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REVIEW ARTICLE

EXTRACTION OF HYDROGEL FROM ALGINATE:ADVANCES IN MARINE ALGAL SOURCES AND SOLUTIONS

Sahana Shree M[1], Shivasakthy Manivasakan[2], Arumugam Muthuvel[3]

[1]BDS, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Pillaiyarkuppam, Pondicherry, India– 607402 drsahanaprostho@gmail.com

[2]MDS., PGDHPE., PhD., Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Pillaiyarkuppam, Pondicherry, India – 607402 shivasakthym@igids.ac.in

[3]Msc. PhD., CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India arucasmb@gmail.com

***Corresponding Author:** Shivasakthy Manivasakan MDS., PGDHPE., PHD., Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Pillaiyarkuppam, Pondicherry, India – 607402 shivasakthym@igids.ac.in

Abstract

The extraction of hydrogel from alginate has garnered significant attention due to its versatile applications in biomedical, pharmaceutical, and environmental fields. Alginate, a naturally occurring polysaccharide derived from brown seaweed, offers a biocompatible, biodegradable, and non-toxic matrix ideal for hydrogel formation. This narrative review explores the various methods for extracting and characterizing alginate-based hydrogels, highlighting the key factors influencing the gelation process, including concentration, pH, ionic strength, and crosslinking agents. By providing an overview of current extraction techniques, material properties, and innovative applications, this review aims to contribute to a deeper understanding of alginate-based hydrogel systems and their potential for future research and development.

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1. INTRODUCTION

In 1753, Linnaeus coined the Latin word "algae," which he called "hepaticae" [2]. It has evolved into a wealth of genetic diversity and novel medicinal compounds over millions of years. Algae are organisms that have chlorophyll. They can be single-celled or form colonies or multicellular structures, and occasionally they can cooperate to make basic tissues [1]. They have unique features in their cells that are not found in plants or animals [2]. From unicellular varieties that range in size from 3 to 10 microns to enormous kelps that may grow up to 70 meters in length and develop as quickly as 50 centimeters per day, they are incredibly diverse [1]. Marine algae grow quickly and do well without freshwater, fertilizers, or arable land [6].

There are two forms of algae: microalgae, which live in both benthic and littoral locations as well as the open ocean as phytoplankton, and macroalgae, or seaweeds, which are located in the littoral zone and comprise green, brown, and red algae [1]. Seaweeds need sunlight, brackish or salty water, and a surface to attach to to flourish. They are usually found in the littoral zone as a result of these factors. Green algae, or Chlorophyceae, comprise species including Chlamydomonas, Chara, and Spirogyra and contain the pigments chlorophyll a and b. Brown algae, or Phaeophyceae, include species including Laminaria, Sargassum, and Dictyota. They contain colors like carotenoids, xanthophyll, and chlorophyll A and C. The red pigment r-phycoerythrin is found in rhodophyceae, or red

algae, which include species such as Porphyra, Gracilaria, and Gelidium [2].

Green algae can be found in freshwater (lakes and rivers) and on surfaces like walls, rocks, and tree bark wherever there is light for photosynthesis, while red and brown algae are mostly found in marine habitats [1,2]. The main component of the algae's cell walls is cellulose. Photosynthesis is carried out by chloroplasts, which are found in algae. In addition, they include mucilage, hemicellulose, pectin, and other substances like calcium carbonate, silica, alginic acid, fucin, and fucoidan. Double membranes make up chloroplasts, which house photosynthetic pigments. Small, thread-like protoplasmic extensions called flagella help in movement [2].

Bioactive substances such as dietary fiber, carotenoids, omega-3 fatty acids, vitamins, minerals, and trace elements are abundant in marine algae. A well-balanced food for the living system, they also supply vital amino acids that are needed in the human diet [2,3], and [4]. For ages, marine algae have been utilized as a vital source of medicine, agricultural feed, fodder, fertilizer, and human nutrition. Porridge made from the red algae Gracilaria edulis is popular in several coastal areas of Tamil Nadu, India [4].

A wide range of medicinal advantages are offered by algae for both internal and external application. In addition to being the primary source of food for all marine life, they also create oxygen[2]. Many types of algae are useful in a variety of businesses, such as the food, dairy, pharmaceutical, and cosmetics sectors. Hydrogen gases, bioethanol, biobutanol, and biodiesel are also abundant in algae [3,4]. Agar, alginate, carrageenan, and biological chemicals including growth hormones and amino acids are among the materials that marine macroalgae provide as a raw material for a variety of industries. Numerous industries, such as textiles, specialty glues, medicinal preparations, and beverage additives, heavily rely on these active substances [5,7].

Strongly controlled bioactive chemicals can be found in marine macroalgae. Flavonoids, carotenoids, phenolic acids, tannins, and polysaccharides are all abundant in marine algae. bromophenols as well. Their polarity and solubility are highlighted by the different biological functions they assist [8].

Stimuli-responsive hydrogels, which change their characteristics in reaction to external variables like pH, temperature, magnetic fields, and light, have attracted a lot of interest in recent decades. Particularly, pH-responsive hydrogels find extensive utility in biomedical applications, including drug delivery, scaffolds for tissue engineering, bioreactors, and biosensors. Sodium alginate is a non-toxic, biodegradable copolymer that shows promise as a pH-responsive hydrogel. In moderate environments, it gels when divalent cations such as Ca2+ are present. This is caused by interactions between Ca2+ and the G-blocks in alginate, which result in the formation of "egg-box junctions." It is ideal for biomedical applications due to its gelation property, hydrophilicity, and biocompatibility [9].

2. EXTRACTION OF HYDROGEL FROM MARINE ALGAE

According to Kurakula et al. (2020), external cross-linking agents, reactive polymers followed by cross-linking, or polymerizing monomers with parallel cross-linking techniques can all be used to create hydrogels. Ionic interactions and covalent bonds, as well as interactions with reactive species and other cross-linking agents, are examples of physical and chemical mechanisms that can cause cross-linking [10].

Alginate adjusts its setting time by forming gels at ambient temperature, as explained by Ray et al. (2020). Alginate gels' textures can be changed from soft to harsh and stretchy by altering their composition. Acid gels, calcium or other multivalent metal salt-induced gels, and a mixture of the two are the three primary forms of alginate gels. Gels are usually formed when calcium or hydrogen ions are released gradually. Other salts, including phosphates, which interact with metal ions, can be added in modest amounts to adjust the gel setting time. In the gelation process, calcium ions interact with alginate's G-block structure to create molecular junctions. Alginate, calcium salts, and sequestrant agents are essential ingredients in alginate gelation; the kind of alginate, counterions, and sequestrants used affects the gel's structure and rate of formation. To obtain the desired hydrogel product, the properties of sequestrants and calcium ions must match the method and formulation, and proper hydration and uniform distribution of alginate are essential for gel formation [15].

Sharma et al. conducted a critical review of the alginate hydrogel preparation procedure. Usually, hydrophilic monomers are used to create a crosslinked network that can absorb water to create hydrogels. Rheological investigations can be used to identify the gel point, which is the point at which the polymer mixture changes from sol to gel. The two main categories of hydrogel production techniques are chemical and physical crosslinking. Physical crosslinking uses hydrogen bonds, ionic contacts, and hydrophobic forces, whereas chemical crosslinking uses methods including grafting, radical polymerization, and click chemistry. Anionic polymers, such as alginate, can be used

to create hydrogels by self-assembly and ion-induced gelation processes. It is possible to add hydrophobic monomers to improve longevity and mechanical strength. Hydrogels can be used in biological and pharmacological applications because crosslinking, which can be accomplished by a variety of processes such as chemical or ionizing radiation, creates networks of polymers that enhance mechanical and viscoelastic qualities [12].

According to Kumar et al. (2024), crosslinking the polysaccharide—which can be done chemically or physically—is how the hydrogel is formed. Hydrogen bonding, aggregation, association, complexation, and crystallization are the processes that crosslink physical hydrogels. Covalently cross-linked hydrogels, also known as chemical hydrogels, are crosslinked via chemical techniques such as polymerization or cross-linkers. While chemically cross-linked hydrogels have strong covalent connections that form irreversible networks, physically cross-linked hydrogels make reversible gels with weak contacts that are easily severed. Hydrogels made of polysaccharides are hydrophilic, meaning they absorb water and swell without disintegrating [19].

Alginate hydrogel was created by Zhang et al. in 2021 using gelation, which is accomplished by ionic or covalent crosslinking. Ionic crosslinking, which is usually carried out at room temperature or up to 100°C using divalent cations, is more popular since it is straightforward and requires mild conditions. Because it is readily available and non-toxic, calcium chloride is the most widely utilized cross-linker. According to the "egg-box" hypothesis, only G-blocks—which are composed of consecutive G residues—can engage in ionic crosslinking because of their advantageous structure [26].

2.1 IONIC CROSSLINKING

Shin et al. (2019) described how to manufacture an alginate nanocomposite hydrogel that releases fluoride ions. Using in situ precipitation and cross-linking, the Alg-CaF2 nanocomposite hydrogel was created using sodium alginate (Alg), calcium carbonate (CaCO3), ammonium fluoride (NH4F), and D-(+)-gluconic acid δ-lactone (GDL). Using distilled water and the proper amounts of NH4F and CaCO3, a 2% Alg solution was made at 37°C. Extra CaCO3 was added for crosslinking, and the CaCO3 to NH4F ratio was fixed at 1:2. For a pH of neutral, 0.5 molar CaCO3 to GDL ratio was reached by adding GDL. CaF2 precipitation and hydrogel crosslinking resulted from the dissolution of CaCO3 and the release of Ca2+ ions upon the addition of GDL. After stirring, the liquid was pipetted into molds and allowed to gel completely for 24 hours at 37°C (11). In 2015, Straccia et al. used CaCO3 as the calcium source to create alginate hydrogels by internal gelation. By releasing calcium ions gradually, this method makes it possible to create gels that are incredibly homogenous and consistent. Different amounts of D-(+)-gluconate-δ-lactone (GDL) and CaCO3 were used [13].

According to Kruk et al. (2022), the distribution of M- and G-residues in the polymer chain as well as the repulsive force of negatively charged sugar residues determine how alginate (ALG) gels. Trivalent ions like aluminum or divalent ions like calcium, strontium, or barium are necessary for the formation of ALG gels. The "egg-box model," which is the most widely used gelation model, states that stronger, more rigid gels are produced when G-blocks link to calcium ions more firmly than M-blocks. M-blocks reduce calcium affinity and provide flexible structures. Both G-blocks and M-blocks can be bound by lanthanum, praseodymium, and neodymium ions. Using lactones such as glucono-δ-lactone to lower pH can also cause ALG gelation. Although calcium-ion-induced gelation is quick and difficult to control, it can be controlled with the use of citrate ions, phosphate buffers, or certain crosslinking agents such as calcium carbonate or gluconolactone. Lowering the temperature or employing freeze-thaw technology can help further regulate the process [16].

To create the hydrogel, Gajic et al. (2023) used an ionic gelation technique, in which calcium ions from a CaCl2 solution mix with alginate. A 26 G needle was used to drip the alginate solution into the CaCl2 gelling solution, which was magnetically stirred, after it had been swirled in water for 24 hours at room temperature. With the use of several polynomial equations, the alginate hydrogels' water retention capacity (WRC) was calculated, and Design-Expert 13 software was used for statistical analysis [24].

2.2 COVALENT CROSSLINKING

Covalent crosslinking, according to Abasalizadeh et al. (2020), is the process of joining two polymer chains together with a crosslinking agent. When crosslinkers such as glutaraldehyde, adipic acid dihydrazide, or poly(ethylene glycol)-diamine react with the functional groups of natural or synthetic polymers (-OH, -COOH, -NH2), this reaction takes place. Covalently crosslinked alginate gels are created when carboxylic groups in various alginate branches react with crosslinkers that include primary diamines. The kind and density of the crosslinking molecules determine the hydrogels' mechanical characteristics and swelling behavior.

Mechanical strength is influenced by crosslinking density, whereas swelling qualities are controlled by the type of crosslinker. Hydrogels with changeable qualities can be used in biomedical applications and other fields because hydrophilic crosslinkers, such as PEG, can restore the hydrophilic nature during crosslinking [27].

2.3 CELL CROSSLINKING

In addition to many physical and chemical techniques for creating alginate gels, Abasalizadeh et al. (2020) indicated that cell involvement in gel creation is important. If certain ligands in the polymer chains attach to cell surface receptors, cells can use the polymers to crosslink. Alginate chains are mechanically robust and biocompatible, however they don't have any bioactive ligands that can anchor cells. Cells can attach to the chains of alginate modified with cell adhesion peptides, like the Arg-Gly-Asp (RGD) sequence, forming a reversible polymer network even in the absence of chemical crosslinking. Cells added to RGD-modified alginate cause homogeneous cell dispersion and receptor-ligand interactions to form a polymer network [27].

2.4 FREE RADICAL POLYMERISATION

Alginate chains undergo free-radical polymerization using a photoinitiator and UV light after being modified with functional groups, such as methacrylates, according to Abasalizadeh et al. (2020). Cells or bioactive compounds can be encapsulated and crosslinked using this technique in physiological settings. Despite the excellent cell viability provided by ionic crosslinking, the hydrogel that results is neither injectable nor stable for clinical usage. Whereas calcium in unmodified alginate creates ionic crosslinking, free-radical polymerization creates covalent crosslinks between methacrylate groups [27].

Alginate and acrylamide were used in the free-radical polymerization process by Varaprasad et al. 2020 to create alginate hydrogels. This procedure involved continuously swirling 0.75 g of alginate and 0.25 g of acrylamide while they were suspended in 10 mL of filtered water. After that, an initiator (ammonium persulfate, 2.191 mM) and a crosslinker (N, N'-methylenebis(acrylamide), 0.648 mM) were added. To create the hydrogel, the mixture was heated to 45°C for 30 minutes. After 480 minutes of soaking and rinsing with filtered water to eliminate any unreacted components, the hydrogel was allowed to dry at room temperature [17].

2.5 PHASE TRANSITION

As demonstrated by poly(N-isopropylacrylamide) (PNIPAM), a well-researched thermosensitive polymer, thermoresponsive phase transition happens when the temperature goes above the low critical solution temperature (LCST), according to Abasalizadeh et al. 2020. The PNIPAM exhibits a reversible phase transition at approximately 32°C, where the polymer precipitates as a solid gel above the LCST and reverts to a liquid below it. Water molecules and PNIPAM's hydrophilic groups form intermolecular hydrogen bonds that weaken with increasing temperature, causing water to be released, while intramolecular hydrogen bonds get stronger, causing aggregation into a solid gel. The branches unfurl to produce a free-flowing polymer solution below the LCST as a result of the reestablished hydrogen bonds between water molecules and the hydrophilic groups of PNIPAM [27].

2.6 MISCALLANEOUS

Liu et al. (2016) prepared alginate hydrogels via solution extrusion and evaluated the drug release behavior. Using a solution extrusion method, alginate hydrogels containing ibuprofen (BF), acetaminophen (AC), or methylthionine chloride (MC) were made at room temperature. In a vacuum mixer, sodium alginate, CaCO3-GDL, and deionized water were combined and allowed to degas for ten minutes. Neutral pH was maintained by using a CaCO3 to GDL molar ratio of 0.5. With a 5% drug loading, the medications were introduced to the alginate solution and swirled for five minutes. After being molded with a rectangular die and extruded through a single-screw extruder, the solutions were submerged in a 3% CaCl2 solution to solidify their surfaces. The sheets were gelled at 25 °C for 20 hours and stored at 5 °C[9].

Modified alginic acid hydrogels were created by Wu et al. to allow for the drug's controlled release. After dissolving 1.5 g of SA in 100 mL of distilled water to create a 1.5% w/v solution, the excess hydrochloric acid (5% w/v) was gradually added while being stirred for an hour, resulting in the formation of a white ALGH precipitate. An additional hour was spent stirring the mixture, after which the precipitate was cleaned with ethanol and distilled water and dried at 35°C in a vacuum oven. P-toluenesulfonic acid (0.1% w/v) was used as a catalyst while ALG-H (1.0 g), 1,10-decanediol (0.25 g), and DMF (25 mL) were agitated in a flask for esterification and crosslinking. Esterification occurred at 80°C for 7 hours, and the water produced was removed by distillation under reduced pressure. The

crosslinked product was washed with distilled water and ethanol, and then dried in a vacuum oven at 35°C[14].

Alginate, Rt extract, and CaO2 solutions were combined in a volume ratio of 1.0:0.3:0.7 (v/v/v) in Tris buffer (pH 8.5) by Nguyen et al. in 2023 to create Alg@Rt hydrogel. Solution A was first created by combining 30 mL of 10 mg/mL Rt extract with 100 mL of 3% alginate solution. After adding 70 mL of solution B (a 3% CaO2) to solution A, the mixture was vortexed until gelation took place. Additionally, a control hydrogel devoid of Rt extract was made. A scanning electron microscope (FE-SEM) was used to view the cross-sections of the Alg@Rt and Alg hydrogels after they had been lyophilized to investigate the porosity structure [18].

A nanocomposite hydrogel containing Linum usitatissimum was created by Mohammadpour et al. in 2021. Wet chemical precipitation was used to create nano hydroxyapatite (nHA). Next, 10 mL of a 1% w/v LOH solution made with distilled water was mixed with 20, 40, and 60 mg of nHA powder. Magnetic stirring and ultrasonication were used to distribute the nHA evenly. After two hours of stirring, alginate was added until the final concentration was 2% w/v. After cross-linking the combination with a 2 M CaCl2 solution, the hydrogels underwent multiple washings to eliminate any remaining unreacted Ca2+. After being pre-frozen for 24 hours at -20°C, the composite materials were lyophilized for 24 hours at -50°C. Before usage, the Alg/nHA nanocomposite gels loaded with LOH were kept at 4°C (20).

Phycocyanin-loaded hydrogels based on alginate were created by Buliga et al. in 2024. 0.1 g of PVA, 0.15 g of sodium alginate, and 10 mL of distilled water were combined in a 15 mL vial. 0.5 g of AM was added to this, and when it had completely dissolved, 0.05 g of MBSA was added as a cross-linking agent. After adding 0.1 g of APS, the reaction was carried out for two hours at 60°C. Using BIOBASE BK-FD10S equipment, the hydrogels were cut into discs and lyophilized by freezing them for 24 hours at -18°C and then freeze-drying them for 24 hours at -60°C. The lyophilized hydrogels (0.075 ± 0.002 g) were soaked in 15 mL of phycocyanin extract (obtained via MAE: 1:15 algae to solvent ratio, 40 min, 40°C, 900 rpm) for 24 hours [21].

To create alginate hydrogel from brown algae, Ziyazadeh et al. (2024) prepared a 2% w/v alginate solution by dissolving alginate in a 1% acetic acid solution. This ensured complete hydration for the best gel formation. The solution was centrifuged for three hours at 25°C and 4000 rpm to eliminate impurities. This allowed the larger particles to settle and the clean supernatant to be collected. The hydrogel was subsequently dried for 24 hours at 37°C in an oven to eliminate any remaining moisture, which was crucial for additional characterization [22].

In 2019, Kavitha et al. used COL from stingray fish skin and purified SA from T. conoides to create hydrogel films. The solvent-casting method was used to create the hydrogels. Stirred at 600 and 400 rpm, respectively, SA (1.5% w/v) and COL (0.5%) were dissolved in distilled water and a 3% acetic acid solution. As a plasticizer, 15% w/w of SA was added to glycerol. With and without glycerol, the SA and COL were mixed at 60:40 and 90:10 ratios. At room temperature, the solution was degassed for 12 hours. Petri dishes (5 cm and 12 cm in diameter) and aluminum foil cubes were tested for casting; the 12 cm Petri dish was determined to be the best for casting microfilms. Approximately 18 mL of the SA mixture was suitable for casting the films with or without glycerol [23].

Poly(acrylamide-sodium alginate) (P(Am-SA)) hydrogel was created by Manjula et al. (2013) at room temperature by dispersing 1 g of acrylamide (Am) and 0.05 g of sodium alginate (SA) in 4 mL of an aqueous solution that contained N, N'-methylene bisacrylamide (MBA), and APS/TMEDA. The liquid was allowed to react overnight to produce a firm gel after being agitated for five minutes at 200 rpm. Similarly, poly(methacrylamide-sodium alginate) (P(MAm-SA)) hydrogel was made, but the reaction was conducted at 45°C. The APS/TMEDA pair was used to start redox polymerization after 1 g of methacrylamide (MAm) and various amounts (0.05–2.50 g) of SA were dissolved in 4 mL of solution containing MBA. The process of redox cross-linked polymerization was used to create the poly(N-isopropylacrylamide-sodium alginate) (P(NIPAAm-SA)) hydrogel. To start polymerization, the reactants were combined and heated. The composition contained 8.83 mM NIPAAm, 0.05 g SA, MBA, APS, and TMEDA [25].

3. CONCLUSION

In conclusion, the methods of extracting hydrogel from alginate involve a variety of techniques aimed at converting the alginate solution into a gel-like substance suitable for various applications. Each of these methods offers unique advantages and can be tailored depending on the desired properties of the final hydrogel product, such as mechanical strength, biocompatibility, and swelling behaviour. The choice of method largely depends on the specific application, whether it is for biomedical, pharmaceutical, or industrial uses.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

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COMPETING INTERESTS

The author declares that they have no competing interests.

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