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# The Using of Ionic Gelation Method Based on Polysaccharides for Encapsulating the Macromolecules– A Review

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**Abstract.** Recently, there has been a considerable research interest in the area of bioactives delivery system using polysaccharides as non toxic biodegradable encapsulates materials. The methode to obtain encapsulates particle from those materials was using ionic gelation that has some benefits i.e. very mild conditions and avoiding harmful organic solvents or high shear forces. The materials have been used to protect the bioavailability and functionality of bioactive from harsh condition of digestive system and to deliver on target site at controlled and sustained rate to the site action. Various biopolymer have been used in research on formulation of encapsulates particle to increase therapeutic benefit, while minimised the disadvantage. We review the using of ionic gelation methods on various formulations of materials and its characteristics, and their application in encapsulation of some important macromolecules.

**Keywords:** ionic gelation method, polysaccharide, encapsulation, delivery system, bioactive

## 1. Introduction

Many of bioactive compounds have several limitation on the extreme conditions including unfriendly environment i.e. oxidation, temperature or light exposure before ingestion as well as impact of some digestive enzymes or another substances during passage in the gastrointestinal tract that can reduce the bioactive functionality. The encapsulation technology may be applied for protecting the bioactives. It is also necessary to control bioaccessibility to target release of bioactive during GI transit [1]. With the appropriate polysaccharides as encapsulates biopolimer and selected of encapsulation method it is possible to release the bioactive at the desire site of action in the body. Succesful adaptation of this can already be found in the use of natural polysaccharides and its derivatives for encapsulating macromolecules e.g. hormon, antibiotic, enzyme, vitamin and probiotic. One method for preparing particle based on polysaccharides for encapsulating those functional macromolecules is ionic gelation/polyelectrolyte complexation [2]. In this review the preparation of particles based on polysaccharides (chitosan, carragenan, gellan gum, pectin, alginate) by ionic gelation method and their medical application are discussed.

## 2. Encapsulation for Protecting the Bioactive Compounds

Encapsulation is defined as a technology for encapsulating/protecting solids, liquids, or gaseous substances in miniature, sealed capsules that can release their contents at controlled rates under specific conditions. Encapsulation involves the incorporation of bioactive substances, cells or other materials in small capsules [3].

Encapsulation technology has drawn increasing attention for its applications with the aim to protect labile compounds from harsh conditions, provide controlled release and target site delivery of bioactives [4]

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within the body after ingestion. The encapsulated substances can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability [5] in uper GI tract. Development the technology of bioactive protecting can be carried out by:

- integration and controlled release of bioactive components from biodegradable and/or sustainable protecting materials,
- encapsulation of bioactive substances either in the capsules and/or during passage in the stomach,
- encapsulating provided with enzymatic activity exerting a health-promoting benefit. The development of functional hybrid bioactive/protecting system will provide more efficient and improve the impact on human health upon consumption.

## 3. Materials for Bioactive Protecting

Many delivery systems have been reported to have the effect of improving the chemical stabilities of natural bioactive for preserving their functionalities [6]. It would be highly desirable to develop biodegradable and sustainable matrixes capable of safe and long shelf-life integration of bioactive components. It might be necessary to design the encapsulation system (Fig. 1) as such bioactive component is released in a specific site of the GI tract. Administration of large structures such as probiotic will require a higher efficiency of encapsulated substances than molecular structures such as vitamins.

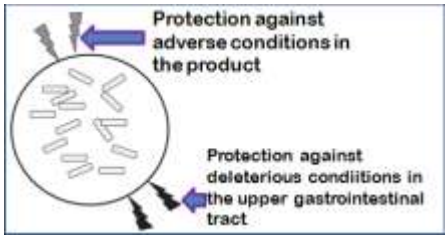


Fig. 1: Capsules system to protect bioactive components against harsh condition [4]

Materials for bioactive protecting would be capable of with holding desired bioactive principles in optimum conditions until their eventual release either through controlled release during passage in digestive tract or deliver in target site. Protected bioactive can impact to consumer health directly by generating safely materials encapsulated. Polysaccharides and its derivatives are thought to be among the most suitable materials for the controlled release of substances. Generally, the components which are incorporated in those materials are vitamins, phytochemicals, proteins, and microbe cells. Vitamins are essential for good health. Some vitamins are destroyed during processing, most of the losses are due to heat [3]. Phytochemicals are non-nutritive plant chemical, use for preventing cell damage, cancer cells replication, and decrease cholesterol levels. A large portion of phytochemicals are removed during processing.

Probiotics is claimed have benefits: alleviation of lactose intolerance, increased resistance to gut invasion by pathogenic species of bacteria, and stimulation of the immune system. However, the probiotic organisms are often not at high levels and their activity may not be optimal in food products [7]. Bacteria are often ruled out as constituents of probiotic foods due to unsuitable technology. Enzyme is protein substance used for reaction catalyse, currently being used in several food processing. The use of free enzyme has some limitations, e.g.resistance to protease or the other denaturing compounds and unstable to heat.

In order to incorporate the bioactives into protecting materials system, it is necessary to select the suitable material to attain the desired release, the optimal temperature/time conditions for mixing the biomaterials with the bioactive, and the method to fabricate the encapsulant. The selection of the materials to be used may be required to exert a protecting function, maintaining and assuring quality and safety of the bioactives. The fabrication of encapsulant has to be adapted to the technological limitations of each bioactive, i.e. sensitivity to high temperatures, light and oxygen.

Many polymers have been used to prepare the encapsulant, but those of natural origin are often preferred because they comply more easily with the requisites of biocompatibility, biodegradability and absence of toxicity that are mandatory in any biomedical application [8], [9]. Among the biomaterials already studied

for encapsulation of bioactive compounds; chitosan, carragenan, gum gellan, pectin, alginate, poly lactic acid (PLA) are very promising materials [3].

## 4. The research of Application of Ionic Gelation Method on Bioactive Encapsulation

The other crucial factor for encapsulating the bioactives is the method of fabrication that determines the structure of material and, therefore, the rate of bioactive substance release. One method for preparing particle based on polysaccharides (and its derivatives) for encapsulating the macromolecule functional substances is ionic gelation/polyelectrolyte complexation [2].

Polyelectrolyte complexation/ionic gelation is a method that uses very mild conditions, avoiding harmful organic solvents or high shear forces [10]. Therefore, it has the general capability of protecting the encapsulated molecules and retaining their activity during the encapsulation, which are its principal advantages [11]. This 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 [2]. Some research on application of ionic gelation method for encapsulating macromolecules was described bellow.

### 4.1. Research on Bioactive Encapsulation Using Some Polysaccharides by Ionic Gelation Method

Mucosal delivery of insulin is one of the most intensively studied subject [2]. A new nanoparticulate delivery carrier for macromolecules insulin hormone which consists of the mucoadhesive chitosan and a negatively charged cyclodextrine (CD) have designed [12]. Resulting particles of biopolymer exhibited nanometer size, a positive zeta potensial and a great capacity for association of insulin. The bioactivity of β-lactamases upon entrapment in calcium-pectinate beads [13]. The encapsulation of enzyme is function of the type of Nonamidated (NAP) and amidated pectin (AP) used but mostly the presence of a large amount of free calcium in beads considerably influences the activity of encapsulated β-lactamases.

Gellan gum can be used to modify the rate of release of bioactive from capsules. Spherical beads containing antibiotic azathioprine were prepared from deacetylated gellan gum [14]. Divalent cations affect the aqueous solubility of azathioprine and encapsulation efficiency of deacetylated gellan gum. Alginate-coated gelatin microspheres were produced to encapsulate the probiotic of *Bifidobacterium adolescentis* 15703T for enhancing survival during exposure to the adverse conditions of the GI tract [15]. Gelatin microspheres were cross-linked with the non-cytotoxic genipin and coated with alginate cross-linked by $Ca^{2+}$ from external or internal sources. The alginate coat prevented pepsin-induced degradation of the gelatin microspheres in simulated gastric juice (pH 2.0). This is a novel encapsulation method, which protects *Bifidobacterium* during exposure to adverse environmental conditions.

The encapsulation technology using κ-carrageenan requires a temperature comprised between 40 and 50$^{o}$C at which the probiotic cells are added to the polymer solution. By cooling the mixture to room temperature, the gelation occurs and then, the particles are stabilised by potassium ions [16]. The encapsulation of probiotic cells in κ-carrageenan beads keeps the cells in a viable state, produced gels are brittle and not able to withstand stresses [17].

### 4.2. Indonesian Research on Encapsulation of Bacteria Using Ionic Gelation Method

The extrusion method for probiotic encapsulation also called as ionic gelation method [18], it seems better to probiotics encapsulation. Indonesian Center for Agricultural Postharvest Research and Development laboratory has been cooperating study with Bogor Agricultural University on encapsulation *Bifidobacterium longum* BF1 (obtained from Reading University, UK) and *Lactobacillus casei* NS (isolated from dadih, Indonesian traditional fermented milk) using ionic gelation method [19]. The material which used was the combination of alginate and skim milk with counterion $CaCl_2$. The research result summarised on Table 1.

Table 1 showed that the bacteria population was reducing after drying. The dry encapsulated *B. longum* BF1 has population 8.60 log cfu/g (reducing 17.94% counted from wet beads), while encapsulated *L. casei* NS has population 12.80 log cfu/g (reducing of 18.21%). It was meaning that population of dry encapsulated

*L. casei* NS higher than dry encapsulated *B. longum* BF1, inline with their survivability (71.50% for *L. casei* NS and 66.00% for *B. longum* BF1). Decreasing population of dry encapsulated bacteria might be caused by water content that removed from beads that indicated by moisture (Table 1). Moisture is one of parameter for describing the impact of water on microbial growth. In addition, it could be resulted from oxygen exposure during drying process. Oxygen is toxic agent for lactic acid bacteria because it will form $H_2O_2$ (hydroxyl radical) [20]. Oxygen capable diffuse across cell membrane and causing oxidative damage on cell lipid membrane, enzymes and DNA. The similar study before has been done on encapsulation *L. casei* NS using biomaterials alginate and skim milk [21]. The encapsulated cells was applied for making cows dadih (Fig. 2). The resulted study was summarised on Table 2.

Table 1. Characteristics of *Bifidobacterium longum* BF1 and *Lactobacillus casei* NS population in biopolymer beads

| Parameters | *B. longum* BF1 | *L. casei* NS |
|---|---|---|
| **Wet Beads characteristics** | | |
| Cell population in wet beads (log cfu/g) | 10.48 ±0.04 | 15.65 ±0.02 |
| Moisture of beads (%) | 97.38 ±1.04 | 97.03 ±0.37 |
| **Drying process (oven, temperature of 40°C)** | | |
| Population of cells encapsulated (log cfu/g) | 8.60 ±0.06 | 12.80 ±0.10 |
| Moisture of dry capsule (%) | 12.38 ±0.13 | 12.03 ±0.37 |
| Survivability (%) (dry base) | 66.0 ±1.6 | 71.5 ±1.0 |

Table 2. Characteristics of *Lactobacillus casei* NS population in biopolymer beads

| Parameters | Alginate 4% (1:0) | Alginate-skim milk (2:1) |
|---|---|---|
| **Wet beads characteristics** | | |
| *L. casei* NS population in wet *beads* (log cfu/g) | 7.76 ±0.01 | 7.68 ±0.03 |
| Encapsulation Efficiency (%) | 52.6 ±1.0 | 35.2 ±3.1 |
| **Drying process (oven, temperature of 40°C)** | | |
| Population of encapsulated *L. casei* (log cfu/g) | < 2.00 | 5.32 ±0.1 |
| Survivability (%) (dry base) | < 22.1 | 58.4 ±0.7 |

The drying process using hot air oven temperature of 40°C for 6 hours was reducing the population both dry encapsulated cell using single alginate 4% and its combination with skim milk in comparison with the wet beads. The decreasing number of >5.76 log cfu/g (from 7.76 log cfu/g in wet beads) was experiencing in encapsulated cells using single alginate while the using of alginate-skim milk combination was reducing 2.36 log cfu/g) (Table 2). The survivability of encapsulated cells using single alginate was <22.10% lower than encapsulted by alginate-skim milk combination. The good result on encapsulation efficiency of 52.6% was using of single alginate comparing with the other one (35.2%).


Fig. 2: Dadih using starter (a) encapsulated *L. casei* directly; (b) releasing *L. casei* from capsule

Fig. 2a showed that wet beads reobtained from cows dadih when dry encapsulated cells was used as starter for resulting milk coagulation. This phenomenon was indicating that life bacteria can release from microcapsule. The capsules must be capable to release their entrapped cells into objective place [22]. The dadih viscousity using releasing cells from micrrocapsule was better than the viscousity using dry encapsulated cell directly i.e 2,563 cps vs 2,147 cps, an opposite result with dadih performance. The good appereance dadih using releasing *L.casei* NS as starter was better than using dry encapsulated cells (Fig. 2b).

## 5. Conclusion

Bioactive protecting is the technology designed to give response to a number of issues related to the feasibility, stability and bioactivity of functional compounds from harsh conditions of environment. This

technology aim to integrate the bioactives within materials for encapsulation and can greatly benefit in the biomedical sectors and food industry, also to obtain the unique properties of biomass derived biopolymers. The using of ionic gelation method for encapsulating bioactives has some reasons that are very simple method and mild process used for designing carriers as possible candidates for 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. Various therapeutic agents such as antibiotics, hormon, enzymes and probiotics have been incorporated in polysaccharides (chitosan, alginate, gellan gum, pectin, carragenan) beads to achieve a controlled release system.

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