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NATURAL BASED POLYSACHHARIDES FOR CONROLL DRUG RELEASE

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ABSTRACT

Considerable research efforts have been directed towards developing safe and efficient Natural based polysaccharide particulate drug delivery systems. The present review outlines the major new findings on the natural based polysaccharides such as Chitosan, Alginate, Pullan,Scleroglucan,Guargum,Gallengum and their characteristics, applications are covered. Chemically modified natural based polysaccharides or its derivatives used in drug delivery research are discussed critically to evaluate the usefulness of these systems in delivering the bioactive molecules. From a literature survey, it is realized that research activities on polysaccharide/nanoparticulate systems containing various drugs for different therapeutic applications and gene therapy have increased at the rapid rate. Hence, the present review is timely.

Keywords Natural Based, Polysaccharide,l Drug Delivery, Gene therapy and Cancer therapy

INTRODUCTION

By far the majority of carbohydrate materials in nature occur in the form of polysaccharides. By our definition, polysaccharides include not only those substances composed only of glycosidically-linked sugar residues, but also molecules that contain polymeric saccharide structures linked via covalent bonds to amino acids, peptides, proteins, lipids and other structures. Polysaccharides, also called glycans, consist of monosaccharides and their derivatives. If a polysaccharide contains only one kind of monosaccharide molecule, it is known as a homo-polysaccharide or homoglycan, whereas those containing more than one kind of monosaccharide are heteropolysaccharides. The most common constituent of polysaccharides is D-glucose, but D-fructose, D-galactose, L-galactose, D-mannose, L-arabinose and D-xylose also appear frequently. Some monosaccharide derivatives found in polysaccharides include amino sugars (D-glucosamine and galactosamine), as well as their derivatives (N-acetylneuraminic acid and N-acetylmuramic acid) and simple sugar acids (glucuronic and iduronic acids). Homo-polysaccharides are often named after the sugar unit they contain; so, glucose homo-polysaccharides are called glucans, while mannose homo-polysaccharides are mannans. Polysaccharides differ not only in the nature of their component monosaccharides, but also in the length of their chains and in the amount of chain branching that occurs. Although a given sugar residue has only one anomeric carbon and, thus, can form only one glycosidic linkage with hydroxyl groups on other molecules, each sugar residue carries several hydroxyls, one or more of which may be an acceptor of glycosyl substituents. This ability to form branched structures distinguishes polysaccharides from proteins and nucleic acids, which occur only as linear polymers. The main functions played by polysaccharides in nature are either storage or structural functions. By far the most common storage polysaccharide in plants is starch, which exists in two forms: α-amylose and amylopectin. Structural polysaccharides exhibit properties that are dramatically different from those of the storage polysaccharides, even though the compositions of these two classes are similar. The structural polysaccharide cellulose is the most abundant natural polymer in the world. Found in the cell walls of nearly all plants, included marine algae, cellulose is one of the principal components, providing physical structure and strength. Chitin is the second-most abundant organic compound in nature after cellulose [1]. Chitin is widely distributed in marine invertebrates, insects, fungi and yeast [2]. However, chitin is not present in higher plants and higher animals.

2. **CHITOSAN**

Chitosan (CS) is a polysaccharide, similar in structure to cellulose. Both are made by linearβ - (1-4)-linked monosaccharides [see Fig. 1 (a)]. However, an important difference to cellulose is that CS is composed of 2-amino-2-deoxy-β-D-glucan combined with glycosidic linkages. The primary amine groups render special properties that make CS very useful in pharmaceutical applications. Compared to many other natural olymers, chitosan has a positive charge and is mucoadhesive [1]. Therefore, it is used extensively in drug delivery applications [2– 6].

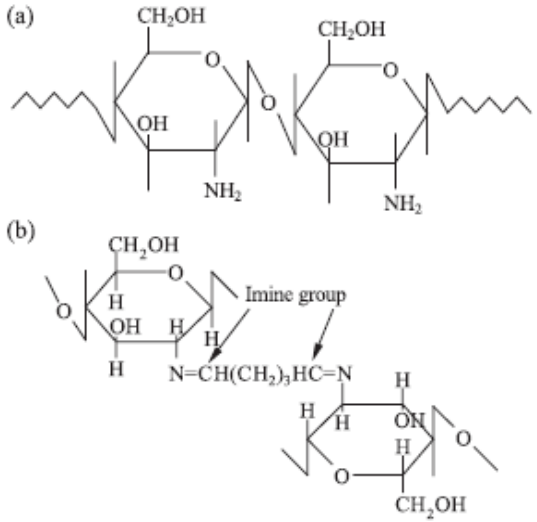

Fig. 1. (a) Structure of chitosan [poly (α1– 4-D-glucosamine)].

(b)Structure of cross-linked chitosan

Sources of Chitin and Chitosan

Chitin is widely distributed in marine invertebrates (Fig. 2), insects, fungi and yeast [3]. However, chitin is not present in higher plants and higher animals. Generally, the shell of selected crustaceans was reported by Knorr [4] to consist of 30–40% protein, 30–50% calcium carbonate and calcium phosphate and 20–30% chitin. Chitin is widely available from a variety of source among which, the principal source is shellfish waste, such as of shrimps, crabs and crawfish [5]. It also exists naturally in a few species of fungi. In terms of its structure, chitin is associated with proteins and, therefore, high in protein contents. Chitin fibrils are embedded in a matrix of calcium carbonate and phosphate that also contains protein. The matrix is proteinaceous, where the protein is hardened by a tanning process [6]. It was also demonstrated that chitin represents 14–27% and 13–15% of the dry weight of shrimp and crab processing wastes, respectively [7].

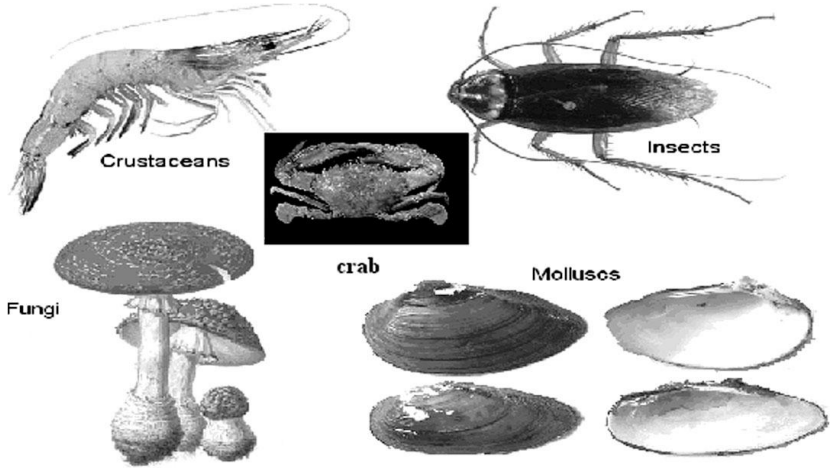

Fig.2 Sources of chitin.

Composition of Chitin, Chitosan and Cellulose

Chitosan is a modified natural carbohydrate polymer derived from chitin which is found in a wide range of natural sources such as crustaceans, fungi, insects and some algae [8]. with strong alkali yields chitosan [9], i.e., 2-amino-2-deoxy-β-D-glucose. A sharp nomenclature with respect to the degree of N-deacetylation has not been defined between chitin and chitosan [6]. In general, chitin with a degree of deacetylation of above 70% is considered as chitosan [10]. Chitosan is insoluble in water but soluble in acidic solvents below pH 6. Organic acids such as acetic, formic and lactic acids are used for dissolving chitosan, and the most commonly used solvent is 1% acetic acid solution. Solubility of chitosan in inorganic acid solvent is quite limited. Chitosan is soluble in 1% hydrochloric acid but insoluble in sulfuric and phosphoric acids. The stability of chitosan in solution is poor above pH 7 due to precipitation or gelation that takes place in alkali pH range. Chitosan solution forms a poly-ion complex with anionic ydrocolloid and provides gel. With regards to its chemical structure (Fig. 3), chitosan is very much similar to cellulose and chitin. Chitin is made up of a linear chain of acetylglucosamine groups, while chitosan is obtained by removing enough acetyl groups (CH3–CO) for the molecule to be soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin [11]. It is also very much similar to cellulose, a plant fiber. As seen in Fig. 3, the only difference between chitosan and cellulose is the amine (–NH2) group in the C-2 position of chitosan instead of the hydroxyl (–OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol,

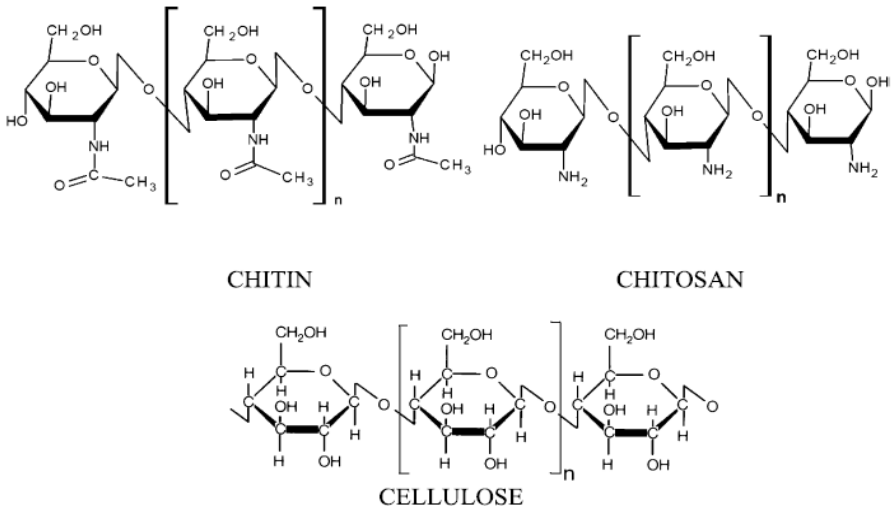

Figure 3. Structure of chitin, chitosan and cellulose

metal ions, proteins and macromolecules [12]. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, ability to form films and to chelate metal ions [13]. Chitosan is a non-toxic, biodegradable polymer of high molecular weight. Chitosan is widely employed inmany biomedical fields [14–18]. Like alginate, chitosan has the characteristic of forming gels in addition to possessing viscosity-related properties, complete biodegradability and even anti-tumor influence [19]. Its bacteriostatic and fungistatic properties are particularly useful for wound treatment. Furthermore, chitosan possesses bioadhesive properties which make it of interest in bioadhesive sustained release formulations [20, 21]. Many chitosan derivatives are also biocompatible and non-toxic with living tissues. Recent studies further indicated that chitosan and its derivatives also are novel scaffold materials for tissue engineering and are promising non-viral vectors for gene delivery [22, 23]. For bone regeneration, several injectable materials based on chitosan and its derivatives have been used. Chitosan–calcium phosphate (CP) composites appear to have a promising clinical application. hemically-modified hyaluronic acid (HA)–chitin and chitosan–HA material were reported to be osteoinductive and exhibited rapid degradation and neovascularization in vivo [24, 25]. Chitosan scaffolds are potentially a useful alternative to synthetic cell scaffolds also for cartilage tissue engineering [11]. Recently, biomineralized alginate/chitosan microcapsules have been proposed as multifunctional scaffolds and delivery vehicles in tissue regeneration of hard and soft tissues [26].

Table 1
Chitosan-based drug delivery systems prepared by different methods for various kinds of drugs

Type of system	Method of preparation	Drug
Tablets	matrix	diclofenac sodium, pentoxyphylline, salicylic acid, theophylline
	coating	propranolol HCl
Capsules	capsule shell	insulin, 5-amino salicylic acid
Microspheres/Microparticles	emulsion cross-linking	theophylline, cisplatin, pentazocine, phenobarbitone, theophylline, insulin, 5-fluorouracil, diclofenac sodium, griseofulvin, aspirin, diphtheria toxoid, pamidronate, suberoylbisphosphonate, mitoxantrone, progesterone
	coacervation/precipitation	prednisolone, interleukin-2, propranolol-HCl
	spray-drying	cimetidine, famotidine, nizatidine, vitamin D-2, diclofenac sodium, ketoprofen, metoclopramide-HCl, bovine serum albumin, ampicillin, cetylpyridinium chloride, oxytetracycline, betamethasone
	ionic gelation	felodipine
	sieving method	clozapine
Nanoparticles	emulsion-droplet coalescence	gadopentetic acid
	coacervation/precipitation	DNA, doxorubicin
	ionic gelation	insulin, ricin, bovine serum albumin, cyclosporin A
	reverse micellar method	doxorubicin
Beads	coacervation/precipitation	adriamycin, nifedipine, bovine serum albumin, salbutamol sulfate, lidocaine–HCl, riboflavin
Films	solution casting	isosorbide dinitrate, chlorhexidine gluconate, trypsin, granulocyte-macrophage colony-stimulating factor, acyclovir, riboflavine, testosterone, progesterone, beta-oestradiol
Gel	cross-linking	chlorpheniramine maleate, aspirin, theophylline, caffeine, lidocaine–HCl, hydrocortisone acetate, 5-fluorouracil

2..Pharmaceutical applications of chitosan particulate systems

Chitosan- based particulate systems are attracting pharmaceutical and biomedical applications as potential drug delivery devices. Some important applications are discussed below.

2.1Colon targeted drug delivery

Chitosan is a promising polymer for colon drug delivery since it can be biodegraded by the colonic bacterial flora [28,29] and it has mucoadhesive character [27]. The pH-sensitive multicore microparticulate system containing CS microcores entrapped into enteric acrylic microspheres was reported [30]. Sodium diclofenac was efficiently entrapped within these CS microcores and then microencapsulated into Eudragit L-100 and Eudragit S-100 to form a multireservoir system. In vitro release study revealed no release of the drug in gastric pH for 3 h and after the lag-time, a continuous release for 8– 12 h was observed in the basic pH.

2.2. Mucosal delivery

Nowadays, mucosal surfaces such as nasal, peroral and pulmonary are receiving a great deal of attention as alternative routes of systemic administration. Chitosan has mucoadhesive properties and therefore, it seems particularly useful to formulate the bioadhesive dosage forms for mucosal administration (ocular, nasal, buccal, gastro-enteric and vaginal-uterine therapy) [31]. Nasal mucosa has high permeability and easy access of drug to the absorption site. The particulate delivery to peroral mucosa is easily taken up by the Peyer's patches of the gut associated lymphoid tissue. Chitosan has been found to enhance the drug absorption through mucosae without damaging the biological system. Here, the mechanism of action of CS was suggested to be a combination of bioadhesion and a transient widening of the tight junctions between epithelial cells [32]. Genta et al. [31] studied the influence of glutaraldehyde on drug release and mucoadhesive properties of CS microspheres. A new in vitro technique was developed based on electron microscopy to study the effect of polymer cross-link density on the mucoadhesive properties of CS microspheres modulating the rate of theophylline release. The ability of insulinloaded CS nanoparticles to enhance the nasal absorption of insulin was investigated in a conscious rabbit model. Chitosan nanoparticles enhanced the nasal absorption of insulin to a greater extent than the aqueous solution of CS [33]. van der Lubben et al. [34] incorporated the model protein ovalbumin into CS microparticles and the uptake of ovalbumin associated with CS icroparticles in murine Peyer's patches was demonstrated using confocal laser scanning microscopy. In a further study, van der Lubben et al. [35] investigated the ability of CS microparticles to enhance both systemic and local immune responses against diphtheria toxoid (DT) vaccine after the oral and nasal administration in mice. Systemic and local IgG and IgA immune responses against DT associated to CS microparticles were strongly enhanced after the oral delivery in mice. Even though oral vaccination has numerous advantages over the parenteral injection, degradation of the vaccine in the gut and low uptake in the lymphoid tissue of the gastrointestinal tract still complicate the development of oral vaccines. In this direction, van der Lubben et al. [36] prepared the CS microparticles and characterized them for size, zeta potential, morphology- and ovalbumin-loading as well as release characteristics. The in vivo uptake of CS microparticles by murine Peyer's patches was studied by using confocal laser scanning microscopy (CLSM). Chitosan microparticles were prepared using a precipitation/coacervation method. The size of CS microparticles was 4.3±±0.7 Am and were positively charged (20±1 mV). Since only microparticles smaller than 10 Am can be taken up by M-cells of Peyer's patches, these microparticles were used as vaccination systems. The CLSM studies showed that the model antigen ovalbumin was entrapped within the CS microparticles. Field emission scanning electron microscopy demonstrated the porous structure of CS microparticles, thus facilitating the entrapment of ovalbumin. Ovalbumin loading in CS microparticles was about 40%. Release studies have shown the low release of ovalbumin within 4 h, but most of ovalbumin (about 90%) remained entrapped in the microparticles. Since CS microparticles are biodegradable, the entrapped ovalbumin was released after intracellular digestion in Peyer's patches. Initial in vivo studies demonstrated that fluorescently labeled CS microparticles can be taken up by the epithelium of the murine Peyer's patches. Since the

uptake by Peyer's patches is an essential step in oral vaccination, these results have shown that the porous CS microparticles developed are most promising vaccine delivery systems

2.3. Cancer therapy

Gadopentetic acid-loaded CS nanoparticles have been prepared for gadolinium neutron-capture therapy [37]. Their releasing properties and ability for longterm retention of gadopentetic acid in the tumor indicated that these nanoparticles are useful as intratumoral injectable devices for gadolinium neutroncapture therapy. The accumulation of gadolinium loaded as gadopentetic acid (Gd-DTPA) in CS nanoparticles designed for gadolinium neutron-capture therapy (Gd-NCT) for cancer have been evaluated in vitro in cultured cells [38]. Using L929 fibroblast cells, Gd accumulation for 12 h at 37 8C was investigated at Gd concentrations lower than 40 ppm. The accumulation leveled above 20 ppm and reached 18.0±2.7 (mean±S.D.) Ag Gd/106 cells at 40 ppm. Furthermore, the corresponding accumulations in B16F10 melanoma cells and SCC-VII squamous cell carcinoma, which were used in the previous Gd- NCT trials in vivo were 27.1±2.9 and 59.8±9.8 Ag Gd/ 106 cells, respectively. This explains the superior growth-suppression in the in vivo trials using SCCVII cells. The accumulation of nanoparticles in these cells was 100– 200 times higher in comparison to dimeglumine gadopentetate aqueous solution (Magnevistw), a magnetic resonance imaging contrast agent. The endocytic uptake of nanoparticles was suggested from TEM. These findings indicated that nanoparticles had a high affinity to cells, thus contributing to the long retention of Gd in tumor tissue leading to significant suppression of tumor growth in in vivo studies. Tokumitsu et al. [39] demonstrated the potential usefulness of Gd-NCT using gadolinium-loaded nanoparticles. The potential of gadolinium neutron-capture therapy (Gd-NCT) for cancer was evaluated using CS nanoparticles as a novel gadolinium device. The nanoparticles incorporated with 1200 mg of natural gadolinium were administered intratumorally twice in mice-bearing subcutaneous B16F10 melanoma. The thermal neutron irradiation was performed for the tumor site, with the fluence of 6.32_1012 neutrons/ cm2, 8 h after the second gadolinium administration. After irradiation, the tumor growth in the nanoparticle- administered group was significantly suppressed compared to that in the gadopentetate solution-administered group, despite radioresistance of melanoma and the smaller Gd dose than that administered in past Gd-NCT trials. Jameela et al. [40] have prepared glutaraldehyde cross-linked CS microspheres containing mitoxantrone. The antitumor activity was evaluated against Ehrlich ascites carcinoma in mice by intraperitoneal injections. The tumor inhibitory effect was followed by monitoring the survival time and change in the body weight of the animal for 60 days. Mean survival time of animals which received free itoxantrone was 2.1 days and this was increased to 50 days when mitoxantrone was given via microspheres. In another study [41], the in vitro release of mitoxantrone was controlled for 4 weeks in phosphate buffer at 27 8C. Mitra et al. [42] have encapsulated doxorubicin– dextran conjugate into long circulating CS nanoparticles. In an attempt to minimize cardiotoxicity of doxorubicin, a conjugate with dextran was prepared and encapsulated in CS nanoparticles. Size of the nanoparticle was 100±10 nm, which favors enhanced permeability and retention effect. Antitumor effect ofevaluated in J774A.1 macrophage tumor cells implanted in Balb/c mice. The in vivo efficacy of these nanoparticles was determined by tumor regression and increased survival time compared to doxorubicin– dextran conjugate and the free drug. These results suggest that the system not only reduced the side effects, but also improved its therapeutic efficacy in the treatment of solid tumors. Janes et al. [43] evaluated the potential of CS nanoparticles as carriers for doxorubicin (DOX). The challenge was to entrap a cationic, hydrophilic molecule into nanoparticles formed by ionic gelation of the positively charged CS. To achieve this objective, the authors have masked the positive charge of DOX by complexing it with dextran sulfate. This modification doubled the DOX encapsulation efficiency relative to controls and enabled real loadings up to 4.0 wt.% of DOX. Authors also investigated the possibility of forming a complex between CS and DOX prior to the formation of particles. Despite low complexation efficiency, no dissociation of the complex was observed upon the formation of

nanoparticles. Fluorimetric analysis of the in vitro drug released showed the initial release phase, the intensity of which was dependent upon the association mode, followed by a very slow release. Evaluation of the activity of DOX-loaded nanoparticles in cell cultures indicated that those containing dextran sulfate were able to maintain cytostatic activity relative to free DOX, while DOX complexed with CS before the nanoparticle formation showed a slightly decreased activity. Additionally, confocal studies showed that DOX was not released in the cell culture medium, but entered the cells while being associated to nanoparticles. These studies have shown the feasibility of CS nanoparticles to entrap DOX and to deliver it to the cells in its active form.

2.4. Gene delivery

Gene therapy is a challenging task in the treatment of genetic disorders. In case of gene delivery, the plasmid DNA has to be introduced into the target cells, which should get transcribed and the genetic information should ultimately be translated into the corresponding protein. To achieve this goal, number of hurdles are to be overcome by the gene delivery system. Transfection is affected by: (a) targeting the these doxorubicin– dextran-loaded nanoparticles was delivery system to target cell, (b) transport through the cell membrane, (c) uptake and degradation in the endolysosomes and (d) intracellular trafficking of plasmid DNA to the nucleus. Chitosan could interact ionically with the negatively charged DNA and forms polyelectrolyte complexes. In these complexes, DNA becomes better protected against nuclease degradation leading to better transfection efficiency. DNA–CS nanoparticles have been prepared [44] to examine the influence of several parameters on their preparation. The transfection efficiency of CSDNA nanoparticles was cell-type dependent. Typically, it was 3 to 4 orders of magnitude, in relative light units, higher than the background level in HEK293 cells, and 2 to 10 times lower than that achieved by LipofectAMINE–Aˆ –DNA complexes. The presence of 10% fetal bovine serum did not interfere with their transfection ability. The study also developed three different schemes to conjugate transferrin or KNOB protein to the nanoparticle surface. The transferrin conjugation only yielded a maximum of 4-fold increase in their transfection efficiency in HEK293 cells and HeLa cells, whereas KNOB conjugated nanoparticles could improve the gene expression level in HeLa cells by 130-fold. Conjugation of PEG on nanoparticles allowed lyophilization without aggregation, and without loss of bioactivity for at least 1 month in storage. The clearance of PEGylated nanoparticles in mice following i.v. administration was slower than the unmodified nanoparticles at 15 min, and with higher depositions in kidney and liver. However, no difference was observed during the first hour.Self-aggregates were prepared [45] by hydrophobic modification of CS with deoxycholic acid in aqueous media. Self-aggregates have a small size (mean diameter of 160 nm) with an unimodal size distribution. Self-aggregates can form charge complexes when mixed with plasmid DNA. The usefulness of self-aggregates/DNA complex for transfer of genes into mammalian cells in vitro has been suggested. Several transfection studies using chemically modified CS have been reported. Trimethyl CS oligomers were examined for their potency as DNA carriers [46]. Chitosan and lactosylated CS carriers were investigated for their transfection efficiencies in vitro [47]. Recently, galactosylated CS-g– dextran– DNA complexes have been prepared [48]. Galactosegroups were chemically bound to CS for liver specificity and dextran was grafted to increase the stability of the complex in water. It was shown that this system could efficiently transfect liver cells. Chew et al. [49] studied the i.m. immunization with full-length Der p 1 cDNA induced significant humoral response to the left domain (approximately corresponding to amino acids 1– 116), but not to the right domain (approximately corresponding to amino acids 117– 222) of Der p 1 allergen. Authors explored the use of CS–DNA nanoparticles for oral immunization to induce the immune responses specific to both left and right domains of Der p 1. DNA constructs pDer p 1 (1– 222) and pDer p 1 (114– 222), which were complexed with CS and delivered orally followed by an i.m. injection of pDer p 1 (1– 222) after 13 weeks. Such an approach has successfully primed Th1-skewed immune responses against both domains of Der p 1. It was suggested that such a strategy could be further optimized for more efficacious gene vaccination for full-length Der p 1. Numerous studies have been reported on prophylactic and therapeutic use of genetic

vaccines for combating a variety of infectious diseases in animal models. Recent human clinical studies with the gene gun have validated the concept of direct targeting of dendritic cells (Langerhan's cells) in the viable epidermis of the skin. However, it is unclear whether the gene gun technology or other needle-free devicesBorchard [50] has recently published a review on the efficient non-viral gene delivery using cationic polymers as DNA-condensing agents. The gene delivery is dependent on several factors such as complex size, complex stability, toxicity, immunogenicity, protection against DNase degradation, intracellular trafficking and processing of the DNA. The review also examined the advances made in the application of CS and CS derivatives to non-viral gene delivery. It gives an overview of the transfection studies performed by using CS as a transfection agent.

2.5. Topical delivery

Due to good bioadhesive property and ability to sustain the release of the active constituents, CS has been used in topical delivery systems. Bioadhesive CS microspheres for topical sustained release of cetyl pyridinium chloride have been evaluated [51]. Improved microbiological activity was shown by these microparticulate systems. Conti et al. [52] prepared microparticles composed of CS and designed as powders for topical wound-healing properties. Blank and ampicillin-loaded microspheres were prepared by spray-drying technique. In vivo evaluation in albino rats showed that both drug-loaded and blank microspheres have shown good wound healing properties. 5.6. Ocular delivery De Campos et al. [53] investigated the potential of CS nanoparticles as a new vehicle to improve the delivery of drugs to ocular mucosa. Cyclosporin A (CyA) was chosen as a model drug. A modified ionic gelation technique was used to produce CyA-loaded CS nanoparticles. These nanoparticles with a mean size of 293 nm, a zeta potential of +37 mV, high CyA association efficiency and loading of 73% and 9%, respectively were obtained. The in vitro release studies, performed under sink conditions, revealed the fast release during the first hour followed by a more gradual drug release during the 24-h period. The in vivo experiments showed that after topical instillation of CyA-loaded CS nanoparticles to rabbits, therapeutic concentrations were achieved in the external ocular tissues (i.e., cornea and conjunctiva) within 48 h while maintaining negligible or undetectable CyA levels in the inner ocular structures (i.e., iris/ ciliary body and aqueous humour), blood and plasma. These levels were significantly higher than those obtained following the instillation of CS solution containing CyA and an aqueous CyA suspension. The study indicated that CS nanoparticles could be used as a vehicle to enhance the therapeutic index of the clinically challenging drugs with potential application at the extraocular level.

3. ALGINATE

Over the last few years, medical and pharmaceutical industries have shown an increased interest in biopolymers in general and in alginates in particular. The reason for this increased interest is their usefulness in specific applications, as it enhances efficient treatment of esophageal reflux, creates multiquality calcium fibers for dermatology and wound healing. They are also used for high- and lowgel strength dental impression materials. Besides this, alginate is an effective natural disintegrant, tablet binder and offers an attractive alternative for sustained-release systems. It offers advantages over synthetic polymers as it forms hydrogels under relatively mild pH and temperature and is generally regarded as non-toxic, biocompatible, biodegradable, less expensive and abundantly available in nature; in addition, alginate meets the important requirement of being amenable to sterilization and storage. All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and other biologically active compounds and for the encapsulation of cells. Calcium alginate is a natural haemostat, so alginate based dressings are indicated for bleeding wounds [54]. The gel forming property of alginate helps in removing the dressing without much trauma. The

biopolymer alginate exhibits, like pectin and others, the effect of ionotropic gelation if multivalent cations diffuse directed from one side into the sol. During this sol-gel-transition channellike pores are created. This effect was already described in the 60s [55]. The dimensions of these pores can be influenced by the chemical conditions, for example concentration of the sol or gelling agent, nature and conformation of the alginate and pH or temperature. Therefore, the phenomenon has lately been explained to be a chemically fixed dissipative structure [56]. The channel-like pores still were obtained if ceramic powder was mixed into the sol to produce ceramic filter membranes consisting of hydroxyapatite or alumina, too [57]. For tissue engineering of bone, the phenomenon of channel-pore structure developed upon cross-linking was introduced as biomaterials by exchanging the toxic copper ions as gelling agent for calcium ions and studying the composite hydrogels [58]. Additionally, nanocrystalline hydroxyapatite could be synchronously precipitated during the sol-gel-transition by adding phosphate ions into the alginate sol [58]. For the reason of higher stability, the content of hydroxyapatite was raised by adding powder in the ratio found inside the bone: one part biopolymer and two parts ceramic phase [59]. Short term softening in relevant media like water, simulated body fluid or DMEM was studied on freeze dried scaffolds and biocompatibility was confirmed by fluorescence microscopy at day 4 of a cell culture experiment with human mesenchymal stem cells (hMSC) even so the scaffolds were stable for three weeks [59]. The proliferation and differentiation of hMSC grown for four weeks has been evaluated on freeze-dried scaffolds with larger pores [59]. The cell number increased four-fold and the osteogenic differentiation marker specific alkaline phosphatase (ALP) activity multiplied three times [60]. To contribute for the repair of osteochondral defects of joints, which affects bone and covering cartilage, biphasic but monolithic alginate scaffolds were produced which divide into a HAP containing part and an alginate/hyaluronic acid composite hydrogel [61]. Even consisting of two parts, the channel-like pores run through the whole scaffold crossing the interface, therefore, fusing of twodifferent scaffolds which might cause stability problems at the interface. This problem was solved by producing monolithic scaffolds.

3.1 Alginate: Structure and chemical modification

Alginate is a high-molecular mass polysaccharide extracted from various species of kelp [62]. Alginates are also produced extracellularly by Pseudomonas aeruginosa and Azetobacter vinilandii [63]. Edward Stanford discovered alginate in 1883 and commercial production, started in 1927, has now expanded to about 50,000 tonnes per year worldwide; 30% of this tonnage is devoted to the food industry, the rest being used in industrial, pharmaceutical and dental applications [64]. The function of alginates in algae is primarily skeletal, with the gel located in the cell wall and intercellular matrix conferring the strength and flexibility necessary to withstand the force of water in which the seaweed grows [65]. Alginate is a linear, anionic block copolymer heteropolysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G). The relative amount and sequential distribution of homogeneous M-M segments (M-blocks), homogeneous G-G segments (G-blocks) and alternating M-G segments (MG-blocks), which represent the primary structure of alginate, depend on the producing species, and for marine sources, on seasonal and geographical variations. The primary structure is generally defined by the FG value, which is the fraction of overall guluronic acid residues in the polymer, and by NG, the number-average of guluronic units in G-blocks. The alginate is known to form a physical gel by hydrogen bonding at low pH (acid gel), and by ionic interactions with divalent (Ca, Sr, and Ba) or trivalent (Fe(III) and Al) ions, that act as crosslinkers between adjacent polymer chains. G-blocks are the ones mainly responsible of such ionic interactions, as in the presence of multivalent cations they can associate to form aggregates of the “egg-box” type. The term “Egg Box” arises from a similitude model in which the cation fits into electronegative cavities like eggs in an egg-box [66]. Hence, an alginate with a higher level of G sequences presents a higher affinity for cross-linking agents than low G-containing alginates [67]. The selective binding of divalent metal ions and the corresponding gel strength were found to increase in the order: MM block<MG block<GG block [68]. The solution viscosity,

molecular weight and primary structure are fundamental to determine the swelling and gelling properties of alginate; the solubility of alginate is also affected by primary structure, ionic strength and pH. Physical properties of alginate gels varies widely, depending on their composition [69], i.e., proportion of G and M residues, the sequential order of these residues, overall molecular weight of the polymer, and calcium ion concentration at the time of gelation. It has been reported that alginates containing a high G content develop a stiffer, more brittle, and more porous gel, which maintains its integrity for long periods of time. During calcium cross-linking, they do not undergo excessive swelling and subsequent shrinking; thus, they can maintain their form in a better manner. Also it has been found that the greater the G content of the gel, the greater is the restriction to solute transport [70]. Conversely, alginates rich in M residues develop softer and less porous beads, which tend to disintegrate easier with time. Alginate with high M content also undergoes a high degree of swelling during calcium crosslinking [66]. Gels with a uniform concentration of alginate can be obtained by cross-linking via the internal setting method [71, 72]. This technique allows a controlled gelation of alginate through a slow release of calcium ions, thus leading to the formation of a very regular gel network. In general, this method uses an inactive form of the crosslinking ion, either bound by a sequestering agent such as phosphate, citrate or EDTA, or as a very low solubility salt, as CaSO4, or as a salt insoluble at neutral pH, for example CaCO3, in association with a slowly hydrolyzing lactone, usually D-glucono-δ-lactone (GDL). Since GDL generates an acidic pH, the calcium ions are gradually released and captured by guluronic residues of alginate. Uniformity and well-controlled material properties are, indeed, necessary in biomedical applications, such as tissue engineering[73,74]. It is worth note that physical gels can gradually loose their mechanical stability in biological fluids, due to an outwards flux of crosslinking calcium ions in the surrounding medium. As a consequence, chemical strategies to introduce stable covalent crosslinks using bifunctional crosslinkers, such as glutaraldehyde, have also been developed [75]. Calcium alginate gels are readily destabilized in the presence of calcium complexants EDTA-sodium citrate [76] or monovalent cations [77] and complex anions such as phosphate, citrate, and lactate, which have high affinity for calcium ions. The instability is also caused by the presence of high concentration of non-gelling ions such as sodium and magnesium. Stabilization can be achieved by adding free calcium ions to the medium while maintaining a Na:Ca ratio of less than 25:1 for high-guluronate alginates and 3:1 for low-guluronate alginates. Stabilization of calcium alginate gels by adding other multivalent ions such as Ti and Al has also been reported [78, 79]. Another important property of alginate gel is that sol-gel transition occurs without any alteration of temperature. The gel can be easily converted into a solution by adding sodium, magnesium, and EDTA.

3.1.1 Chemical modification

Aqueous carbodiimide chemistry, using 1-ethyl-(dimethylaminopropyl)carbodiimide (EDC) as water soluble carbodiimide, is widely used to couple carboxylic groups on alginate with molecules containing primary or secondary amines [80,81] as well as dihydrazides [82] (FIG.4). A coreactant as N-hydroxysulfosuccinimide (sulfo-NHS) is often used to stabilize the reactive EDCintermediates against hydrolysis, raising the efficiency of amide bond formation [83].

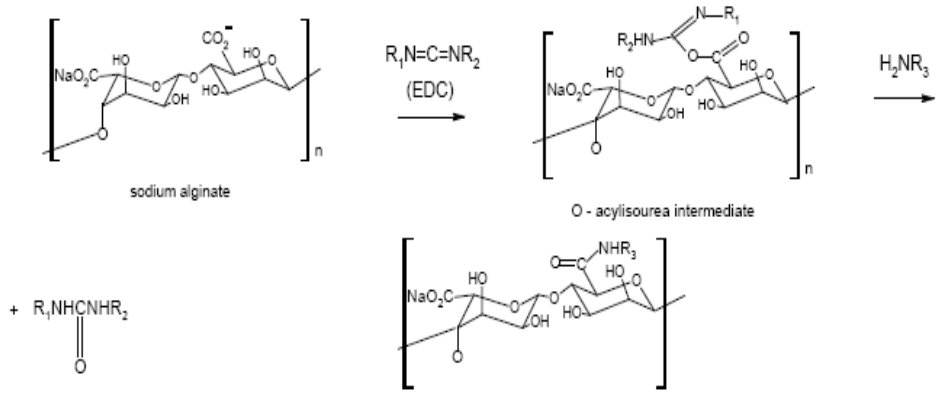

Fig.4

Such a reaction, however, consumes the carboxyl groups, essential for the gelation process. To overcome this inconvenient, the introduction of aldehydic groups, more reactive than hydroxyl or carboxylic ones, onto sodium alginate via periodate oxidation represent a selected approach to activate the polysaccharide for the successive chemical modifications [84-86]. Periodate oxidation selectively cleaves the vicinal glycols in polysaccharides to form their dihaldeyde derivatives (Fig5) The reaction proceeds with significant depolymerization of alginate. As the depolymerization is a freeradical mediated reaction due to oxidation of impurities present, the addition of aliphatic alcohols, usually isopropanol, that act as radical scavenger prevents the depolymerization giving rise to xidized alginate of higher Mw [87,88]. Depolymerization also depends on primary structure; as a matter of fact, the oxidation is still more degradative when the content of mannuronic and guluronic alternating blocks (MG-blocks) is high, as chain scission preferentially takes place at atipical sugar units. The subsequent step involves the condensation of the aldehydic groups with amines via a reductive amination reaction.

(fig 6). The most frequently employed reducing agents are sodium borohydride (NaBH4) and sodium cianoborohydrurecyanoborohydride (NaCNBH3). The lastlatter has the advantage to giveof affording a rapid reduction of the intermediates imine groups at pH 6-7, while the competitive reduction of carboxylic groups to the corresponding alcohols is negligible in this pH range.

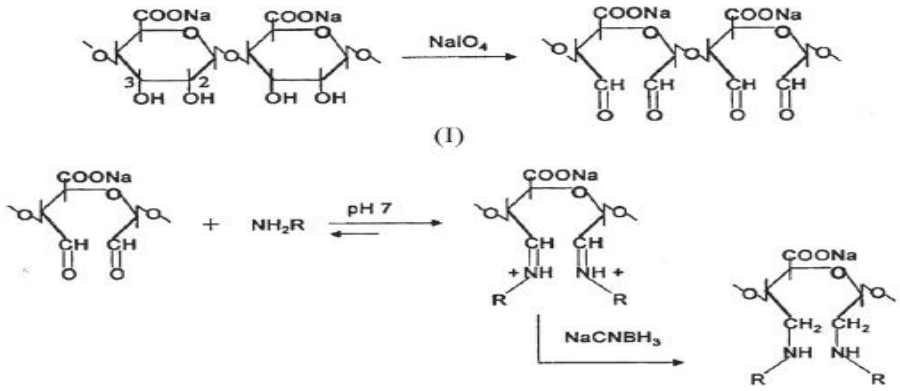

Fig.5

The reaction is often used for hydrophobization of alginate by reaction with medium-long chain (C8 - C16) alkylic amines. Hydrophobized derivatives of alginate demonstrate amphiphilic properties in aqueous medium and have been widely investigated for a variety of applications, such as a material for immobilizing enzymes. Moreover, hydrophobization of alginate by insertion of alkylic chains is commonly reported to promote protein absorption and, consequently, cell anchorage. For several applications, as additive in food and cosmetics, the gelling ability of alginate may be inhibited by chemical modification with propylene oxide.

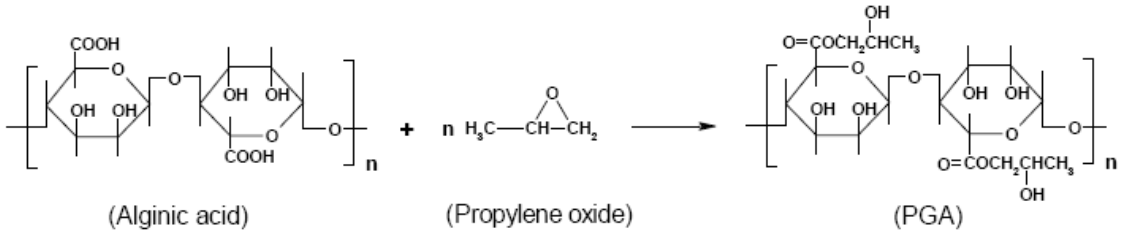

Fig.6

PGA (propylene glycol alginate) is the only, commercially available, chemically modified alginate (coded as E405). PGA is made by contacting a partially neutralised alginic acid with propylene oxide gas under pressure (Scheme 3). The propylene oxide reacts exothermically with the alginic acid to form a mixed primary/secondary ester. The partial or total substitution of acid groups with hydroxyester brings about a reduced or absent capacity of gelling, and thus the alginate can also be used as densifier in acidic solutions.

3.2 Alginate-based materials for drug-delivery applications

Alginate has been successfully used as a matrix for the entrapment and/or delivery of biological agents, such as drugs and proteins. In particular proteins can be loaded and released by alginate matrices without loss of their biological activity because of the relatively mild gelation process of alginate. In pharmaceutical formulations, the alginate gel can be prepared prior to use, or it can spontaneously form in situ in physiological fluids, by low pH and/or calcium ions naturally present in the site of administration. Alternatively, the gelling agent can be added either as a part of the formulation or separately administered. The microencapsulation technique has been specifically developed for the oral delivery of proteins, as they are quickly denaturated and degraded in the hostile environment of the stomach. The protein is encapsulated in a core material that, in turn, is coated with a biocompatible, semi permeable membrane, which controls the release rate of the protein while protecting it from biodegradation. Several examples are reported, in which alginate is used in combination with polyethyleneglycol (PEG). Alginate gels can act as core materials in this application, while PEG, which exhibits certain useful properties such as protein resistance, low toxicity and immunogenicity, together with the ability to preserve the biological properties of proteins [89, 90], can act as a coating membrane. A chitosan/PEG-alginate microencapsulation process [91], applied to biological macro-molecules such as albumin or hirudin, was reported to be a good candidate for oral delivery of bioactive peptides [92]. In general, drugs with non-favourable solid state properties, such as low solubility, benefit from encapsulation in an amorphous gel matrix. Recently, the synthesis of alginate bearing cyclodextrin (CD) molecules covalently linked on polymer chains for a sustained release of hydrophobic drugs has been reported [82,93]. Such CD-derivatives of alginate are promising as they exhibit cumulative properties of size specificity of CD and transport properties of polymer matrix. Solid preparations based on alginate such as oral tablets [94], microcapsules [95], implants [96], topical delivery systems [97,98] are currently disposable. The oral route is considered the preferred ministration route. Tablets are the most abundant dosage form, due to their convenience, easy of preparation and handling. The simpler tablet formulations are prepared by direct compression of a mechanical mixture of the various ingredients, without any need of granulation or coating. Tablets based on alginate have been prepared by direct compression, as well as wet or dry granulation and coating with various techniques [94, 99, 100]. In monolithic tablets made from alginate (in which the drug is homogeneously dispersed), drug release is controlled by the formation of a viscous hydrated layer round the tablet, in which water penetrates, that acts as a diffusional barrier. Water soluble drugs are mainly released by diffusion across this gel layer, while poorly soluble drugs are mainly released by erosion of the tablet. Micro- and nanocapsules can be prepared from alginate. Microcapsules (typically > 200 μm) are simply obtained by dropping an aqueous solution of alginate into a gelling solution, either acid (pH< 4) or, more usually, containing calcium chloride (CaCl2) as cross-linking agent. Microspheres of lower dimension (< 10 μm) are produced by a water-in-oil emulsification process using an ltrasonicator [101]. To obtain a stable water-in-oil emulsion a surfactant agent is used. An aqueous CaCl2 solution is then added to the emulsion under stirring to allow ionotropic gelation of the particles. The main shortcomings of alginate devices are their rapid erosion at neutral pH and low adhesion to mucosal tissues, which is further reduced upon crosslinking. Bioadhesive formulations [100, 102, 103], or formulations with prolonged gastric residence times [104] made from alginate have been reported. In these works, alginate was used in combination with chitosan, polylysine or vegetable oils. More recently, alginate beads for floating drug delivery systems (FDDS) have been prepared [105]. FDDS have a lower density than gastric fluids, so their gastric residence time is longer. Floating alginate beads are easily obtained by dropping an alginate solution containing a foaming agent such as CaCO3 or NaHCO3 in CaCl2/acetic acid. The CO2 gas produced remains entrapped inside the beads, which show low density and high porosity. Modification of polysaccharides by introducing acrylic polymer chains is used to

obtain a finer control over drug release rate and to improve adhesion to biological substrates [106-112]. Hydrogels based on crosslinked poly(acrylic acid) [113, 114] have been reported to adhere to mucus providing a barrier against irritations and inflammations of membranes of the gastrointestinal system. Acrylic polymers containing amine functionality, as poly(dimethylaminoethylacrylate, DMAEA), in combination with glycolic residues have been demonstrated to how good bioadhesion and mucoadhesion [115].

3.2.1 Novel alginate-acrylic polymers as a platform for drug delivery

Following the above reported examples, the chemical modification of alginate with both poly(acrylic acid) (AA-pAcrAc) and polyDMAEA (AA-pDMAEA) through radical grafting of acrylic monomers has been reported, with the aim to modulate the time of erosion, the rate of release of drugs and the adhesion to substrates [116]. Acrylamide and acrylic acid modified chitosan obtained by a redox- type initiation are described. [106, 107, 111]. The radical polymerization of an acrylic monomer initiated by peroxide is normally performed directly in the presence of the polysaccharide. In our approach, an original two-step procedure was used to minimize secondary reactions such as, for example, the free homopolymerization reaction of the acrylic monomer; besides, the high viscosity of alginate solution might hinder the polymerization because of the slow diffusion rate of monomers and growing chains, with consequent low conversion. To overcome these problems, as a first step acrylic monomers have been pre-polymerized at 100°C, to promote the disproportionation reaction as the prevalent termination process. It is in fact reported that at this temperature the bimolecular reaction between two acrylic polymeric radicals prevails, that brings to the transfer of a hydrogen atom with formation of a double bond at the end of the acrylic chain [117]. In the second step, coupling of such vinyl-terminated acrylic chains with sodium alginate in presence of potassium persulphate was performed. As the literature on acrylic modified polysaccharides does not clearly indicate the mechanism of grafting (the reported data refer either to a template type polymerization [108, 109], or to a true chemical grafting process [118, 119], an investigation aimed at lucidating the chemical structure (graft copolymer or inter-polymer complex) of the obtained polymers via diffusion-ordered NMR spectroscopy (DOSY) [120] was performed. This original technique is an innovative convenient way of displaying the molecular self-diffusion information in a bi-dimensional array, with the NMR spectrum in one dimension and the self-diffusion coefficient in the other one. DOSY has been successfully used for the analysis of mixtures [121], for the characterization of aggregates [122], for the molecular weight determination of uncharged polysaccharides [123]. It is believed that DOSY is an appropriate technique also to distinguish between copolymers and interpolymer complexes. The DOSY maps obtained in the case of AA-pDMAEA sample, together with those of plain alginate and plain pDMAEA as comparison, are shown in Figure 1. It is evident that the two components retain different values of the diffusion coefficient in the AA-pDMAEA sample. As a consequence, we may safely assess that no covalent neither ionic bond exists between the two components of the mixture, namely alginate and polyDMAEA. Analogous results were obtained for acrylic acid-grafted alginate (AA-pAcrAc). Moreover, in the DOSY map it is also possible to evidence the presence of low molecular weight compounds showing a fast value of the diffusion coefficient. This is not unexpected, as peroxides may cause a degradation of AA as side reaction [124], and the formation of oligomers during the radical polymerization of acrylics is also well known. In conclusion, we may hypothesize in both cases the formation of a stable interpolymer complex based on non-ionic inter-chain interactions between the two polymers.

4. Pullulan

4.1 Introduction

Pullulan is a water soluble, neutral linear polysaccharide consisting of α–1, 6-linked maltotriose residues. It is a fungal exopolysaccharide produced from starch by Aureobasidium pullulans[125]. The early observation on this exopolymer was made by Bauer in 1938 and this exopolysaccharide was named as pullulan by Bender et al in 19592. Molecular weights of pullulan range from thousands to 2,000,000 daltons depending on the growth conditions of the organism Aureobasidium pullulans. Pullulan is biodegradable, impermeable to oxygen, non-hygroscopic and non-reducing. Pullulan is easily soluble in hot and cold water to make clear and viscous solution and also has high adhesion and film forming abilities. Pullulan films are thermally stable and possess anti-static and elastic properties andcan be developed into compression mouldings[126,127]. These properties of pullulan are attributed to the unique linking it possesses with structural flexibility3. The principal advantages of pullulan are that it is a nonionic polysaccharide and is blood compatible, biodegradable non-toxic, nonimmunogenic, non-mutagenic and noncarcinogenic[127- 130]. Pullulan productuion was started commercially by Hayashibara Company in 1976 and they are still the main source of pullulan

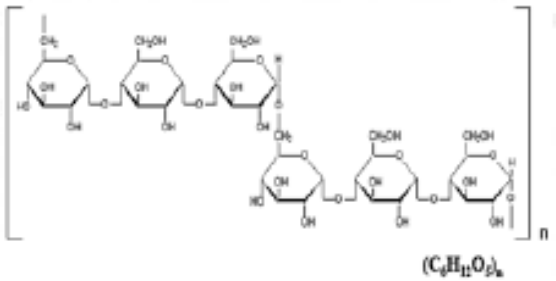

Fig.7 PULLULAN

Pullulan is currently used extensively in the food industry[128]. It is a slow digesting macromolecule which is tasteless as well as odorless hence it is used as a low-calorie food additive providing bulk and texture. Pullulan possess oxygen barrier property, good moisture retention and also it inhibits fungal growth[128]. These properties make it excellent material for food preservation and are used extensively in the food industry. There is a twenty year history of safe use in Japan as a food ingredient and as a pharmaceutical bulking agent. FDA had estimated that daily intake of pullulan would be up to 10 g per day for a person based on food categories and usage[128]. However, recently pullulan is also being investigated for its biomedical applications in various aspects like targeted drug and gene delivery, tissue engineering, wound healing and also even in diagnostic imaging using quantum dots. Pullulan is highly water soluble; hence for drug delivery applications, mostly hydrophobized pullulan is used as drug delivery carriers. These hydrophobized pullulan molecules can form colloidally stable nanoparticles upon self-aggregation in water with monodispersity. Pullulan due to its film forming properties can entrap biological molecules and due to its excellent oxygen barrier properties these molecules remain stable with

enhanced shelf-life. Pullulan can be chemically modified to produce derivatives with low solubility or a modified polymer that is completely insoluble in water[127]. Pullulan derivatives are developed and their applications towards the above mentioned aspects were also studied by various groups[131-133]. This review focuses on the recent developments in the biomedical application of pullulan.

4.2Biomedical Applications of Pullulan

Pullulan is now extensively studied for various applications in iomedical field. This is mainly due to its non-toxic, nonimmunogenic and biodegradable properties. In comparison to a similar but more popular polysaccharide, dextran, the degradation rate of pullulan in serum is faster than that of dextran. The degradation index is 0.7 after 48 hour incubation in comparison with 0.05 for dextran. The degradation rate can be reduced or regulated by varying degrees of chemical modification[134]. Some of the major areas in which pullulan is investigated for is discussed in the following section.

4.2.1 Targeted drug/gene delivery and imaging

Liver targeting

On the sinusoidal surface of the hepatocytes, asialoglycoprotein receptors are expressed abundantly which removes the asialo (galactose-terminal) glycoproteins from the sinusoidal circulation by internalizing the bound protein via endocytosis. Kaneo et al has reported the strong binding of pullulan to the asialoglycoprotein receptor with high affinity and the bound molecule is internalized to the hepatocyte via receptormediated endocytosis[135]. Pullulan accumulates in the liver in significantly higher amounts than other water soluble polymers. This property of pullulan is widely exploited for targeted drug/gene delivery to liver. Interferon (IFN) is used as the conventional monotherapy of hepatic virus C induced liver diseases. But the efficiency of current interferon therapies is reported to be clinically insufficient. An attempt by Suginoshita et al was made to target IFN to liver by complexing it with pullulan DTPA[136]. They reported that intravenous administration of this IFN-DTPA-Pullulan conjugate in mice showed enhanced IFN activity than the free IFN. It was concluded from this study that this enhanced activity is due to the liver targeting ability of pullulan and it seems to be a promising interferon therapy.

4.2.2 Nanoparticles for drug/gene delivery and cancer therapy

Recently the role of polysaccharides in developing controlled drug delivery systems has increased significantly and pullulan is gaining lot of attraction towards this application. Self-assembling nanoparticles from hydrophoboized pullulan, pH sensitive derivatised pullulan nanoparticles, anionic/amphiphilic icroparticles[131],[137-138] are some of the examples. Akiyoshi et al developed insulin delivery system of the size 20-30 nm by complexing the hydrogel nanoparticle of cholesterol bearing pullulan14. These complexed nanoparticles were stable and protected insulin from the enzymatic degradation and suppressed insulin aggregation. It was proved in vivo that the biological activity of the complexed insulin remained intact. Carboxymethylation introduces negative charge in pullulan and this derivative unlike pullulan has low affinity for asialoglycoprotein receptors[139]. The liver uptake clearance of pullulan was decreased by more than hundred fold. This derivative was then investigated for application in chemotherapy. The authors conjugated doxorubicin, a known chemotherapy drug used in various cancers, via a peptide linker to carboxymethylated pullulan. Conjugating such small molecular drugs to polysaccharides make them inactive and is referred to as macromolecular prodrugs. The

conjugated drug to be pharmacologically active should get released from the prodrug. The conjugation reduces the free drug plasma concentration and drug exposure to other susceptible tissues. Compared to free drug these prodrugs have long half-life. This increased half-life will lead to passive accumulation of prodrugs in the tumor. This is because of the enhanced permeability of the prodrugs to tumor due to its leaky vasculature and the retention of these macromolecular conjugates due to decreased lymphatic drainage. Nogusa et al[140] in their in vivo study the authors established that the conjugated drug was more effective than the free drug. They tried both the conjugate and free drug on murine carcinoma models, solid tumor (lung carcinoma and reticulosarcoma) and nonsolid tumor (P388 leukemia). The conjugate was more effective in reducing the tumor volume and increasing the survival rate than the free drug. The conjugate but did not have any effect on leukemia cells indicating that it is effective only against solid-tumors. Hydrophobically modified pullulan is known to from self-assembled nanoparticles. Na et al developed pH sensitive self-assembled nanoparticles of succinylated pullulan acetate/sulfonamide (PA/SDM) conjugates which are responsive to even minute pH variations[133]. These particles were of the size range <70 nm and showed good stability but shrank and aggregated below pH 7.0. These particles were developed for targeting solid tumors and inflammatory regions where the extracellular pH (pH 6.5-7.2) is lower than the normal tissues and blood. These particles were tested for loading and release properties with adriamycin (doxorubicin). The drug release rate from the PA/SDM nanoparticles was pH-dependent nd it was significantly enhanced below a pH of 6.8. The authors conclude that these pHresponsive PA/SDM nanoparticles may provide some advantages for targeted anticancer drug delivery due to the particle aggregation and enhanced drug elease rates at tumor pH. Gene therapy is another area where the application of pullulan is being explored. Gene therapy is thought to be a cure for various inherited disorders and cancer[141-142]. Gene delivery is usually achieved by endocytic pathway. Efforts for gene therapy using virus have been performed but viruses are known to be immunogenic and can be hazardous. So attempts to develop non-viral vectors are taken and cationic derivatives of natural polymers are investigated towards this purpose. Recently pullulan being biocompatible and non-toxic is investigated for gene delivery application. Hosseinkhani etal developed pullulan-DTPA derivative which has metal chelating residues and mixed with a plasmid DNA in aqueous solution containing Zn2+ ions to obtain the conjugate of pullulan derivative and plasmid DNA with Zn2+ coordination[141]. Pullulan is known for its specificity for liver and this property is exploited for liver targeting. The authors observed that the conjugate enhanced the level of gene expression at the liver paranchymal cells and the enhanced gene expression lasted for a period of 12 days after the injection.

4.3Medical imaging

Recently nanotechnology is being investigated for successful and earlier detection of cancerous growths in the body. Quantum dots are nano-size semiconductor particles which has currently attracted lots of attention in the biological field[143]. They are used as fluorescent probes for long-term cell-tracking as it is highly photostable with strong fluorescence. Endocytosis of these QD's into the cell is usually low and for bioimaging purposes there should be detectable amount of the QD's. Recently there are numerous literatures reporting the efficiency of polysaccharides in the drug/gene delivery and imgaing agents. Hasegawa et al developed cholesterol pullulan and amino-group-modified cholesterol pullulan nanogel as a novel carrier to deliver QD into cells in comparison to conventional cationic liposome which has the disadvantage of forming aggregates once it is internalized in the cell [144]. Nanoparticles were prepared by simple mixing with nanogels of cholesterol-bearing pullulan modified with amino groups and quantum dots. The size of these hybrid particles were about 38 nm. They reported that the intensity of fluorescence per cell of CHPNH2–QD nanoparticle was comparable to that of liposome–QD complex and particles with higher number of amino groups showed fluorescence up to 3.4 times than that of the control. The

authors conclude that the chemical modification of CHP by introducing cationic groups significantly enhances its cellular uptake and simultaneously the QD's better than the conventional cationic liposomes and that these nanoparticles could be a promising fluorescent probe for bioimaging.

4.4 Tissue engineering

Tissue engineering requires scaffold or artificial extracellular matrix that can accommodate cells and regulate their growth leading to three-dimensional tissue regeneration. Na et al developed carboxymethyl pullulan and conjugated it with heparin and investigated its properties towards tissue engineering applications[145]. Heparin-conjugated pullulan inhibited the proliferation of smooth muscle cells (SMCs) in vitro. Heparin-conjugated pullulan material can thus be used for the proliferation of vascular endothelial cells and to inhibit the proliferation of SMCs

4.5 Molecular chaperons

Chaperon like activity- able to catch and release proteins. Molecular chaperons selectively bind denatured proteins in order to prevent irreversible aggregation due to host-guest interaction. Then the host chaperon releases the protein in its refolded form. Water soluble polymers such as PEO were tried to increase the recovery yield of the native protein during refolding[146]. These polymers block the exposed hydrophobic surface on the denatured proteins in such a way which just preven ts the aggregation ofproteins. Excessively strong binding to intermediate would prevent folding to the native confirmation. Nomura et al developed hydrophobized pullulan nanogels possessing properties of molecular chaperons[147]. The complexed proteins were effectively released from the nanogels in their refolded forms in presence of cyclodextrins. They concluded that these amphiphilic nanogels selectively traps denatured proteins and cyclodextrin acts as a effector molecule to control the binding ability of host to proteins and that this nanogel system is a promising technique for protein refolding. The chaperon like property i.e the binding and release of proteins in the active form is further being explored towards tissue engineering applications

4.6 Plasma expander

Like dextrans pullulan was also explored as a potential blood-plasma substitute. Only highly water soluble polymers can be used as plasma expanders and pullulan is highly water soluble. Blood plasma xpander operates via the colloidal osmotic pressure induced by the macromolecules. It is reported that pullulan to be used as a plasma blood expander should have a Mw of about 60 kDa [148]. They observed that pullulan with higher molecular weight range increased venous pressure where as low molecular weight gets rapidly excluded from the organism followed by the development of secondary hemorrhagic shock. Therefore, the pullulan for this particular purpose should be in the effective therapeutic Mw range free from low and high molar mass fractions. Shingel et al developed an anionically modified pullulan via γ-irradiation which was used as base for blood-plasma substitute[149- 151]. Introduction of carboxyl and carbonyl groups increases the resistance of pullulan to degradative action amylase. This substitute was studied in dogs under the conditions of experimental shock. An isovolumetric replacement with this new derivative of pullulan resulted in a rapid recovery of the animal and the blood microcirculation was normalized.

4.7 Surface modification

Yet another promising application of this versatile polymer is the use in surface modification as evidenced by work by Hasuda et al[152]. The authors synthesized photoreactive pullulan, azidophenylpullulan (Az-pullulan) and ph otoimmobilised on polystyrene, polyethylene and silane coupled glass surfaces by micropatterning method. These surfaces which have different contact angles showed same contact angle for all the surfaces. Interaction of these modified surfaces with proteins like albumin and cells having macrophage like properties which can adhere to various surfaces[153] (RAW 264) was then studied. The authors observed that the adherence of both the cells and protein was more pronounced on the unmodified surface. The authors suggest that the pullulan forms a hydrophilic non-ionic surface layer which reduces the protein adsorption. There was no cell adhesion also on to Az-pullulan modified surface. Hydrophilic surfaces reduce cell adhesion. Authors consider Azpullulan as a promising candidate for bioinert surfaces due to reduced interaction with protein and cell. Targeted drug delivery with magnetic nanoparticles is possible with the use of external magnetic fields target the particle to the site of interest. Magnetic nanoparticles are also of interest in diagnostic imaging as well. But since the magnetic nanoparticles being hydrophobic gets easily destroyed or cleared from the circulation and these particles are also cytotoxic. Hydrophilic surface modification of these particles prolongs the half-life in the circulation. Gupta et al coated prepared superparamagnetic iron oxide nanoparticles (SPION) and coated with pullulan (Pn-SPION) [154]. They studied the effect of pullulan coating on the cytotoxicity and the cellular uptake of the nanoparticles. The cytotoxicity studies were done on fibroblasts and it was observed that with uncoated particles (SPION) the cell death was 60% and with Pn-SPION there were no cytotoxic effects. Similarly cell adhesion test also showed that the attached cell number was decreased upto 64% in SPION but for pullulan coated it was comparable with the control cell population. The authors attribute the low toxicity of Pn-SPION to the hydrophilicity of pullulan. By transmission electron microscopy the cellular uptake of the particles was also established. These pullulan coated magnetic particles is thought to be useful for medical imaging like vascular compartment imaging, lymph node, receptor, perfusion and target specific imaging.

5. Scleroglucan: A Versatile Polysaccharide for Modified Drug Delivery

5.1 Introduction

Among these macromolecules, scleroglucan (Sclg) [155] also seems to be potentially useful for the formulation of modified release dosage forms and numerous studies have been devoted to this specific topic. Interest in this polysaccharide was first aroused in 1967 [156]. Sclg is a general term used to designate a class of glucans of similar structure produced by fungi, especially those of the genus Sclerotium. The commercial product is termed Scleroglucan, but it is also known with other names according to the company that produces the polysaccharide (e.g., Actigum, Clearogel, Polytetran, Polytran FS, Sclerogum). Because of its peculiar rheological properties and its resistance to hydrolysis, temperature and electrolytes, Sclg has various industrial applications, especially in the oil industry for hickening, drilling muds and for enhanced oil recovery [157, 158]. Other industrial uses include the preparation of adhesives, water colors, printing inks and liquid animal feed composition [159]. In the cosmetic industry, Sclg may be used in hair control compositions [160] and in various skin care preparations, creams and protective lotions [161, 162]. In pharmaceutical products, Sclg may be used as a laxative [163], in tablet coatings [164] and in general to stabilize suspensions. In the food industry, numerous Japanese patents describe quality improvements of frozen foods [165], Japanese cakes [166], steamed foods [167], rice crackers [168] and bakery products [169]. The use of Sclg as an antitumor, antiviral and antimicrobial compound has also been investigated [170-173]. Sclg has shown immune stimulatory effects [174]

compared with other biopolymers, and its potential contribution to the treatment of many diseases should be taken into account in therapeutic regimens.

5.2 Structure of Scleroglucan

Sclg is a branched homopolysaccharide that gives only D-glucose upon complete hydrolysis. The polymer consists of a main chain of (1→3)-linked ß-D-glucopyranosyl units; every third unit it bears a single ß-D- lucopyranosyl unit linked (1→6) Fig.8

Scheme 1. Repeating unit of Scleroglucan.

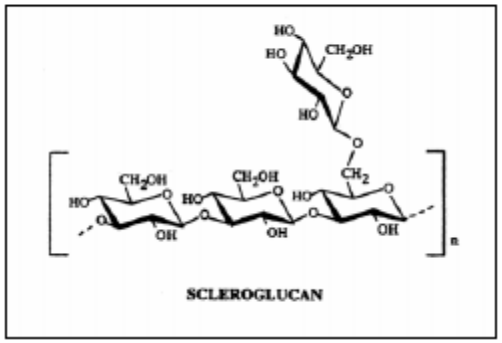

fig.8

Oriented-fiber X-ray diffraction indicates that Sclg has a triple-helical backbone conformation [24] and also that dissolved Sclg chains assume a rod-like triple helical structure [25, 26], in which the Dglucosidic side groups are on the outside and prevent the helices from coming close to each other and aggregating. In dimethyl sulfoxide, or in solutions of pH=12.5 or higher, the reduced viscosity, the specific rotation and the sedimentation coefficient indicate, in each case, that Sclg molecules are monodispersed in a single-chain random coil [175-177]. In this paper we will give an overview of the main lines of research carried out with Sclg, and its derivatives, as a potential matrix for sustained drug release of bioactive molecules [178, 179]. In particular the presentation will be divided in six sections: the first five refer to the different strategies used for the preparation of the systems while the sixth one reports drug release and related theoretical calculations.In fact, some features of this polymer, like the solubility in aqueous media, the biocompatibility and the good chemical versatility due to the hydroxyl moiety easily accessible for grafting reactions, allow for its quite widespread potential use, both as native polymer and/or after derivatization reactions.

5.3 Crosslinked Scleroglucan

The reactions leading to the chemical hydrogels starting from scleraldehyde, the intermediate product of the oxidation, and from sclerox, are shown in fig(a) and fig(b) Reactions schemes to obtain the cross-linked polymers from Scleraldehyde and from Sclerox

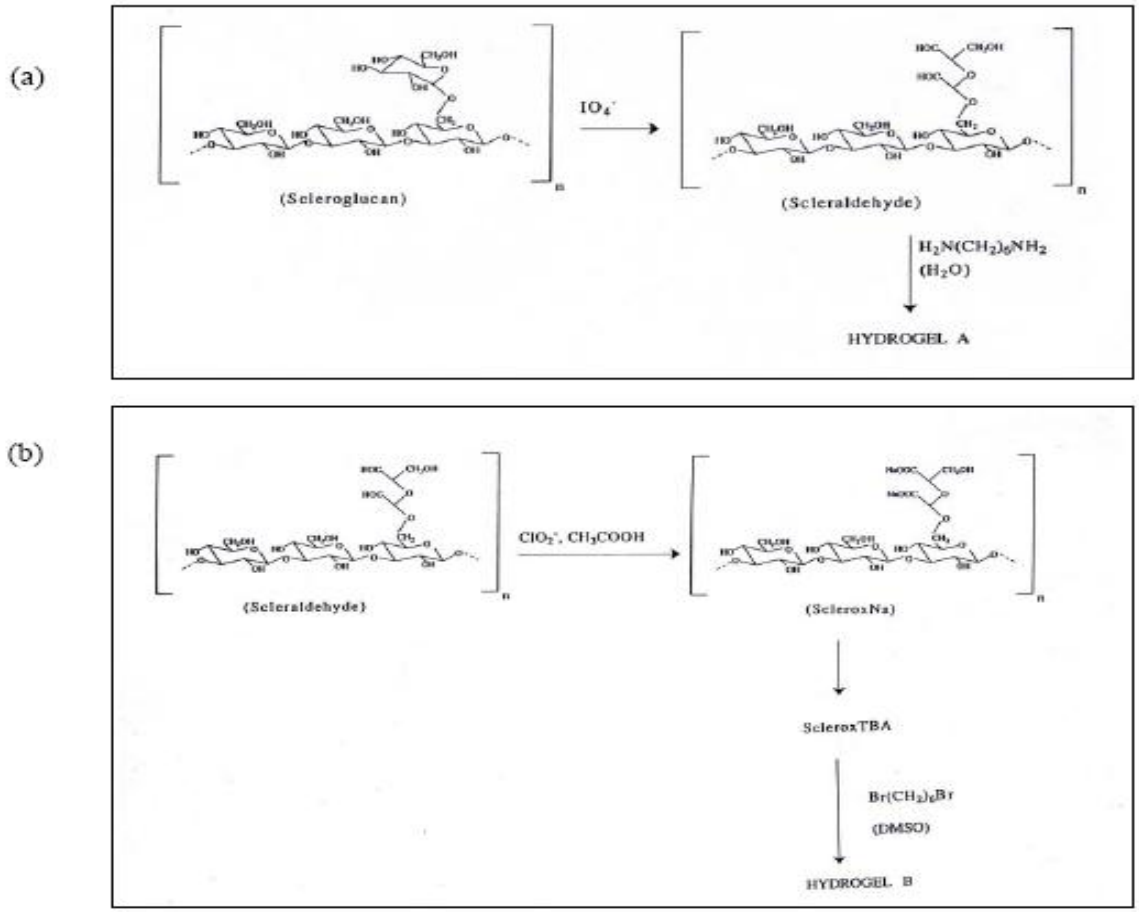

fig.9

The scleraldehyde with a low degree of oxidation (10 and 20%), prepared by a controlled oxidation of Sclg, was found to retain essentially a triple-stranded helical conformation, while the triple-stranded chain disentangles in single chains with increasing the degree of oxidation (40 and 100%). The hydrogel prepared from scleraldehyde with a low degree of oxidation, according to SAXS profiles analysis, can be represented by a network composed of randomly oriented triple helices interlinked at the sites where the aldehyde groups are present [180, 181]. According to the degree of oxidation of the polysaccharide, to the length of the crosslinking agent and to the reagent/polymer" ratio, products with different properties were obtained. Both crosslinked polymers gave hydrogels, i.e. systems capable to swell in aqueous medium to a different extent:therefore their structure together with their suitability as matrices for controlled delivery of bioactive materials were studied. In particular, the hydrogel obtained from sclerox [182] using hexamethylene dibromide as a crosslinking agent, had a remarkable increase of weight when it was soaked in water. In the case of the lower degree of crosslinking an appreciable percentage of carboxylic groups did not react with the dihalide, thus this hydrogel, keeping markedly the characteristic of the starting polyelectrolyte, was noticeably affected by environmental conditions such as ionic strength.

5.4 Drug release from polymeric matrices

During the study of these polysaccharide matrices an important factor to be taken into account was the diffusion of the model drug through the network. In fact, when polymeric matrices are brought in contact with an external release medium (usually water or a physiological solution) the matrix network swelling begins and the system internal structure changes accordingly from a glassy to rubbery state. In the rubbery region, drug molecules eventually embedded in the matrix can diffuse through the net meshes and the macroscopic drug release occurs. During the polymeric swelling phenomenon three moving boundaries may form: the swelling front, the erosion front and the diffusion front [183-185]. The swelling front separates the glassy portion of the system from the rubbery one, and moves towards the glassy core. The erosion front separates the rubbery region from the external release liquid. Finally, the diffusion front separates the regions of undissolved and dissolved drug, and follows the swelling front in its motion. If the drug concentration used is well below its solubility threshold in the incoming release liquid, the swelling front and the diffusion front coincide. The characteristics of the drug release kinetics may be highly influenced by the interaction of the dry matrix with the surrounding environment. At the swelling front, a molecular rearrangement of the polymeric chains takes place due to the transition from glassy to rubbery state. The time required for this rearrangement depends on the relaxation time tr of the given polymer/liquid interface which, in turn, is a function of both local release medium concentration and temperature. If tr is much lower than the characteristic time of diffusion td of the liquid - defined as the ratio of the release liquid diffusion coefficient at equilibrium and the square of a characteristic length (radius in case of spherical matrices) - the release liquid absorption may be described by means of Fick's law with a concentrationdependent diffusion coefficient. On the contrary, if tr is much greater than td, a Fickian release liquid absorption with constant diffusivity takes place. In both these cases, however, the diffusion of the drug molecule in the swelling network may be described by Fick's law with a non-constant diffusion coefficient and the macroscopic drug release is said to be Fickian. When tr ≅ td, the release liquid absorption does not follow Fick's law of diffusion [186-188]. In such cases, the macroscopic drug release becomes anomalous

5.Guar Gum

5.1 Inrroduction

Guargum has its unique rheology modifying properties, it is being idely used across a broad spectrum of industries viz. oil well drilling, textile, paper, paint, cement, cosmetic, food, pharmaceutical etc. India is the major producer of guar in the world and its contribution to the world- production is around 80%. The most important property of guar gum is its ability to hydrate rapidly in cold water to attain uniform and very high viscosity at relatively low concentrations. Apart from being the most cost-effective stabilizer and emulsifier it provides texture improvement and water binding, enhances mouth feel and controls crystal formation. The main properties of guar gum are: It is soluble in hot & cold water but insoluble in most organic solvents. It has strong hydrogen bonding properties. It has excellent thickening, emulsion, stabilizing and film forming properties. It has excellent ability to control rheology by water phase management. The viscosity of guar gum is influenced by temperature, pH, presence of salts and other solids.

5.2 Chemical structure of gour gum

Naturally and abundantly available guar gum for colon targeted drug delivery. Guar gum is a natural nonionic polysaccharide derived from seeds of Cya- mopsis tetragonolobus (Family: Leguminaciae). It con- sists of linear chains of (1→4)-□-D mannopyranosyl units with □-D-galactopyranosyl units attached by (1→6) linkages [189]. Guar gum is used as a binder (up to 10%) and disintegrating agent in solid dosage forms. It is also used as a suspending, thickening and stabilis- ing agent (up to 2.5%) in liquid oral and topical prod- ucts. Guar gum contains about 80% galactomannan, 12% water, 5% protein, 2% acid insoluble ash, 0.7% ash and 0.7% fat Chemically, guar gum is a straight chain galactomannan, which is 75-85% of the endosperm, has a chain of (1→4)-linked-β-D-mannopyranosyl units with single α-Dgalactopyranosyl units connected by (1→6) linkages to, on the average, every

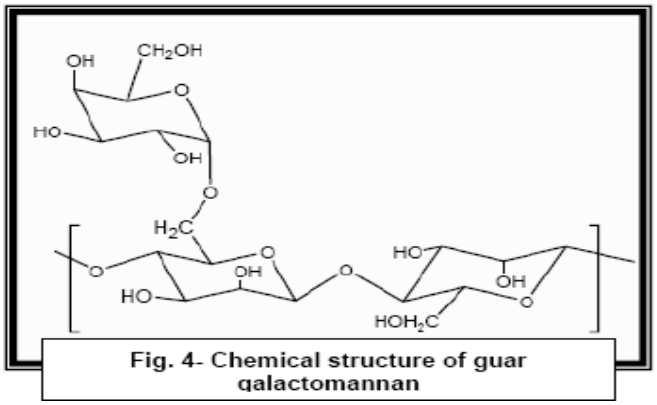

Fig. 4- Chemical structure of guar galactomannan

Fig. 10 Chemical structure of guargalactomannan

second main chain unit (Fig. 4). The ratio of D-mannopyranosyl to Dgalactopyranosyl units is about 1.8:1. The average molecular weight of guaran is in the range of 1-2 ×106 dalton. The cis-position is important since adjacent hydroxyl groups reinforce each other in hydrogen bonding reactions.

5.2.1 Chemical modification of gourgum

Guar gum was chemically modified by sulphonation using chlorosulphonic acid (ClSO3H) as a reagent. Activated partial thromboplastin time (APTT) assay showed that the guar gum sulphate could inhibit the intrinsic coagulant pathway. The anticoagulant activity strongly depended on the degree of substitution (DS) and molecular weight (Mw) of polysaccharides. DS>0.56 was essential for anticoagulantactivity. The guar gum sulphate with the DS of 0.85 and the Mw of 3.40×104 had the best blood anticoagulant activity. The optimum reaction conditions for affording maximum percentage of grafting for grafting of acrylonitrile (AN) onto sodium salt of partially carboxymethylated guar gum (DS 0.497) using ceric ammonium nitrate (CAN) as a redox initiator, in an aqueous medium, by successively varying reaction conditions such as concentrations of nitric acid, ceric ammonium nitrate, monomer (AN) as well as reaction time, temperature and amount of substrate was also established by an expert. The IR-spectroscopic, thermal (TGA/DSC) and scanning electron microscopic (SEM) techniques were used for the characterization of the graft copolymer. Using microwave (MW) irradiation grafting of polyacrylonitrile (PAN) onto guar gum in water was done without using any radical initiator or catalyst within a very short reaction time. The extent of grafting could be adjusted by controlling the reaction conditions and maximum percentage grafting (%G) of about 188% was obtained under optimum conditions in 1.66 minutes. Grafting of acrylamide onto guar gum is achieved by Ce(IV) induced free-radical polymerization to prepare interpenetrating polymer network (IPN) beads of polyacrylamide-g-guar gum with sodium alginate by crosslinking with glutaraldehyde.Two widely used pesticides, solid chlorpyrifos and liquid fenvelarate, were loaded up9 to 60-70% efficiency in the IPN beads. Equilibrium swelling experiments indicate thatthe swelling of the beads decreases with an increase in crosslinking, as well as an increase in pesticide loading. The action of a cationic polyelectrolyte (ammonium hydroxy-propyl-trimethyl chloride of the polysaccharide guar gum, commercially know as cosmedia guar, CG) in aqueous alumina suspension was investigated. This polymer was used aiming to find alternatives for synthetic polymers, as for instance, sodium polyacrylate-PANa, normally used as a deflocculating agent of alumina suspension. The measurements of particle size, as a function of time, showed that the addition of this polyelectrolytic macromolecule (CG) keeps the particles dispersed for a longer time, in comparison with the suspension containing only alumina. The ceric-ammonium-nitrate-initiated graft copolymerization of polyacrylamide onto hydroxypropyl guar gum by solution polymerization technique was studied. The synthesized products were then characterized by various instrumental techniques like viscometry, elemental analysis, IR, thermal, XRD and SEM studies. The percentage of grafting increases with increasing catalyst concentration and decreases with monomer concentration taking other parameters constant A mild method for microencapsulation of sensitive drugs, such as proteins, employing a suitably derivatized carboxymethyl guar gum (CMGG) and multivalent metal ions like Ca2+ and Ba2+ was reported. The swelling data of Ca2+ and Ba2+ crosslinked beads suggest that Ba2+ crosslinks CMGG much more efficiently than Ca2+. The drug loading efficiency of these Ba2+/CMGG beads, as a function of concentration of both metal ion as well as drug, was then determined using Bovine Serum Albumin as a model drug. Results indicated that Ba2+ crosslinked carboxymethyl guar gum beads could be used for gastrointestinal drug delivery

5.3Interpenitrating network of gourgum

Guar gum / poly (acrylic acid) semi-interpenetrating polymer network (IPN) hydrogels have been prepared via free radical polymerization in the presence of a crosslinker of N,N′-methylene bisacrylamide (MBA). Hydrogels showed enormous swelling in aqueous medium and displayed swelling characteristics, which were highly dependent on the chemical composition of the hydrogels and pH of the medium (ionic strength I = 0.15 mol/L) in which hydrogels were immersed. On increasing the guar gum concentration the grafting parameters increase. The optimum time and temperature for the grafting reaction was 120 minutes and 35 °C respectively. The water uptake behavior of barium ions crosslinked sodium alginate/carboxymethyl guar gum bipolymeric beads in the media of varying pH was also studied. The beads swelled to nearly 15±4% in simulating gastric fluid (SGF) of pH 1.2 in 3 h. On transferring the hydrogel into simulated intestinal fluid (SIF) of pH 7.4, the swelling was enhanced to nearly 310±12%. When loaded with the model drug vitamin B12, the 8 total release in SGF in 3 h was nearly 20%, while nearly 70% was released in SIF in the next 7 h. The percent entrapment was nearly 50% when the beads were crosslinked with a 5-6% (w/v) BaCl2 solution.

5.4 Drug delivery in Gour gum

Guar gum hydrates and swells in cold water forming viscous colloidal dispersion or sols [190-192]. This gelling retards the drug release from the tablets [193-195]. Guar gum is being used to deliver drug to colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine [196-198]. The anaerobic bacteria that are responsible for the degradation of guar gum in the colon areBacteroides species (B. fragilis, B. ovatus, B. Variabilis, B. uniformis, B. distasonis and B. thetaio- taomicron). Metronidazole and tinidazole are the drugs of choice in the treatment of amoebiasis, and are also effective against the anaerobic microorganisms [199]. The con- comitant use of metronidazole or tinidazole with guar gum based colon targeted formulations (e.g. guar gum matrix tablets of 5-ASA) may not be uncommon. It is to be noted that the utility of guar gum as a colon-spe- cific drug delivery carrier is based on its degradation by colonic bacteria [200-202]. The colon is rich in anaerobic bacteria [203]. It implies that guar gum in the form of either a matrix tablet or as a compression coat over the drug core might have been degraded to a larger extent by the action of anaerobic microbial population of large intestine [200-202].. Since metronidazole and tinida- zole are active against anaerobic bacteria [199], the use-fulness of guar gum on concomitant administration of these drugs with guar gum based formulations in pro- viding colon-specific drug delivery is not known. In the light of this information, it is planned to study the influence of metronidazole and tinidazole on the use- fulness of guar gum as a carrier for colon-specific drug delivery using matrix tablets of albendazole containing 20% guar gum as model formulations. It was reported that matrix tablets of albendazole containing 20% of guar gum were found to be potential colon-specific drug delivery systems in the treatment of helminthiasis[204]

5.4 **Reaserch Application of Gour Gum**

Guar gum is always a favorite agro-based commodity, attracting wide interest of researchers all over the world. Following is the research work conducted during the last 10 years on guar gum in many national and international laboratories to prepare value added products from this renewable source of hydrocolloid. Grafting of poly (N-isopropylacrylamide) (PNIPAAm) was carried out onto Ocarboxymethyl- O-hydroxypropyl guar gum (CMHPG) in aqueous solutions by using potassium persulfate (KPS) and N,N,N,N′-tetramethylethylene diamine (TMEDA) as the initiation system, resulting in new stimuli-responsive grafted polysaccharides. The resulting grafted polysaccharides showed lower critical solution temperatures in aqueous media.

5.5. Pharmaceutical application of gour gum

Guar gum or its derivatives are used in pharmaceutical industries as gelling / viscosifying / thickening, suspension, stabilization, emulsification, preservation, water retention / water phase control, binding, clouding/bodying, process aid, pour control for suspensions, anti-acid formulations, tablet binding & disintegration agent, controlled drug delivery systems, slimming aids, nutritional foods etc. Guar gum is an important non-caloric source of soluble dietary fiber. Guar gum powder is widely used in capsules as dietary fiber. Fiber is a very important element of any healthy diet. It is useful in clear the intestinal system since fiber cannot be digested. This keeps the intestines functioning properly and also improves certain disorders and ailments. All natural fiber diet works with body to achieve a feeling of fullness and to reduce hunger. Its synergistic mix of guar gum and fiber mixture when taken with water expands in stomach to produce a feeling of fullness.

6.Gellan gum and tamarind xyloglucan

6.1Introduction

Some polysaccharides are used to enhance the quality of product by thickening and gelling, and by reducing the undesired defect of water release (syneresis) in some products, and by stabilizing emulsion and suspension [205]. The polysaccharides used in these ways are called texture modifier and creation of various kinds of texture modifier is required to make the products with desirable texture. Gelling ability is an important property as a texture modifier and some polysaccharides can form a gel at low concentrations (~1%). There are only a few polysaccharides which have a gelling ability by themselves at low concentration whereas a lot of non-gelling polysaccharides are used as a thickener and stabilizer [206]. Gellan gum is a polysaccharide which can form a gel at low concentrations. Gelation mechanism and gel properties of gellan have not been clarified well at the present stage. Tamarind xyloglucan, a polysaccharide obtained from tamarind seed, is a valuable thickener and stabilizer. We regarded tamarind xyloglucan as a representative of non-gelling polysaccharides. Understanding novel gelling conditions of tamarind xyloglucan would lead to create various kinds of texture modifier.

6.2Gellan gum

Gellan gum is an extracellular polysaccharide produced by micro-organism Sphingomonas elodea (ATCC 31461) previously known as Pseudomonas elodea. Being a fermentation product, it can be produced on demand and with consistent quality. The primary structure of gellan gum is composed of a linear tetrasaccharide repeat unit: 1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, and 1,4-α-L-rhamnose as reported by O'Neil et al. and Jannson et al. [207,208]. Gellan gels in the presence of appropriate amount of cations are transparent, resistant to heat in the wide range of pH [209]. Brittle gellan gels have a good flavor release, suitable for gel products with new texture. For example, an assembly of brittle gel particle, called a microgel, has a specific texture like fluid gel which was produced from agarose gel [210]. Because of industrial importance of

gellan gels, gelation and gel properties have been understood briefly. Some fundamental questions still remain to be answered. For example, what is the gelation mechanism like? In order to further understand gelation and gel properties of gellan gum, a collaborative

research group was organized conjunction with the research group of polymer gels affiliated to the Society of Polymer Science, Japan, in 1989. The common gellan was used to study its properties with various techniques. Light scattering and osmotic pressure measurements showed that gellan changes from two single chains to one double helix on cooling and changes from one double helix to two single chains on heating [211, 212]. Rheological measurements showed that a gel formation occurs after the coil-to-helix transition on further cooling under appropriate conditions [213]. Proposed gelation mechanism is described in those special issues. Gel properties also remain to be clarified. We found that rheological properties change dramatically under gel state at a certain temperature. At this characteristic temperature single peak appeared in differential scanning calorimetry (DSC) and spectra changed in circular dichroism. These thermally-induced physicochemical changes are believed to be due to a helix-coil transition of gellan; thus, we concluded that a helix-coil transition can occur even in gel state. Since gellan is apolyelectrolyte, gel properties are influenced by the salts. When the gellan gels wereimmersed in NaCl solutions, the elastic modulus of the gel increased and circular dichroism spectra changed as shown in Fig.1. This was also attributed to the helix-coil ransition of gellan.

6.3Tamarind xyloglucan

Xyloglucan is a major structural polysaccharide in the primary cell walls of higher plants. Tamarind xyloglucan is obtained from the endosperm of the seed of the tamarind tree, Tamarindus indica, a member of the evergreen family, that is one of the most important and common trees of Southeast Asia and widely indigenous to India, Bangladesh, Myanmar, Sri Lanka, and Malaysia [214]. Purified, refined tamarind xyloglucan is produced in Japan and is permitted as a thickening, stabilizing, and gelling agent.Tamarind xyloglucan has a (1→4)-β-D-glucan backbone that is partially substituted at the O-6 position of its glucopyranosyl residues with α-D-xylopyranose [215]. Some of the xylose residues are β-D-galactosylated at O-2 [215]. Although tamarind xyloglucan itself does not form a gel, gel can be obtained under appropriate conditions, such as by adding some substances or removing substituents. Tamarind xyloglucan forms a gel in the presence of 40-65% sugar over a wide pH range [216]. It also forms a gel in the presence of alcohol [216] or by removing galactose residues from tamarind xyloglucan [217, 218]. In order to seek novel gelling condition of tamarind xyloglucan, we prepared a mixture of tamarind xyloglucan and epigallocatechin gellate (EGCG), a polyphenol abundant in tea leaves. The mixture formed a translucent or opaque gel [219]. Rheological and DSC studies showed that the gelation occurred on cooling and gel melted on subsequent heating [219]. EGCG was most likely bound to tamarind xyloglucan chains for a gel network, which was detected as a DSC peak and two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY) [219]. Since it has been reported that some mixtures of polysaccharides can form a gel by specific interaction, a mixture of tamarind xyloglucan and gellan was prepared in order to test whether or not the mixture shows a specific interaction leading to a synergistic gelation. From viscoelastic measurements we confirmed that the mixture formed a gel under the condition where individual polysaccharide does not form a gel at the experimental concentrations, indicating the synergistic gelation occurred [220]. In DSC measurements, the gelation was detected as a peak that appeared at higher temperatures than a peak arising from helix-coil transition of gellan alone [220]. It was also detected as a change in circular dichroism which was not observed in tamarind xyloglucan alone and gellan alone [220]. Judging from the results it was concluded that tamarind xyloglucan and gellan might associate to form a gel network. modifiers suitable for social requirement.

CONCLUSION

It has been shown how the most abundant natural based polysaccharides i.e.chitosan, alginate, pullan, scleroglucan, guargum, gallengum and its derivative are employed in many biomedical applications, both alone as well as in composites and in blends, and how their structure can be modified in order to improve deficiencies or to impart them innovative properties. The possibility of producing a variety of chemically modified derivatives makes these polysaccharides versatile biomaterials in almost all fields of biomedical interest. It is possible to forecast that diversified chemical modification approaches will open more and more new perspectives and potential applications in the future. It is also worth mentioning that improving the performance of natural polymers is an opportunity for the medical and pharmaceutical industry, as the time-to-market of the said polymers is reduced, when compared to synthetic biodegradable polymers. The application of the principles of chemical modification to natural polysaccharides will allow for a technological development competitive with that of polymers from petroleum sources.The biomedical application of polysaccharide with special reference to controlled delivery systems is an exciting field of research with unlimited future prospects which will show beacon light in the areana of nanomedicine research in the future.

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