowledgements

The financial support of the Ministerio de Ciencia e Innovación of Spain FIS(PS09/01513) and MAT2010-21509-C03-03, and the FPI grant from UCM to A. Martínez are gratefully acknowledged.

## 7. References

- Ahmad, Z.; Pandey, R.; Sharma, S. & Khuller, G. K. (2006). Pharmacokinetic and pharmacodynamic behaviour of antitubercular drugs encapsulated in alginate nanoparticles at two doses. *Int J Antimicrob Agents*, 27, 5, pp. (409-416), 0924-8579 (Print) 0924-8579 (Linking).
- Akbuga, J. & Durmaz, G. (1994). Preparation and evaluation of crosslinked chitosan microspheres containing furosemide. *International Journal of Pharmaceutics*, 11, pp. (217-222).
- Akhtar, S.; Hughes, M. D.; Khan, A.; Bibby, M.; Hussain, M.; Nawaz, Q.; Double, J. & Sayyed, P. (2000). The delivery of antisense therapeutics. *Adv Drug Deliv Rev*, 44, 1, pp. (3-21), 0169-409X (Print) 0169-409X (Linking).
- Akiyoshi, K.; Kobayashi, S.; Shichibe, S.; Mix, D.; Baudys, M.; Kim, S. W. & Sunamoto, J. (1998). Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a

- carrier of protein drugs: complexation and stabilization of insulin. *J Control Release*, 54, 3, pp. (313-320), 0168-3659 (Print) 0168-3659 (Linking).
- Alonso-Sande, M.; Cuna, M. & Remunan-Lopez, C. (2006). Formation of new glucomannan-chitosan nanoparticles and study of their ability to associate and deliver proteins. *Macromolecules*, 39, pp. (4152-4158).
- Allison, D. D. & Grande-Allen, K. J. (2006). Review. Hyaluronan: a powerful tissue engineering tool. *Tissue Eng*, 12, 8, pp. (2131-2140), 1076-3279 (Print) 1076-3279 (Linking).
- Ameller, T.; Legrand, P.; Marsaud, V. & Renoir, J. M. (2004). Drug delivery systems for oestrogenic hormones and antagonists: the need for selective targeting in estradiol-dependent cancers. *J Steroid Biochem Mol Biol*, 92, 1-2, pp. (1-18), 0960-0760 (Print) 0960-0760 (Linking).
- Amidi, M.; Romeijn, S. G.; Borchard, G.; Junginger, H. E.; Hennink, W. E. & Jiskoot, W. (2006). Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. *J Control Release*, 111, 1-2, pp. (107-116), 0168-3659 (Print) 0168-3659 (Linking).
- Aumelas, A.; Serrero, A.; Durand, A.; Dellacherie, E. & Leonard, M. (2007). Nanoparticles of hydrophobically modified dextrans as potential drug carrier systems. *Colloids Surf B Biointerfaces*, 59, 1, pp. (74-80), 0927-7765 (Print) 0927-7765 (Linking).
- Azizi, E.; Namazi, A.; Haririan, I.; Fouladdel, S.; Khoshayand, M. R.; Shotorbani, P. Y.; Nomani, A. & Gazori, T. (2010). Release profile and stability evaluation of optimized chitosan/alginate nanoparticles as EGFR antisense vector. *Int J Nanomedicine*, 5, pp. (455-461), 1178-2013 (Electronic) 1176-9114 (Linking).
- Bae, H.; Ahari, A. F.; Shin, H.; Nichol, J. W.; Hutson, C. B.; Masaeli, M.; Kim, S. H.; Aubin, H.; Yamanlar, S. & Khademhosseini, A. (2011). Cell-laden microengineered pullulan methacrylate hydrogels promote cell proliferation and 3D cluster formation. *Soft Matter*, 7, 5, pp. (1903-1911), 1744-6848 (Electronic) 1744-683X (Linking).
- Balazs, E. A. (2004). *Viscoelastic properties of hyaluronan and its therapeutics use*, Elsevier, Amsterdam
- Barbosa, M.; Granja, P.; Barrias, C. & Amaral, I. (2005). Polysaccharides as scaffolds for bone regeneration. *ITBM-RBM*, 26, pp. (212-217).
- Barbucci, R.; Pasqui, D.; Favalaro, R. & Panariello, G. (2008). A thixotropic hydrogel from chemically cross-linked guar gum: synthesis, characterization and rheological behaviour. *Carbohydr Res*, 343, 18, pp. (3058-3065), 1873-426X (Electronic) 0008-6215 (Linking).
- Barratt, G. M. (2000). Therapeutic applications of colloidal drug carriers. *Pharm Sci Technol Today*, 3, 5, pp. (163-171), 1461-5347 (Electronic) 1461-5347 (Linking).
- Bernardo, M. V.; Blanco, M. D.; Sastre, R. L.; Teijon, C. & Teijon, J. M. (2003). Sustained release of bupivacaine from devices based on chitosan. *Farmaco*, 58, 11, pp. (1187-1191), 0014-827X (Print) 0014-827X (Linking).
- Berthold, A.; Cremer, K. & Kreuter, J. (1996). Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. *Journal of Controlled Release*, 39, pp. (17-25).

- Bertholon, I.; Lesieur, S.; Labarre, D.; Besnard, M. & C., V. (2006). Characterization of Dextran-Poly(isobutylcyanoacrylate) Copolymers Obtained by Redox Radical and Anionic Emulsion Polymerization. *Macromolecules*, 39, pp. (3559-3567).
- Blanco, M. D.; Gomez, C.; Olmo, R.; Muniz, E. & Teijon, J. M. (2000). Chitosan microspheres in PLG films as devices for cytarabine release. *Int J Pharm*, 202, 1-2, pp. (29-39), 0378-5173 (Print) 0378-5173 (Linking).
- Bodnár, M.; Daróczy, L.; Batta, G.; Bakó, J.; Hartmann, J. F. & Borbély, J. (2009). Preparation and characterization of cross-linked hyaluronan nanoparticles. *Colloid & Polymer Science*, 287, pp. (991-1000).
- Bodnar, M.; Hartmann, J. F. & Borbely, J. (2005). Preparation and characterization of chitosan-based nanoparticles. *Biomacromolecules*, 6, 5, pp. (2521-2527), 1525-7797 (Print) 1525-7797 (Linking).
- Braga, T. P.; Chagas, E. C.; Freitas de Sousa, A.; Villarreal, N. L.; Longhinotti, N. & Valentini, A. (2009). Synthesis of hybrid mesoporous spheres using the chitosan as template. *Journal of Non-Crystalline Solids*, 355, pp. (860-866).
- Burdick, J. A. & Prestwich, G. D. (2011). Hyaluronic acid hydrogels for biomedical applications. *Adv Mater*, 23, 12, pp. (H41-56), 1521-4095 (Electronic) 0935-9648 (Linking).
- Cafaggi, S.; Russo, E.; Stefani, R.; Parodi, B.; Caviglioli, G.; Sillo, G.; Bisio, A.; Aiello, C. & Viale, M. (2011). Preparation, characterisation and preliminary antitumour activity evaluation of a novel nanoparticulate system based on a cisplatin-hyaluronate complex and N-trimethyl chitosan. *Invest New Drugs*, 29, 3, pp. (443-455), 1573-0646 (Electronic) 0167-6997 (Linking).
- Cetin, M.; Aktas, Y.; Vural, I.; Capan, Y.; Dogan, L. A.; Duman, M. & Dalkara, T. (2007). Preparation and in vitro evaluation of bFGF-loaded chitosan nanoparticles. *Drug Deliv*, 14, 8, pp. (525-529), 1071-7544 (Print) 1071-7544 (Linking).
- Coviello, T.; Matricardi, P.; Marianecchi, C. & Alhaique, F. (2007). Polysaccharide hydrogels for modified release formulations. *J Control Release*, 119, 1, pp. (5-24), 1873-4995 (Electronic) 0168-3659 (Linking).
- Cui, Z. & Mumper, R. J. (2001). Chitosan-based nanoparticles for topical genetic immunization. *J Control Release*, 75, 3, pp. (409-419), 0168-3659 (Print) 0168-3659 (Linking).
- Chen, Q.; Hu, Y.; Chen, Y.; Jiang, X. & Yang, Y. (2005). Microstructure formation and property of chitosan-poly(acrylic acid) nanoparticles prepared by macromolecular complex. *Macromol Biosci*, 5, 10, pp. (993-1000), 1616-5187 (Print) 1616-5187 (Linking).
- Chen, X. G.; Lee, C. M. & Park, H. J. (2003). O/W emulsification for the self-aggregation and nanoparticle formation of linoleic acid-modified chitosan in the aqueous system. *J Agric Food Chem*, 51, 10, pp. (3135-3139), 0021-8561 (Print) 0021-8561 (Linking).
- Choi, K. Y.; Chung, H.; Min, K. H.; Yoon, H. Y.; Kim, K.; Park, J. H.; Kwon, I. C. & Jeong, S. Y. (2010). Self-assembled hyaluronic acid nanoparticles for active tumor targeting. *Biomaterials*, 31, 1, pp. (106-114), 1878-5905 (Electronic) 0142-9612 (Linking).
- Davidenko, N.; Blanco, M. D.; Peniche, C.; Becherán, L.; Guerrero, S. & Teijón, J. M. (2009). Effects of different parameters on characteristics of chitosan-poly(acrylic acid) nanoparticles obtained by the method of coacervation. *Journal of Applied Polymer Science*, 111, pp. (2362-2371).

- de la Fuente, M.; Ravina, M.; Paolicelli, P.; Sanchez, A.; Seijo, B. & Alonso, M. J. (2010). Chitosan-based nanostructures: a delivery platform for ocular therapeutics. *Adv Drug Deliv Rev*, 62, 1, pp. (100-117), 1872-8294 (Electronic) 0169-409X (Linking).
- de la Fuente, M.; Seijo, B. & Alonso, M. J. (2008). Design of novel polysaccharidic nanostructures for gene delivery. *Nanotechnology*, 19, 7, pp. (075105), 0957-4484 (Print) 0957-4484 (Linking).
- de Martimprey, H.; Vauthier, C.; Malvy, C. & Couvreur, P. (2009). Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. *Eur J Pharm Biopharm*, 71, 3, pp. (490-504), 1873-3441 (Electronic) 0939-6411 (Linking).
- De, S. & Robinson, D. (2003). Polymer relationships during preparation of chitosan-alginate and poly-L-lysine-alginate nanospheres. *J Control Release*, 89, 1, pp. (101-112), 0168-3659 (Print) 0168-3659 (Linking).
- Douglas, K. L. & Tabrizian, M. (2005). Effect of experimental parameters on the formation of alginate-chitosan nanoparticles and evaluation of their potential application as DNA carrier. *J Biomater Sci Polym Ed*, 16, 1, pp. (43-56), 0920-5063 (Print) 0920-5063 (Linking).
- Drogoz, A.; David, L.; Rochas, C.; Domard, A. & Delair, T. (2007). Polyelectrolyte complexes from polysaccharides: formation and stoichiometry monitoring. *Langmuir*, 23, 22, pp. (10950-10958), 0743-7463 (Print) 0743-7463 (Linking).
- Fernandez-Hervas, M. & Fell, J. (1998). Pectin/chitosan mixtures as coatings for colon-specific drug delivery: an in vitro evaluation. *International Journal of Pharmaceutics*, 169, pp. (115-119).
- Gavory, C.; Durand, A.; Six, J. L.; Nouvel, C.; Marie, E. & Leonard, M. (2011). Polysaccharide-covered nanoparticles prepared by nanoprecipitation. *Carbohydrate Polymers*, 84, pp. (133-140).
- George, M. & Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan--a review. *J Control Release*, 114, 1, pp. (1-14), 0168-3659 (Print) 0168-3659 (Linking).
- Gonzalez Ferreiro, M.; Tillman, L.; Hardee, G. & Bodmeier, R. (2002). Characterization of alginate/poly-L-lysine particles as antisense oligonucleotide carriers. *Int J Pharm*, 239, 1-2, pp. (47-59), 0378-5173 (Print) 0378-5173 (Linking).
- Guerrero, S.; Teijón, C.; Muñoz, E.; Teijón, J. M. & Blanco, M. D. (2010). Characterization and in vivo evaluation of ketotifen-loaded chitosan microspheres. *Carbohydrate Polymers*, 79, pp. (1006-1013).
- Gullotti, E. & Yeo, Y. (2009). Extracellularly activated nanocarriers: a new paradigm of tumor targeted drug delivery. *Mol Pharm*, 6, 4, pp. (1041-1051), 1543-8384 (Print) 1543-8384 (Linking).
- Gurwitz, D. & Livshits, G. (2006). Personalized medicine Europe: health, genes and society: Tel-Aviv University, Tel-Aviv, Israel, June 19-21, 2005. *Eur J Hum Genet*, 14, 3, pp. (376-380), 1018-4813 (Print) 1018-4813 (Linking).
- Han, S. K.; Lee, J. H.; Kim, D.; Cho, S. H. & Yuk, S. H. (2005). Hydrophilized poly(lactide-co-glycolide) nanoparticles with core/shell structure for protein delivery. *Science and Technology of Advanced Materials*, 6, pp. (468-474).
- Heinze, T.; Liebert, T.; Heublein, B. & Hornig, S. (2006). Functional Polymers Based on Dextran. *Advances in Polymer Science*, 205/2006, pp. (199-291).



- Hornig, S. & Heinze, T. (2008). Efficient approach to design stable water-dispersible nanoparticles of hydrophobic cellulose esters. *Biomacromolecules*, 9, 5, pp. (1487-1492), 1526-4602 (Electronic) 1525-7797 (Linking).
- Hu, F. Q.; Ren, G. F.; Yuan, H.; Du, Y. Z. & Zeng, S. (2006). Shell cross-linked stearic acid grafted chitosan oligosaccharide self-aggregated micelles for controlled release of paclitaxel. *Colloids Surf B Biointerfaces*, 50, 2, pp. (97-103), 0927-7765 (Print) 0927-7765 (Linking).
- Ito, T.; Iida-Tanaka, N.; Niidome, T.; Kawano, T.; Kubo, K.; Yoshikawa, K.; Sato, T.; Yang, Z. & Koyama, Y. (2006). Hyaluronic acid and its derivative as a multi-functional gene expression enhancer: protection from non-specific interactions, adhesion to targeted cells, and transcriptional activation. *J Control Release*, 112, 3, pp. (382-388), 0168-3659 (Print) 0168-3659 (Linking).
- Janes, K. A.; Calvo, P. & Alonso, M. J. (2001). Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv Drug Deliv Rev*, 47, 1, pp. (83-97), 0169-409X (Print) 0169-409X (Linking).
- Kang, J.; Lee, M. S.; Copland, J. A., 3rd; Luxon, B. A. & Gorenstein, D. G. (2008). Combinatorial selection of a single stranded DNA thioaptamer targeting TGF-beta1 protein. *Bioorg Med Chem Lett*, 18, 6, pp. (1835-1839), 1464-3405 (Electronic) 0960-894X (Linking).
- Katas, H. & Alpar, H. O. (2006). Development and characterisation of chitosan nanoparticles for siRNA delivery. *J Control Release*, 115, 2, pp. (216-225), 0168-3659 (Print) 0168-3659 (Linking).
- Kaul, G. & Amiji, M. (2002). Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. *Pharm Res*, 19, 7, pp. (1061-1067), 0724-8741 (Print) 0724-8741 (Linking).
- Kim, D. G.; Jeong, Y. I.; Choi, C.; Roh, S. H.; Kang, S. K.; Jang, M. K. & Nah, J. W. (2006). Retinol-encapsulated low molecular water-soluble chitosan nanoparticles. *Int J Pharm*, 319, 1-2, pp. (130-138), 0378-5173 (Print) 0378-5173 (Linking).
- Kogan, G.; Soltes, L.; Stern, R. & Gemeiner, P. (2007). Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol Lett*, 29, 1, pp. (17-25), 1573-6776 (Electronic) 0141-5492 (Linking).
- Kratz, F. (2008). Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *J Control Release*, 132, 3, pp. (171-183), 1873-4995 (Electronic) 0168-3659 (Linking).
- Kumbar, S. G. & Aminabhavi, T. M. (2003). Synthesis and characterization of modified chitosan microspheres: effect of the grafting ratio on the controlled release of nifedipine through microspheres. *Journal of Applied Polymer Science*, 89, pp. (2940-2949).
- Lan, S. F. & Starly, B. (2011). Alginate based 3D hydrogels as an in vitro co-culture model platform for the toxicity screening of new chemical entities. *Toxicol Appl Pharmacol*, pp. (1096-0333 (Electronic) 0041-008X (Linking).
- Lassalle, V. & Ferreira, M. L. (2007). PLA nano- and microparticles for drug delivery: an overview of the methods of preparation. *Macromol Biosci*, 7, 6, pp. (767-783), 1616-5187 (Print) 1616-5187 (Linking).
- Leach, J. B. & Schmidt, C. E. (2005). Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue

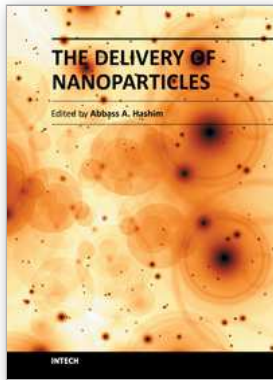
- engineering scaffolds. *Biomaterials*, 26, 2, pp. (125-135), 0142-9612 (Print) 0142-9612 (Linking).
- Learoyd, T. P.; Burrows, J. L.; French, E. & Seville, P. C. (2008). Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. *Eur J Pharm Biopharm*, 68, 2, pp. (224-234), 0939-6411 (Print) 0939-6411 (Linking).
- Leathers, T. D. (2003). Biotechnological production and applications of pullulan. *Appl Microbiol Biotechnol*, 62, 5-6, pp. (468-473), 0175-7598 (Print) 0175-7598 (Linking).
- Lee, J. W.; Park, J. H. & Robinson, J. R. (2000). Bioadhesive-based dosage forms: the next generation. *J Pharm Sci*, 89, 7, pp. (850-866), 0022-3549 (Print) 0022-3549 (Linking).
- Lemarchand, C.; Couvreur, P.; Besnard, M.; Costantini, D. & Gref, R. (2003a). Novel polyester-polysaccharide nanoparticles. *Pharm Res*, 20, 8, pp. (1284-1292), 0724-8741 (Print) 0724-8741 (Linking).
- Lemarchand, C.; Couvreur, P.; Vauthier, C.; Costantini, D. & Gref, R. (2003b). Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *Int J Pharm*, 254, 1, pp. (77-82), 0378-5173 (Print) 0378-5173 (Linking).
- Letchford, K. & Burt, H. (2007). A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur J Pharm Biopharm*, 65, 3, pp. (259-269), 0939-6411 (Print) 0939-6411 (Linking).
- Li, Y.; Nagira, T. & Tsuchiya, T. (2006). The effect of hyaluronic acid on insulin secretion in HIT-T15 cells through the enhancement of gap-junctional intercellular communications. *Biomaterials*, 27, 8, pp. (1437-1443), 0142-9612 (Print) 0142-9612 (Linking).
- Li, Y. L.; Zhu, L.; Liu, Z.; Cheng, R.; Meng, F.; Cui, J. H.; Ji, S. J. & Zhong, Z. (2009). Reversibly stabilized multifunctional dextran nanoparticles efficiently deliver doxorubicin into the nuclei of cancer cells. *Angew Chem Int Ed Engl*, 48, 52, pp. (9914-9918), 1521-3773 (Electronic) 1433-7851 (Linking).
- Liu, L.; Fishman, M. L.; Kost, J. & Hicks, K. B. (2003). Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials*, 24, 19, pp. (3333-3343), 0142-9612 (Print) 0142-9612 (Linking).
- Liu, L.; Won, Y. J.; Cooke, P. H.; Coffin, D. R.; Fishman, M. L.; Hicks, K. B. & Ma, P. X. (2004). Pectin/poly(lactide-co-glycolide) composite matrices for biomedical applications. *Biomaterials*, 25, 16, pp. (3201-3210), 0142-9612 (Print) 0142-9612 (Linking).
- Liu, Z.; Jiao, Y.; Wang, Y.; Zhou, C. & Zhang, Z. (2008). Polysaccharides-based nanoparticles as drug delivery systems. *Adv Drug Deliv Rev*, 60, 15, pp. (1650-1662), 1872-8294 (Electronic) 0169-409X (Linking).
- Lu, B.; Xiong, S. B.; Yang, H.; Yin, X. D. & Zhao, R. B. (2006). Mitoxantrone-loaded BSA nanospheres and chitosan nanospheres for local injection against breast cancer and its lymph node metastases. I: Formulation and in vitro characterization. *Int J Pharm*, 307, 2, pp. (168-174), 0378-5173 (Print) 0378-5173 (Linking).
- Martínez, A.; Iglesias, I.; Lozano, R.; Teijón, J. M. & Blanco, M. D. (2011). Synthesis and characterization of thiolated alginate-albumin nanoparticles stabilized by disulfide bonds. Evaluation as drug delivery systems. *Carbohydrate Polymers*, 83, 3, pp. (1311-1321).

- Mendichi, R. & Soltes, L. (2002). Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid. *Inflamm Res*, 51, 3, pp. (115-116), 1023-3830 (Print) 1023-3830 (Linking).
- Misaki, A.; Torii, M.; Sawai, T. & Goldstein, I. J. (1980). Structure of the dextran of *Leuconostoc mesenteroides* B-1355. *Carbohydrate Research*, 84, pp. (273-285).
- Moghimi, S. M.; Hunter, A. C. & Murray, J. C. (2005). Nanomedicine: current status and future prospects. *FASEB J*, 19, 3, pp. (311-330), 1530-6860 (Electronic) 0892-6638 (Linking).
- Muzzarelli, R. A. A. & Muzzarelli, C. (2005). Chitosan chemistry: Relevance to the biomedical sciences Polysaccharides 1: Structure, characterization and use. *Advances in Polymer Science*, 186, pp. (151-209).
- Naessens, M.; Cerdobbel, A.; Soetaert, W. & Vandamme, E. J. (2005). *Leuconostoc* dextransucrase and dextran: production, properties and applications. *Journal of Chemical Technology & Biotechnology*, 80, pp. (845-860).
- Nair, L. S. & Laurencin, C. T. (2007). Biodegradable polymers as biomaterial. *Progress in Polymer Science*, 6, pp. (762-798).
- Oh, E. J.; Park, K.; Kim, K. S.; Kim, J.; Yang, J. A.; Kong, J. H.; Lee, M. Y.; Hoffman, A. S. & Hahn, S. K. (2010). Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives. *J Control Release*, 141, 1, pp. (2-12), 1873-4995 (Electronic) 0168-3659 (Linking).
- Opanasopit, P.; Apirakaramwong, A.; Ngawhirunpat, T.; Rojanarata, T. & Ruktanonchai, U. (2008). Development and characterization of pectinate micro/nanoparticles for gene delivery. *AAPS PharmSciTech*, 9, 1, pp. (67-74), 1530-9932 (Electronic) 1530-9932 (Linking).
- Orive, G.; Ponce, S.; Hernandez, R. M.; Gascon, A. R.; Igartua, M. & Pedraz, J. L. (2002). Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. *Biomaterials*, 23, 18, pp. (3825-3831), 0142-9612 (Print) 0142-9612 (Linking).
- Pan, Y.; Li, Y. J.; Zhao, H. Y.; Zheng, J. M.; Xu, H.; Wei, G.; Hao, J. S. & Cui, F. D. (2002). Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin in vivo. *Int J Pharm*, 249, 1-2, pp. (139-147), 0378-5173 (Print) 0378-5173 (Linking).
- Pandey, R. & Ahmad, Z. (2011). Nanomedicine and experimental tuberculosis: facts, flaws, and future. *Nanomedicine*, 7, 3, pp. (259-272), 1549-9642 (Electronic) 1549-9634 (Linking).
- Pandey, R. & Khuller, G. K. (2005). Antitubercular inhaled therapy: opportunities, progress and challenges. *J Antimicrob Chemother*, 55, 4, pp. (430-435), 0305-7453 (Print) 0305-7453 (Linking).
- Perera, G.; Barthelmes, J. & Bernkop-Schnurch, A. (2010). Novel pectin-4-aminothiophenole conjugate microparticles for colon-specific drug delivery. *J Control Release*, 145, 3, pp. (240-246), 1873-4995 (Electronic) 0168-3659 (Linking).
- Pliszczak, D.; Bourgeois, S.; Bordes, C.; Valour, J. P.; Mazoyer, M. A.; Orecchioni, A. M.; Nakache, E. & Lanteri, P. (2011). Improvement of an encapsulation process for the preparation of pro- and prebiotics-loaded bioadhesive microparticles by using experimental design. *Eur J Pharm Sci*, 44, 1-2, pp. (83-92), 1879-0720 (Electronic) 0928-0987 (Linking).

- Prestwich, G. D. (2008). Engineering a clinically-useful matrix for cell therapy. *Organogenesis*, 4, 1, pp. (42-47), 1547-6278 (Print) 1547-6278 (Linking).
- Qiu, L. Y. & Bae, Y. H. (2006). Polymer architecture and drug delivery. *Pharm Res*, 23, 1, pp. (1-30), 0724-8741 (Print) 0724-8741 (Linking).
- Raju, H. B. & Goldberg, J. L. (2008). Nanotechnology for ocular therapeutics and tissue repair. *Expert Review of Ophthalmology*, 3, pp. (431-436).
- Rehm, B. H. A. (Ed). (2009). *Alginates: Biology and Applications*, Springer, 978-3-540-92678.
- Reis, C. P.; Ribeiro, A. J.; Houng, S.; Veiga, F. & Neufeld, R. J. (2007). Nanoparticulate delivery system for insulin: design, characterization and in vitro/in vivo bioactivity. *Eur J Pharm Sci*, 30, 5, pp. (392-397), 0928-0987 (Print) 0928-0987 (Linking).
- Reis, C. P.; Ribeiro, A. J.; Veiga, F.; Neufeld, R. J. & Damge, C. (2008). Polyelectrolyte biomaterial interactions provide nanoparticulate carrier for oral insulin delivery. *Drug Deliv*, 15, 2, pp. (127-139), 1071-7544 (Print) 1071-7544 (Linking).
- Rekha, M. R. & Chandra, P. S. (2007). Pullulan as a promising biomaterial for biomedical applications: A perspective. *Trends in Biomaterials & Artificial Organs*, 20, pp. (116-121).
- Romberg, B.; Hennink, W. E. & Storm, G. (2008). Sheddable coatings for long-circulating nanoparticles. *Pharm Res*, 25, 1, pp. (55-71), 0724-8741 (Print) 0724-8741 (Linking).
- Rotureau, E.; Leonard, M.; Dellacherie, E. & Durand, A. (2004). Amphiphilic derivatives of dextran: adsorption at air/water and oil/water interfaces. *J Colloid Interface Sci*, 279, 1, pp. (68-77), 0021-9797 (Print) 0021-9797 (Linking).
- Sakamoto, J.; Annapragada, A.; Decuzzi, P. & Ferrari, M. (2007). Antibiological barrier nanovector technology for cancer applications. *Expert Opin Drug Deliv*, 4, 4, pp. (359-369), 1742-5247 (Print) 1742-5247 (Linking).
- Sarmah, J. K.; Bhattacharjee, S. K.; Mahanta, R. & Mahanta, R. (2009). Preparation of cross-linked guar gum nanospheres containing tamoxifen citrate by single step emulsion in situ polymer cross-linking method. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 65, pp. (329-334).
- Sarmah, J. K.; Mahanta, R.; Bhattacharjee, S. K. & Biswas, A. (2011). Controlled release of tamoxifen citrate encapsulated in cross-linked guar gum nanoparticles. *Int J Biol Macromol*, 49, 3, pp. (390-396), 1879-0003 (Electronic) 0141-8130 (Linking).
- Sarmiento, B.; Martins, S.; Ribeiro, A.; Veiga, F.; Neufeld, R. & Ferreira, D. (2006). Development and comparison of different nanoparticulate polyelectrolyte complexes as insulin carriers. *International Journal of Peptide Research and Therapeutics*, 12, pp. (131-138).
- Singh, V.; , P., S.; Singh, S. K. & Sanghi, R. (2009). Removal of cadmium from aqueous solutions by adsorption using poly(acrylamide) modified guar gum-silica nanocomposites. *Separation and Purification Technology*, 67, pp. (251-261).
- Sinha, V. R. & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. *Int J Pharm*, 224, 1-2, pp. (19-38), 0378-5173 (Print) 0378-5173 (Linking).
- Sinha, V. R.; Singla, A. K.; Wadhawan, S.; Kaushik, R.; Kumria, R.; Bansal, K. & Dhawan, S. (2004). Chitosan microspheres as a potential carrier for drugs. *Int J Pharm*, 274, 1-2, pp. (1-33), 0378-5173 (Print) 0378-5173 (Linking).
- Sinha, V. R. & Trehan, A. (2003). Biodegradable microspheres for protein delivery. *J Control Release*, 90, 3, pp. (261-280), 0168-3659 (Print) 0168-3659 (Linking).

- Skiba, M.; Wouessidjewe, D.; Puisieux, F.; Duchène, D. & Gulik, A. (1996). Characterization of amphiphilic fl-cyclodextrin nanospheres. *International Journal of Pharmaceutics*, 142, pp. (121-124).
- Soumya, R. S.; Ghosh, S. & Abraham, E. T. (2010). Preparation and characterization of guar gum nanoparticles. *Int J Biol Macromol*, 46, 2, pp. (267-269), 1879-0003 (Electronic) 0141-8130 (Linking).
- Srinophakun, T. & Boonmee, J. (2011). Preliminary Study of Conformation and Drug Release Mechanism of Doxorubicin-Conjugated Glycol Chitosan, via cis-Aconityl Linkage, by Molecular Modeling. *Int J Mol Sci*, 12, 3, pp. (1672-1683), 1422-0067 (Electronic) 1422-0067 (Linking).
- Sujata, V. B. (2002). *Biomaterials*, Springer, 0-7923-7058-9, India.
- Tasciotti, E.; Liu, X.; Bhavane, R.; Plant, K.; Leonard, A. D.; Price, B. K.; Cheng, M. M.; Decuzzi, P.; Tour, J. M.; Robertson, F. & Ferrari, M. (2008). Mesoporous silicon particles as a multistage delivery system for imaging and therapeutic applications. *Nat Nanotechnol*, 3, 3, pp. (151-157), 1748-3395 (Electronic) 1748-3387 (Linking).
- Thebaud, N. B.; Pierron, D.; Bareille, R.; Le Visage, C.; Letourneur, D. & Bordenave, L. (2007). Human endothelial progenitor cell attachment to polysaccharide-based hydrogels: a pre-requisite for vascular tissue engineering. *J Mater Sci Mater Med*, 18, 2, pp. (339-345), 0957-4530 (Print) 0957-4530 (Linking).
- Tibbals, H. F. (2010). *Medical Nanotechnology and Nanomedicine*, CRC Press, 978-1-4398-0876-4, USA.
- Tirafferri, A.; Chen, K. L.; Sethi, R. & Elimelech, M. (2008). Reduced aggregation and sedimentation of zero-valent iron nanoparticles in the presence of guar gum. *J Colloid Interface Sci*, 324, 1-2, pp. (71-79), 1095-7103 (Electronic) 0021-9797 (Linking).
- Tiyaboonchai, W. & Limpeanchob, N. (2007). Formulation and characterization of amphotericin B-chitosan-dextran sulfate nanoparticles. *Int J Pharm*, 329, 1-2, pp. (142-149), 0378-5173 (Print) 0378-5173 (Linking).
- Tiyaboonchai, W.; Woiszwilllo, J.; Sims, R. C. & Middaugh, C. R. (2003). Insulin containing polyethylenimine-dextran sulfate nanoparticles. *Int J Pharm*, 255, 1-2, pp. (139-151), 0378-5173 (Print) 0378-5173 (Linking).
- Tokumitsu, H.; Ichikawa, H. & Fukumori, Y. (1999). Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsion-droplet coalescence technique and characterization. *Pharm Res*, 16, 12, pp. (1830-1835), 0724-8741 (Print) 0724-8741 (Linking).
- Varum, F. J.; McConnell, E. L.; Sousa, J. J.; Veiga, F. & Basit, A. W. (2008). Mucoadhesion and the gastrointestinal tract. *Crit Rev Ther Drug Carrier Syst*, 25, 3, pp. (207-258), 0743-4863 (Print) 0743-4863 (Linking).
- Vauthier, C. & Bouchemal, K. (2009). Methods for the preparation and manufacture of polymeric nanoparticles. *Pharm Res*, 26, 5, pp. (1025-1058), 1573-904X (Electronic) 0724-8741 (Linking).
- Vauthier, C. & Couvreur, P. (2000). Development of nanoparticles made of polysaccharides as novel drug carrier systems, In: *Handbook of Pharmaceutical Controlled Release Technology*, D. L. Wise, (Ed), pp. (413-429), Marcel Dekker, New York.
- Vila, A.; Sanchez, A.; Tobio, M.; Calvo, P. & Alonso, M. J. (2002). Design of biodegradable particles for protein delivery. *J Control Release*, 78, 1-3, pp. (15-24), 0168-3659 (Print) 0168-3659 (Linking).

- Wang, N. & Wu, X. S. (1997). Preparation and characterization of agarose hydrogel nanoparticles for protein and peptide drug delivery. *Pharm Dev Technol*, 2, 2, pp. (135-142), 1083-7450 (Print) 1083-7450 (Linking).
- Wang, S.; Mao, X.; Wang, H.; Lin, J.; Li, F. & Wei, D. (2011). Characterization of a novel dextran produced by *Gluconobacter oxydans* DSM 2003. *Appl Microbiol Biotechnol*, 91, 2, pp. (287-294), 1432-0614 (Electronic) 0175-7598 (Linking).
- Wang, Y. S.; Liu, L. R.; Jiang, Q. & Zhang, Q. Q. (2007). Self-aggregated nanoparticles of cholesterol-modified chitosan conjugate as a novel carrier of epirubicin. *European Polymer Journal*, 43, pp. (43-51).
- Yang, J. S.; Xie, Y. J. & He, W. (2011). Research progress on chemical modification of alginate: A review. *Carbohydrate Polymers*, 84, pp. (33-39).
- Yang, X.; Zhang, Q.; Wang, Y.; Chen, H.; Zhang, H.; Gao, F. & Liu, L. (2008). Self-aggregated nanoparticles from methoxy poly(ethylene glycol)-modified chitosan: synthesis; characterization; aggregation and methotrexate release in vitro. *Colloids Surf B Biointerfaces*, 61, 2, pp. (125-131), 0927-7765 (Print) 0927-7765 (Linking).
- Yip, G. W.; Smollich, M. & Gotte, M. (2006). Therapeutic value of glycosaminoglycans in cancer. *Mol Cancer Ther*, 5, 9, pp. (2139-2148), 1535-7163 (Print) 1535-7163 (Linking).
- Yoksan, R.; Matsusaki, M.; Akashi, M. & Chirachanchai, S. (2004). Controlled hydrophobic/hydrophilic chitosan: colloidal phenomena and nanosphere formation. *Colloid & Polymer Science*, 282, pp. (337-342).
- You, J. O. & Peng, C. A. (2004). Calcium-alginate nanoparticles formed by reverse microemulsion as gene carriers. *Macromolecular Symposia*, 219, pp. (147-153).
- Yuan, Z. (2007). Study on the synthesis and catalyst oxidation properties of chitosan bound nickel(II) complexes. *Journal of Agricultural and Food Chemistry*, 21, 5, pp. (22-24).
- Zhang, J.; Chen, X. G.; Li, Y. Y. & Liu, C. S. (2007). Self-assembled nanoparticles based on hydrophobically modified chitosan as carriers for doxorubicin. *Nanomedicine*, 3, 4, pp. (258-265), 1549-9642 (Electronic) 1549-9634 (Linking).
- Zhi, J.; Wang, Y. J. & Luo, G. S. (2005). Adsorption of diuretic furosemide onto chitosan nanoparticles prepared with a water-in-oil nanoemulsion system. *Reactive and Functional Polymers*, 249-257, pp.),
- Zhou, B.; McGary, C. T.; Weigel, J. A.; Saxena, A. & Weigel, P. H. (2003). Purification and molecular identification of the human hyaluronan receptor for endocytosis. *Glycobiology*, 13, 5, pp. (339-349), 0959-6658 (Print) 0959-6658 (Linking).



## **The Delivery of Nanoparticles**

Edited by Dr. Abbass A. Hashim

ISBN 978-953-51-0615-9

Hard cover, 540 pages

**Publisher** InTech

**Published online** 16, May, 2012

**Published in print edition** May, 2012

Nanoparticle is a general challenge for today's technology and the near future observations of science. Nanoparticles cover mostly all types of sciences and manufacturing technologies. The properties of this particle are flying over today scientific barriers and have passed the limitations of conventional sciences. This is the reason why nanoparticles have been evaluated for the use in many fields. InTech publisher and the contributing authors of this book in nanoparticles are all overconfident to invite all scientists to read this new book. The book's potential was held until it was approached by the art of exploring the most advanced research in the field of nano-scale particles, preparation techniques and the way of reaching their destination. 25 reputable chapters were framed in this book and there were alienated into four altered sections; Toxic Nanoparticles, Drug Nanoparticles, Biological Activities and Nano-Technology.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

A. Martínez, A. Fernández, E. Pérez, M. Benito, J.M. Tejjón and M.D. Blanco (2012). Polysaccharide-Based Nanoparticles for Controlled Release Formulations, *The Delivery of Nanoparticles*, Dr. Abbass A. Hashim (Ed.), ISBN: 978-953-51-0615-9, InTech, Available from: <http://www.intechopen.com/books/the-delivery-of-nanoparticles/polysaccharide-based-nanoparticles-for-controlled-release-formulations>

# **INTECH**

open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.