We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 4

Alginate and Sericin: Environmental and Pharmaceutical Applications

Thiago Lopes da Silva,
Jacyara Moreira Martins Vidart,
Meuris Gurgel Carlos da Silva,
Marcelino Luiz Gimenes and
Melissa Gurgel Adeodato Vieira

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65257

Abstract

Alginate is a polysaccharide that, for commercial purposes, is extracted exclusively from marine brown algae. In this chapter, we discuss the main sources of alginate and sodium alginate manufacturing, its chemical structure and physicochemical properties, the alginate modifications, and blend formation. We also present applications of alginate and sericin blend in the pharmaceutical and environmental fields as well as case studies.

Keywords: alginate, alginate production, alginate properties, alginate modification, alginate pharmaceutical applications, alginate environmental applications

1. Introduction

"Alginate" is the term usually used for the salts of alginic acid (carboxylic salts), but it can also refer to all the derivatives of alginic acid and alginic acid itself [1]. Alginates are biopolymers that have two main sources: bacteria and seaweed (brown algae). This biomaterial is a natural polysaccharide that occurs as structural components in the cell wall of marine brown algae (*Phaeophyceae*) as well as capsular polysaccharides in some bacteria (*Azotobacter* and *Pseudomonas*). Commercial alginates are extracted exclusively from marine algae sources although the microbial fermentation is technically feasible for this biopolymer production [2].

INTECH
open science | open minds

© 2017 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

Alginate is composed of blocks of mannuronic acid residues (M-blocks), blocks of guluronic acid residues (G-blocks), and blocks with alternating M and G residues (MG-blocks). The source of brown seaweed, growth, location, tissue used in the alginate extraction, age of the tissue used for alginate preparation, and season of the year, among other conditions, are important factors that contribute to vary the chemical composition and sequence of M and G units what implies in different alginate properties, like viscosity, gelation, solubility, among others [3, 4].

Alginate, polymer with polyelectrolyte nature, is considered low or nontoxic, nonimmunogenic, biocompatible, and biodegradable. The industrial applications of alginate are linked to its ability to retain water, and its gelling, viscosifying, and stabilizing properties [5, 6]. As biopolymer with important properties, it has large uses in several industrial fields, including textiles, food industry, agri-foods, pharmaceuticals, cosmetics, paper [6], and medical supplies, among others. The physical properties significantly control the stability of the gels, the rate of drug release from gels, and the phenotype and function of cells encapsulated in alginate gels [7].

The gelling property, which is the ability of alginate to form gels in the presence of multivalent cations (e.g., Ca^{2+}), is one of its main biofunctional properties [5, 6, 8, 9]. Emerging biotechnological applications are based on this unique property, which allow specific biological effects of the alginate molecule. The gel formation and the almost temperature-independent sol/gel transition in the presence of multivalent cations make alginate suitable for the development of biomaterial that can be used in cell immobilization, tissue engineering, drug delivery, controlled release, immobilization of microorganism, and matrix for living cell, among other applications [10].

2. Alginate sources

At present, commercial alginates are exclusively extracted from marine brown algae found in coastal waters around the globe [7].

The brown algae are an important assemblage of plants that are classified in about 265 genera with more than 1500 species. They derive their characteristic color from the large amounts of the carotenoid fucoxanthin (which yields a brown color) contained in their chloroplasts and the presence of various pheophycean tannins. Brown algae flourish in temperate to subpolar regions where they exhibit the greatest diversity in species and morphological expression [11].

Brown algae belong to Phaeophyta class. Typical algal cell walls of Phaeophyta are composed of a fibrillar skeleton (made from cellulose material) and as amorphous embedding matrix. The Phaeophyta algal-embedding matrix is predominately alginic acid or alginate (the salt of alginic acid) with a smaller amount of sulfated polysaccharide (fucoidan) [11, 12].

The main commercial sources are species of *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis, Sargassum,* and *Turbinaria* [13]. Of these, the most important species are *Laminaria, Macrocystis,* and *Ascophyllum*. The alginate content in algae can reach values of 40% of its dry

http://dx.doi.org/10.5772/65257

weight. On a dry weight basis, the alginate contents are 22–30% for *A. nodosum*, 25–44% for *L. digitata,* and 17–33 and 25–30%, respectively, for the fronds (leaves) and stems of *L. hyperborea* [14].

Algae species	Monomer M (mannuronic acid) (%)	Monomer G (guluronic acid) (%)	M/G ratio
Ascophyllum nodosum	60.0	40.0	1.50
Laminaria digitate	59.0	41.0	1.43
Laminaria japonica	69.3	30.7	2.26
Macrocystis pyrifera	61.0	39.0	1.56
Laminaria hyperborean, fronds	56.0	44.0	1.28
Laminaria hyperborean, stems	30.0	70.0	0.43
Sargassum filipendula	16.0	84.0	0.19
S. polycystum	17.4	82.6	0.21
S. muticum	23.7	76.3	0.31
S. oligocystum	38.3	61.7	0.62
S. horneri	39.0	61.0	0.64
S. miyabei	43.2	56.8	0.76
S. thunbergii	43.8	56.2	0.78
S. henslowianum	45.1	54.9	0.82
S. hemiphyllum	51.5	48.5	1.06
S. siliquastrum	53.1	46.9	1.13
S. vulgare (high viscosity)	53.9	46.1	1.17
S. fluitans (Cuba)	34.2	65.8	0.52
S. fluitans (Florida)	54.1	45.9	1.18
S. pallidum	55.8	44.2	1.26
S. maclurei	59.5	40.5	1.47
S. tenerrimum	60.5	39.5	1.53
S. vulgare (low viscosity)	60.9	39.1	1.56
S. patens	61.4	38.6	1.59
Turbinaria ornata	47.1	52.9	0.89

Table 1. Percentages of monomers M and G (mannuronic acid and guluronic acid) and M/G ratio of some alginate extracted from brown seaweeds [14, 18, 19].

Chemical composition and sequence of M and G units in alginate may vary widely among species and even in different parts of the algae [2]. The time of the year (season) when the algae

is harvested, the location of growth, and the age of the tissue used for alginate preparation also influence the composition and sequence of the unit monomer of the polysaccharide [6, 15–17].

The ratio M/G is an important factor because the properties of alginate solution, gels, and its produced biomaterials depend on the G and M contents. **Table 1** shows the M and G contents of alginate extracted from common species of brown seaweed.

From **Table 1**, it can be seen that same species can present different composition of M and G monomers depending on the local harvest (*S. fluitans* – Cuba and Florida) and the tissue utilized in alginate extraction (*L. hyperborean* – fronds and stems). The ratio M/G varies largely ranging from 0.19 to 2.26 indicating wide differences in species composition. Depending on the alginate material that must be developed, this is a critical feature in choosing the raw alginate source.

Alginates with more extreme compositions containing up to 100% mannuronate can be isolated from bacteria. Alginates with a very high content of guluronic acid can be prepared from special algal tissues such as the outer cortex of old stipes of *L. hyperborea*, by chemical fractionation, or by enzymatic modification in vitro using mannuronan C-5 epimerases from bacteria [5].

3. Sodium alginate manufacturing

Alginates occur in brown algae in the intracellular matrix as gels containing sodium, calcium, magnesium, strontium, and barium ions, such that the counterion composition is determined by the ion-exchange equilibrium with seawater [20]. The extraction process of sodium alginate is relatively simple and can be divided into two categories: calcium alginate process and alginic acid process. At first, the key intermediate products formed are calcium alginate and alginic acid, while the second only alginic acid is formed. The calcium alginate process has the advantage of easy separation of both calcium alginate and the alginic acid that are precipitates in fibrous form. Furthermore, although the process of alginic acid has one step less as compared to the calcium alginate process, it should be noted that the overall losses of alginic acid in this process are greater than in the calcium alginate process, due to the fact that the alginic acid precipitated forms a gelatinous precipitate which is very difficult to separate [13].

Figure 1 shows the steps involved in the manufacture of sodium alginate. Initially, the ions (Na^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+}) are removed by protons exchange by adding a dilute mineral acid, such as HCl, which will result in the formation of insoluble salts of alginic acid. Treatment with formaldehyde is carried out for the removal of phenolic compounds and also to bleach the material. Then, an alkaline extraction is performed by adding Na_2CO_3 or NaOH, yielding soluble sodium alginate and insoluble seaweed residue. Alkaline extraction is the main step as it corresponds to the extraction phase itself [21]. A separation process is employed to separate the sodium alginate solution of the extraction residue. From the sodium alginate solution, it is possible to employ the precipitation method which generates both calcium alginate and alginic acid as intermediates (calcium alginate process) or generate only alginic

http://dx.doi.org/10.5772/65257

acid as an intermediate (alginic acid process). In calcium alginate process, $CaCl_2$ is added to the sodium alginate solution to form insoluble calcium alginate, which is converted into insoluble alginic acid by the addition of dilute mineral acid (e.g., HCl). Alginic acid is finally converted to sodium alginate by the addition of Na_2CO_3 or NaOH. In the case of alginic acid process, sodium alginate solution is treated with dilute mineral acid, giving rise to the insoluble alginic acid. Alginic acid is suspended in alcohol (ethanol or methanol), and NaOH or Ca_2CO_3 solution is added for obtaining sodium alginate [13, 20–23].

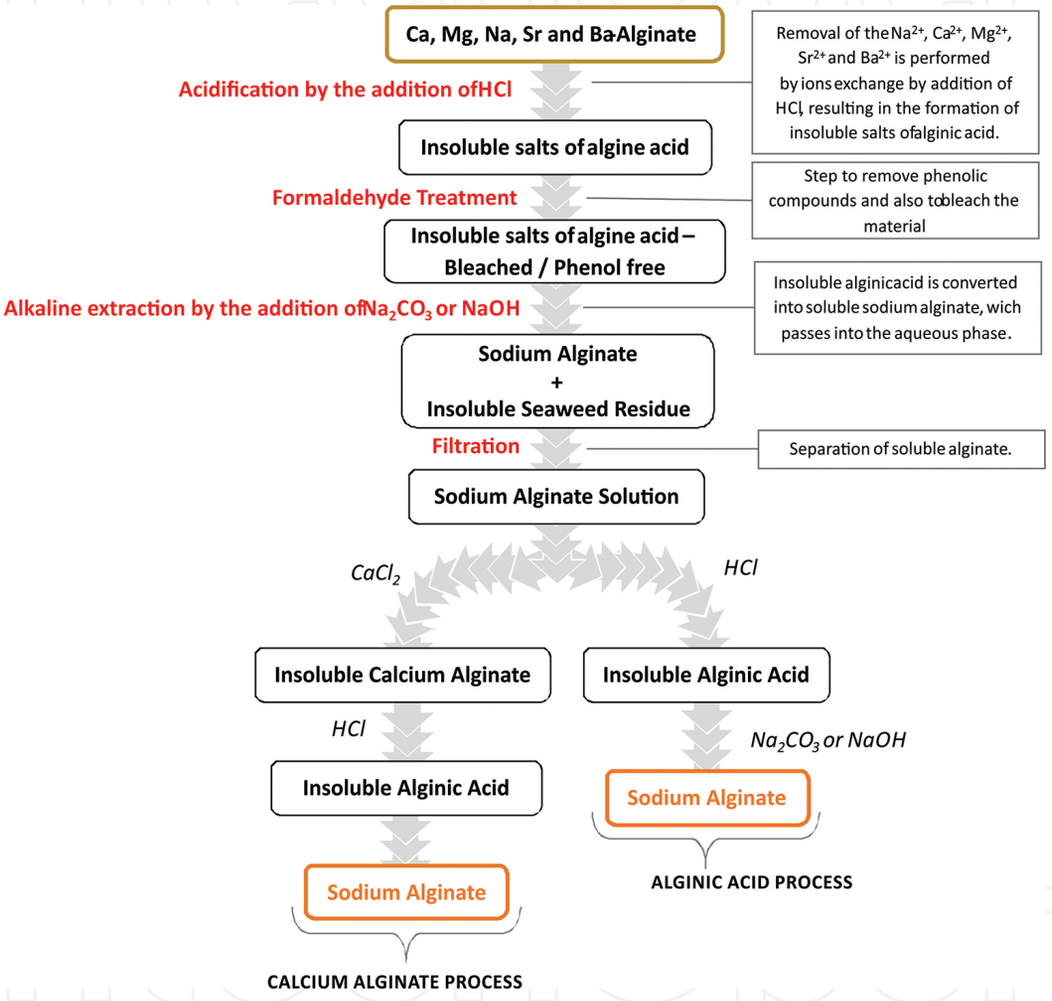


Figure 1. Scheme of sodium alginate production from brown algae.

4. Chemical structure

Alginates constitute a family of linear binary unbranched copolymers composed of 1,4-linked β-D-mannuronic acid (monomer M) and α-L-guluronic acid (monomer G) residues [3, 6]. These two acid residues (saccharide unit) present stereochemically differences at C-5 [5, 14]. Alginate

presents a number of free hydroxyl and carboxyl groups distributed along the backbone which allow reactions and chemical functionalization [10].

The alginate polymer accepts different conformation of M and G saccharides in its chain. The chain can be composed of homopolymeric regions of β-D-mannuronic acid residues (M-blocks: MMMMMM), homopolymeric region of β-D-mannuronic acid residues (G-blocks: GGGGGG), and heteropolymeric regions where G and M exist in alternating sequence (MG-block: MGMGMG) [24, 25]. **Figure 2** presents both M and G monomers and the chain conformation of alginate.

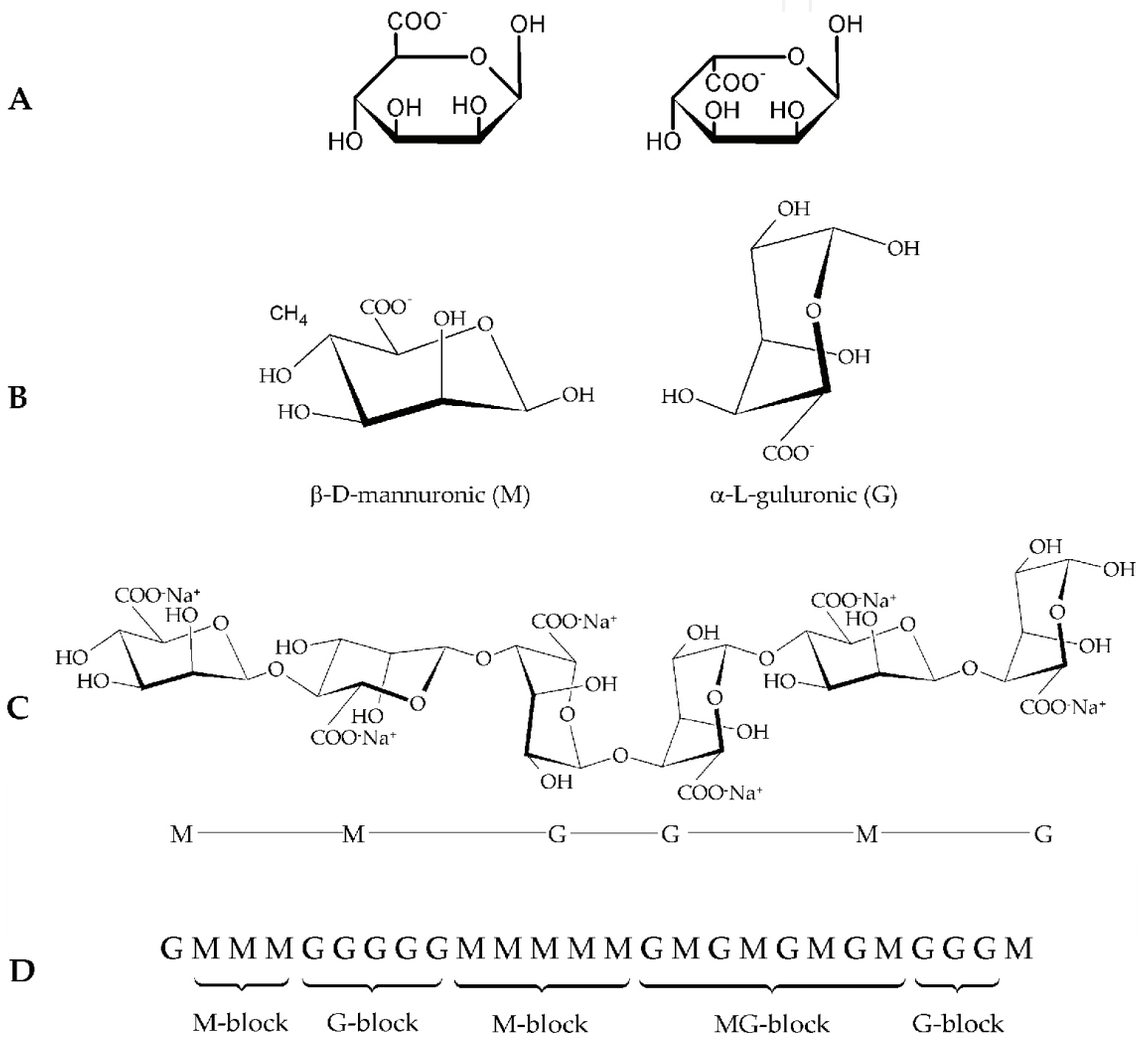


Figure 2. Structural characteristics of alginates: (A) alginate monomers, (B) chemical structure of monomers, (C) chain conformation, and (D) block distribution.

Because of differences in chemical structure of alginates, the properties of alginate can vary depending on the source of brown seaweed. The proportion and sequential arrangement of M and G (uronic acid residues) in the alginate chain, that is, the proportion of the three types of blocks, leads to differences in the physical properties of the respective alginate products [6].

http://dx.doi.org/10.5772/65257

Properties of alginates depend on the relative proportion of three types of uronic blocks; for industrial utilization of any particular alginate, it is quite important to quantify the relative proportions of the uronic acids. Methods such as H NMR and C NMR (proton nuclear magnetic resonance spectroscopy) have been developed to measure the ratio M/G, as well the MM, GG, and MG/GM contents [3, 6, 26]. The ratio of mannuronic acid to guluronic acid, although the number and size of blocks are not provided, provides a practical estimate to evaluate the composition and quality of the alginate to a particular use.

Alginates extracted from different sources differ in M and G contents as well as the size of each block, and nowadays more than 200 different alginates are currently being manufactured [7].

Alginates have no regular repeating distribution of the monomers along the polymer chain. That is why this distribution cannot be described by Bernoullian statistics, which implies that the knowledge of the monomeric composition is not sufficient to determine the sequential structure of alginates [2, 5]. It was suggested that a second-order Markov model would be required for a general approximate description of the monomer sequence in alginates [5].

Alginate contains all four possible glycosidic linkages within the alginate molecule: diequatorial linkages connect mannuronic acid residues in M-blocks, diaxial linkages connect guluronic acid residues in G-blocks, and equatorial-axial (MG) and axial-equatorial (GM) glycosidic bonds connect both uronic residues in MG blocks [5, 24]. Due to this kind of linkages, the M-bock is a relatively straight polymer, such as a flat ribbon, while G-block presents a buckled arrangement. The conformation of linkages and chain alginate is reported in **Figure 2**.

The diaxial linkage in G-blocks results in a large hindered rotation around the glycosidic linkage, which combined with the polyelectrolyte nature of the alginate molecule may account for its stiff and extended nature [2]. G-blocks are stiffer than alternating blocks, which in turn are more soluble at low pH [27]. In the uronic blocks, the rigidity decreases along the series GG > MM > MG [28]. The electrostatic repulsion between the charged groups on the polymer chain also will increase the chain extension and hence the intrinsic viscosity [5].

5. Physicochemical properties

5.1. Ionic cross-linking

The most important feature of alginate properties is its ability to form hydrogels with divalent cations. The alginate chelation with multivalent cations is the basis for gel formation. Selective binding of earth metal ions increases significantly with the increase of G content in the alginate backbone chain.

Gel formation is driven by the interactions between G-blocks, which associate to form tightly held junctions in the presence of divalent cations [20]. The divalent cations, such as Ca^{2+}, act as cross-links between the functional groups of alginate chain [10], "zipping" the G-blocks in alginate chain, that is, the G-block of one polymer forms junctions with the G-block of adjacent polymer chain through interactions with the carboxylic groups in the sugars, which leads to

the formation of a gel network. Because of the structural form of the G-block, the metal chelation-binding chain is called the egg-box model of cross-linking. **Figure 3** shows the egg-box model for alginate gel formation.

It was believed that only G-blocks of alginate participate in intermolecular cross-linking with divalent cations to form hydrogel, but some researches indicate that MG-blocks also participate in this process [7, 29]. The participation of MG-block is less important to hydrogel formation because these blocks form weak junctions [20]. The linkage of long alternating sequences in secondary MG/GM junctions is suggested to account for the shrinking of alginate gels in view of its dependence on the length of the MG-blocks [29]. Gels prepared from alginate with a high content of G residues (high M/G ratio) exhibit higher stiffness than those with a low amount of G residues [7].

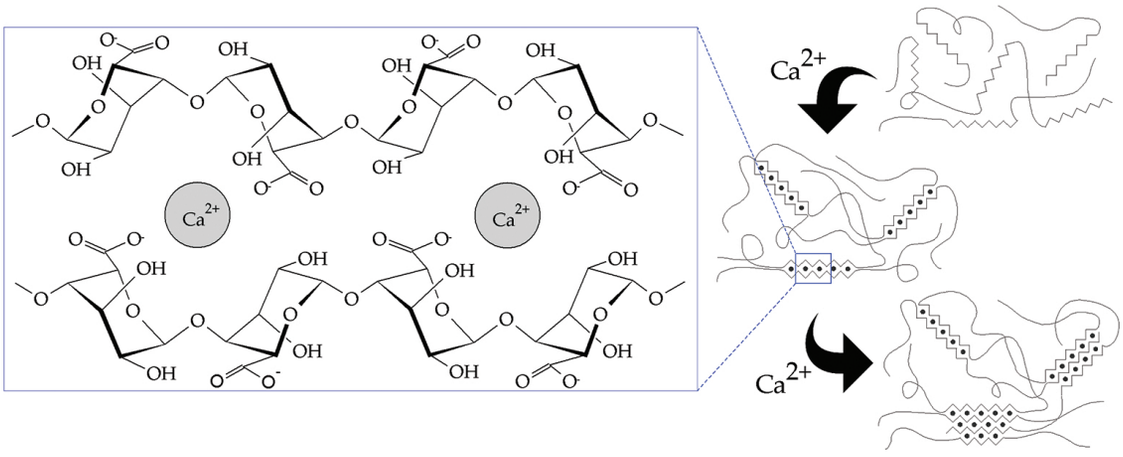


Figure 3. The "egg-box" model of gelation of alginate by calcium.

Alginate's affinity toward the different divalent ions has been shown to decrease in the following order: Pb > Cu > Cd > Ba > Sr > Ca > Co, Ni, Zn > Mn. Since the composition and block structure varies greatly in different types of alginates, it follows that both the gel and ion-binding properties of alginate are influenced by the choice of alginate material and cross-linking ion [30]. Despite the variety of cations, because of the cost and no toxicity, Ca^{2+} is the most used ion to produce alginate gel.

Concerning alginate particles, the preparation method of calcium alginate particles also interferes with their physical properties, such as the porosity, volume of water, sphericity, and elasticity [31]. Methods such as atomization [32], emulsification [31], and dripping [33] are also employed. Calcium cross-linking of alginates can be performed by mainly two methods: diffusion method and internal setting method. In the "diffusion" method, the ions diffuse into the alginate solution from an outside reservoir. In the "internal setting" method, the ion source is located within the alginate solution and a controlled trigger (typically pH or solubility of the ion source) sets off the release of cross-linking ions into the solution. The diffusion method

http://dx.doi.org/10.5772/65257

yields gels having a Ca^{2+} ion concentration gradient across the thickness, while internal setting gives gels with uniform ion concentrations throughout [2, 20].

The gelation rate is an important factor that affects the uniformity and strength of hydrogels. Lower rates can be achieved by temperature control (lower temperature implies slower ionic cross-linking), alginate composition (high G content implies higher stiffness), pH control, and Ca^{2+} concentration of calcium solution source.

Calcium chloride ($CaCl_2$) is one of the most frequently used agents to ionically cross-linking alginate. However, it typically leads to rapid and poorly controlled gelation due to its high solubility in aqueous solutions [7]. The fast gelation rate with $CaCl_2$ results in varying cross-linking densities and a polymer concentration gradient within the gel bead. By contrast, the use of $CaCO_3$ and $CaSO_4$, at internal setting method, which has very low solubility in pure water, allows its uniform distribution in alginate solution before gelation occurs [34].

The ionic gelation process (by diffusion method) to produce alginate beads usually is performed by dripping a sodium alginate solution into a $CaCl_2$ bath. This process has been used in both drug delivery and cell encapsulation [34], and to produce adsorbent beads from a blend of sericin-protein/alginate [33, 35]. **Figure 4** shows the schematic process for producing alginate beads.

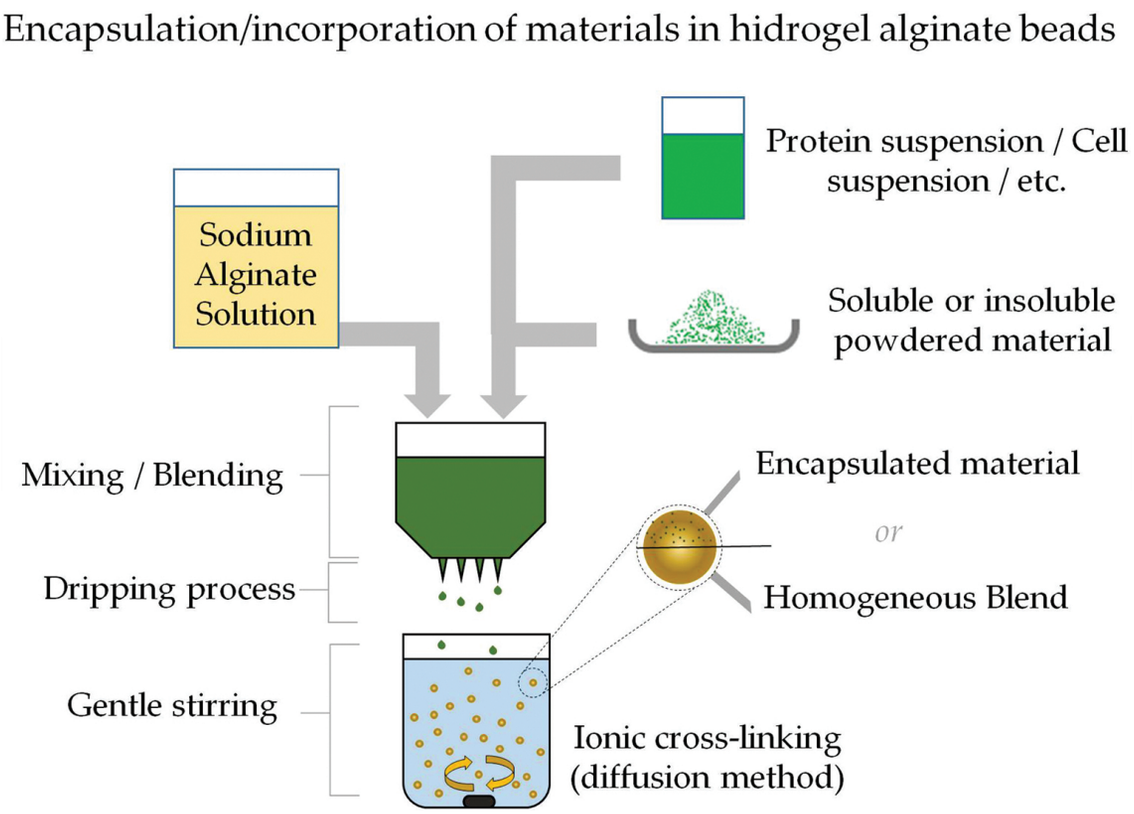


Figure 4. Alginate beads preparation by diffusion setting method.

5.2. Covalent cross-linking

Alginate hydrogels have been attractive for a variety of biomedical applications, but they possess limited mechanical properties when ionically cross-linked with divalent cations [36]. Covalent cross-linking is applied in order to improve physical properties of alginate gels compared with those ionically cross-linked ones [37]. This kind of cross-linking is typically formed by the reaction between carboxylic groups in alginate chains and a cross-linking molecule possessing primary diamines [38]. The use of cross-linking reagents must be carefully investigated because many of them can be toxic and unreacted cross-linkers need to be thoroughly removed from gels [37].

The stress-relaxation behavior of hydrogels is strongly affected by how the polymers are cross-linked. In gels with ionic cross-links, stress relaxes mainly through breaking and subsequent reforming of the ionic cross-links, while in gels with covalent cross-links, stress relaxes mainly through migration of water [38]. The ionic cross-linking stresses lead to plastic deformation of the alginate gel, while the covalent cross-linking leads to a stress relaxation allowing a significant elastic deformation [7].

Covalent cross-linking of alginate with poly(ethylene glycol) (PEG)-diamines of various molecular weights (MWs) generates hydrogels with a range of mechanical properties. Hydrogels with a range of elastic moduli could be generated by controlling either the chain length of the cross-linking molecule or the cross-linking density. The elastic modulus increased gradually with an increase in cross-linking density or weight fraction of PEG in the hydrogel [36].

The introduction of hydrophilic cross-linking molecules as second macromolecules (such as PEG) compensates for the loss of hydrophilic groups in the alginate backbone [37].

Maiti and Sa [39] used ionotropic gelation method for the preparation of ibuprofen-loaded calcium alginate (CALG) and ethylenediamine (EDA)-treated calcium alginate (EDA-CALG) microspheres to investigate drug delivery and the retard of the drug release to some extent. The reduction in drug entrapment efficiency by a maximum of 44.60% for EDA-CALG microspheres compared to untreated CALG microspheres was observed. EDA-CALG microspheres released almost all of its contents within 7 h in pH 6.8 phosphate buffer; however, CALG microspheres were found to release the same within 3 h.

5.3. Alginic acid gels

Alginates may also gel following a third and ion-independent way in that they form acid gels at pH values below the pKa values of the uronic residues [2, 40]. When the pH of alginate solutions is lowered below the pKa of the uronic acids in a highly controlled fashion, acid gels are formed. Such gels, often called “acid gels,” are stabilized by an intermolecular hydrogen-bonding network [20]. With the exception of some pharmaceutical uses, the number of applications of acid gels is rather limited to date [2]. Two methods are generally used to make acid gels: In the first method, a slowly hydrolyzing lactone such as glucono delta-lactone (GDL) is added to a solution of Na-alginate, and in the second method, preformed Ca-alginate gels are converted to acid gels by proton exchange [40].

http://dx.doi.org/10.5772/65257

6. Physical properties

6.1. Molecular weight (MW)

Alginates, like polysaccharides in general, are polydisperse with respect to MW. In this aspect, they resemble synthetic polymers rather than other biopolymers such as proteins and nucleic acids. Because of this polydispersity, the MW of an alginate is an average over the whole distribution of MW [5].

The MW distribution can have implications for the uses of alginates, as low-molecular-weight fragments containing only short G-blocks may not take part in gel-network formation and consequently do not contribute to the gel strength. Furthermore, in some high-tech applications, the leakage of mannuronate-rich fragments from alginate gels may cause problems, and a narrow molecular-weight distribution therefore is recommended [5].

The MW of commercially available sodium alginates ranges between 32,000 and 400,000 g/mol [7]. Usually, a higher MW would result in a higher level of interchain bonding and a greater mechanical gel strength and viscosity [14].

The use of alginate with high MW can improve the physical properties of its gels. However, an alginate solution formed from high-MW polymer becomes greatly viscous, which is often undesirable in processing [7, 41].

For the successful use of hydrogels as cell immobilization/delivery vehicles, a key property that must be satisfied is the maintenance of the viability of the cells through the gel-preparation process. The high viscosity may not be desirable in terms of maintaining cell viability during the pre-gel/cell-mixing process, as a high-solution viscosity would lead to cells being exposed to high shear forces during the mixing. Cell membranes are highly labile to shear forces, and the mixing can lead to damage or cell death [42].

Manipulation of the MW and its distribution can independently control the pre-gel solution viscosity and postgelling stiffness. The elastic modulus of gels can be increased significantly, while the viscosity of the solution minimally raises, by using a combination of high- and low-MW alginate polymers [7, 8].

6.2. Viscosity

Alginates can be prepared with a wide range of molecular weights (50–100,000 kDa), and aqueous solutions of alginates have non-Newtonian characteristics, that is, the viscosity decreases with increasing shear rate (shear thinning). The viscosity of an alginate solution depends on the concentration of the polymer, the MW distribution [27], pH, and G- and M-residues content of alginate.

The viscosity of the alginate solution increases as the molecular weight increases making it difficult to dissolve a high concentration of alginate in a given amount of water. Alginate manufacturers can control the MW (or the degree of polymerization, DP) by varying the severity of the extraction conditions to produce products with viscosities in a 1% solution ranging from 10 to 1000 mPa, with a DP range of 100–1000 units [14].

The viscosity of the alginate solution increases sharply as the concentration increases. However, high-solution viscosities make it difficult to remove bubbles in the solution brought in during the mixing process. For practical purposes, aqueous solutions of alginate have a maximum content of 5–6% of sodium alginate. The temperature of solution also interferes in the viscosity solution. The viscosity decreases as temperature increases (at a rate of 2.5% per degree Celsius). Since viscosity drops sharply on heating, it is useful to heat a solution during the dissolution process. Also, the heating is beneficial since the reduced viscosity helps the bubbles to rise from the solution. However, if alginate solutions are maintained above 50°C for several hours, depolymerization may occur, giving a permanent loss of viscosity and MW [14].

The viscosity of alginate solutions is unaffected over the range pH = 5–11. Below pH = 5, the free $–COO^-$ ions in the chain start to become protonated, to –COOH, and as the electrostatic repulsion between chains is reduced, they are able to come closer and form hydrogen bonds, producing higher viscosities. When the pH is further reduced, a gel will form, usually between pH = 3 and 4. Above pH = 11, slow depolymerization occurs on the storage of alginate solutions, giving a fall in the viscosity [14].

7. Alginate modification

One of the most effective ways to design high-performance biomaterials is by chemically reacting the functional groups available on the alginate backbone [43]. Alginate has the ease of chemical functionalization due to the presence of free hydroxyl and carboxyl groups distributed along the backbone. By forming alginate derivatives through functionalizing available hydroxyl and carboxyl groups, the properties such as solubility, hydrophobicity, and physicochemical and biological characteristics may be modified. Techniques such as oxidation, sulfation, esterification, and amidation can be employed to perform chemical modification of alginate [10]. These chemical modifications allow tailored physical and chemical properties in the modified alginate.

7.1. Oxidation

Alginates may form physical gels under specific conditions, and their functional groups (–OH and –COOH) also allow different chemical and physical modifications. The oxidation on its groups can be performed and the features of new material present important responses. The oxidized alginate presents more reactive groups implying on the modification on its properties, such as a faster degradation, which is important when these ones are used in support for drug-controlled delivery, for instance [44].

Although alginate is an attractive material due to its biocompatibility and ability to form hydrogels, its slow and uncontrollable degradation can be an undesirable feature [45, 46]. Ionically cross-linked alginate hydrogels exhibit a remarkably slow degradation rate, which is typically months to years for their complete removal from injection sites. The alginate oxidation can accelerate the degradation rate improving its property for medical purposes [47].

http://dx.doi.org/10.5772/65257

Oxidized alginates present more reactive groups and a faster degradation when these ones are used in supports for drug-controlled delivery, for example [44].

The periodate oxidation has been used for alginate oxidation and extensively reviewed in literature. Periodate-oxidized alginates are highly susceptible to biodegradation, and therefore oxidized alginates have the potential to be used in a number of biomedical applications wherein biocompatibility and biodegradability are important criteria. Oxidized alginates could also function as potential nontoxic and biodegradable cross-linking agents for proteins in the preparation of hydrogels [48].

When alginate is oxidized by reacting with sodium periodate, the carbon-carbon bonds of the cis-diol groups in the uronate residues are cleaved and changed to dialdehyde groups. Varying the oxidation degree, the degradation rate can be controlled increasing the vulnerability of alginate hydrogels to hydrolysis [47]. The oxidation reaction with sodium periodate on –OH groups at C-2 and C-3 positions of the uronic units of sodium alginate is presented in **Figure 5**.

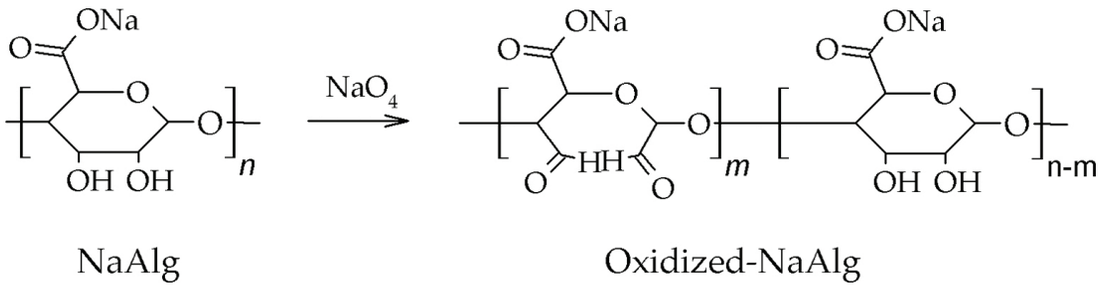


Figure 5. Oxidation of sodium alginate.

The periodate oxidation reactions on –OH groups of the uronic units of alginate leads in a rupture of carbon-carbon bond, to the formation of two aldehyde groups in each oxidized monomeric unit. Therefore, larger rotational freedom and new reactive groups along the backbone are obtained [10].

Gomez et al. [44] conducting a study about the characterization of the oxidized derivatives of sodium alginate (alginate oxidation by sodium periodate) found that the molar mass decreases rapidly until an oxidation of 10 mol% and then remains nearly constant. In addition, the polymers with a degree of oxidation higher than 10 mol% were no more able to form gels with calcium ions. A decrease in the cooperative interactions between calcium ions and carboxylate groups was observed due to the decrease in molar mass and the number of unreacted G units.

7.2. Sulfation

Sulfation of polysaccharides, both enzymatically in nature and by chemical methods, is known to provide blood compatibility and anticoagulant activity [20]. When alginate is sulfated, it will show high blood compatibility because of the structural similarity to that of heparin, which has been widely used for anticoagulant therapy [10]. **Figure 6** presents the sulfation of sodium alginate using chlorosulfonic acid in formamide.

After sulfated modification, the sodium alginate would contain sulfate and carboxyl groups, as the nearest structural analogs of the natural blood anticoagulant heparin [49]. Heparin from animal sources had the potential to induce disease-affecting mammals, such as the avian influenza virus and bovine spongiform encephalopathy [50]. These reasons strongly motivated the necessity to find new anticoagulants and antithrombotics to replace heparin [49].

Ronghua et al. [51] reported the sulfation of sodium alginate using chlorosulfonic acid in formamide. The in vitro coagulation assay of human plasma containing the sulfates indicated that alginate sulfates had considerably high anticoagulant activity especially to the intrinsic coagulation pathway.

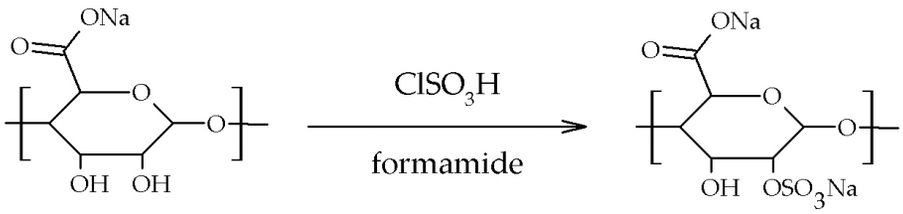


Figure 6. Sulfation of sodium alginate.

7.3. Esterification

Esterification involves the reaction of –COOH groups with –OH groups producing an ester as final product. As shown in **Figure 7**, alginate can be modified by direct esterification with several alcohols in the presence of catalyst and the alcohol is present in excess to ensure that the equilibrium is in favor of product formation. This method was successfully used by researchers to modify native alginate, increasing its hydrophobic nature by the addition of alkyl groups to the backbone of the native alginate [10].

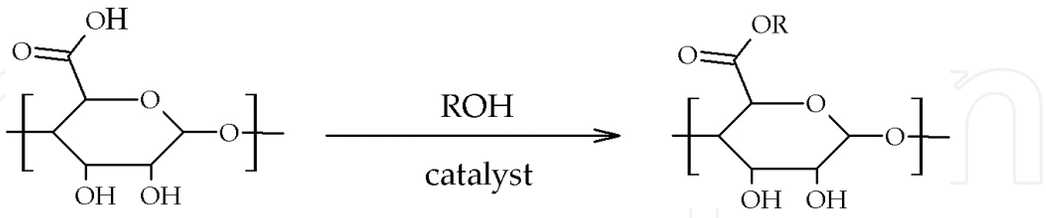


Figure 7. Esterification of alginate.

The only synthetic derivative of alginic acid to find wide use, and acceptance as a food additive, is propylene glycol alginate. This is formed by reacting propylene oxide with moist alginic acid. Esterification occurs at the carboxylic acid groups on the alginate chain, mainly with the primary hydroxyl group of propylene glycol. Depending on the reaction conditions, such as reaction temperature and ratios of propylene oxide to alginic acid, varying degrees of esterification can be achieved. A product with about 60–70% esterification is satisfactory for

most purposes but up to about 90% esterification can be achieved and this type of product (80–90%) is useful in very acidic, short-term applications [13].

7.4. Ugi reaction

Pure alginate has its inherent drawbacks, such as rigid backbone, poor mechanical strength, uncontrolled degradation, and extensive water uptake properties, which restricts its practical applications. As a result of available hydroxyl and carboxyl groups, chemical modification of alginate could be achieved mostly at the two secondary hydroxyl positions (C-2 and C-3) or the one carboxyl (C-6) position via the acetylation, phosphorylation, sulfation oxidation, esterification, amidation, and Ugi reaction [52].

The Ugi reaction is an important reaction used in combinatorial chemistry, and it is a multi-component reaction in organic chemistry involving a ketone or an aldehyde, an amine, an isocyanide, and a carboxylic acid to form a bisamide [10, 53]. Among the chemical modification methods, the Ugi four-component condensation reaction is the most effective and unique that could endow alginate with specific property without the aid of the catalyst [52]. The Ugi multicomponent reactions lead a hydrophobic behavior to the modified alginate [10].

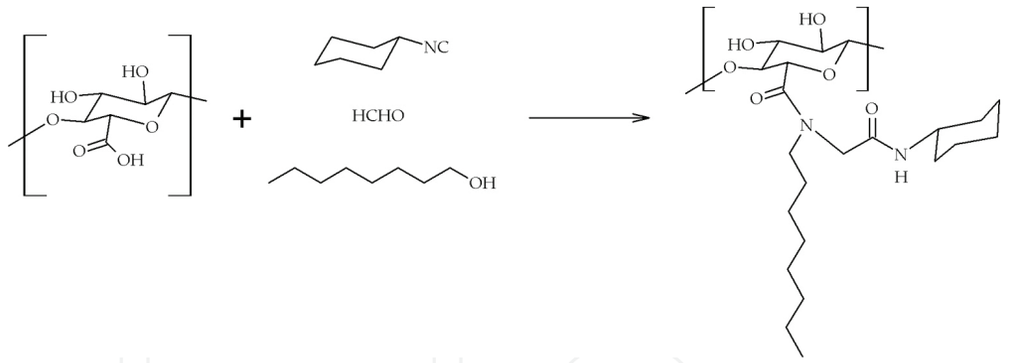


Figure 8. Ugi four-component reaction.

Amphiphilic alginate derivative modified by introducing the hydrophobic groups onto its hydrophilic backbone could enhance its affinity for the hydrophobic drug, thus making it an attractive candidate for drug delivery [52].

The Ugi reaction procedure involves the preparation of aqueous alginate solution followed by acidification (HCl, pH 3.6) to allow the Ugi reaction. The n-octylamine groups are added with respect of molar amount of carbohydrate monomers. Formaldehyde, n-octylamine, and cyclohexyl isocyanide are added to the solution successively. After the addition, the solution is stirred for 24 h. Unreacted monomers and other low-molecular weight impurities are removed from solution by dialysis, and thereafter the solution is freeze-dried. **Figure 8** presents the Ugi four-component reaction.

8. Blend formation

Alginates are established among the most versatile biopolymers, used in a wide range of applications [54]. It can be easily modified in any form, such as hydrogels, microspheres, microcapsules, sponges, foams, and fiber [55], and can cross-link, copolymerize, and blend with other polymers due its polar side chain made of hydroxyl and carboxyl groups [33, 56].

The possibility of alginate modifications (including blending) can increase the applications of alginate in various fields such as tissue engineering, drug delivery [57], environmental [33], and others. The combination of favorable properties of each constituent polymer results in a new hybrid system with the properties that are often significantly improved or substantially different from those of the individual polymers [58]. Alginate beads can be prepared easily through simple and economic procedures; these beads suffer from low drug encapsulation and poor mechanical property in the intestinal pH, which lead to rapid drug release [59]. Therefore, modified alginate beads using sodium alginate and natural polysaccharide blends have been investigated to improve drug encapsulation, swelling, mucoadhesion, drug release, and others. Okra (*Hibiscus esculentus*) is an annual plant cultivated throughout the tropical and subtropical areas of the world, and it is chemically inert, nonirritant, biodegradable, and biocompatible. Okra gum is already investigated as useful excipients in the development of pharmaceutical formulations, and its highly viscous property leads to the usefulness of it as a drug-release-retarding polymer [60]. Sinha et al. [60] developed controlled drug release Zn^{2+}-ion-induced alginate-okra gum blend beads by ionic-gelation-cross-linking method using zinc sulfate ($ZnSO_4$) as a cross-linker in an aqueous environment. These beads exhibited sustained in vitro drug release over a prolonged period of 8 h and followed controlled-release pattern. The swelling and degradation of the optimized beads was influenced by the pH of test media, which might be suitable for intestinal drug delivery.

Alginate application in wastewater treatment is limited due its tendency to swell in water and other mechanical weakness. In this way, alginate can be blended with other polymers to overcome the drawbacks of alginate and to combine the good characteristics of both polymers [61]. Chitosan is a polysaccharide biopolymer derived from chitin [62]. It is well established as an excellent natural adsorbent due to the presence of the amino ($–NH_2$) and hydroxyl (–OH) groups, and has also other useful features such as being polycationic, nontoxic, biodegradable, and antibacterial properties. However, due to its weak mechanical property, chemical and physical modifications are carried out on chitosan [61]. Alginate can be easily blended with chitosan by the strong electrostatic interaction between the amino groups of chitosan and the carboxyl groups of alginate [63]. Dubey et al. [64] developed chitosan-alginate nanoparticles using microemulsion method for the removal of Hg (II) ions from aqueous solution. The results obtained in this study proved that the prepared biopolymer nanomaterial could be an effective and economically viable adsorbent for the removal of Hg (II) ions. Moreover, the nanoparticles can be regenerated and reused subsequently for the metal removal.

Sericin is a water-soluble globular protein that is easily soluble in hot or boiling water and is extracted from the silkworm *Bombyx mori* cocoons [65]. Most of the sericin is removed during the silk processing and it is usually discarded in the wastewater [66]. This fact leads to

http://dx.doi.org/10.5772/65257

environmental contamination due to the high oxygen demand for its degradation by microbes. Therefore, finding viable means to recover sericin would bring environmental and economic benefits [67]. Sericin isolated presents weak structural properties, but the presence of polar side chain made of hydroxyl, carboxyl, and amino groups in this protein enables easy cross-linking, copolymerization, and blending with other polymers to obtain biodegradable products with better properties [33, 56]. The use of blends provides an improvement in the physical characteristics of the materials produced with the protein, and the use of alginate has an advantage because it has the affinity for a variety of cations [33]. Polymers such as silk sericin and alginate can undergo a sol-gel transition by thermal cross-linking (by heating) [68], photo-cross-linking, and with pH variation, depending on β-sheet content in case of sericin [69].

Other applications of the alginate and sericin blend will be discussed in the following topics.

9. Applications of alginate and sericin blend in the pharmaceutical and environmental fields

In the work of Khandai et al. [70], sericin was evaluated as release retardant along with sodium alginate in aceclofenac-sustained release mucoadhesive microsphere formulation. Micro-particulate drug delivery of aceclofenac was prepared by gelation technique using a blend of sodium alginate and sericin as release retardant. All the formulations developed showed diffusion type of release mechanism in a sustained manner. Thus, the aceclofenac microsphere helped to increase the patient compliance, decrease the dosing frequency, and also prevent gastric hemorrhage which is commonly found to be associated with conventional dosage form.

Khampieng et al. [71] assessed the anti-inflammatory efficacy and preparation of silk sericin-loaded alginate nanoparticles that were prepared by the emulsification method followed by internal cross-linking. This study confirms the hypothesis that the topical application of silk sericin-loaded alginate nanoparticle gel can inhibit inflammation induced by carrageenan.

Sericin-alginate microbeads were developed by Nayak et al. [72] via ionotropic gelation under high voltage, and the beads were coated with chitosan and cross-linked with genipin, for the purpose of encapsulating hepatocytes for advanced cellular functions. This study suggests that the developed sericin-alginate-chitosan microcapsule contributes toward the development of cell encapsulation model. It also offers to generate enriched population of metabolically and functionally active cells for the future therapeutics especially for hepatocytes transplantation in acute liver failure.

Silva et al. [33] studied the adsorption of copper and zinc by particles produced from silk sericin and alginate blend. The results obtained suggest the potential use of sericin-alginate particles, cross-linked by ionic gelation and by heat, to adsorption processes of toxic metals, zinc and copper.

10. Case study

10.1. Case study 1: incorporation and release of diclofenac sodium in sericin and alginate blend

10.1.1. Drug incorporation and preparation of particles

In this study, sericin was extracted from silkworm cocoons (*B. mori*), and the concentration of the sericin solution (SS) extracted was adjusted to 2.5% (w/v). In order to prepare the sericin/alginate blend, the sodium alginate was added to the sericin solution and the mixture was stirred at 4000 rpm. The drug incorporation was performed by adding diclofenac sodium (DS) to the sericin/alginate blend and dispersed with an Ultraturrax® (T18, IKA, USA) at 8000 rpm until homogeneity was obtained. The ionic gelation method was used to prepared particles with different compositions [73]. For this, a mixture of sericin, alginate, and DS was added dropwise to a calcium chloride solution (3% w/v) and stirred continuously. After dripping, the particles were stirred at 100 rpm for 30 min, and then washed with deionized water and dried at room temperature.

10.1.2. Determination of incorporation efficiency

In order to determine the incorporation efficiency, accurately weighed 0.1 g of dried particles was added to 500 mL of phosphate buffer (pH 6.8), and kept overnight. Hence, the suspension was subjected to agitation for 15 min in a sonicator (1510RMTH, Branson, USA) and filtered through a 0.45-μm filter. The DS content in the filtrate was determined by spectrophotometer (UVmini1240, Shimadzu, Japan) at 276 nm. All determinations were carried out in triplicate. The incorporation efficiency was calculated by

$$\text{Incorporation} = \frac{\text{practical DS content}}{\text{theoretical DS content}} \times 100 \quad (1)$$

Table 2 shows the effect of sericin and alginate concentration in the DS incorporation efficiency of the formulations developed. It was noticed that the incorporation efficiency increased by increasing sericin proportion in the blend; therefore, sericin significantly contributes to the incorporations of the DS.

Formulation	Sericin (% w/v)	Alginate (% w/v)	DS (% w/v)	Incorporation efficiency (%)
F1	2.5	1.25	2.0	91.1 ± 2.4
F2	2.5	2.60	2.0	82.5 ± 3.6
F3	2.5	3.30	2.0	77.9 ± 2.1
F4	–	4.00	2.0	75.5 ± 2.1

Table 2. Effect of blend composition in the DS incorporation efficiency [73].

http://dx.doi.org/10.5772/65257

The surface morphology of the sericin/alginate/DS particles (F1, F2, and F3) and alginate/DS particles (F4) was visualized by scanning electron microscopy (SEM) with the magnification of 150× and is presented in **Figure 9**. By analyzing the F1 micrograph, it was found that it has no clear-cut boundaries and has poorly defined shape, which does not favor the reproducibility of these particles. Spherical particles with a rough and rugged surface were observed in F2 and F3 micrographs. F4 micrographs indicated oval particles and a very rough and rugged surface too. Thus, it was evident from the SEM micrographs that balanced concentrations of sericin and alginate, as in F2 and F3, favor the sphericity of the particles. Besides, it is verified that roughness increased by increasing alginate proportion in the blend. It can be inferred that the F4 particles (alginate/DS) possibly release the drug in its dissolution medium (gastric or enteric) more rapidly when compared to particles containing sericin in its composition, since the surface has greater roughness and therefore its contact surface is higher. Consequently, particles containing sericin can contribute to the sustained release of the DS present in the matrix and maybe reduce the drug side effects.

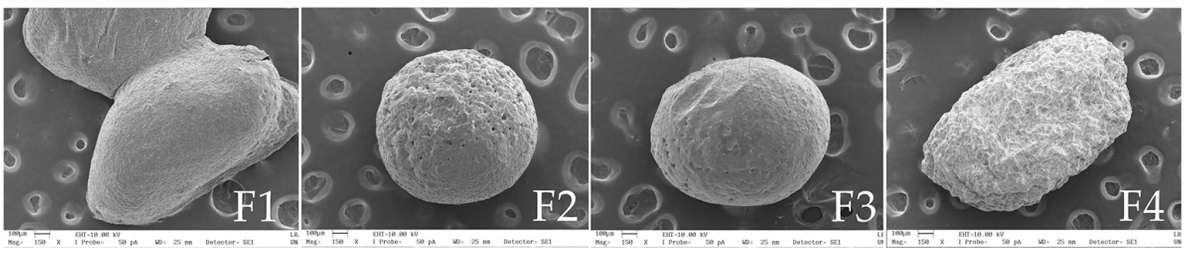


Figure 9. Micrographs of formulations evaluated (as shown in **Table 2**).

10.1.3. In vitro drug release study

According to the methodology recommended by the US Pharmacopeia, the *in vitro* release of DS was tested using a dissolution apparatus. The dissolution was measured at 37 ± 1°C under 50 rpm speed. Accurately weighed quantities of sericin/alginate/DS particles equivalent to 50 mg DS were added to 900 mL of dissolution medium. The test was carried out in 0.1 M HCL (pH 1.2) for 2 h, and then continued in phosphate buffer (pH 6.8) for the next 10 h. Aliquots of 5 mL were collected at regular time intervals, and the same amounts of fresh dissolution medium were replaced into a dissolution vessel. The collected aliquots were filtered, and suitably diluted to determine the absorbance using an ultraviolet-visible infrared spectroscopy (UV-VIS) spectrophotometer at 276 nm.

Table 3 shows the percentage of DS released after 2 h of the dissolution test in acid medium, for F2, F3, and F4. Whereas F1 is nonreproductive, dissolution tests were not conducted for this formulation.

It was found that all formulations were resistant to the gastric medium. Among all studied formulations, F4 presented the lowest drug release in gastric medium. Because F4 has only alginate and DS in its formulation, the low drug release observed in other formulations can be attributed to the presence of this polysaccharide in all composition.

Formulation	DS release (%)
F2	2.63 ± 0.66
F3	1.92 ± 0.36
F4	1.49 ± 0.26

Table 3. Diclofenac sodium release in acid medium, pH 1.2.

Figure 10 shows the diclofenac sodium dissolution profiles for F2, F3, and F4. It can be seen that the presence of sericin in the formulations causes a prolonged drug release compared to the formulation without sericin (F4), which releases all incorporated drug at 45 min. Formulations containing sericin in the composition (F2 and F3) showed the total drug release between 240 and 300 min of dissolution in simulating enteric medium (phosphate buffer, pH 6.8).

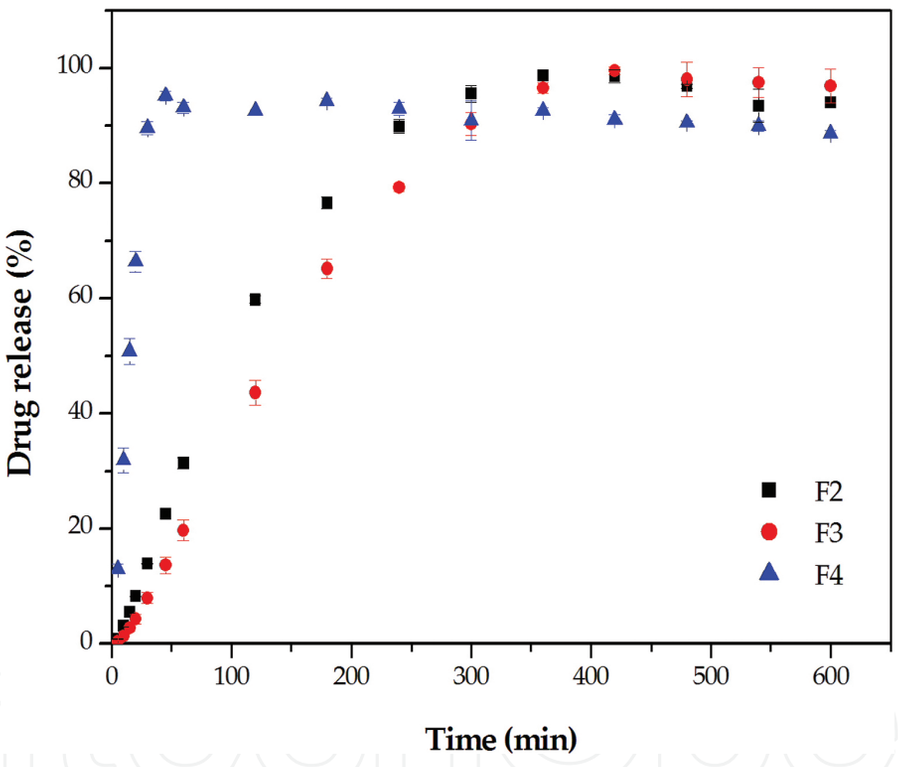


Figure 10. Diclofenac sodium dissolution profile in pH 6.8.

10.2. Case study 2: metallic affinity of toxic and noble metals by particles produced from blends of sericin, alginate and poly(ethylene glycol) diglycidyl

In this study, particles were produced from sericin-alginate blend, by diffusion method, and the evaluation of metallic affinity toward toxic and noble metals by particles was performed. Also, the influence of the presence of the poly(ethylene glycol) diglycidyl ether, as cross-linking agent, in the blend was investigated. The particles were produced dripping the blends in aqueous and alcoholic (ethanol) solutions of $CaCl_2$ and $Ca(NO_3)_2$, providing different sources

of divalent ion, and then the particles were dried first at 40°C (24 h) and then at 100°C (24 h). The metallic affinity for toxic metals: copper (Cu^{2+}), nickel (Ni^{2+}), cadmium (Cd^{2+}), zinc (Zn^{2+}), lead (Pb^{2+}), and chromium (Cr^{3+}), and noble metals: palladium (Pd^{2+}), platinum (Pt^{4+}), gold (Au^{3+}), and silver (Ag^{+}) were investigated in this work [74].

10.2.1. Particles preparation

The sericin was obtained by autoclave extraction (40 min, 1 kgf/cm^2) from silkworm cocoons. All cocoons were cleaned, washed (in deionized water), and cut into pieces about 1 cm^2 before the extraction procedure (the ratio of cocoons and ultrapure water was 40 g of cocoons to 1000 mL of water). The extracted sericin solution, still hot, was filtered to remove the fibers of fibroin and stored in a sealed bottle at room temperature for 12 h [35, 74]. After this period, the SS solution was frozen for, at least, 24 h in a conventional refrigerator (-4°C) and then it was thawed at room temperature. This procedure was performed in order to separate the sericin from diluted solution. The freezing procedure promotes the precipitation of sericin, which can be recovered by filtration. The sericin was heated in autoclave (120°C for 10 min) to solubilize again the protein and then the concentration was adjusted by dilution to 25 g/L.

Particle	Blend formulation (g/L) [sericin/alginate/PEG]	Calcium solution
1	25/20/0	Aqueous solution of $CaCl_2$
2	25/20/0	Alcoholic solution of $CaCl_2$
3	25/20/0	Aqueous solution of $Ca(NO_3)_2$
4	25/20/0	Alcoholic solution of $Ca(NO_3)_2$
5	25/20/5	Aqueous solution of $CaCl_2$
6	25/20/5	Alcoholic solution of $CaCl_2$
7	25/20/5	Aqueous solution of $Ca(NO_3)_2$
8	25/20/5	Alcoholic solution of $Ca(NO_3)_2$

Table 4. Formulations and methods of preparations of the produced particles.

The sodium alginate (Sigma-Aldrich brand) was added in adjusted SS in a concentration of 20 g/L. The blend was mixed at 5000 rpm (Ultraturrax® T18—USA) until it was homogeneous. In the particle formulations containing PEG, the cross-linking agent was added in the blend in a concentration of 5 g/L, being mechanically agitated for 30 min. The particles were prepared by ionic gelation technique (diffusion method) where the blend was dripped with a peristaltic pump in alcoholic (ethanol) and aqueous solutions of $CaCl_2$ (3% m/V) and $Ca(NO_3)_2.4H_2O$ (6.4% m/V) (same concentration of Ca^{2+} in both solutions). The particles were agitated in Jar test for 12 h in Ca^{2+} solution. The produced particles were rinsed in deionized water and dried at a continuous flow oven at 40°C, and then submitted to thermal cross-

linked at 100°C for 24 h [33]. **Table 4** presents the blend formulation and the respective calcium solution used to produce particles.

10.2.2. Metal affinity tests of single components

Metal solutions of 1 mmol/L of toxic metals: copper (Cu^{2+}), nickel (Ni^{2+}), cadmium (Cd^{2+}), zinc (Zn^{2+}), lead (Pb^{2+}), and chromium (Cr^{3+}), and noble metals: palladium (Pd^{2+}), platinum, gold, and silver (Ag^{+}) were prepared and for the respective affinity tests 0.5 g of each particle formulated was immersed in 50 mL of each metal solution. The particles were maintained in contact with metal solutions under agitation (200 rpm) for 24 h at 25°C. The metal concentrations, before and after adsorption process, were measured at atomic absorption spectroscopy (AAS – 7000A – Shimadzu) according to equipment instructions.

The percentage of metal removal (%*R*) of each was determined by Eq. (2)

$$\%R = \frac{C_0 - C_e}{C_0} \times 100 \tag{2}$$

10.2.3. Adsorption results

From **Table 5**, it can be seen that the adsorption process to toxic metal, Cu, Cd, Pb, and Cr, presents high percentage reduction. Obtained results of low-removal values indicate that the adsorption of nickel and zinc by sericin/alginate and sericin/alginate/PEG particles is not very effective. With exception to silver, which results were slightly lower than the general results observed for Cu, Cd, Pb, and Cr metal ions, the results observed to noble metals (Pd, Pt, and Au) showed greater values than the ones observed to toxic metals.

Particle	Pd	Pt	Au	Ag	Cu	Cd	Ni	Pb	Zn	Cr
1	87.1	73.1	98.9	–	65.0	62.2	14.8	65.9	15.6	73.2
2	88.7	71.8	99.2	–	73.9	70.7	15.5	82.0	21.9	71.0
3	88.6	66.4	99.4	61.3	74.4	72.1	23.5	80.0	24.1	74.4
4	88.9	70.3	99.2	60.4	71.4	73.6	14.0	83.2	28.5	72.0
5	86.8	74.4	99.3	–	74.9	70.7	19.7	82.3	17.7	73.3
6	86.9	73.7	99.7	–	72.8	70.9	12.0	81.8	22.3	71.8
7	88.9	71.6	99.7	63.4	73.1	79.6	23.7	82.8	27.8	73.7
8	89.0	73.2	99.7	61.0	74.3	79.6	13.3	83.3	30.2	69.6

Table 5. Percentage removal (%*R*) of the metal concentration in solution by sericin/alginate/PEG particles.

In general, the particles containing PEG in its formulation (i.e., particles 5–8), exhibit a slightly higher range of %*R*. For example, copper is removed within the observed range (65.0–73.9%) when particles without PEG were used and comparatively within the range (72.9–74.9%) for

the particles with PEG. PEG possesses as amphipathic behavior, and the introduction of this cross-linking agent develop modifications in particle structure. Thus, the modifications promoted by PEG in the structure of the particles seem to improve the capacity of the adsorbent.

10.2.4. Considerations

Sericin consists of 17–18 kinds of amino acids with large amount of polar side chains made of hydroxyl, carboxyl, and amino groups. Alginate presents the polar groups hydroxyl and carboxyl along the chain backbone. The presence of these kinds of groups enables this polymer to ionic and covalent cross-linking, allowing interactions and linkages between the proteins and polysaccharide chains, and also allows interactions with pollutants (such as toxic metal) and pharmaceutical.

Despite the interesting properties that sericin and alginate present, there are few works dedicated to study biomaterials that use the blend of both polymers as raw material.

Concerning pharmaceutical field, the development of a new pharmaceutical form of diclofenac sodium-modified release based on alginate-sericin blends is intended to solve current problems related to its therapeutic administration. These problems are related to the development of gastric irritations and decrease of therapeutic effects by partial degradation in acid medium that occurs in the usual forms.

As a bioadsorbent material, or a matrix to drug incorporation or to improve the therapeutic effects of some pharmaceuticals, there are many potential uses for the sericin-alginate blend.

Acknowledgements

The authors thank the BRATAC Company for providing the silkworm cocoons, Geolab® Indústria Farmacêutica S/A for providing the drugs, CNPq (Proc. 470615/2013-3, 473808/2012-9 and 300986/2013-0), CAPES, FAPESP (Proc. 2015/13505-9, 2014/26355-2 and 2011/51824-8), and Fundação Araucária (Proc. 22597-257/12) for financial support.

Author details

Thiago Lopes da Silva[1], Jacyara Moreira Martins Vidart[1], Meuris Gurgel Carlos da Silva[1], Marcelino Luiz Gimenes[2] and Melissa Gurgel Adeodato Vieira[1*]

*Address all correspondence to: melissagav@feq.unicamp.br

1 Department of Process and Products Design, Chemical Engineering School, University of Campinas, Campinas, SP, Brazil

2 Department of Chemical Engineering, State University of Maringá, Maringá, PR, Brazil

References

[1] McHugh DJ. A guide to the seaweed industry. FAO Fisheries Technical Paper. 2003. 105 p.

[2] Draget KI, Taylor C. Chemical, physical and biological properties of alginates and their biomedical implications. Food Hydrocoll. 2011;25(2):251–6. http://dx.doi.org/10.1016/j.foodhyd.2009.10.007.

[3] Grasdalen H, Larsen B, Smidsrød O. 13C NMR studies of alginate. Carbohydr Res. 1977;56:C11–5. DOI: 10.1016/S0008-6215(00)83369-8.

[4] Haug A, Larsen B, Smidsrød O. A study of the constitution of alginic acid by partial acid hydrolysis. Acta Chem Scand. 1966;20:183–90. DOI: 10.3891/acta.chem.scand.20-0183.

[5] Draget K, Smidsrød O, Skjåk-Bræk G. Alginates from algae. Biopolym Online. 2005;6:1–30. http://onlinelibrary.wiley.com/doi/10.1002/3527600035.bpol6008/full.

[6] Grasdalen H, Larsen B, Smidsrød O. A p.m.r. study of the composition and sequence of uronate residues in alginates. Carbohydr Res. 1979;68(1):23–31. DOI: 10.1016/S0008-6215(00)84051-3.

[7] Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012;37(1):106–26. http://dx.doi.org/10.1016/j.progpolymsci.2011.06.003.

[8] Kong H-J, Lee KY, Mooney DJ. Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration. Polymer. 2002;43(23):6239–46. http://dx.doi.org/10.1016/S0032-3861(02)00559-1.

[9] Grant GT, Morris ER, Rees DA, Smith PJC, Thom D. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Lett. 1973;32(1):195–8. http://dx.doi.org/10.1016/0014-5793(73)80770-7.

[10] Yang JS, Xie YJ, He W. Research progress on chemical modification of alginate: A review. Carbohydr Polym. 2011;84(1):33–9. http://dx.doi.org/10.1016/j.carbpol.2010.11.048.

[11] Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 2003;37(18):4311–30. http://dx.doi.org/10.1016/S0043-1354(03)00293-8.

[12] Kim SK. Handbook of marine macroalgae: Biotechnology of applied phycology. 2nd ed. John Wiley & Sons; 2011. 736 p.

[13] McHugh DJ. Production and utilization of products from commercial seaweeds. (288) ed. FAO Fisheries Technical Paper, editor. 1987. 189 p.

[14] Qin Y. Alginate fibers: An overview of the production processes and applications in wound management. Polym Int. 2008;57:171–80. DOI: 10.1002/pi.2296.

[15] Latifi AM, Nejad ES, Babavalian H. Comparison of extraction different methods of sodium alginate from brown alga *Sargassum* sp. Localized in the southern of Iran. J Appl Biotechnol Rep. 2015;2(2):251–5.

[16] Sanchez-Machado DI, Lopez-Cervantes J, Lopez-Hernandez J, Paseiro-Losada P, Simal-Lozano J. Determination of the uronic acid composition of seaweed dietary fibre by HPLC. Biomed Chromatogr. 2004;18(2):90–7. http://dx.doi.org/10.1002/bmc.297.

[17] Haug A, Larsen B, Fykse O, Block-Bolten A, Toguri JM, Flood H. Quantitative determination of the uronic acid composition of alginates. Acta Chem Scand. 1962;16:1908–18. DOI: 10.3891/acta.chem.scand.16-1908.

[18] Torres MR, Sousa APA, Silva Filho EAT, Melo DF, Feitosa JPA, de Paula RCM, et al. Extraction and physicochemical characterization of Sargassum vulgare alginate from Brazil. Carbohydr Res. 2007;342(14):2067–74. http://dx.doi.org/10.1016/j.carres.2007.05.022.

[19] Minghou J., Yujun W., Zuhong X, It I. Studies on the M:G ratios in alginate. Hydrobiologia. 1984;116(1): 554–6. DOI:10.1007/BF00027745.

[20] Pawar SN, Edgar KJ. Alginate derivatization: A review of chemistry, properties and applications. Biomaterials. 2012;33(11):3279–305. http://dx.doi.org/10.1016/j.biomaterials.2012.01.007.

[21] Vauchel P, Kaas R, Arhaliass A, Baron R, Legrand J. A new process for the extraction of alginates from *Laminaria digitata*: Reactive extrusion. Food Bioprocess Technol. 2008;1(3):297–300. DOI: 10.1007/s11947-008-0082-x.

[22] Ruvinov E, Cohen S. Alginate biomaterial for the treatment of myocardial infarction: Progress, translational strategies, and clinical outlook. From ocean algae to patient bedside. Adv Drug Deliv Rev. 2016;96:54–76. http://dx.doi.org/10.1016/j.addr.2015.04.021

[23] Bertagnolli C, Uhart A, Dupin JC, da Silva MGC, Guibal E, Desbrieres J. Biosorption of chromium by alginate extraction products from *Sargassum filipendula*: Investigation of adsorption mechanisms using X-ray photoelectron spectroscopy analysis. Bioresour Technol. 2014;164:264–9. http://dx.doi.org/10.1016/j.biortech.2014.04.103.

[24] Daemi H, Barikani M. Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. Sci Iran. 2012;19(6):2023–8. http://dx.doi.org/10.1016/j.scient.2012.10.005.

[25] Morris ER, Rees AD, Thom D. Characterisation of alginate composition and block structure by circular dichroism. Carbohydr Res. 1980;81:305–14. DOI:10.1016/S0008-6215(00)85661-X.

[26] Haug A, Larsen B, Smidsrød O. Uronic acid sequence in alginate from different sources. Carbohydr Res. 1974;32(2):217–25. DOI:10.1016/S0008-6215(00)82100-X.

[27] Augst AD, Kong 1 HJ, Mooney DJ. Alginate hydrogels as biomaterials. Macromol Biosci.2006;6(8):623–33. DOI: 10.1002/mabi.200600069.

[28] Jang LK, Harpt N, Grasmick D, Vuong LN, Geesey G. A two-phase model for determining the stability constants for interactions between copper and alginic acid. J Phys Chem. 1990;94(1):482–8. DOI: 10.1021/j100364a083.

[29] Donati I, Holtan S, Mørch YA, Borgogna M, Dentini M, Skjåk-Bræk G. New hypothesis on the role of alternating sequences in calcium-alginate gels. Biomacromolecules. 2005;6(2):1031–40. DOI: 10.1021/bm049306e.

[30] Mørch ÝA, Donati I, Strand BL, Skjåk-Bræk G. Effect of Ca^{2+}, Ba^{2+}, and Sr^{2+} on alginate microbeads. Biomacromolecules. 2006;7(5):1471–80. DOI: 10.1021/bm060010d.

[31] Silva MGC, Canevesi RLS, Welter RA, Vieira MGA, da Silva EA. Chemical equilibrium of ion exchange in the binary mixture Cu^{2+} and Ca^{2+} in calcium alginate. Adsorption. 2015;21(6):445–58. DOI: 10.1007/s10450-015-9682-8.

[32] Tu J, Bolla S, Barr J, Miedema J, Li X, Jasti B. Alginate microparticles prepared by spray coagulation method: Preparation, drug loading and release characterization. Int J Pharm. 2005;303(1):171–81. http://dx.doi.org/10.1016/j.ijpharm.2005.07.008.

[33] Silva TL, da Silva AC, Vieira MGA, Gimenes ML, da Silva MGC. Biosorption study of copper and zinc by particles produced from silk sericin–alginate blend: Evaluation of blend proportion and thermal cross-linking process in particles production. J Clean Prod. 2015;1–9. http://dx.doi.org/10.1016/j.jclepro.2015.05.067.

[34] Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. Biomaterials. 2001;22(6):511–21. http://dx.doi.org/10.1016/S0142-9612(00)00201-5.

[35] Silva TL, da Silva AC, Vieira MGA, Gimenes ML, da Silva MGC. Production and physicochemical characterization of microspheres made from sericin and alginate blend. Chem Eng Trans. 2014;39:643–8. DOI: 10.3303/CET1439108.

[36] Eiselt P, Lee KY, Mooney DJ. Rigidity of two-component hydrogels prepared from alginate and poly(ethylene glycol)-diamines. Macromolecules. 1999;32(17):5561–6. DOI: 10.1021/ma990514m.

[37] Reis RL, Neves NM, Mano JF, Gomes ME, Marques AP, Helena S. Azevedo. Natural-based polymers for biomedical applications. Woodhead Publishing Series in Biomaterials; 2008. 832 p.

[38] Zhao X, Huebsch N, Mooney DJ, Suo Z. Stress-relaxation behavior in gels with ionic and covalent crosslinks. J Appl Phys. 2010;107(6):1–5. DOI: 10.1063/1.3343265.

[39] Maiti S, Sa B. Preparation and characterization of ibuprofen-loaded alginate microspheres using ethylenediamine as a crosslinker. Orient Pharm Exp Med. 2008;8(2):178–86. DOI 10.3742/OPEM.2008.8.2.178.

[40] Draget KI, Bræk GS, Smidsrød O. Alginic acid gels: The effect of alginate chemical composition and molecular weight. Carbohydr Polym. 1994;25(1):31–8. DOI: 10.1016/0144-8617(94)90159-7.

[41] LeRoux MA, Guilak F, Setton LA. Compressive and shear properties of alginate gel: Effects of sodium ions and alginate concentration. J Biomed Mater Res. 1999;47(1):46–53.

[42] Kong HJ, Smith MK, Mooney DJ. Designing alginate hydrogels to maintain viability of immobilized cells. Biomaterials. 2003;24(22):4023–9. http://dx.doi.org/10.1016/S0142-9612(03)00295-3.

[43] Pawar SN, Edgar KJ. Alginate esters via chemoselective carboxyl group modification. Carbohydr Polym. 2013;98(2):1288–96. http://dx.doi.org/10.1016/j.carbpol.2013.08.014.

[44] Gomez CG, Rinaudo M, Villar MA. Oxidation of sodium alginate and characterization of the oxidized derivatives. Carbohydr Polym. 2007;67(3):296–304. http://dx.doi.org/10.1016/j.carbpol.2006.05.025.

[45] Gao C, Liu M, Chen J, Zhang X. Preparation and controlled degradation of oxidized sodium alginate hydrogel. Polym Degrad Stab. 2009;94(9):1405–10. http://dx.doi.org/10.1016/j.polymdegradstab.2009.05.011.

[46] Boontheekul T, Kong HJ, Mooney DJ. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials. 2005;26(15):2455–65. DOI: 10.1016/j.biomaterials.2004.06.044.

[47] Jeon O, Alt DS, Ahmed SM, Alsberg E. The effect of oxidation on the degradation of photocrosslinkable alginate hydrogels. Biomaterials. 2012;33(13):3503–14. DOI: 10.1016/j.biomaterials.2012.01.041.

[48] Balakrishnan B, Lesieur S, Labarre D, Jayakrishnan A. Periodate oxidation of sodium alginate in water and in ethanol-water mixture: A comparative study. Carbohydr Res. 2005;340(7):1425–9. http://dx.doi.org/10.1016/j.carres.2005.02.028.

[49] Fan L, Jiang L, Xu Y, Zhou Y, Shen Y, Xie W, et al. Synthesis and anticoagulant activity of sodium alginate sulfates. Carbohydr Polym. 2011;83(4):1797–803. http://dx.doi.org/10.1016/j.carbpol.2010.10.038.

[50] Mendes SF, Santos O dos, Barbosa AM, Vasconcelos AFD, Aranda-Selverio G, Monteiro NK, et al. Sulfonation and anticoagulant activity of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 grown on fructose. Int J Biol Macromol. 2009;45(3):305–9. http://dx.doi.org/10.1016/j.ijbiomac.2009.06.004.

[51] Ronghua H, Yumin D, Jianhong Y. Preparation and in vitro anticoagulant activities of alginate sulfate and its quaterized derivatives. Carbohydr Polym. 2003;52(1):19–24. http://dx.doi.org/10.1016/S0144-8617(02)00258-8.

[52] Yan H, Chen X, Li J, Feng Y, Shi Z, Wang X, et al. Synthesis of alginate derivative via the Ugi reaction and its characterization. Carbohydr Polym. 2016;136:757–63. http://dx.doi.org/10.1016/j.carbpol.2015.09.104.

[53] Ugi I. The α-addition of immonium ions and anions to isonitriles accompanied by secondary reactions. Angew Chem. 1962;I(1):8–21. DOI: 10.1002/anie.196200081.

[54] Tønnesen HH, Karlsen J. Alginate in drug delivery systems. Drug Dev Ind Pharm. 2002;28(6):621–30. http://dx.doi.org/10.1081/DDC-120003853.

[55] Sun J, Tan H. Alginate-based biomaterials for regenerative medicine applications. Materials. 2013;6(4):1285–309. DOI:10.3390/ma6041285.

[56] Dash BC, Mandal BB, Kundu SC. Silk gland sericin protein membranes: Fabrication and characterization for potential biotechnological applications. J Biotechnol. 2009;144(4):321–9. http://dx.doi.org/10.1016/j.jbiotec.2009.09.019.

[57] Venkatesan J, Bhatnagar I, Manivasagan P, Kang K, Kim S. Alginate composites for bone tissue engineering: A review. Int J Biol Macromol. 2014;72C:269–81. http://dx.doi.org/10.1016/j.ijbiomac.2014.07.008.

[58] Zhang Y, Liu J, Huang L, Wang Z, Wang L. Design and performance of a sericin-alginate interpenetrating network hydrogel for cell and drug delivery. Sci Rep. 2015;5(4):12374. DOI:10.1038/srep12374

[59] Singh B, Sharma V, Chauhan D. Gastroretentive floating sterculia-alginate beads for use in antiulcer drug delivery. Chem Eng Res Des. 2010;88(8):997–1012. http://dx.doi.org/10.1016/j.cherd.2010.01.017.

[60] Sinha P, Ubaidulla U, Hasnain MS, Nayak AK, Rama B. Alginate-okra gum blend beads of diclofenac sodium from aqueous template using $ZnSO_4$ as a cross-linker. Int J Biol Macromol. 2015;79:555–63. http://dx.doi.org/10.1016/j.ijbiomac.2015.04.067.

[61] Ngah WSW, Fatinathan S. Adsorption of Cu(II) ions in aqueous solution using chitosan beads, chitosan-GLA beads and chitosan-alginate beads. Chem Eng J. 2008;143(1):62–72. http://dx.doi.org/10.1016/j.cej.2007.12.006.

[62] Gotoh T, Matsushima K, Kikuchi KI. Preparation of alginate-chitosan hybrid gel beads and adsorption of divalent metal ions. Chemosphere. 2004;55(1):135–40. http://dx.doi.org/10.1016/j.chemosphere.2003.11.016.

[63] Vijaya Y, Popuri SR, Boddu VM, Krishnaiah A. Modified chitosan and calcium alginate biopolymer sorbents for removal of nickel (II) through adsorption. Carbohydr Polym. 2008;72(2):261–71. http://dx.doi.org/10.1016/j.carbpol.2007.08.010.

[64] Dubey R, Bajpai J, Bajpai AK. Chitosan-alginate nanoparticles (CANPs) as potential nanosorbent for removal of Hg (II) ions. Environ Nanotechnol Monit Manag. 2016;6:32–44. http://dx.doi.org/10.1016/j.enmm.2016.06.008

[65] Cao T-T, Zhang Y-Q. Processing and characterization of silk sericin from Bombyx mori and its application in biomaterials and biomedicines. Mater Sci Eng C. 2016;61:940–52. http://dx.doi.org/10.1016/j.msec.2015.12.082.

[66] Zhang YQ. Applications of natural silk protein sericin in biomaterials. Biotechnol Adv. 2002;20(2):91–100. http://dx.doi.org/10.1016/S0734-9750(02)00003-4.

[67] Aramwit P, Siritientong T, Srichana T. Potential applications of silk sericin, a natural protein from textile industry by-products. Waste Manag Res. 2012;30(3):217–24. DOI: 10.1177/0734242X11404733.

[68] Mohammed ZH, Hill SE, Mitchell JR. Covalent crosslinking in heated protein systems. J Food Sci. 2000;65(2):221–6. http://dx.doi.org/10.1111/j.1365-2621.2000.tb15983.x.

[69] Gasperini L, Mano JF, Reis RL. Natural polymers for the microencapsulation of cells. J R Soc Interface. 2014;11(100):20140817. http://dx.doi.org/10.1098/rsif.2014.0817.

[70] Khandai M, Chakraborty S, Sharma A, Pattnaik S, Dinda SC, Sen KK, et al. Preparation and evaluation of algino-sericin mucoadhesive: An approach for sustained drug delivery. J Adv Pharm Res. 2010;1:48–60.

[71] Khampieng T, Aramwit P, Supaphol P. Silk sericin loaded alginate nanoparticles: Preparation and anti-inflammatory efficacy. Int J Biol Macromol. 2015;80:636–43. http://dx.doi.org/10.1016/j.ijbiomac.2015.07.018.

[72] Nayak S, Dey S, Kundu SC. Silk sericin-alginate-chitosan microcapsules: Hepatocytes encapsulation for enhanced cellular functions. Int J Biol Macromol 2014;65:258–66. http://dx.doi.org/10.1016/j.ijbiomac.2014.01.042.

[73] Vidart JMM, Nakashima M, da Silva TL, Rosa PCP, Gimenes ML, Vieira MGA, et al. Sericin and alginate blend as matrix for incorporation of diclofenac sodium. Chem Eng Trans. 2016;52 (*In Press*).

[74] Silva TL, Meinerz VH, Vidart JM, Gimenes ML, Vieira MGA, Silva MGC. Metallic affinity of toxic and noble metals by particles produced from sericin, alginate and poly (ethylene glycol). Chem Eng Trans. 2016;52 (*In press*).

IntechOpen

IntechOpen