# POLYSACCHARIDES BASED ON MICRO- AND NANOPARTICLES OBTAINED BY IONIC GELATION AND THEIR APPLICATIONS AS DRUG DELIVERY SYSTEMS

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The preparation technique of nano- and microparticles based on ionotropic gelation process is extremely mild and involves the mixing of two aqueous phases at room temperature. This review emphasizes the present state of the synthesis of nano- and microparticles by the ionotropic gelation method as well as the applications of these macromolecular supports in the pharmaceutical field.

## INTRODUCTION

One of the most important goals of drug delivery research is the design of nano- and microsystems able to deliver drugs at the right place in the body sites at the rate required for a specific treatment and at the right dosage forms.

The application of polymeric materials for medical purposes is growing very fast. Polymers have found applications in various biomedical fields such as: implantation of medical devices and artificial organs, tissue engineering, prostheses, ophthalmology, dentistry, bone repair, drug delivery systems.

Among them, the use of the natural biopolymers for diversified applications in life science has advantages as biocompatibility and biodegradability, leading therefore to ecological safety and possibility of preparing a variety of chemically and enzymatically modified derivatives for specific uses. Polysaccharides [1, 2] as a class of natural polymers are extremely bioactive, biocompatible and are generally derived from agricultural feedstock or crustacean shell wastes.

Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as: microspheres, nanoparticles, lipo-somes, etc., which modulates the release and absorption characteristics of the drug. Micro- and nanoparticles constitute an important part of particulate drug delivery systems by virtue of their small size and efficient carrier characteristics.

Nano- and microparticles have been prepared using several different methods. One method for preparing nano- and microparticles based on polysaccharides is the ionotropic gelation method.

In the ionotropic gelation method polysaccharides (alginate, gellan and pectin) are dissolved in water or in weak acidic medium (chitosan). These solutions are then added dropwise under constant stirring to the solutions containing other counterions (Figure 1). Due to the complexation between oppositely charged species, polysaccharides undergo ionic gelation and precipitate to form spherical particles. The beads are removed by filtration, washed with distilled water and dried.

The counterions used for ionotropic gelation can be divided into two major categories:

Low molecular weight counterions (e.g. $CaCl_2$, $BaCl_2$, $MgCl_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolyphosphate, hexametaphosphate and $[Fe(CN)_6]^{-4}$ / $[Fe(CN)_6]^{-3}$;

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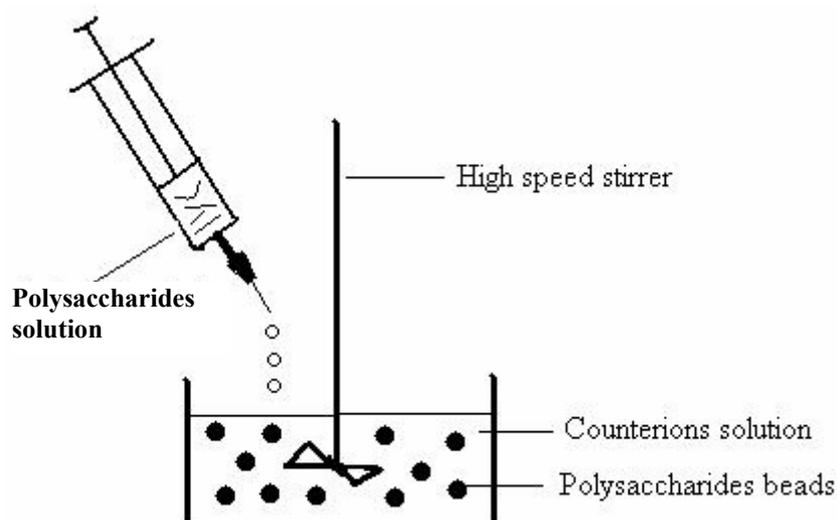* Corresponding author: marpopa@ch.tuiasi.ro

Fig. 1 – Schematic representation of preparation of polysaccharides particles by ionic gelation method.

High molecular weight ions (*e.g.* octyl sulphate, lauryl sulphate, hexadecyl sulphate, cetylstearyl sulphate).

The ionotropic gelation method is very simple and mild. In addition, reversible physical crosslinking by electrostatic interaction instead of chemical crosslinking avoids the possible toxicity of reagents and other undesirable effects.[3]

In this review the preparation of nano- and microparticles based on polysaccharides (chitosan, alginate, gellan, pectin) by ionotropic gelation method, as well as their medical applications are discussed.

## POLYSACCHARIDES USED IN PREPARATION OF MICRO AND NANOPARTICLES BY IONOTROPIC GELATION METHOD

**1. Chitosan.** Chitosan is a biopolymer that has received much attention and has been extensively studied for micro- and nonoparticles preparation.[4] Properties like biodegradability, low toxicity and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations [5-7] as antidiabetic agents, anti-inflammatory drugs, immobilization of enzymes and protein, ophthalmology.

Chitosan, a linear polyaminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose.[8, 9] Chitin is the main component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *Aspergillus* and mucor. Chitin is a homopolymer composed of $\beta$-(1,4)-linked N-acetyl-glucosamine units while chitosan comprises copolymers of glucosamine and N-acetyl-glucosamine.[10] Chitosan has one primary amino and two free hydroxyl groups for each $C_6$ building unit (Figure 2).

Due to the different ways of applications, chitosan has been formulated as powder, gels and films,[11] sponges, [12] intragastric floating tablets [13] and especially spherical particles (micro- and nanoparticles).[14-17]

The ionotropic gelation method is commonly used to prepare chitosan nanoparticles. In acidic solution, the $-NH_2$ group of chitosan molecule is protonated and interacts with different gelation agents. [18]
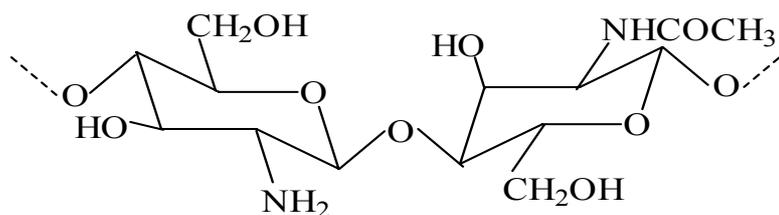


Fig. 2 – Structure of chitosan.

Bodmeier et al.[19] was the first to report the ionotropic gelation of chitosan with tripolyphosphate (TPP) while Alonso et al.[20] developed chitosan nanoparticles adding a solution containing TPP into an acidic phase (pH 4-6) containing chitosan. The study of Wu et al.[21] showed that the formation of nanoparticles is possible only within specific, moderate concentrations of chitosan and TPP. Furthermore, the chitosan/TPP weight ratio should be within the range 4:1-6:1 in order to obtain a high yield of nanoparticles.[22]

Grenha et al.[23] reported chitosan nanoparticles prepared with different concentrations of chitosan solutions. Their results showed that the higher concentration chitosan solution formed larger size particle. Gan et al.[24] reported that variations in chitosan molecular weight, concentration, chitosan to TPP weight ratio, and solution pH were examined systematically for their effects on nanoparticle size, amount of surface charge, and tendency of particle aggregation so as to enable a rapid fabrication of chitosan nanoparticles with predetermined properties. They reported that at the same concentration of chitosan solution (between 0.5 and 0.3 mg/mL), the mean particle size of chitosan–TPP nanoparticles increased with increasing $M_w$ of chitosan used.

Biro et al.[25] prepared chitosan nanoparticles using two different gelation agents (sodium sulphate and TPP). Their results showed that the mean size of the nanoparticles was strongly influenced by the applied gelation agent. Much smaller particles were obtained with TPP (162 nm) than with sodium sulphate (517 nm). The explanation of this phenomenon could be that nanoparticles prepared with sodium sulphate may have a porous nature as their higher swelling ability found by Shu and Zhu[26] shown.

Dambies et al.[27] prepared chitosan gel beads using molybdate as gelling agent.

It was observed that this new gelation technique led to a structure different from the one produced during alkaline coagulation of a chitosan solution. Instead of a morphology characterized by large open pores, gel beads produced in a molybdate solution, under optimum conditions (pH = 6, molybdate concentration, 7g / L) were found to have a double layer structure corresponding to a very compact 100 μm thick external layer and an internal structure of small pores.

Tang, Huang, and Lim[28] reported a method to manipulate the nanoparticle size after chitosan nanoparticles were prepared by ionotropic gelation:

ultrasonication at increasing reaction time or radiation amplitude to decrease the mean diameter and polydispersity of the nanoparticles.

The study of Tsai et al.[29] showed that the particle size of chitosan–TPP prepared with the ionotropic gelation method can be influenced by using different mechanical energies such as ultrasonic radiation or mechanical shearing, different treatment times, different chitosan concentrations, and different solution temperatures. Ultrasonic radiation or mechanical shearing treatment resulted in different nanoparticle sizes and different reduction rate of nanoparticle during treatment. This may be due to different degradation mechanisms of chitosan molecules and different size of degraded chitosan molecules participating in the ionotropic gelation with TPP molecules. The degradation of chitosan molecules by ultrasonic radiation is mainly caused by a cavitation effect, whereas the degradation by mechanical shearing is mainly caused by a tearing and/or stretching effect. Mean diameter of nanoparticle decreased with increasing solution temperature.

The study of Calvo et al.[30] showed that the size and zeta potential of chitosan-polyethylene oxide nanoparticles can be conveniently modulated by varying the ratio chitosan/ethylene oxide or propylene oxide.

Krauland and Alonso[31] have designed a new nanoparticulate delivery carrier for macromolecules which consists of the mucoadhesive chitosan and a negatively charged cyclodextrin (CD), via the very mild ionotropic gelation technique. Briefly, 1 mL of an aqueous solution of carboxymethyl-β-cyclodextrin (CM-β-CD) (3 - 10.5 mg/mL) or 1 mL of a mixture of the aqueous solutions of TPP (0.375-1.125 mg/mL) and CM-β-CD (0.75-10.5mg/mL) were added to 3 mL of a chitosan solution (0.2 %, w/v, pH 4.9) under magnetic stirring at room temperature maintained for 10 min to allow the complete stabilisation of the system. The nanoparticles were isolated by centrifugation in a glycerol bed and then resuspended in 100 μL of ultrapure water by shaking using a vortex. The final composition of the system (chitosan/CD ratio) can be modified by adjusting the formulation conditions. Moreover, the resulting nanoparticles exhibited a small size, a positive zeta potential and a great capacity for the association of macromolecule insulin. Insulin was dissolved in the CM-β-CD/TPP or TPP phase in a concentration of 2.4 mg/mL.

**2. Sodium alginate.** Alginates have a long history of use in numerous biomedical applications, including drug delivery systems, as they are biodegradable, biocompatible and mucoadhesive polymers.[32] Alginate polymers are also hemocompatible, have not been found to accumulate in any major organs and show evidence of *in vivo* degradation.[33] Sodium alginate is used in a variety of oral and topical pharmaceutical formulations and it has been specifically used for the aqueous microencapsulation of drugs, in contrast to more conventional solvent based systems.[33, 34]

Sodium alginate is a sodium salt of alginic acid, a naturally occurring polysaccharide obtained from marine brown algae. Alginate contains two uronic acids, α-L-guluronic and β-D-mannuronic acids, and is composed of homopolymeric blocks and blocks with an alternating sequence [35] (Figure 3).
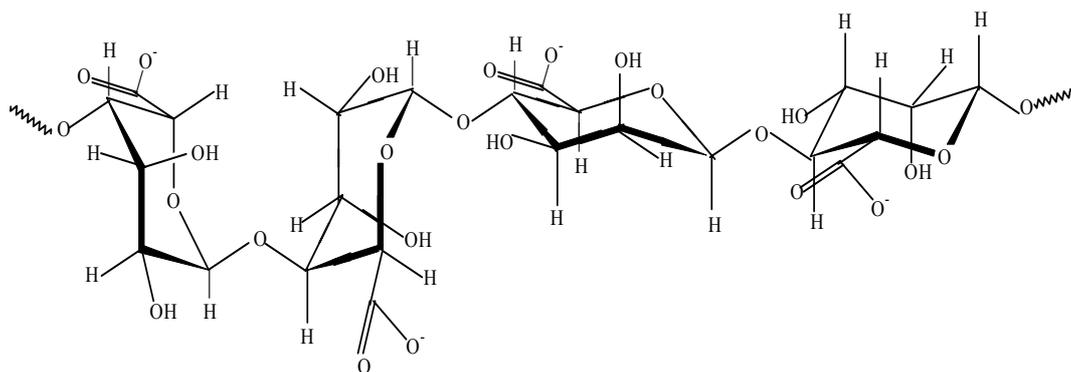


Fig. 3 – Structure of alginate.

In the case of beads prepared from pure sodium alginate, the formation of beads takes place due to the ionotropic gelation of spherical drops by $M^{n+}$. The polyguluronate units in the alginate molecules form a chelated structure with metal ions, called an "egg-box" junction with interstices in which the cations may pack and be coordinated.[36] The junction between the chains formed in this way is kinetically stable towards dissociation while the polymannuronate units show the normal polyelectrolyte characteristics of cations binding.[37] The two interactions thus result in formation of spherical beads, as depicted in Figure 4.
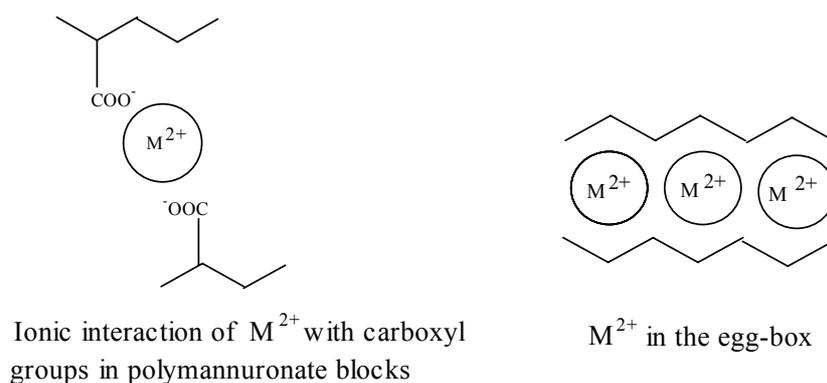


Ionic interaction of $M^{2+}$ with carboxyl groups in polymannuronate blocks

$M^{2+}$ in the egg-box

Fig. 4 – Bonding interactions between $M^{2+}$ and –COO⁻ group in the alginate beads.

The mechanical and swelling properties of alginate beads produced by ionotropic gelation with cations depend upon a number of factors like valence of ions, size of ions, etc. For example, monovalent cations and $Mg^{2+}$ do not induce gelation[38] and $Ba^{2+}$ produce stronger beads[39] than $Ca^{2+}$. Bajpani *et al.*[40] describe a detailed investigation of swelling/degradation behaviour of alginate beads crosslinked with different metal ions (e.g. $Ca^{2+}$, $Ba^{2+}$ and $Al^{3+}$). The results obtained can be explained on the basis of the extent of crosslinking in the beads and the size of the cations involved in the crosslinking process. Since barium and calcium ions are divalent, their bonding to alginate is expected to occur in a planar two-dimensional manner inside the beads.[41] But since

barium ion has the largest radius (1.74 Å) compared to the other two cations (i.e. 1.14 Å for $Ca^{2+}$ and 0.68 Å for $Al^{3+}$), it is supposed it fills a larger space between the alginate molecules, thus producing a tight arrangement with smaller voids.

The $Al^{3+}$ crosslinked beads display somewhat embarrassing results. The trivalent $Al^{3+}$ is accepted to form a three-dimensional network by a trivalent bonding structure with the sodium alginate.[41] This three-dimensional bonding results in extended

crosslinking through the whole bead. This is because the crosslinking occurs in two different planes at the same time resulting in compacting the alginate molecules (Figure 5). Because of the smallest size of $Al^{3+}$ among the three crosslinking cations (i.e. 0.68 Å) its diffusion from the beads into the outer solution is relatively faster as compared to that of $Ba^{2+}$. This consequently results in a lower water uptake than of the beads crosslinked with $Ba^{2+}$.
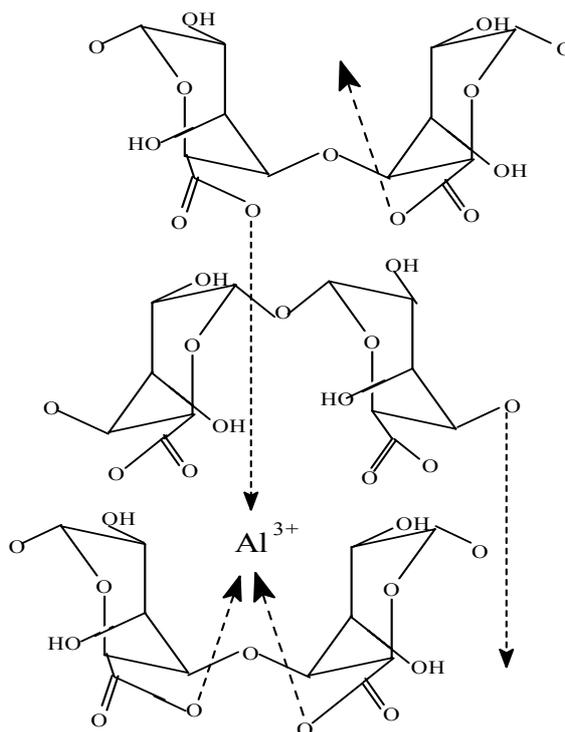


Fig. 5 – Expected three-dimensional interaction between $Al^{3+}$ and alginate molecules.

Hence it can be concluded that the nature of crosslinking cations exerts a great influence on the swelling and degradation behaviour of beads. Moreover, the beads crosslinked with $Ba^{2+}$ exhibit a fair stability with minimum water uptake.

**3. Gellan gum.** Gellan gum can be used to produce easy-to-swallow solid dosage forms, such as gels and coated tablets, and to modify the rate of release of active ingredients from tablets and capsules. It is also conveniently used for controlled or sustained release of various drugs [42-46] and also for the preparation of the microcapsules.[47]

Gellan gum is an exocellular anionic heter-opolysaccharide produced by the aerobic fermentation of the bacterium *Sphingomonas elodea* (formerly known as *Pseudomonas elodea*). There are two chemical forms of gellan gum: native or natural form, which has high acyl

contents, and low or deacetylated form. Both forms have a similar linear structure made up of the repeating units of tetrasaccharide which is composed of β-D-glucose, β-D-glucuronic acid and α-L-rhamnose residues in the molar ratio of 2:1:1 (Figure 6). The native form contains two acyl substituents, namely acetate and glycerate, both being located on the same glucose residue and, on the average, there is one glycerate and a half acetate group per every tetrasaccharide repeating unit (Figure 6). This difference in substitutions leads to a difference in the gelling potential.[48]

Some investigators have elucidated the gelation mechanism of gelan gum in the presence of various monovalent and divalent cations.[49-52] The gelling of gellan gum is inducible by cations and is temperature dependent. In aqueous solution, the gelation of gellan gum is usually accompanied by a

two-step process, which involves formation of double helices from random coil chains ('coil-to-helix transition') and an aggregation of pairs of double helices. Furthermore, the coil-helix transition is greatly affected by the electrostatic interaction with co-existing cations in the solution [52], whereas the aggregation behaviour appears to be affected by the pH.[53]
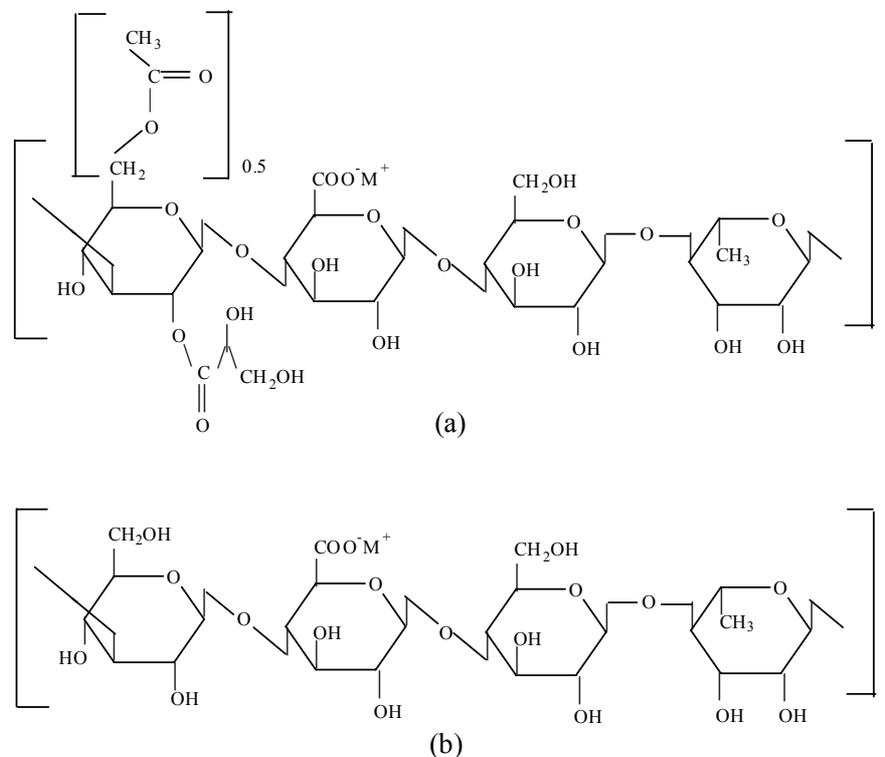


Fig. 6 – Chemical structures of gellan gum ($M^+$ = metal ion):
(a) native or high acyl gellan gum: (b) low acyl gellan gum.

Gellan gum forms gels in the presence of mono- and divalent cations; however, its affinity for divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ is much higher than for monovalent ones such as $Na^+$ and $K^+$. [49] This difference in efficiency has been attributed to the difference in their gel-inducing mechanisms. In the case of monovalent cations, the gelation is mainly contributed by the screening of the electrostatic repulsion between the ionized carboxylate groups ($COO^-$) on the gellan chains.[54] On the other hand, in the case of divalent cations, the gelation and aggregation of gellan gum occur via a chemical bonding between divalent cations and two $COO^-$ groups belonging to glucuronic acid molecules in the gellan chains; in addition the screening effect appears.[52]

**4. Pectin.** Calcium pectinate gel beads have been used in various ways in the gastrointestinal tract because it is a gentle and simple encapsulation technique, for example for sustained release of drugs [48-50], and for targeting drugs to the colon.[55, 57, 58]

Pectin, a natural polysaccharide, is a non-toxic product extracted from citrus peels or apple pomaces. Pectin consists mainly of linearly connected α-(1-4)-D-galacturonic acid residues (Figure 7).
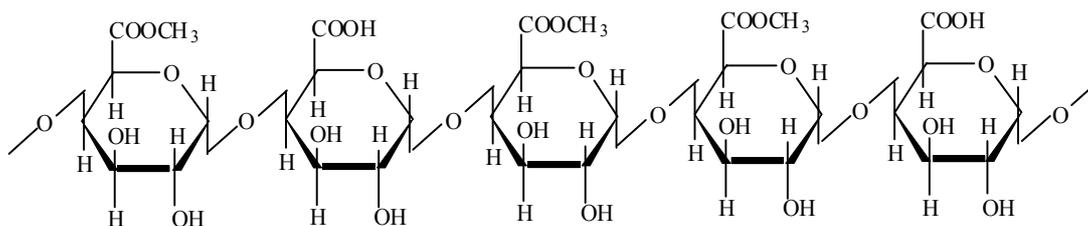


Fig. 7 – Structure of pectin.

The degree of esterification (DE), which is expressed as a percentage of carboxyl groups (esterified) is an important means to classify pectin. The properties of pectin depend on the degree of esterification: low-methoxyl pectin (DE < 50 %) form rigid gels by the action of calcium, which cross-links the galacturonic acid chains.[59]

An aqueous solution of pectin was dropped into calcium chloride solution and gelled spheres were formed instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules as described in many reports.[55, 56, 60] The calcium ions, as a cross-linker, induced an interfacial cross-linking reaction of pectin. Therefore, an insoluble calcium pectinate membrane was formed around the pectin droplets. At the very early stage of bead formation, the calcium pectinate shell/membrane with the liquid core was obtained. Besides, the internal liquid core containing pectin might be complexed with the calcium ions that migrated into the core, providing relatively strong beads with a breaking force of about 5-6 N after 2 min of gelation. The rate of further membrane formation (especially in the first few minutes) is probably controlled by the rate of diffusion of calcium through the calcium pectinate membrane, which may be very rapid. This is similar with the calcium pectinate gel coated pellets in which the cross-linking reaction occurred after cores containing calcium salts were immersed in pectin solution.[61] As the gelation time increases, the thickness of the calcium pectinate shell/membrane may increase according to the formation of cross-linked pectin structure and be accompanied by an increase in mechanical strength of the beads formed. The mechanical strength value becomes constant after about 20 min of cross-linking. This is probably due to molecular re-arrangements and saturation of the carboxyl groups with calcium ions. Gel beads strength increases with increasing $Ca^{2+}$ concentration but reduces with temperature and acidity increase (pH<3).

The gelling ability of the pectins with the divalent cations is similar to that found with the alginates: $Mg^{2+} << Ca^{2+}$, $Sr^{2+} < Ba^{2+}$, with $Na^+$ and $K^+$ not gelling. The similarity with the behavior of the alginates is due to the poly-α-(1-4)-D-galacturonic acid which is almost the mirror image of poly-α-(1-4)-L-guluronic acid, the only difference being that the 3-hydroxyl group is axial in the latter.

## SOME APPLICATIONS OF MICRO-AND NANOPARTICLES BASED ON POLYSACCHARIDES

Drug loading in microparticle/nanoparticle system can be done by two methods, i.e. during the preparation of particles (incorporation) and after their formation (incubation). In these systems drug is physically embedded into the matrix or adsorbed through the surface. Various methods of loading have been developed to improve the efficiency of loading, which largely depends upon the method of preparation as well as on the physicochemical properties of the drug. Maximum drug loading can be achieved by incorporating the drug during the formation of particles, but it may get affected by the process parameters such as the preparation method, presence of additives, etc.

Microparticulate systems based on polysaccharides are attracting as potential drug delivery devices in the pharmaceutical and biomedical applications. Some important applications are discussed below.

**Different categories of drugs microencapsulated.** Microparticles and nanoparticles based on polysaccharides prepared by ionotropic gelation method are extensively investigated for immobilization of various classes of drugs. The findings of these studies are summarized in the following sections.

**a. Antidiabetic agents.** Mucosal delivery of insulin is one of the most intensively studied subjects, among which achieving oral delivery has been an elusive goal for many investigators. Pan *et al.*[62] prepared the insulin-loaded chitosan nanoparticles by ionotropic gelation of chitosan with TPP anions. Insulin loaded chitosan nanoparticles have been prepared by mixing insulin with TPP solution and then adding this to chitosan-solution under constant stirring. The ability of chitosan nanoparticles to enhance the intestinal absorption of insulin and the relative pharmacological bioavailability of insulin was investigated by monitoring the plasma glucose level of alloxan-induced diabetic rats after the oral administration of various doses of insulin-loaded chitosan nanoparticles. The *in vitro* release experiments indicated an initial burst effect, which is pH-sensitive. The chitosan nanoparticles enhanced the intestinal absorption of insulin to a greater extent than the aqueous solution of chitosan *in vivo*. After administration of 21 I.U./kg insulin in the chitosan nanoparticles, hypoglycemia was prolonged over 15 h.

The study of Ma [63, 64] showed that the association and *in vitro* release of insulin were strongly influenced by the pH of the chitosan-insulin nanoparticle dispersion. Insulin association with chitosan nanoparticles at pH 5.3 was 50% less efficient than that at pH 6.1. However, the insulin association at pH 5.3 was stronger, where more than 75% of the associated insulin remained intact *in vitro* release studies. In contrast, insulin association at pH 6.1 was highly labile, it rapidly and completely dissociating when the chitosan nanoparticles were diluted with aqueous media. These differences at pH formulation between the 5.3 and 6.1 could have an impact on the *in vivo* pharmacological activity of the chitosan-insulin nanoparticles.

Gliclazide, 1-(3-azabicyclo-[3,3,0]-oct-3-yl)3-(p-tolyl sulfonyl)urea, is a second generation sulfonyl urea drug oral hypoglycaemic which is useful for a long-term treatment of non-insulin dependent diabetes mellitus (NIDDM). Al-Kassas *et al.*[65] prepared gliclazide loaded Ca-alginate beads using ionotropic gelation method as descried by Takeshita *et al.*[6] and Sugawara *et al.*[67] Gliclazide powder was added to a solution of sodium alginate and dispersed homogeneously then dropped into the solution of $CaCl_2$ (0.1 M). The authors also investigated the ability of the system to incorporate and control the release of gliclazide through variation in the processing conditions such as polymer concentration, stirring speed, internal phase volume and type of surfactant in the external phase. Alginate beads can control, improve and prolong the systemic absorption of the gliclazide through their mucoadhesive properties. This effect results in maintaining tight blood glucose level and improved patient compliance.

**b. Antiinflammatory drugs.** Spherical pellets of poorly soluble drugs (ibuprofen, indometacin, ketoprofen, piroxicam, sodium diclofenac) were prepared by dispersing the drug in solution of ionic polysaccharides: chitosan, sodium alginate or pectin, and then dropping these dispersions into solutions containing the respective counterions TPP or $CaCl_2$.[56, 58, 68-70]

The droplets instantaneously formed gelled spheres by ionotropic gelation. Strong spherical beads with a narrow particle size distribution and low friability could be prepared with high yield and a drug content approaching 98 %.

**c. Ocular delivery.** De Campos *et al.*[71] investigated the potential of chitosan nanoparticles as a new vehicle to improve the delivery of drugs to ocular mucosa. Cyclosporin A (CyA) was chosen as a drug model. A modified ionic gelation technique was used to produce CyA-loaded chitosan nanoparticles. The *in vivo* experiments showed that after topical instillation of CyA-loaded chitosan 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 chitosan solution containing CyA and an aqueous CyA suspension. The study indicated that chitosan nanoparticles could be used as a vehicle to enhance the therapeutic index of the clinically challenging drugs with potential application at the extraocular level.

Chitosan nanoparticles of dorzolamide hydrochloride (Dorzo) were prepared by the ionotropic gelation method and their *in vitro* properties were studied by Papadimitriou *et al.*[72] Based on wide angle X-Ray diffractometry (WAXD) data, Dorzo was dispersed in the nanoparticles in crystalline form, probably due to the weak interaction developed between Dorzo and chitosan/TPP matrix as FT-IR data indicated. The nanoparticles exhibited mucoadhesive properties which diminished with increasing drug content. *In vitro* drug release was observed with the Dorzo-loaded chitosan nanoparticles in PBS (pH 7.4) in simulated intestinal fluid. The results suggest that the Dorzo-loaded chitosan nanoparticles could be further evaluated for the controlled ocular delivery of Dorzo.

**d. Antibiotics.** Spherical beads containing azathioprine were prepared from deacetylated gellan gum by ionotropic gelation method by Singh and Kim.[48] Divalent cations affect both the aqueous solubility of azathioprine as well as encapsulation efficiency of deacetylated gellan gum. The pH of the ionotropic medium does not seem to affect the solubility of azathioprine, wheareas it affects the encapsulation efficiency of gellan gum in a negative and significant manner. The encapsulation efficiency of gellan is much higher in the presence of transition elements ($Cu^{2+}$ and $Zn^{2+}$) comparatively to alkaline earth metal ions ($Ca^{2+}$, $Mg^{2+}$ and $Ba^{2+}$) when used at the same concentration level. Higher concentrations of $Ca^{2+}$ tend to decrease the percentage encapsulation efficiency, which may be related to a decrease in gel strength.

**e. Proteins and enzymes.** The bioactivity of β-lactamases upon entrapment in calcium-pectinate beads was evaluated by Bourgeois *et al.*[73] Non-amidated (NAP) and amidated pectin (AP) beads were prepared according to the ionotropic gelation method using $CaCl_2$ as gelling agent, washed and dried at $37^0C$ in an oven for 2 h. The encapsulation of the protein is function of the type of pectin used (NAP or AP) but mostly the presence of a large amount of free calcium in beads considerably influences the activity of encapsulated β-lactamases. A drastic elimination of free $CaCl_2$ from Ca-pectinate network reduces moisture content in beads and avoids the risk of protein hydrolysis. Finally, the drying process of beads also modified the activity of encapsulated protein. However, such process and formulation parameters can be easily controlled in order to preserve the activity of encapsulated β-lactamases.

Chitosan is often used as support material for enzyme immobilization.[74-76] Biró *et al.*[25] used as different sized chitosan support particles formed by ionotropic gelation method with two different gelation agents (sodium sulphate and TPP) for enzyme immobilization. β-Galactosidase was selected for immobilization on support. Surprisingly, much lower enzyme activity was found on the obviously smaller nanoparticles obtained with TPP as gelation agent. A possible explanation of the fivefold higher specific activity obtained with sodium sulphate is that among various proteins present in the Maxilact LX 5000 preparation, β-galactosidase was more selectively attached by these nanoparticles, as it was able to bind also to the active groups located in the pores. Another reason of the lower activity of the biocatalyst prepared using nanoparticles obtained with TPP should be a partial inhibition of the enzyme by the gelation agent.

## CONCLUSION

The ionotropic gelation method is a very simple and mild process used for designing nano- and microcarriers as possible candidates for immobilization and controlled release of various bioactive compounds.

By dropwise addition of a polysaccharide solution into different counterions solution under constant stirring, the spherical polysaccharide microspheres were performed.

The influence of polysaccharide molecular weight, polysaccharide solutions concentration, polysaccharide/gelling agent ratio and solution pH of polysaccharide on the mean size of nano-/microparticles and also on the drug loading and drug release processes were discussed.

Various therapeutic agents such as antidiabetic, anti-inflammatory, antibiotics, proteins and enzymes have been incorporated in polysaccharides (chitosan, alginate, gellan, pectin) beads to achieve a controlled release system.

## REFERENCES

1. A. J. Domb, A. Benitolila and D. Teomin, *Acta Polym.*, **1998**, *49*, 526-533.
2. T. Hayashi, *Prog Polym Sci.*, **1994**, *19*, 663-702.
3. S. A. Agnihotri, N. N. Mallikarjuna and T. M. Aminabhavi, *J. Control. Rel.*, **2004**, *100*, 5-28.
4. K. L. Douglas and M. J. Tabrizian, *J. Biomater. Sci. Polymer Edn.*, **2005**, *16*, 43-56.
5. T. Chandy and C. P. Sharma, *Artif. Cells Artif. Organs*, **1990**, *18*, 1-5.
6. L. Ilum, I. Jabbal-Gill, M. Hinchcliffe, A. N. Fisher and S. S. Davis, *Adv. Drug Deliv. Rev.,***2001**, *51*, 81-96.
7. O. Felt, P. Furrer, J. M. Mayer, B. Plazonnet, P. Buri and R. Gurny, *Int. J. Pharm.,* **1999**, *180*, 185-193.
8. R. A. A. Muzzarelli, "Chitin", Pergamon Press, New York, 1977, p. 1-37.
9. G. A. F. Roberts, "Chitin Chemistry", Mac Millian Press, Houndmills, 1992, p. 1-50.
10. Y. Kato, H. Onishi and Y. Machida, *Curr. Pharm. Biotechnol.*, **2003**, *4*, 303-309.
11. M. Fujita, M. Ishihara, M. Simizu, K. Obara, T. Ishizuka, Y. Saito, H. Yura, Y. Morimoto, B. Takase, T. Matsui, M. Kikuchi and T. Machara, *Biomaterials*, **2004**, *25,* 699-706.
12. F. L. Mi, S. S. Shyu, Y. B. Wu, S. T. Lee, J. Y. Shyong and R. N. Huang, *Biomaterials*, **2001**, *22*, 165-173.
13. T. Koizumi, G. C. Ritthidej and T. Phaechamud, *J. Control Release*, **2001**, *70,* 277-284.
14. S. Vasiliu, M. Popa and M. Rinaudo, *Eur. Polym. J.,* **2005**, *41*, 923-932.
15. S. Vasiliu, C. Luca and M. Popa, *Chem. Bull. "Politehnica" Univ. (Timisoara),* **2002**, *48,* 1-6.
16. C. Luca, S. Vasiliu and M. Popa, *Cellulose Chem. Technol.*, **2005**, *39,* 55-63.
17. S. Vasiliu, M. Popa and C. Luca, *Eur. Polym. J.*, **2008**, *44*, 3894-3898.
18. S. T. Lee, L. F. Mi, Y. J. Shen and S. S. Shyu, *Polymer*, **2001**, *42*, 1879-1892.
19. R. Bodmeier, H. Chen and O. Paeratakul, *Pharm. Res.*, **1989**, *6*, 413-417.
20. M. J. Alonso, A. Sanchez, *J. Pharm. Pharmacol.,* **2003**, *55*, 1451-1463.
21. Y. Wu, W. Yang, C. Wang, J. Hu and S. Fu, *Int. J. Pharm..*, **2005**, *295*, 235-245.
22. H. Zhang, M. Oh, C. Allen and E. Kumacheva, *Biomacromolecules*, **2004**, *5*, 2461-2468.
23. A. Grenha, B. Seijo and C. Remuñán-López, *Eur. J. Pharm. Sci.*, **2005**, *25*, 427-437.
24. Q. Gan, T. Wang, C. Cochrane and P. McCarron, *Colloid Surface B: Biointerfaces*, **2005**, *44*, 65-73.

25. E. Biró, A. Sz. Németh, C. Sisak, T. Feczkó and J. Gyenis, *J. Biochem. Biophys Methods*, **2008**, *70*, 1240-1246.
26. X. Z. Shu and K. J. Zhu, *Int. J. Pharm.*, **2002**, *233*, 217-225.
27. L. Dambies, T. Vincent, A. Domard and E. Guibal, *Biomacromolecules*, **2001**, *2*, 1198-1205.
28. E. S. K. Tang, M. Huang and L. Y. Lim, *Int. J. Pharm.*, **2003**, *265*, 103-114.
29. M. L. Tsai, S. W. Bai and R. H. Chen, *Carbohydr. Polym.*, **2008**, *71*, 448-457.
30. P. Calvo, C. Remuñán-López, J. L. Vila-Jato and M. J. Alonso, *J. Appl. Polym. Sci.*, **1997**, *63*, 125-132.
31. A. H. Krauland and M. J. Alonso, *Int. J. Pharm.*, **2007**, *340*, 134-142.
32. S. De and D. Robinson, *J. Control Release*, **2003**, *89*, 101-112.
33. M. Rajaonarivony, C. Vauthier, G. Couarraze, F. Puisieux and P. Couvreur, *J. Pharm. Sci.*, **1993**, *82*, 912-917.
34. R. Bodmeier and J. Wang, *J. Pharm. Sci.*, **1993**, *82*, 191-194.
35. K. I. Draget, "Handbook of Hydrocolloids", G. O. Philips and P. A. Williams (Eds.), Woodhead Publishing, Cambridge, **2000**, p. 379-395.
36. K. Park, W. S. W. Sharaby and H. Park, "Biodegradable Hydrogels for Drug Delivery", Technomic Publishing, P. A. Lancaster, 1993, p. 99-140.
37. O. Smidsrod, *Chem. Soc.*, **1974**, *57*, 263-270.
38. C. K. Kim and E. J. Lee, *Int. J. Pharm.*, **1992**, *79*, 11-19.
39. A. R. Kulkarni, K. S. Soppimath, T. M. Aminabhavi and A. M. Dave, *J. Appl. Polym. Sci.*, **2002**, *85*, 911-918.
40. S. K. Bajpai and S. Sharma, *React. Funct. Polym.*, **2004**, *59*, 129-140.
41. S. Al. Musa, D. A. Fara and A. A. Badwan, *J. Control. Release*, **1999**, *57*, 223-232.
42. W. Kubo, S. Miyazaki and D. Attwood, *Int. J. Pharm.*, **2003**, *258*, 55-64.
43. F. Paul, A. Morin and P. Monsan, *Biotechnol. Adv.,* **1986***, 4*, 245-259.
44. F. Alhaique, M. Carafa, T. Coviello, E. Murtas, M. Dentini and F.M. Riccieri, *Proc. Control Release Soc.,* **1995***, 22*, 28-34.
45. T. Coviello, M. Dentini, G. Rambone, P. Desideri, M. Carafa, E. Murtas, F. M. Riccieri and F. Alhaique, *J. Control Release,* **1998***, 55*, 57-66.
46. E. Santucci, F. Alhaique, M. Carafa, T. Coviello, E. Murtas and F. M. Riccieri, *J. Control Release,* **1996***, 42*, 157-164.
47. G. R. Chilvers and V. J. Morris, *Carbohydr. Polym.*, **1987**, *7,* 111-120.
48. B. N. Singh and K. H. Kim, *J. Microencapsul.*, **2005**, *22*, 761-771.
49. G. R. Sanderson and R. C. Clark, *Food Technol.*, **1983**, *37*, 63-69.
50. H. Grasdalen and O. Smidsrød, *Carbohydr. Polym.*, **1987**, *7*, 371-393.

51. J. Tang, M. A. Tung and Y. Zeng, *J. Food Sci.*, **1997**, *62*, 688-712.
52. S. Kanesaka, T. Watanable and S. Matsukawa, *Biomacromolecules*, **2004**, *5*, 863-868.
53. J. I. Horinaka, K. Kani, Y. Hori and S. Maeda, *Biophys. Chem.*, **2004**, *111*, 223-227.
54. K. Nakajima, T. Ikehara and T. Nishi, *Carbohydr. Polym.*, **1996**, *30*, 77-81.
55. O. Munjeri, P. Hodza, E. E. Osim and C. T. Musabayane, *J. Pharm. Sci*, **1998**, *87*, 905-908.
56. P. Sriamornsak and J. Nunthanid, *Int. J. Pharm.*, **1998**, *160*, 207-212.
57. P. Sriamornsak and J. Nunthanid, *J. Microencapsul.*, **1999**, *16*, 303-313.
58. O. Chambin, G. Dupuis, D. Champion, A. Voilley and Y. Pourcelot, *Int. J. Pharm.*, **2006**, 3*21*, 86
59. C. Rolin, "Industrial Gums: Polysaccharides and Their Derivatives", R. L. Whistler, J. N. Bemiller (Eds), Academic Press, New York, 1993, p. 257-293.
60. P. Sriamornsak and N. Thirawong, *Int. J. Pharm.*, **2003**, *267*, 151-156.
61. P. Sriamornsak, M. A. Burton and R. A. Kennedy, *Int. J. Pharm.*, **2006**, *326*, 80-88.
62. Y. Pan, Y. J. Li, H. Y. Zhao, J. M. Zheng, H. Xu, G. Wei, J. S. Hao and F. D. Cui, *Int. J. Pharm.*, **2002**, *249*, 139-147.
63. Z. Ma, H. H. Yeoh and L. Y. Lim, *J. Pharm. Sci.*, **2002**, *91*, 1396-1404.
64. Z. Ma, T. M. Lim and L. Y. Lim, *Int. J. Pharm.*, **2005**, *293*, 271-280.
65. R. S. Al-Kassas, O. M. N. Al-Gohary and M. M. Al-Faadhel, *Int. J. Pharm.*, **2007**, *341*, 230-237.
66. S. Takeshita, S. Sugawara, T. Imai and M. Otagiri, *Biol. Pharm. Bull.*, **1993**, *16*, 420-424.
67. S. Sugawara, T. Imai and M. Otagiri, *Pharm. Res.*, **1994**, *11*, 272-277.
68. R. Bodmeier and O. Paeratakul, *J. Pharm. Sci.*, **1989**, *78*, 964-967.
69. S. Puttipipatkhachorn, T. Pongjanyakul and A. Priprem, *Int. J. Pharm.*, **2005**, *293*, 51-62.
70. V. R. Sinha, A. K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K. Bansal and S. Dhawan, , *Int. J. Pharm.*, **2004**, *274*, 1-33.
71. A. M. De Campos, A. Sanchez and M. J. Alonso, *Int. J. Pharm.*, **2001**, 22*4*, 159-168.
72. S. Papadimitriou, D. Bikiaris, K. Avgousatkis, E. Karavas and M. Georgarakis, *Carbohydr. Polym.*, **2008**, *73*, 44-54.
73. S. Bourgeois, M. Gernet, D. Pradeau, A. Andremont and E. Fattal, *Int. J. Pharm.*, **2006**, *324*, 2-9.
74. G. Spagna, F. Andreani, E. Salatelli, D. Romagnoli and P. G. Pifferi, *Process Biochem,* **1998***, 33*, 57-62.
75. M. Portaccio, S. Stellato, S. Rossi, U. Bencivenga, M. S. Mohy Eldin, F. S. Gaeta and D.G.Mita, *Enzyme Microb. Technol.,* **1998***, 23,* 101-106.
76. G. Spagna, R. N. Barbagallo, E. Greco, I. Manenti and P. G. Pifferi, *Enzyme Microb. Technol.,* **2002***, 30,* 80-89.