10.17951/aa.2019.74.1.109-124

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXXIV, 1 SECTIO AA 2019

Alginates - structure, properties, applications

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Alginates are salts of alginic acid, natural polymers and polysaccharides. They are usually obtained from marine algae. Their solutions often take the form of a gel. The first references to alginates appeared in 1881, so over the years these compounds have been thoroughly studied. Alginates have a lot of valuable properties, which is why they are used in many industries, from stabilizers in the food industry through additives for wound dressings to substances used in bone and muscle regeneration in medicine.

Keywords: alginates, alginate gels, alginic acid, gelling technologies, stability, industry, modern materials

1. INTRODUCTION

Alginates are natural anionic polysaccharides usually salts of alginic acid. They consist of linear copolymers of d-mannuric acid connected by β (1-4) bonds and units of l-guluronic acid connected by β (1-4) bond [1]. Alginates are mainly obtained from marine algae, usually brown algae. They're also produced extracellularly by some bacterial species: *Azotobacter vinelandii, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* [1, 2]. Alginate was first described in literature in 1881 by the British E.C.C. [3]. Stanford as the most

abundant polysaccharide in brown algae, which contains up to 40% dry matter. It builds their cell walls, more precisely it is found in the intercellular matrix as a gel containing sodium, calcium, magnesium, strontium and barium ions [4]. These substances are mainly used in the food industry as emulsions and suspensions. In the production of confectionery (e.g. jellies, puddings) they're used as thickeners [1, 3].

1.1. Structure of alginates

Alginates have a wide variety of compositions and fractions [5]. The division of the sequential structures of these compounds was done by Haug and his colleagues in 1964–1967 [3, 4]. Alginates are divided into three fractions, which differ significantly in composition. Two fractions contained almost homopolymeric particles of guluronic (G) and mannuronic acid (M), while the third fraction contained both monomers with a large number of dimeric residues in equal proportions. It can be concluded from the research that alginate was a real block copolymer, which consisted of homopolymeric M and G regions, called M and G blocks, respectively, associated with regions of alternating structure (MG blocks) [3, 4]. Studies from later years have shown that alginates do not have regularly repeating units, and that the distribution of monomers along the polymer chain cannot be described by Bernoulli statistics [5, 6]. It can be concluded that knowledge of the monomer composition is not sufficient to determine the sequential structures of alginates. After checking alginates using 1H and 13C NMR spectroscopy, more detailed information on their structure was obtained [6, 7]. It is important to remember that neither the alginate molecules nor the composition or sequence of each chain overlap and are the same [4].

Another method of studying the structure of alginates is to determine the diadetic composition using circular dichroism (CD) [8]. With this technique, the diadic frequencies of the three alginate fractions (F_{GG}, F_{MM} and $F_{GM + MG}$) can be determined from 1H NMR spectra [8, 9]. These studies are very important because of the high ability of alginates to form ionotropic gels. The dyadic frequencies of the alginate fraction have a great impact on stability, porosity and strength of gels, so the exact structure characteristics of alginates are important, and the circular dichroism method is sensitive, fast and reliable [8].

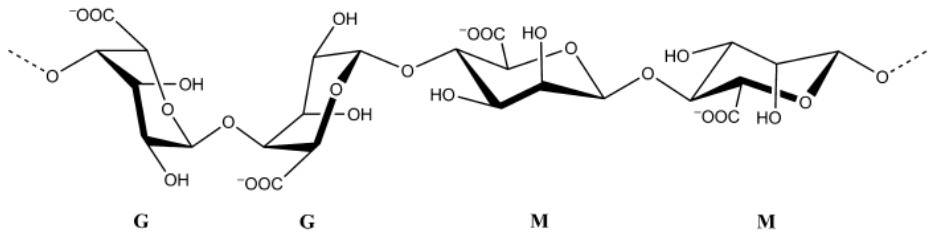

Fig. 1. Structure of alginates [4].

An example block structure of alginate:

MMMMM GGGGGGG MGM GGGGGGG MGMGMGMGMG
block M block G block G block MG

1.2. Properties of alginates

The most popular alginate is sodium alginate, the structure of which is presented below:

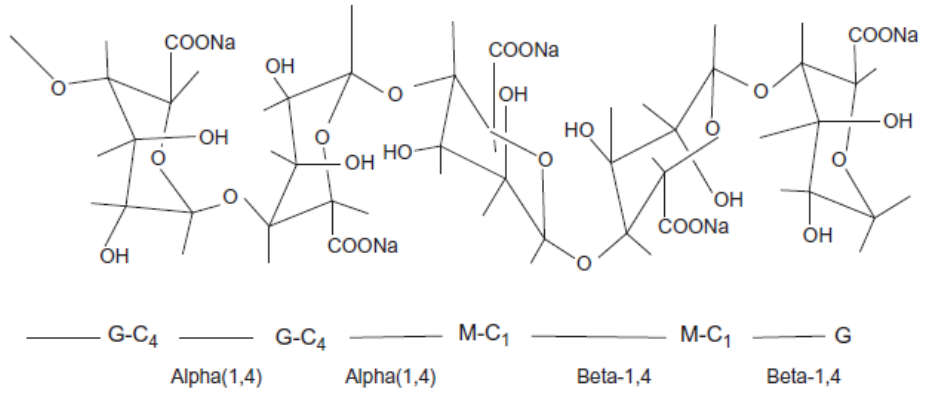

Fig. 2. Sodium alginate [6].

The most common commercially available sodium alginates are in the range of 32 000 to 4 000 000 g/mol. The molecular weight distribution is extremely important in the context of potential applications. Fragments with a low molecular weight are those that contain only G blocks that do not participate in gelation [7, 10]. Sodium alginates form colloidal solutions insoluble in organic solvents, whose pH is less than 3.0, alcohols and water-alcohol solutions with an alcohol content greater than 30%, while slowly

soluble in cold water. In contrast, calcium alginate is practically insoluble in organic solvents and water, but it dissolves in sodium citrate solution [6, 11].

Ion binding by alginates is the basis for their gelling properties. The affinity of alginates for metals increases in the order Mg << Ca <Sr <Ba. This is a unique property for alginates compared to other polyanions [6].

To determine the solubility of alginates in water you need to use three basic parameters: total ionic strength, pH of the solution and the content of gelling ions, i.e. water hardness (in this case). The main problem is the high content of Ca^{2+} ions. The dissociation constants of mannuric and glucuronic acid monomers are 3.38 and 3.65, respectively, and the pKa of the alginate polymer differs only slightly from the monomeric residues. Alginate solutions have the ability to lower pH [4, 7]. This can happen in two ways. The precipitation of alginic acid molecules causes a sudden decrease in pH, whereas the controlled and slow release of protons can contribute to the formation of an "alginic acid gel" [12]. The mechanism of precipitation of alginic acid molecules has been thoroughly studied and it turned out that the addition of a small amount of acid to the alginate solution causes precipitation in a small pH range, and it depends on the chemical composition, sequence and molecular weight of alginate [4]. A characteristic feature of alginates is quite high stability and durability. Dry alginate powder, e.g. sodium alginate stored in a cool, dark and dry place has a shelf life of up to several months, if we put it in the freezer, its shelf life increases to several years, and in addition we will not observe a drastic decrease in molecular weight. In contrast, dried alginic acid, which may undergo intramolecular catalysed degradation, is very unstable at room temperature. Before we use alginate for applications in various industries, we need to consider what factors will affect it, whether it will not affect its stability, and whether subjecting it to chemical reactions will not cause degradation. This is very important because in conditions of degradation, the relative viscosity of alginate drops very quickly. Glycosidic bonds are also sensitive to acid and base degradation and oxidation by free radicals [4, 6].

1.3. Applications of alginates

Alginates find many interesting applications. The most important are as follows:

1.3.1. Bone alginate scaffolding

Bone scaffolds must have high mechanical strength needed for bone regeneration in the place where the implant is placed, so it is important that they are very porous. Alginate / hydroxyapatite (HA) composite scaffoldings are prepared in the process of internal gelatinization and subsequent freeze-drying, thanks to which the appropriate porosity is obtained [13, 14]. Porous HA implants play important roles *in vivo*: they control the bone regeneration process, affect the mechanical performance of the implant and the path of bone regeneration [15]. Implant-building nanoparticles are prepared in the presence of chitosan by modification with lactose, resulting in a colloidal solution that is adsorbed on to bone scaffolding and acts as a temporary implant [13, 14].

The basic method of bone tissue engineering, culture of osteogenic cells used to build bone scaffolds, is considered an alternative therapeutic technique [16]. The material used for scaffolding production should be compatible with bone material, have good electrical conductivity, be thermally and electrically stable. A good combination is polypyrrole (PPy), which is compatible with a wide range of cells and alginate, which allows cell encapsulation and efficient penetration of cells into the matrix. To increase the interaction between cells, chitosan was added to the mixture of polypyrrole and alginate, which ensured the mechanical stability of the system [16].

1.3.2. Encapsulating agents from alginates

Animal studies have shown that the alginate molecule itself can have different effects on biological systems and can give different technological properties in the liquid phase. The reason for this is the large number of possible chemical compositions and molecular weights of alginate. The biological effect can be observed in the first attempts to transplant closed islets of Langerhans, which are aimed at controlling diabetes, especially type I [17]. There is an overgrowth of alginate capsules by phagocytes and fibroblasts, which really resembles an inflammatory reaction to a foreign body introduced into the body, and this induced response depends primarily on the

content of mannuronate residues. These alginate fragments, rich in mannuronate, which would not participate in the gel network, are washed out of the capsules and trigger a direct immune response that can be linked to (1-4) glycosidic linkages [18, 19]. This is possible because other polyuronates, like D-glucuronic acid, also have this property. The ability of alginate to encapsulate islets *in vivo* is still unclear because studies show that diabetes has been "cured" in many animals, and this is not medically possible. In addition, tissues and microspheres were subjected to XPC testing, which did not show a correct glucose response. It can be assumed that the failure was caused by insufficient tissue implantation, which confirms the belief that work must be developed to increase stability of biomaterials [20].

In the food industry, appropriate sensory profiles, stability, delayed release and thermal protection are important features that would be difficult to achieve without microencapsulation techniques. Taking advantage of the gelation capability, alginate-based capsules have many applications in the food industry, including encapsulation of reactive or volatile molecules such as acidifying substances, fats and flavors [4, 21]. In the food industry, alginate encapsulation and immobilization technologies are used for a variety of purposes, including food processing, food functionality and product acceptance. Immobilization or encapsulation technology is used to produce a wide range of bacterial metabolites, including enzymes, amino acids, organic acids and alcohols. Because alginate spontaneously forms ionotropic gels at low temperature, it is ideal for trapping enzymes or whole cells that would otherwise be damaged under more stringent conditions [4]. The latest research into the use of alginate in the food industry has focused on encapsulating live probiotic cells in order to deliver them with food to the large intestine in the human body [4, 6]. Probiotics can change microflora through implantation or colonization in the host compartment, and thus can have a beneficial effect on host health. Simple and inexpensive technologies for creating gel microcapsules from alginates under conditions that will not damage the bacterial cells they contain are now readily available. High G block alginates are the best alginates for forming microcapsules because of their high mechanical stability, high porosity and salt tolerance. In these applications, alginates work to increase the survival of bacterial cells during food storage, and also

reduce cell destruction under adverse conditions in the stomach and small intestine [17].

1.3.3. Gels and gel technologies

Alginate gels are cold-binding, which means that gelation is not temperature dependent. This feature distinguishes them from other polysaccharides. However, the kinetics of the gelation reaction can be influenced by temperature changes [21, 22]. The temperature at which alginate gels were formed also affects a variety of their properties, e.g. thermal stability - they can be heat treated at high temperatures and as it turns out they are not fusible. That is why they are used in the production of baking creams. The most important factors affecting the gelation process are alginate concentration, sequence, chemical composition, as well as the ratio of gelling and non-gelling ions and the presence of complexing factors such as phosphates or citrates [23]. It is also extremely important to remember that alginate is a polyelectrolyte, i.e. it can interact with other charged polymers under appropriate conditions. An example would be proteins in mixed systems that cause phase transition or an increase in viscosity. This property is used in the restructuring of food and animal feed [4].

1.3.4. Modern dressing materials made of alginates

For many years, alginic fibers were produced only for textile purposes. Currently, they are widely used in medicine, primarily as modern dressing materials. They can be divided according to the fiber structure, including fibers from: alginic acid, zinc alginate, copper alginate, sodium alginate, calcium alginate, calcium alginate with the addition of nanosilica (SiO_2), as well as mixed fibers from Ca/Na and Ca/Zn alginate [24, 25]. To obtain the abovementioned fibers, sodium alginate is most often used as the starting polymer. The fibers are formed using the wet solution method. The spinning fluid is most often an aqueous solution of sodium alginate (at a concentration of 5-8%). The coagulation bath is a solution of polyvalent metal salts ($ZnCl_2$, $CuCl_2$, $CaCl_2$), with a small addition of HCl. The solidification process occurs as a result of chemical reactions consisting in the exchange of Na^+ ion for divalent ions, most often Ca^{2+}. The connection of adjacent macromolecules with main bonds results in the formation of a divalent metal alginate insoluble in water

to a degree depending on the substitution of sodium ions. After the solidification process in the coagulation bath and the subsequent stretching process, further technological operations are carried out. The obtained fibers are characterized by good sorptive properties, especially a small internal surface and small pore volume. These features allow the retention of a large amount of water into the fiber, but also binding it in the capillaries. This mechanism is important for the ability to absorb large amounts of secretion from the wound by fibers used in dressing materials [3, 24].

1.3.5. Alginate for satiety

Aqueous solutions of alginates form gels in the reaction with acid or calcium ions. The human stomach has an acidic pH, which means that you can give the patient alginate in a solution and it will gel when it reaches the stomach. High viscosity and gel strength are often associated with low organoleptic tolerance of food, but high viscosity in the stomach is associated with increased satiety [22, 24]. The unique structure of alginate and the possibility of spontaneous gelation in the stomach has been used for the production of satiety enhancing preparations. The 10-day treatment with alginate or placebo was performed on stomach function, appetite and the feeling of satiety associated with it. The patients were overweight or obese adults. Studies show that after adding alginate to a liquid meal, the subjects experienced a significantly lower postprandial hunger. In addition, it has also been proven that alginate increases stomach volume by slowing digestion of gastric contents, but without affecting the rate of gastric emptying. [24, 26].

1.3.6. Cell cultures

Alginate gels are used in biomedical research as a model system for growing mammalian cells. Alginate hydrogel systems are designed to control cell function. Alginate scaffolds also serve as cell carriers in 3D cell cultures [27, 28]. They can act as 2-D systems physiologically in 3-D culture. Alginates do not have mammalian cell receptions, and in addition, proteins are poorly adsorbed in alginate gels. allows this. Therefore, these materials can be used as an ideal example in which one can check highly specific and quantitative modes of cell adhesion. An additional advantage of alginates is that from *in vitro* tests it is easy to switch to *in vivo* tests, because alginates are biocompatible

and do not cause problems with their introduction into living organisms [29, 30]. In recent years, alginate gels have been used to work on the micro-environment of 3-D cultures in which vascular system cancer cells were cultured. These studies focused on the use of integrin in the 3-D tumor microenvironment, which is called RGD-alginate gel encapsulation [31]. It influenced the way of signaling the recruitment of blood vessels. In the future, this can be used to develop new forms of cancer therapy [11, 29].

1.3.7. Alginate gels as protein carriers

Over the past few years, intensive research has been carried out on the use of alginate gels as carriers of cell populations or individual proteins that can affect tissue and organ regeneration in the human body. During these tests a wide range of gelation methods, degradative behavior of various materials, and physical and chemical properties were used. Even in the absence of degradation of the alginate gel, a large proportion of proteins diffuse out of it, although studies show that degradation of the gel accelerates release. Protein particles that are too large for their release to be based on diffusion can still be transported, but gel degradation is required, then the cells can migrate freely from the alginate hydrogel. Work is also underway on nanoporous alginate gels and qualitative cell migration that has not been quantified. The number of externally migrating cells was quantified as a function of porosity and RGD presentation in macroporous alginate gels. Similarly, changes in cell migration rate were observed in the macroporous RGD-alginate gel and the surrounding ECM gel [11, 29]. Synthetic extracellular matrices (ECM) are polymeric biomaterials with covalent coupling specific for ligand cells or extracellular signaling molecules. They have many advantages: they control the growth of cells in biomaterials, increase the deposition of these cells and additionally affect their behavior and differentiation. Mooney et al. introduced a ligand responsible for increasing adhesion containing RGD to alginate to create a three-dimensional system for isolating cells in skeletal muscle. The modification ensured cell proliferation, adhesion and expression in tissue engineering [11, 32]. ECM are fully hydrated gels, which is why their wettability plays a key role in mimicking the aquatic environment in vivo, as a result of which hydrogels can be used as materials for the production of 3D matrices [33].

1.3.8. Muscles, nerves, pancreas and liver

Alginate gels are also tested for their regenerative properties in tissue and organ reconstruction (pancreas, liver), as well as skeletal muscles and nerves [34]. Tissues are currently transplanted, growth factors are supplied to them, or both are combined to improve the end result. Alginate gels are very helpful in these methods. Localized and long-term supply of VEGF and insulin-like growth factor-1 (IGF-1) obtained from alginate gels to the body to modulate both angiogenesis and myogenesis has resulted in considerable muscle regeneration and functional formation. These factors were monitored for the activation and proliferation of satellite cells, as well as for the protection of cells against apoptosis, i.e. controlled cell death caused by released factors [35, 36]. Constant administration of the aforementioned factors and external displacement of myoblasts to damaged muscle tissue *in vivo* from RGD-alginate gels led to virtually complete muscle tissue reconstruction and restoration of muscle fibers at the wound site. Alginate gels have been used in the study of peripheral and central nervous system repair [35, 37]. Alginate-based anisotropic gels have been used to introduce acute and major cervical spine lesions in adult rats. These gels have been integrated into the spinal parenchyma in such a way that they do not cause any inflammatory reactions and directed axonal regrowth. Alginate gels, covalently crosslinked with ethylenediamine, have also been found to be useful in restoring a 50-millimeter gap in the cat's sciatic nerves and contributed to the development of regenerating axons and astrocyte responses on the trunk of a cut spinal cord in young rats. Alginates were also used as a binder to repair peripheral nerve fractures that could not be sutured. Alginate gels may find use in neuronal therapies that are cell-based, since neuronal stem cells grown from mice in calcium alginate beads have the ability to differentiate multiline cells into glial cells and neurons. Recombinant alginate gels containing the Tyr – Ile – Gly – Ser – Arg sequence improved cell adhesion of neuroblastoma NB2a and neurite outgrowth from cells. It depended on the peptide density in alginate gels [36].

In the work of Donati et al. alginate was modified with carbodiimide by introducing 1-amino-1-deoxy-β-D-galactose via the imide linkage with the carboxyl group to improve the encapsulation and adhesion of hepatocytes in biomaterial engineering. Hepatocytes are liver cells that perform a lot of metabolic activities, but outside of

the body lose their functions and low vitality. The solution to the problem may be anchoring hepatocytes in the alginate matrix. In addition, a high-density cell culture system can be created by encapsulating hepatocytes in alginate capsules [32].

As mentioned earlier, the liver is one of the most important organs and liver cell disease can be life-threatening, which is why science focuses on creating *in vitro* hydrogel models. Sun et al. presented 3D bioprinting (DIW) hydrogel constructions: gelatin/chitosan embedded in hepatocytes, all encapsulated in the alginate capsule [38]. Hepatocytes inoculated with collagen, laminia or fibronectin proteins promote the development and spread of vessels and form sandwich structures. Sandwich configurations most often create ECM-based cultures that mimic the liver environment in vivo: collagen connected to the hepatic tissue matrix and semi-synthetic hydrogels, including heparin PEG. Heparin added to hydrogels allows you to control the binding and release of HGF – hepatocyte growth factor to regulate liver cell function and regeneration [38].

1.3.9. Alginates in *in vitro* cancer models

Cancer is one of the main causes of death and intensive research is ongoing into the causes, stages of the disease, and treatment therapies. *In vitro* models can be very helpful in these studies, because thanks to them you can learn the biology and physiology of tumors, the mechanisms of their angiogenesis, as well as check the effectiveness of treatment [38]. *In vitro* models of liver, ovarian, brain, breast and lung cancers are most commonly performed. The traditional strategy of tissue engineering is to seed cells on porous scaffolds or gel surfaces. Zhang et al. they constructed a porous chitosan and alginate (CA) scaffold and then inoculated glioma cells on it. The tests were carried out in mice and the results were compared with the growth of tumor cells on standard 2D Matrigel matrices. The results showed that CA scaffolds showed greater tumor volume and angiogenesis, and thus more malignancy than 2D matrices [38, 39].

Bioprinting is a new approach to creating 3D cancer models [38]. The hydrogel in bioprinting acts as a matrix supporting and regulating the action of cells enclosed within the matrix [27]. Alginate and gelatin hydrogels proved to be the best for this type of extrusion. Dai et al. they created a three-dimensional cell model of glioma by

modifying a porous gelatin/alginate/fibrinogen hydrogel. Considering the disordered and specific nature of cancer cells, materials such as gelatin, collagen and fibrinogen are helpful to provide a suitable cancer model environment [38, 40].

1.3.10. Alginate in modification of textiles

Textile materials (sportswear, underwear, hospital clothes) are a great medium for the accumulation and development of bacteria and microorganisms. For this reason, the textile industry is looking for solutions to modify the surfaces of textiles while maintaining their strength and provide comfort to users. Chemical treatment is a complicated, long-lasting, expensive and has a bad environmental impact. For this reason, a new, environmentally friendly method of modification without toxic chemicals is sought [11]. The solution to this problem may be the use of alginate, which is a biocompatible material with high gelling capacity. Currently, three techniques are known for using alginate for antibacterial modification of textile materials:

- nanocomposite coatings – the most commonly used antibacterial agents in the field of textiles take away silver. Scientific research confirms that silver nanocrystalline very quickly destroys microorganisms, so Zahran et al. they created silver nanoparticles (AgNP) with antibacterial effect on the bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [11, 41]. The fabric is coated with a substance formed by reducing silver nitrate hydrolyzed with an alkaline alginate solution [11]. The discovery of recent years are nanocomposites zinc oxide - sodium alginate - cellulose. ZnO - SACNF fibers have very good antibacterial properties against Escherichia coli and have found application in the production of bacterial resistant wound dressings [11, 42].
- ionic crosslinking coatings – Grace et al. they dipped cotton fibers into an aqueous sodium alginate solution and performed ion crosslinking of algae with Cu^{2+} ions to make copper cellulose alginate cotton (CACC) [11, 43]. The fibers formed in this way were characterized by antibacterial activity and excellent mechanical strength, therefore they can be used for the production of dressings [11, 44].

– layer by layer – layer by layer (LbL) is one of the methods for producing multilayer polyelectrolytes (PEM) in the process of alternating adsorption of oppositely charged polyelectrolytes by immersing the material in a polyelectrolyte solution [11]. PEM alginate coatings are used in textile modification. Gomes et al. using LbL they created a cotton coating coated with antibacterial polyelectrolyte chitosan and alginate, which during SEM showed an effective antibacterial effect against *Staphylococcus aureus* and *Klebsiella pneumonia* [11, 45].

1.3.11. Alginates in a controlled release drug delivery system

Controlled release (CR) drug delivery systems are a very important element of health care and have many advantages: biodegradability, increased effectiveness of drug therapy, less toxicity and high patient comfort. The release of the drug from the alginate ball occurs at the appropriate pH in the process of diffusion through the matrix [46, 47]. Studies confirm that the release of a drug depends on its solubility in the environment, e.g. drugs with high solubility in an acidic environment release much better at acidic pH. In addition, the drug release process can be controlled by coating the matrix with sodium alginate beads, which acts as a diluent in CR capsules [47, 48]. In order to achieve sustained release of the drug, it was proposed to use an external chitosan coating on alginate beads, which can be a matrix for the controlled release of vaccines, polypeptide drugs and even proteins [47, 49].

2. SUMMARY

Alginates have great potential and are used as materials in many areas: food industry, biomedicine and tissue engineering. They have many attractive features, which include: mild gelation conditions, biocompatibility and simple modifications enabling the production of alginate derivatives with new, valuable properties. Alginates are also used as wound healing agents as well as pharmaceutical ingredients, e.g. in the treatment of type 1 diabetes. Chemically modified alginate is widely used as a carrier in bone regeneration. Paying attention to the wide ranges of cross-linking strategies available, various configurations of molecules, as well as their structures and molecular weights, alginate gels can be obtained that are suitable for use in

various fields. It can be concluded that in the future alginates have a good chance of even greater interest of scientists and the extension of their applications in many fields.

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