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ALGINATE GENERAL CHARACTERISTICS AND PROPERTIES

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Abstract: *Alginates are anionic natural polysaccharides, salts of alginic acid. Alginate is being extracted from algal or bacterial sources, as for commercial production, algal sources are preferred. It is a linear copolymer composed of acids and acid residues, and its structure is mainly defined by sequence pattern - the G-block length, M/G ratio, and molecular weight are key in determining its physicochemical and technological properties. The diverse physical and chemical properties of alginate lead to the performing of derivatization reactions on the polysaccharide backbone. To meet the specific needs, alginates may need modification by acetylation, phosphorylation, sulfation, hydrophobic modification, covalent crosslinking of alginates, graft copolymerization of alginates, or other methods. Due to their properties and characteristics, alginates have been widely applied and explored in many fields, such as food science, pharmacy, textiles, cosmetics and many others.*

Keywords: *alginate, physical properties, chemical properties, derivatization, modification methods, gelling, emulsifying, encapsulation*

INTRODUCTION

Alginate was firstly described by a British chemist E.C.C. Stanford, as a naturally occurring polysaccharide isolated from brown seaweed. Alginate is abundant in brown seaweed *(Phaeophyceae)* consisting of up to 40% (dry matter) (Draget, K. I., Phillips, G. O., & Williams, P. A., 2009). The polysaccharide was first isolated from marine microalgae last century, but it was approximately 80 years later that a bacterial source *(P. aeruginosa)* was identified (Linker, A., & Jones, R. S., 1966). Commercially, alginate is mainly extracted from brown seaweed as soluble sodium alginate. Annual world production of brown algae in dry weight is estimated to be approximately 85 thousand tons, from which about 23 thousand tons of alginate is obtained (Bertagnolli, C., da Silva, M. G. C., & Guibal, E., 2014). Among the food resources available in the oceans, seaweed has been identified as one of the 50 future foods that will contribute to transforming our global food system (Tagliapietra, B. L. & Clerici M., 2023). Differences in the nutritional composition of brown algae are related to species, cultivation place, atmospheric conditions, harvest period, and seasonal variations (Peñalver, R. *et al*., 2020). Due to their excellent properties on renewability, biocompatibility, biodegradation, antimicrobial activity, non-immunogenicity, gelation capacity, and processing, alginates have been widely applied and explored in many fields (Hurtado, A., *et al*., 2022). Alginate has a wide range of applications in food industry, pharmaceutical industry, medicine, tissue engineering, packaging, wastewater treatment and others. It is generally recognized as safe when used in accordance with good manufacturing practice. The focus of this review is to provide in brief the general characteristics and properties of alginate that premise the further application.

GENERAL CHARACTERISTICS OF ALGINATE

Based on literature survey, the most commonly used protocol of alginate production from algal sources is soaking milled seaweed in formaldehyde, and then collecting the solid for acid pre-

treatment with HCl. Next, the solid residue from the acid pre-treatment is extracted using Na_2CO_3. Then the liquid portion is precipitated by ethanol. Finally, the solid output is dried in oven. Novel extraction methods using ultrasound, microwave, enzymes and extrusion improved the extraction yield and alginate properties, but the financial benefits have not been fully justified yet (Saji, S., Hebden, A., Goswami, P., & Du, C., 2022).

Alginate consists of a linear block copolymer sequence of α-L-guluronic acid (G) and β-D-mannuronic acid (M) bonded by 1→4 linkages. The difference at C-5 in the uronic acids makes guluronic acid and mannuronic acid stereochemically different to each other (Maurstad, G., *et al*., 2008). These uronic acids can form homogeneous blockchains of MM (M blocks) or GG units (G blocks) and chains with alternate blocks of mannuronic acid and guluronic acid (MG blocks) (Makarova, A. O., *et al.*, 2023). The physical properties of alginate depend on a number of different key factors in which M and G contents play a crucial part. The blocks are present on (Fig. 1) (Lee, K. Y., & Mooney, D. J., 2012). The first report on the chemical structure of alginates appeared as early as 1966 from B. Larsen *et al.* described in detail the partial hydrolysis of alginates followed by fractionation to obtain alginates containing different copolymer compositions (Haug, A., Larsen, B., & Smidsrod O., 1966).

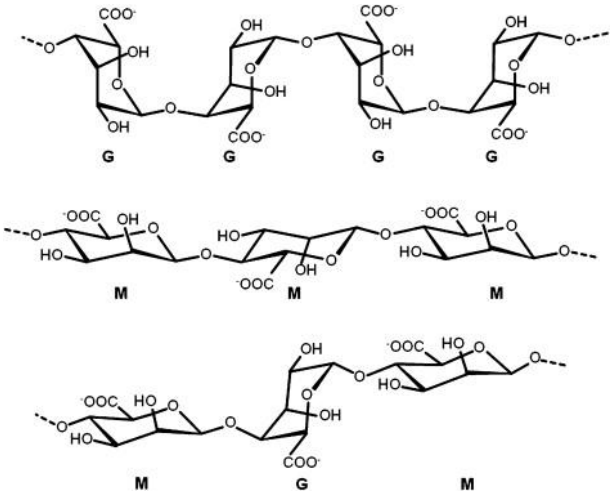

Fig. 1. Chemical structures of G-block, M-block, and alternating block in alginate.

The molecular formula of sodium alginate is $(C_6H_7O_6Na)_n$, relative molar mass is about 32 kDa–200 kDa (Hagen, A., Skjak, B. G., & Domish M., 1996). Its theoretical value of molar mass of structural unit is 198.11, the molar mass has a great influence on the physicochemical properties of alginate. Sodium alginate with high molar mass is forming a high viscosity solution (Kong, H. J., Lee, K. Y., & Mooney, D. J., 2002).

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 Bernoullli statistics. 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 ^{13}C NMR spectroscopy, more detailed information on their structure is obtained from Venkatesan (Venkatesan, J., *et al*., 2014). Another method of studying the structure of alginates is by using circular dichroism (CD). 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 (Donati I., *et. al*., 2003). The copolymer composition, sequence and molecular weights vary with the source and species that produce the copolymer (Draget, K. I., Phillips, G. O., & Williams, P. A., 2009). Therefore, alginate has been the subject of research in various fields related to the exploitation of marine biological

resources, and it has been found that the structure and composition of alginate significantly influence its biological activity (Li, L., Zhu, B., Yao, Z., & Jiang J., 2023). It is also essential that different molecular characteristics of alginates, such as uronic acid (M/G) ratio, alginate chain's block structure, molecular weight, and the degree of polymerization, significantly influence their chemical and physical properties (Bojorges, H. *et al.*, 2023).

GENERAL PROPERTIES OF ALGINATE

Fundamentally, alginates are characterized by their molecular mass (M_w, M_n), polydispersity index (M_w/M_n), macromolecular parameter (e.g., intrinsic viscosity ([η]), critical concentration (C*), gyration (Rg) and hydrodynamic qualities (Rh)), as well as by their M/G ratio and number and length of monad (G and M), dyad (GG, MM, MG or GM), and triad (MMG, GGM, MGM) frequencies, which provide structural information that is easily correlated with their rheological properties (gelling or/and thickening) in a solution or in the presence of mono- (K^+, Na^+) and di-valent (Ca^{2+}, Mg^{2+}) salts (Hentati, F., *et al*., 2018).

The solubility of alginates in water is governed by three parameters – pH of the solvent, ionic strength of the medium, and presence of gelling ions in the solvent. To make alginates soluble it is essential that the pH be above a certain critical value and the carboxylic acid groups be deprotonated (Pawar, S. N., & Edgar, K. J., 2011).

Sodium alginate and thickeners (xanthan gum, guar gum, tragacanth gum), synthetic polymer medicinal materials (such as carbohydrates), sugars, fats, waxes, some surfactants and some organic solvents (such as glycerin, propylene glycol, ethylene glycol, etc.) are compatible. It is incompatible with acridine derivatives, crystal violet, phenylmercury vinegar (nitrate), calcium salts, heavy metals, and ethanol with a concentration higher than 5% (Pawar, S. N., & Edgar, K. J., 2012).

The most important feature of alginate's physical properties is the selective binding of multivalent cations, which is the basis for gel formation, and the fact that the sol/gel transition of alginates is not particularly influenced by temperature. Selective binding of certain alkaline earth metals ions (e. g. strong and cooperative binding of Ca^{2+} relative to Mg^{2+}) increases markedly with increasing content of α-l-guluronate residues in the chains. Polymannuronate blocks and alternating blocks are almost without selectivity (Smidsrod, O., 1973). Some chelation of multivalent cations obviously takes place as a result of structural features in the G-blocks, with the so-called “egg-box” model (Grant, G. T., *et al.*, 1973) being an initial attempt at explaining the phenomen.

***Hydrogels formation by alginate chelates with divalent cations*.** Gel formation is driven by the interactions between G-blocks which associate to form tightly held junctions in the presence of divalent cations (Sikorski, P., *et al*., 2007). In addition to G-blocks, MG blocks also participate, forming weak junctions (Donati, I., *et al.*, 2005). Alginate has been found to exhibit different affinities for certain divalent ions, e.g., $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+}$ (Xu, Y. J., *et al.*, 2021). However, calcium is the most commonly used cation to produce gels. Diffusion set gels are typically made by dropping a Na-alginate solution into a $CaCl_2$ bath.

The encapsulation of cells within alginate gels is a preferred technique for immunoprotection and has been reviewed in several publications (Zimmermann, H., *et al*., 2007; Ghidoni, I., *et al*., 2008; Lee, K. Y., & Mooney, D. J., 2012). When the pH of alginate solutions is lowered below the pK_a of the uronic acids in a highly controlled way, acid gels are formed. (Siddhesh, N. P., & Kevin J. E., 2012). Polysaccharides undergo hydrolytic cleavage under acidic conditions.

The mechanism of acid hydrolysis of the glycosidic bond has been described by Timell (Timell, T. E., 1964). The enzymatic degradation of alginates by lyase occurs by a β-elimination mechanism resulting in unsaturated compounds (Tsujino, I., & Saito, T., 1961; Preiss J, & Ashwell, G., 1962). A similar degradation route is followed when they are subjected to strongly alkaline environments. The rate of degradation increases rapidly above pH 10.0 and below pH 5.0. Above a pH of 10.0, the degradation arises mostly from the β-elimination mechanism, while below 5.0 the degradation is mostly due to acid catalyzed hydrolysis (Haug, A. L., Larsen, & B., Smidsrød O. 1963).

The derivatization and design strategies for alginates depend on three important parameters: solubility, reactivity and characterization (Siddhesh, N. P., & Kevin J. E., 2012). The earliest known report addressing the chemical modification of alginates was published by Chamberlain et al., wherein acetylation of the hydroxyl groups of alginic acid was described (Chamberlain N. H., Cunningham G. E., Speakman J. B., 1946). Alginates as biosynthesized by bacteria are partially acetylated (Franklin, M. J., & Ohman, D. E. 2002; Franklin, M. J., Douthit, S. A., & McClure M. A., 2004; Franklin, M. J., & Ohman, D. E., 1993). Acetylation of alginates coupled with a detailed analysis of the acetyl substitution pattern along the backbone was first reported by Skjåk-Bræk *et al.* (Skjåk-Bræk, G., Paoletti, S., & Gianferrara, T, 1989). The synthesis of phosphorylated alginate derivatives was described to evaluate the ability to induce hydroxyapatite nucleation and growth (Coleman, R. J., e*t al*, 2011). Sulfation of polysaccharides, both enzymatically in nature as well as by chemical methods is known to provide blood-compatibility and anticoagulant activity (Alban, S., Schauerte, A. & Franz, G. 2002). Hubert *et al.* attempted the hydrophobic modification of sodium alginate by covalent attachment of short polyether chains to the alginate backbone (Carré, M. C., *et al*., 1991). Hubert et al. also reported the synthesis of hydrophobically modified alginate derivatives starting from Na-alginate (Babak, V. G., *et al*., 2000). Dellacherie et al. reported the physico-chemical properties of hydrophobically modified alginates in aqueous solution (Pelletier, S., *et al.*, 2000). Production of microspheres based on hydrophobically modified alginates by a dispersion gelation method was reported by Leonard *et al*. (Leonard, M., *et al.* 2004). The creation of synthetic derivatives has the potential to empower the next generation of applications for alginates (Siddhesh, N. P., & Kevin J. E., 2012). Alginate biocompatibility varies at the level of its purity and has been extensively evaluated in-vivo as well as in-vitro (Wu, T., *et al*., 2018). In mammal's alginate is inherently non-degradable due to lack of the alginase enzyme, which is responsible to cleave the polymer chains. To make Alginate degradable in physiological environment includes partial oxidation of alginate chains (Jeon, O., *et al.,* 2010). Alginates are reported nontoxic according to plenty of studies and crosslinked with sodium/calcium are nontoxic to cells, even not harmful for eyes and skin (Podgórna, K., *et al*., 2017).

Alginate has found numerous applications in the domain of pharmaceutical science, paper and textile industry, food industry, scaffolding, biomedical and engineering due to its cost-effective nature, film forming ability, gelling, biocompatibility, biodegradability, nontoxic, non-immunogenic, readily availability and antimicrobial nature (Kumar, A. *et al.*, 2023).

CONCLUSION

The information reviewed shows that alginate is a biocompatible natural polymer frequently applied in various industrial and scientific areas, because of its versatility, non-toxicity and diverse physicochemical properties. The most important properties of alginate include its gel-forming ability, the ability to chemical modifications and the ability to interact with di- and trivalent cations. The advanced research within these areas is promoting a further detailed understanding of the general properties of alginate and its fields of application. As a biomaterial obtained from algal sources the development of alginate research and application can contribute to transforming our global approach in many industries and areas of life.

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