Review Article

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Methods of extraction, physicochemical properties of alginates and their applications in biomedical field – a review

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Abstract: In this paper, the current state-of-art of extraction of alginates and the determination of their physico-chemical properties as well as their overall applications focussing on biomedical purposes has been presented. The quality and quantity of the alginate obtained with a variable yield prepared from brown seaweeds as a result of many factors, such as type of algae, extraction methods, chemical modification and others. Alginates are mainly extracted by using conventional alkaline extraction. However, novel extraction techniques such as microwave and ultrasound assisted extractions have gained a lot of interest. The extraction parameters (e.g., temperature and time of extraction) have critical impact on the alginate physiochemical and mechanical properties and thus, their potential applications. By controlling a chemical process makes it possible get various forms of alginates, such as fibres, films, hydrogels or foams. It is important to characterise the obtained alginates in order to their proper applications. This article presents several techniques used for the analysis of alginate properties. These natural polysaccharides are widely used in the commercial production, as a food ingredient, in the pharmaceutical industry due to their antibacterial, anticancer and probiotic properties. Their gelling characteristic and absorbable properties enable using alginates as a wound management material. Moreover, they are also biocompatible, non-toxic and biodegradable, therefore adequate in other biomedical applications.

Keywords: brown seaweeds; alginates; biopolymers; extraction; physicochemical properties; analytical techniques; applications; wound dressings; healing wounds; chronic wounds; hydrogels.

1 Introduction

Brown seaweeds (known also as macroalgae) are abundant in many coastlines and in some of them they represent an almost unexploited but very valuable marine resource. This biomass constitutes a production system of bioactive compounds such as polysaccharides, proteins, minerals, lipids including polyunsaturated fatty acids, pigments, vitamins, antioxidants, etc., which are known to have antibacterial, antifungal, antiviral, antioxidative, anti-inflammatory and antitumor properties [1]. In this review paper we describe alginates that are natural polysaccharides extracted mainly from brown seaweeds. They are composed of two hexuronic acids: β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by 1–4 bonds [2, 3]. These units are randomly distributed in a linear chain. They can be also arranged as homogeneous blocks MM or GG and heterogeneous or alternating as MG, as shown in Figure 1 [3, 4]. Alginates are salts of long-chain alginic acids, that in brown seaweeds are present mainly as the calcium salt of alginic acid, although magnesium, potassium and sodium salts are also present [5]. Among them, sodium alginates are water soluble polymers, which give highly viscous solutions [6]. In the presence of polyvalent cations, such as calcium, sodium alginate has the ability to form gel [2, 7].

Alginates are used widely due to their rheological properties [8, 9], as well as biocompatibility,

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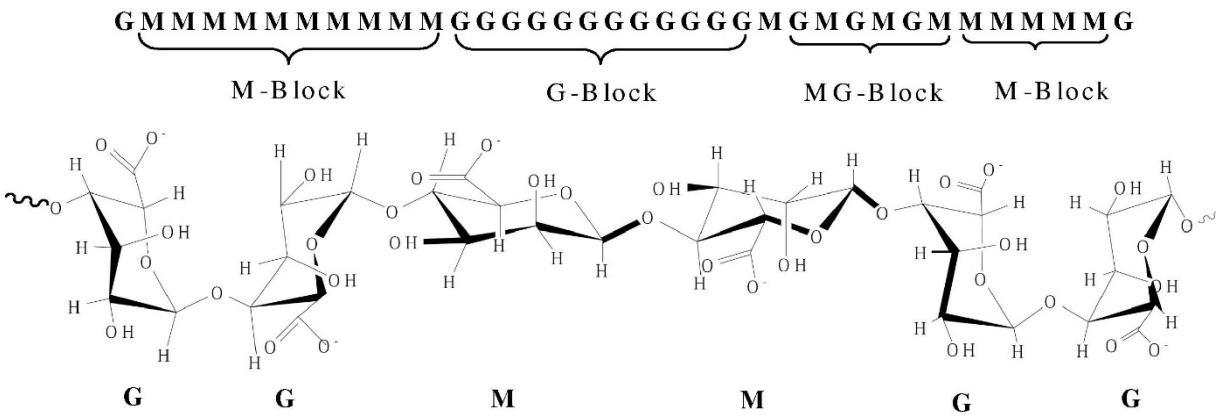

Figure 1: Alginate chain sequence and the macromolecular conformation [4].

biodegradability and lack of toxicity [10, 11]. The proportion of the three types of blocks – MM, GG and MG determines the physical properties of alginates – alginates with high G have higher gelling properties, whereas those with high M have higher viscosity [3, 4]. Assessment of the M/G ratio is also fundamental – for alginates with a high M/G ratios alginates provide elastic gels, whereas for alginate with a low M/G ratios generate brittle gels [4, 12].

Alginates are also characterised by unique biological and pharmacological properties [11]. In the food and pharmaceutical industry they mainly act as a gelling agent, stabilizer and thickener [2, 13]. Nowadays, several applications of alignates have been seen, mainly as food additives to stabilize and improve food consistency (e.g., baked food, fruit industry, jellies, jams, ice cream, mayonnaise) [11, 14–16]. Moreover, these biopolymers are commonly considered as healthy food elements due to their anticancer and prebiotic properties [17]. Alginates additives could also be used as a complementary cure for obesity. Furthermore, these hydrocolloids are a potential beverage ingredient (e.g., beer foam stabilizers, dairy products, drinks for lowering blood sugar level) [11, 18]. Another use of alginates in food industry is product packaging [14].

Alginates find also applications in a wide range of biomedical engineering (controlled drug release, cells encapsulation, immunostimulatory agents, scaffolds etc.) [3, 14, 18, 19], in a pharmaceutical industry (e.g., cosmetic, toothpaste) [15], in agriculture as an elicitation of plant growth [12], in textiles (e.g., additives for textile prints) and medical textiles – alginate fibres as a wound management material (wound dressing), bandages [3, 14, 20–22], pill disintegrators and dental impression material [3], tissue engineering – alginate scaffolds (e.g., bone, regeneration of cells in soft tissues) [23] and many others.

Alginates are also known as biosorbents of metal ions – they are able to bind metal ions from aqueous solutions due to the ion-exchange mechanism [24–27]. These biopolymers carry carboxyl groups capable of metal ions binding [28]. Several authors examined biosorption properties of alginates, for example Cd(II) ions sorbed by *Sargassum* spp. alginate [29], Pb(II), Cu(II), Cd(II) and Zn(II) ions by alginate extracted from *Sargassum filipendula* [30], Cu(II) and Pb(II) by *Laminaria digitata* alginate [31].

The aim of the present paper is to present the current trends in extraction of alginates from brown seaweeds. Conventional and novel methods of alginate extraction have been described, as well as process parameters that enable you to receive the optimal amount of alginate. The process efficiency is dependent not only on extraction parameters, but also other factors, such as species and origin of algae, preparation of samples, as well as the biomass pre-treatment. The type and procedure of extraction is significant due to the impact of chemical modification on the alginates form and their mechanical properties. It is an important aspect, because mechanical and physicochemical properties determine the alginate application. In the present article, the characteristics of alginates has been described, as well as their application in biomedical fields, but also in a sector of wound management materials based on alginates and their influence on wounds healing. Figure 2 shows a graphical representation of the present paper.

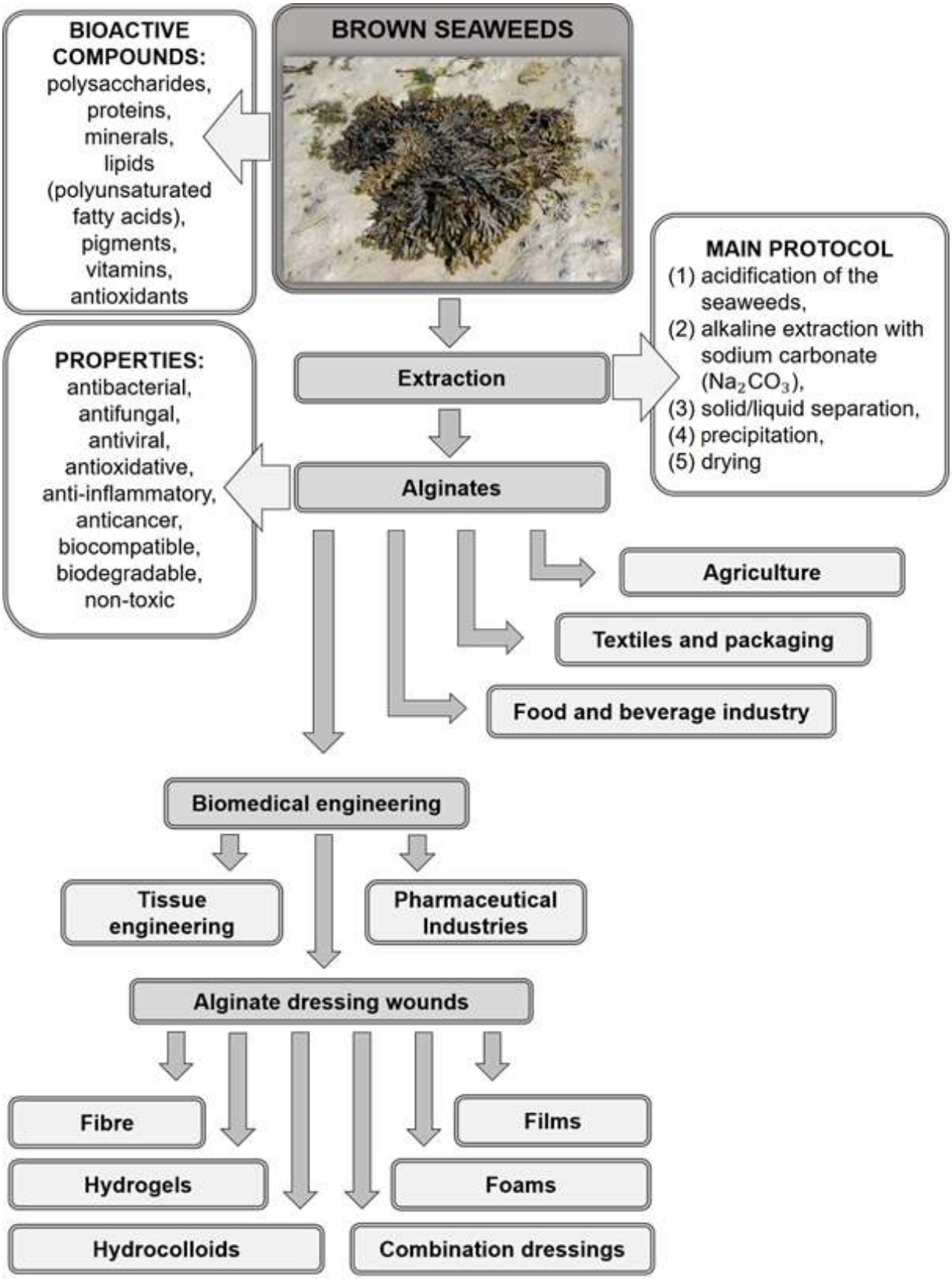

Figure 2: Alginates processing scheme with potential applications.

2 Brown seaweeds as a source of alginates

Alginates are produced industrially from marine seaweeds that belong to the taxonomic group of brown algae (class Phaeophyceae) [11]. Alginate-yielding seaweeds, also called "alginophytes", are mainly harvested from wild populations, although they can also originate from the artificial cultivation of algae – for example kelp *Saccharina japonica* [11, 32]. Nowadays, commercial alginates are produced mainly from brown seaweeds of the following genera *Ascophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia*, *Macrocystis* and *Saccharina* [16, 33]. A detailed list of species of brown seaweeds that are used in various countries for the alginate production is presented in a book chapter in a book chapter of Peteiro [11].

Alginates are a part of the cell wall and inter-cellular matrix of brown seaweeds and provide the flexibility and the mechanical strength in order to survive in the water reservoirs [3, 6, 34]. Seaweed alginates exist as an insoluble mixed salt of all cations that are found in a sea water such as calcium, sodium and magnesium [16, 34]. The alginate biopolymer can represent up to 40% of dry matter of brown seaweeds [30]. The quantity and quality of the alginates in brown seaweeds depend on many factors such as algae species, season of their harvesting, the type and age of the tissues [14, 25]. Kumar and Sahoo [35] revealed that the alginate content in a biomass fluctuated as a function of seasonality (the highest content was in *Sargassum wightii* collected from Mandapam, Tamil Nadu, India in March, amongst other periods – January, May, July, September, November), as well as different vegetative parts of the thallus (the highest was in the main axis, among other parts such as whole thallus, young and old blades). In the case of *Sargassum filipendula* collected from Cigarras beach in Brazil, the highest extraction yield of alginates was for algae harvested in fall – May and spring – November and the smallest during summer – February [27]. On the other hand, Zubia et al. [36] found that the alginate yield from *Sargassum mangarevense* (Arue, Tahiti, French Polynesia) and *Turbinaria ornata* (Punaauia, Tahiti, French Polynesia) displayed spatial variations, but no significant seasonal changes (algae collected in February and July). A detailed description of genera and species used for alginate production, as well as natural habitats of brown seaweeds, harvesting methods of wild seaweeds, their quantities and cultivation methods is presented in the work of McHugh [32].

3 Conventional extraction of alginates from brown seaweeds

Generally, the protocol concerning extraction of alginates is composed of 5 steps: (1) acidification of the seaweeds, (2) alkaline extraction with sodium carbonate (Na_2CO_3), (3) solid/liquid separation, (4) precipitation and (5) drying [5, 8, 11, 37, 38]. All these steps were described in details in a series of publications dedicated to the pilot plant scale extraction of alginates from *Macrocystis pyrifera*: effect of pre-extraction treatments on yield and quality of alginate [5], extraction conditions and methods of separating the alkaline-insoluble residue [39], precipitation, bleaching and conversion of calcium alginate to alginic acid [40] and conversion of alginic acid to sodium alginate, drying and milling [41]. The results of the experiments carried out in a pilot plant presented in the mentioned publications are especially important because they are closer to those that can be expected at an industrial scale [39]. Some of the procedures of the brown seaweeds pre-treatment, alginates extraction and their purification mainly in the laboratory scale are presented in Table 1.

Algal biomass before extraction of alginates can be pre-treated not only chemically (e.g., acidification), but also physically. First of all, the biomass used for the alginates extraction must contain 83% of a dried matter (17% moisture) and less than 3% sand [3]. Another important parameter is a biomass size. Reduction of the size of a raw material facilitates the subsequent processing of algae [38]. Fertah et al. [4] used for the extraction of alginates from *Laminaria digitata* ground biomass with the size <1 mm and 1–5 mm. It was shown that a higher yield was obtained for the biomass with lower particle size what can be explained by its higher surface area. Santagata et al. [42] showed also that the using a low-frequency ultrasounds induced seaweed (*Sargassum*) cell wall disruption, causing the improvement of the extraction yield of alginates.

Several authors [4, 12, 13, 27, 29, 36, 39, 43] before extraction of alginates from brown seaweeds performed soaking of algae in formaldehyde/formalin (usually 1–12 h) in order to soften the seaweed tissue, bleaching algal biomass and avoid alginate pigmentation [3, 5, 27]. The solution of formaldehyde reacts with algal phenolic compounds, polymerize and make the colouring substances insoluble [43]. Additionally, then the extraction yield of alginates can be higher [3]. In the work of Andriamanantoanina and Rinaudo [2] it was shown that the bleaching of brown algae before extraction (e.g.,

Table 1: Conventional extraction procedures of alginates.

Seaweed species	Extraction technique	Extraction yield (% dry weight - d.w.)	Characteristic	Reference
Sargassum mangarevense *Turbinaria ornata* Arue and Punaauia, Tahiti, French Polynesia	stirring sun-dried alga for 12 h in 1% formaldehyde washing with deionized water prior to acidification (0.2N H_2SO_4, 4 h, 25°C) washing with deionised water prior to alginates extraction extraction: stirring of algae in 1% Na_2CO_3 (12 h, 25°C) filtration and precipitation of alginates in 95% ethanol (1:2 *v/v*) washing precipitate with absolute ethanol then with acetone drying (24 h, 40°C)	*S. mangarevense*: 9.3±1.7% *T. ornata*: 19.2±1.3	phenolic content and antioxidant activity antimicrobial activity	[36]
Sargassum wightii Mandapam, Tamil Nadu, India		max. 33.1±0.3%	biochemical composition of leftover pulp after extraction (total carbohydrate, protein, lipid, ash, minerals, total phenolic content)	[35]
Laminaria digitata Portsall, Brittany, France	acidification (0.5M H_2SO_4, at least one night, 4°C) and rinsing with distilled water to eliminate acid excess batch extraction: soaking of acidified algae in 4% (*w/w*) Na_2CO_3 solution and stirring or reactive extrusion extraction (corotative twin-screw extruder): 5 min., 4% Na_2CO_3 solid/liquid separation – centrifugation precipitation with sulphuric acid and drying	batch extraction: 33±2% reactive extrusion extraction: 39±2%	rheological characteristic (viscosity), higher for reactive extrusion extraction (0.8±0.1 L/g) than for batch extraction: 0.30±0.05 L/g	[8]
Laminaria digitata Portsall, Brittany, France	like Vauchel et al., 2008a extraction duration: 1, 1.5, 2, 2.5, 3, 3.5, 4 h	extraction for 4 h is required to reach the max. sodium alginate content	decrease in alginate dynamic viscosity after 2 h of extraction the molecular weight of sodium alginate solution decreased with the increase of extraction duration	[9]
Sargassum duplicatum *Sargassum crassifolium* Pamengpeuk shore of Banten, Indonesia	rinsing and immersing pieces of algae leaves in water until they were expanded and then immersing in a 0.3% HCl for 1 h extraction: algae pieces were rinsed with distilled water and soaked in 4% (*w/w*) Na_2CO_3 under stirring at least 2 h separation of supernatant from the solution acidification with 10% HCl and rinsing the alginic acid with water and drying addition of 10% NaOH to produce sodium alginate	n.a.	characteristic of alginates: SEM, XRF, FTIR	[23]
Sargassum natans *Sargassum vulgare* *Padina gymnospora* *Padina antillarum* Ghana *Laminaria digitata* *Macrocystis pyrifera* Denmark	soaking of algae overnight in 2% (*w/w*) formaldehyde washing of seaweeds with water and adding 0.2M HCl (overnight) washing and adding 2% Na_2CO_3 at 99°C for 3 h filtration and precipitation of sodium alginate in 80% isopropanol	from 16±0.7% for *P. gymnospora* to 29±4.2% for *L. digitata*	rheological properties (gel-strength properties)	[13]

Continued **Table 1:** Conventional extraction procedures of alginates.

Seaweed species	Extraction technique	Extraction yield (% dry weight - d.w.)	Characteristic	Reference
Zonaria sp., *Chnoospora sp.* - Fort-Dauphin; *Sargassum* sp., *Spatoglossum* sp. – Toliary; *Sargassum* sp. - Fenerive-Est, Madagascar	washing of algae with water, soaking in ethanol to inactivate enzymes, drying overnight at 35°C and grounding bleaching treatment of ground algae to eliminate pigments with aqueous solution of sodium hypochlorite with active chlorine extraction: 1M Na_2CO_3 at 60°C for 2h, pH~11 centrifugation, filtration and neutralization of the suspension with 1N HCl till pH~7-8 precipitation with 50% ethanol	from 9.2% for *Chnoospora* sp. to 26.6% for *Sargassum* sp. (unbleached algae) and from 17.4% for *Spatoglossum* sp. to 50.8% for *Chnoospora* sp. (chlorine bleached algae)	the extraction after bleaching of algae with sodium hypochlorite gave white alginates with higher yield than after direct extraction	[2]
Sargassum angustifolium Bushehr, Iran	removing non-target compounds (pigments and lipids) from milled algae with 85% ethanol, overnight, room temperature rinsing of the biomass with acetone and drying at room temperature treating the dried biomass with distilled water (65°C, 3 h) or HCl (0.1 M, pH 2, 65°C, 3 h) or alcalase (5% w/w, pH 8, 50°C, 24 h) or cellulase (5% w/w, pH 4.5, 50°C, 24 h) extraction of alginates with 3% Na_2CO_3, pH 11, 65°C, 3 h precipitation with 75% ethanol	3.3% for water treated seaweeds 3.4% for acid pre-treatment 3.5% for alcalase and cellulase treatments	the use of enzymes considerably reduced protein and polyphenol contaminations of alginates compared to those of water and acid extraction method did not affect the structure of the obtained alginates (FT-IR) the highest molecular weight was found in acid treated alginate, the minimum for cellulase assistant alginate all alginates showed antioxidant activity – the highest for enzyme-treated algae	[16]
Sargassum baccularia *Sargassum binderi* *Sargassum siliquosum* *Turbinaria conoides* Port Dickson, Negeri Sembilan, Malaysia	*cold method:* soaking of air-dried seaweeds in 1% $CaCl_2$ (27°C, overnight ~18 h) and washing seaweeds with distilled water (for *hot method* – storing time was 3 h at 50°C) storing seaweeds in 5% HCl solution for 1 h and washing with distilled water storing algae in 3% Na_2CO_3 solution for 1 h and adding water before it was left to stand overnight separation of the viscous mixture from its residue by centrifuging precipitation of sodium alginate with ethanol/water mixture (1:1, *v/v*) filtration, washing with ethanol and drying of the precipitate *hot method:* - similarly as the cold method, except first step: storing time was 3 h at 50°C in 1% $CaCl_2$	*S. baccularia*: hot method – 26.7%; cold – 23.9% *T. conoides*: hot – 41.4%; cold – 40.5%; *S. binderi*: hot – 38.9%; cold – 28.7%; *S. siliquosum*: hot – 49.9%; cold – 38.9%	higher extraction yield was for hot method the intrinsic viscosity, as well as molecular weight of alginates from seaweeds was lower when it was extracted through the hot method	[47]

Continued **Table 1:** Conventional extraction procedures of alginates.

Seaweed species	Extraction technique	Extraction yield (% dry weight - d.w.)	Characteristic	Reference
Cystoseira trinode *Cystoseira myrica* *Sargassum dentifolium* *Sargassum asperifolium* *Sargassum latifolium* Egypt	treating of ground algae with formaldehyde before extraction with 0.2M HCl suspension of residue in distilled water, neutralization (pH 7-8) and filtration* extraction of the neutral residue with 3% Na_2CO_3 precipitation of alginate with ethanol *filtrate (neutral extract) was also mixed with ethanol to precipitate alginate (without Na_2CO_3)	*C. trinode* – 3.3% *C. myrica* – 2.2% *S. dentifolium* – 3.3% *S. asperifolium* – 12.1% *S. latifolium* – 4.3%	yield of alginate for *C. trinode* extracted in the neutral step was 1.1% (three times lower than in the case of extraction of alginate by Na_2CO_3), but for *S. latifolium* – 17.2 (four times higher than for extraction with Na_2CO_3)	[34]
Sargassum fluitans Guanabo Beach, Havana, Cuba *Sargassum oligocystum* Goold Island, Australia	*neutral extraction*: extraction 3 times of ground algae in 0.2N HCl, filtration, washing with water re-suspension of solids in distilled water and NaOH to neutralise the alginic acid and maintain the pH between 6.5 and 7.5, overnight, filtration and extraction once more filtration, reduction of volume by vacuum roto-evaporation addition of sodium chloride precipitation of alginate with ethanol, washing with ethanol and ethyl ether and drying 30–40°C *room temperature (22°C) alkaline extraction*: soaking the ground algae overnight in a 5% formaldehyde extraction 3 times in 0.2N HCl, filtration, washing with water re-suspension of solids in distilled water and excess NaOH, gently stirring overnight neutralization of filtrate with HCl and volume reduction by vacuum roto-evaporation precipitation and washing of alginate as above *high-temperature alkaline extraction*: algae with and without a formaldehyde pre-treatment extraction of biomass three times in 0.2 N HCl, filtration, washing with water re-suspension of solids in a 2% Na_2CO_3, 2 h, 80°C, water bath, constant stirring Na-alginate precipitated with dilute HCl was separated by filtration	alginate yield was independent of the temperature, the extraction method and pre-treatment with formaldehyde from 21.1 to 22.8% for *S. fluitans* From 18.9 to 20.5% for *S. oligocystum*	heavy-metal sorption obtained alginates have similar metal binding capacities (uptake ranging from 1.59 to 1.81 mmol Cd/gram of biomass)	[29]
Sargassum turbinarioides Nosy Be, Madagascar	soaking of dried algae in 2% formaldehyde (24 h, room temp.) washing of solids with water and adding 0.2 M HCl (24 h) washing of solids and extraction with 2% Na_2CO_3 (3 h, 100°C) collection of a soluble fraction by centrifugation precipitation of alginate with ethanol 95% and washing with acetone	10%	the extracted alginate was a polymer of uronic acids (no neutral monosaccharide and sulphate were detected)	[12]

Continued **Table 1:** Conventional extraction procedures of alginates.

Seaweed species	Extraction technique	Extraction yield (% dry weight - d.w.)	Characteristic	Reference
Laminaria digitata Atlantic coast, Western Morocco	size of milled algae: <1 mm and 1-5 mm soaking of dried algae in 2% formaldehyde for one night washing of solids with water and adding 0.2M HCl (24 h) washing of solids and extraction with 2% Na_2CO_3 (5 h, 25, 40, 60°C) centrifugation and precipitation of alginate with ethanol purification of alginate with ethanol, methanol and acetone	for size <1 mm: 25°C – 38.3%, 40°C–51.8%, 60°C–43.2%; for size 1–5 mm: 25°C –35.3%, 40°C – 44.0%, 60°C – 40.2%	higher extraction yield was for lower algae size and the temperature 40°C it is suggested that it can be used as a polyelectrolyte complexes for the production of drug delivery micro-and nanoparticles	[4]
Sargassum vulgare Pacheco Beach, Caucaia, Ceara, Brazil	soaking of dried algae in 2% formaldehyde for 24 h washing of solids with water and adding 0.2M HCl (24 h) washing of solids and extraction with 2% Na_2CO_3 (25, 60 and 80°C, 1, 2, 3, 4 and 5 h) centrifugation and precipitation of alginate with ethanol purification of alginate with ethanol and acetone	the best yield – 16.9% was after 5 h of extraction at 60 or 80°C. This yield is 40% higher than the yield for the sample extraction at 25°C	increase in the temperature of the extraction resulted in higher solution viscosity	[49]
Sargassum muticum Nykøbing Mors, Denmark	soaking of algae in 0.2%formaldehyde, 24 h, 16°C washing of solids with water and adding 0.2M HCl washing of solids and extraction with Na_2CO_3 at 0.5% (pH 11.7), 1% (pH 11.8), 2% (pH 11.97), 3% (pH 12), 4% (pH 12.2), 5% (pH 12.4), different temperatures (50, 60, 70, 80, 90 and 100°C) with varying times (1, 1.5, 2, 2.5, 3 and 3.5 h) centrifugation and precipitation of alginate with aqueous ethanol	maximum yield: 13.6±0.1% for 86°C, alkali 3% and solvent 93%	yield increased with the increase of the extraction period – maximum in 3 h (for 2% alkali and 0.2M HCl); the highest yield was for temp. between 90 and 100°C (for 2% alkali, 0.2M HCl and extraction time 3 h); the residual supernatant of the extract is rich in polyphenols and antioxidants	[15]
Sargassum sp. *Turbinaria* sp. *Hormophysa* sp. Madagascar	suspension of ground algae in distilled water and addition of 0.1N HCl under strong stirring to get pH 3 (24 h, at room temperature) washing of biomass with distilled water and its suspension in water addition of 1M Na_2CO_3 and EDTA, the pH of the is 11 (80°C, 2 h) acidification with 6N HCl (pH 3), collection of precipitate by centrifugation and suspension in distilled water addition of an 1M NaOH (pH 10) – precipitation of sodium alginate with ethanol in the presence of NaCl and washing with ethanol/water mixtures	31% – *Sargassum* sp. 31% – *Turbinaria* sp. 30% – *Hormophysa* sp	in absence of EDTA, the alginate yield is lower (10-13%) in relation with the presence of calcium complexed in the cell wall	[26]

Continued **Table 1:** Conventional extraction procedures of alginates.

Seaweed species	Extraction technique	Extraction yield (% dry weight - d.w.)	Characteristic	Reference
Sargassum filipendula Cigarras beach, Brazil Collection: fall (May), spring (November) and summer (February)	soaking of dried algae in 0.4% formaldehyde for 0.5 h, washing with distilled water and addition of 0.1M HCl for 2 h washing of bleached algae with distilled water and extraction with 2% Na_2CO_3 (5 h, 60°C) separation of alginate from the crude extract by ethanol precipitation	fall: 17.0±0.1% spring: 17.2±0.3% summer: 15.1±0.1%	extraction yield depended on the season of algae harvesting	[27]

n.a. – not available

with chlorine) favoured the yield of extraction possibly due to the swelling of the algae cell walls.

The treatment of seaweeds before extraction of alginates with diluted mineral acids is considered by many authors as an essential step that makes the alginate more readily soluble in an alkaline solution [5, 8, 9, 13, 23, 35, 36] etc., (see Table 1). More precisely, the acidification of algae can have several goals, for example it is necessary (1) to remove external salts and residual formaldehyde [3], (2) to remove non-target compounds such as polyphenols and other polysaccharides such as fucoidans and laminarins [27, 44] and (3) to provide acidic condition up to pH 4 where alginate salts, for example with calcium, sodium, potassium are converted to alginic acid which is then more easily converted into soluble sodium alginate during extraction with sodium carbonate [3, 13, 44]. In order to remove non-target compounds such as pigments and lipids, Borazjani et al. [16] treated the milled algae with 85% ethanol overnight at room temperature. Youssouf et al. [10] used pre-treatment of *Sargassum binderi* and *Turbinaria ornata* with 80% ethanol at room temperature in order to eliminate other compounds such as pigments, polyphenols and fatty acids. Leal et al. [43] performed pre-treatment of algae (*Lessonia flavicans*, *Desmarestia ligulata* and *Desmarestia distans*) with petroleum ether to remove fats. Borazjani et al. [16] proposed pre-treatment of algal biomass (*Sargassum angustifolium*) before extraction with (1) distilled water: 65°C, 3 h, (2) HCl: 0.1M, pH 2, 65°C, 3 enzymes: (3) alcalase: 5% *w/w*, pH 8, 50°C, 24 h or (4) cellulase: 5% *w/w*, pH 4.5, 50°C, 24 h and examined its effect on the chemical composition and molecular properties of alginates.

Another approach was proposed by Lorbeer et al. [44] who used an acidic treatment (HCl solution) of the brown alga *Ecklonia radiata* in order to extract fucoidan (a sulphated polysaccharide found mainly in brown seaweeds) and at the same time to facilitate the efficient sequential extraction of alginates. In the case of the extraction of fucoidan from seaweeds, acid treatment disrupts hydrogen bonds between polysaccharides and liberates fucoidan. At the same time, alginate is converted into an insoluble alginic acid and do not contaminate the fucoidan extract [45]. This is an example of a sequential biorefinery extraction process of two polysaccharides [44, 45]. Yuan and Macquarrie [46] also described a step-by-step biorefinery process of a seaweed *Ascophyllum nodosum*, which was designed to obtain fucoidan, alginates, sugars and biochar using microwave-assisted extraction. It was shown that brown seaweeds could be potentially used as a feedstock for a biorefinery process in order to produce valuable chemicals and fuels. By using an acid pre-treatment of brown seaweeds it is possible to extract separately alginate and fucoidan. Fenoradosoa et al. [12] found that the alginate extracted from *Sargassum turbinarioides* after its pre-treatment with HCl was a polymer of uronic acids (0.3% *w/w*). It was not associated with sulphated fucans which can occur together with alginates as matrix polysaccharides in *Sargassum* species.

The central step in the extraction protocol is the alkaline extraction which aims at the conversion of the original insoluble calcium and magnesium salts into soluble purified sodium alginate [5]. If the seaweeds (an original calcium alginate) are treated with alkali without prior acid pre-treatment of biomass, then the extraction is due to an ion-exchange [5]. In the extraction process it is necessary to optimize the temperature, extraction time, alkali concentration and consumption of solvent used for precipitation of alginates [15].

The conventional extraction process of alginates is based mainly on thermal treatment [38]. During extraction

process, seaweeds together with sodium carbonate are heated to higher temperature (from room temperature till 100°C) [3]. A slight increase of treatment temperature can lead to the rise in the extraction yield but also to the decrease of viscosity. Normally, the extraction temperature ranges between room temperature and 50°C [47]. Fertah et al. [4] showed that among three tested temperatures – 25, 40 and 60°C, the highest extraction yield of alginates from *Laminaria digitata* was achieved for 40°C. However, alternative results were obtained by Mazumder et al. [15] who extracted alginates from *Sargassum muticum* at following temperatures – 50, 60, 70, 80, 90 and 100°C. It was found that the extraction yield increased significantly above 70°C and the highest yield was observed when the temperature was between 90 and 100°C. Hernández-Carmona et al. [39] tested the effect of temperature on the extraction of alginates from *Macrocystis pyrifera* at 70, 80 and 90°C. It was found that the yield at 90°C – 21.9 % was significantly higher than the yield at 70°C – 19.4%.

There is also an important role of extraction time acting as a critical variable that influences the yield of alginate [15]. The extraction process usually requires several hours (Table 1). In the work of Mazumder et al. [15] it was shown that there was a direct relationship between extraction time – 1, 1.5, 2, 2.5, 3 and 3.5 h of alginates from *Sargassum muticum* and the extraction yield. Maximum yield was reached for 3 hours. Also Hernández-Carmona et al. [39] examined the effect of time – from 1 to 9 h of the alkaline extraction of *Macrocystis pyrifera* on the alginate yield. It was found that the yield of alginate increased with time and maximum was reached after 3.5 h of extraction.

In the work of Vauchel et al. [8] it was shown that the rheological properties of alginates (e.g., dynamic viscosity) can be decreased after 2 hours of alkaline treatment. Also Truus et al. [48] indicated that longer the extraction process and higher the temperatures will cause the production of alginates with lower viscosity, because of depolymerisation of the polymeric chain of the alginate. In the work of Torres et al. [49] alginates extracted from *Sargassum vulgare* for 1 h at 60°C were denoted as "low viscosity", whereas for 5 h at 60°C as "high viscosity" – increase in the temperature during alginates extraction resulted in higher viscosity of solution, probably due to the dissolution of macromolecules with a high molar mass. The M/G ratio values were 1.56 and 1.27, respectively, what is higher than the ratio for most *Sargassum* spp. alginates which is in a range 0.19–0.82.

For the extraction of alginates from brown seaweeds, alkali are used and they change the alginic acid to soluble sodium alginate that appears like a viscous gel [12, 15, 29]. Alginates are extracted mainly with sodium carbonate which is also added to reach pH ~10 [3, 15]. Rahelivao et al. [26] used the aqueous solution of 1M Na_2CO_3 and EDTA in order to increase pH to 11. The basic pH leads to the formation of a water soluble sodium alginate [10]. Mazumder et al. [15] examined the effect of alkali concentration (0.5–5%) on the extraction yield of alginates (for constant other parameters – 0.2M HCl, 3 h extraction time, 100°C, that also influence extraction yield) from *Sargassum muticum*. It was noted that the extraction yield increased rapidly up to 3% of alkali and decreased significantly after this concentration, probably due to the depolymerization of the alginate structure.

The last stage in the production of alginates is their precipitation and purification. Gomez et al. [6] studied three routes of precipitation of sodium alginate from the extract obtained from *Macrocystis pyrifera* – with ethanol, HCl and $CaCl_2$. In the first route, sodium alginate is directly precipitated from the extract wit ethanol. In the HCl route, sodium alginate extract is mixed with HCl. The obtained precipitate of alginic acid is separated by centrifugation and mixed with distilled water and Na_2CO_3 to obtain soluble sodium alginate. It is precipitated then with ethanol. $CaCl_2$ route involves its utilization for the precipitation of sodium alginate from the extract. The obtained calcium alginate after washing with water is mixed with HCl to obtain insoluble alginic acid, which is separated by centrifugation. Later, the procedure is the same as for HCl route. Gomez et al. [6] showed that ethanol route had the lowest number of steps and displayed the best performance – the highest yield and rheological properties of the obtained sodium alginate. Treatment of the sodium alginate extract with HCl caused degradation of the polymer chain. In this research it was also shown that $CaCl_2$ route gave alginates with the lowest molecular weight and poor mechanical properties [6]. As can be seen from Table 1, ethanol route is the most often used for the precipitation of sodium alginate.

The effect of the ethanol concentration on the precipitation of alginates from the solution after extraction is randomly studied. Mazumder et al. [15] presented interesting data which showed that the extraction yield of alginate from *Sargassum muticum* was negligible for 50 and 60% ethanol. The precipitation range increased slightly at 70% and reached a maximum between 90 and 100%.

Taking into account the application of alginates for some food/pharmaceutical/medical applications, it is also necessary to bleach the obtained product. Part of the compounds that impair the colour of alginates is removed in the previous steps – treatment with formalin/formaldehyde and acid treatment, but the final colour of

alginate can be still dark brown. In the literature it was shown that the best stage to bleach the product is as calcium alginate ($CaCl_2$ route) since it is more resistant to the degradation than alginic acid (HCl route) [50]. Usually, for bleaching a sodium hypochlorite solution (12%) is added to a suspension of the calcium alginate in water [39].

4 Novel extraction techniques of alginates from brown seaweeds

Nowadays, there is a demand for new, eco-friendly methods which will improve extraction process (e.g., yield, experimental conditions). Such a technique can be ultrasound assisted extraction (UAE) which limits energy consumption by the reduction of the extraction time, as well as the volume of solvents used, thus making it a "greener" process [10, 42]. Youssouf et al. [10] showed that the extraction yield of alginates from *Sargassum binderi* and *Turbinaria ornata* depended on algae/water ratio, pH and the time of exposure to ultrasounds. The highest extraction yield of alginates equal to 54% was obtained for the following experimental conditions: algae/water ratio of 10 g/L, pH 12, ultrasounds for 40 minutes and ultrasound power 150 W. Conventional extraction methods permit to obtain 25% alginate yield in 2 h [10]. As can be seen, the time of the extraction of alginates was significantly reduced – from about 2 h for a conventional method to 15–30 minutes with ultrasound assistance. Also microwave-assisted extraction (MAE) is proposed as a novel technique for the extraction of alginates since it possesses enormous potential to overcome major drawbacks that accompany the conventional extraction – thermal and/or solvent based techniques [38, 46]. In the work of Silva et al. [38] it was demonstrated that the determination of the optimal acid pre-treatment conditions (40 mL of 0.1M HCl per g of dried seaweed with constant stirring at 20°C during 14 h) of *Saccorhiza polyschides* significantly enhanced MAE of alginates. The examples of the extraction of alginates with the use of novel techniques are presented in Table 2.

5 Characteristic of alginates obtained from brown seaweeds

What is important, the extracted alginates should be characterised in details – their morphology, content of chemical elements and functional groups, etc., in order to use them appropriately for several applications. The techniques that can be used for the analysis of properties of extracted alginates are briefly described below.

^{1}H-NMR (nuclear magnetic resonance) is used to determine composition, structure, M/G ratio and block distribution of alginates (e.g., 2, 4, 6, 10, 12, 13, 15, 16, 24, 26, 27, 29, 34, 46, 49). This analysis is considered to be the most reliable method to determine composition and block structure of the alginate [12]. Additionally, it provides insight into the relation between rheological functionality and chemical structure of alginates [13].

Chromatography found its application to determine a molecular weight of the extracted compounds (e.g., 2, 6, 10, 12, 16, 42, 46). High performance anion exchange chromatography can be used to determine the carbohydrate composition (monosaccharide analysis), whereas size-exclusion chromatography to analyse alginates [2, 12, 13].

Rheological characteristic is an important parameter for the application of polysaccharides (including alginates) in the industry [12] and is considered in terms of:

(a) intrinsic viscosity (capillary viscosimetry) (e.g., 4, 9, 12, 24, 26, 34, 47, 49). The intrinsic viscosity is directly linked to the average molecular weight – the higher viscosity, the higher molecular weight [8]. This parameter depends also on the extraction technique – for example, for the sodium alginate obtained by batch extraction it was 0.3 L/g, whereas for reactive extrusion extraction 0.8 L/g [8]. Additionally, bleaching of seaweeds before extraction can decreases the intrinsic viscosity of alginate [2].
(b) gel strengths of the alginate (gel cylinders) (e.g., 2, 34). This technique is used for gel characteristic in the presence of calcium ions [2]. Bleaching of seaweeds before extraction process can favour the gel formation even if the molecular weight is decreased [2].
(c) oscillatory rheology (gelation kinetics of hydrocolloids – rheometer) (e.g., 6, 13, 26).

Another important parameter in terms of application in food and biomedical field is alginate purity. It can be determined by measuring the level of phenolic compounds (Folin-Ciocalteu reagent e.g., 16; fluorescence spectroscopy e.g., 4, 15) and proteins (Lowry method) introduced to the extracted alginate [16]. Fluorescence spectroscopy can be applied to alginates, because they are strongly fluorescent due to small amounts of polyphenolic residues [4]. Protein content in the alginate can be reduced by the pre-treatment of seaweeds before extraction with HCl or protease and carbo-hydrase

Table 2: Novel extraction techniques of alginates.

Seaweed species	Extraction technique	Extraction yield (% d.w.)	Examination	Reference
Saccorhiza polyschides Angeiras beach, Portugal	conventional acid pre-treatment of soaked algae with 1M HCl to pH 4, 15 minutes, filtration, washing with water, repetition of procedure once more and finally stirring of the mixture at the same acid conditions overnight. Examined periods - 0.5, 7.25 and 14 h, temp. – 20, 30 and 40°C addition of 10% Na_2CO_3 to the acidified seaweed in water – adjustment pH to 10 ***microwave-assisted extraction*** – in a Microwave Accelerated Reaction System for Extraction and Digestion (100°C, 20 minutes) precipitation of alginate soluble fraction with 10% $CaCl_2$ conversion of calcium alginate into alginic acid 1M HCl treatment of fibres of alginic acid were treated with ethanol/H_2O adjustment of pH to 8 with 10% Na_2CO_3 to obtain sodium alginate	from 7.5 to 23.8%	optimal acid pre-treatment conditions 40 mL of 0.1M HCl per g of dried seaweed with constant stirring at 20°C during 14 h	[38]
Ascophyllum nodosum Shetland, UK	pre-extraction of dry algae with 80% ethanol under stirring at room temperature, 18 h and then 70°C, 4 h, centrifugation and drying suspension of algae in 0.1M HCl ***microwave-assisted extraction*** of alginate in a microwave synthesizer: 0.1M Na_2CO_3, 10 minutes, 100°C precipitation of alginate with ethanol	18.2%	biorefinery process – production from *A. nodosum* beside alginates, also fucoidan, sugars and biochar	[46]
Sargassum binderi *Turbinaria ornata* Mauritius	treating of dried seaweeds overnight with 80% ethanol, room temperature washing of biomass with distilled water ***ultrasound assisted extraction*** – PEX3 Sonifier (frequency of 25 kHz at the base with maximum input power of 150 W), 30 min., pH 12, temp. 30, 50, 70 and 90°C, ultrasound power: 75, 150 W – obtained sodium alginate solution filtration and precipitation of alginates by adding (1) ethanol or (2) $CaCl_2$ (transformation of sodium alginate into calcium alginate, then addition of 1N HCl up to pH 4 in order to convert alginates into the insoluble alginic acid. The formed precipitate was filtered, washed and suspended in water – alginic acid was changed into sodium alginates with 0.5% Na_2CO_3. Finally sodium alginate was precipitated with ethanol)	27% at 90°C	yield of extracted alginates increased with temperature and increase of ultrasound power from 75 to 150 W alginates can be used in various fields of industry such as food, cosmetics and pharmaceuticals alginates also display a good potential for drug delivery applications	[10]

enzymes [16]. Other analyzes include determination of the uronic acid content (spectrophotometry) (e.g., 9, 12) and the chemical elements in alginates (SEM-EDX) (e.g., 31). Some impurities (e.g., presence of Al) can be associated with the alginate extraction [31].

Structural characteristic of extracted alginates can be performed using SEM, XRF and FTIR techniques. SEM (Scanning Electron Microscopy) is used to study the morphology of alginates (e.g., 16, 23, 27, 28, 30, 31, 42). XRF (X-ray Fluorescence) is applied to quantify chemical elements in the alginate, for example Na, Ca, Si, Fe, S (e.g., 23). The major elements in alginates from *Sargassum duplicatum* and *S. crassifolium* were Na and then Ca [23]. The presence of sulphur can be associated with residues of fucoidan present in the brown seaweeds, from which alginate is extracted [31]. To confirm the existence of functional groups in the alginate FTIR (Fourier Transform Infra-Red Spectroscopy) analysis is used (e.g., 4, 10, 12, 13, 16, 22, 23, 28, 30, 42, 43). FTIR spectra of alginates are usually performed in the band range of 4000–450 cm^{-1}. Carboxyl (COO^-) at about 1626-1623 cm^{-1} and at 1421 cm^{-1} and hydroxyl groups (O-H bending of guluronic acid

units) at about 1025 cm^{-1} are evident in the FTIR spectra of alginates [13, 23]. A band around 1100 cm^{-1} is typical for mannuronic acid units [13]. Carboxyl groups are from the mannuronate and guluronate moieties in alginate present in the sodium salt form [13].

Alginates are also examined in terms of their biological properties such as phenolic content (the Folin-Ciocalteu reagent) (e.g., 15, 36), antioxidant activity determined using DPPH radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl) (e.g., 16, 22, 36) and reducing power (TCA; tricarboxylic acid) (e.g., 15, 16), as well as antimicrobial activity (bacteria, yeast, mould) (e.g., 22). Borazjani et al. [16] showed that alginates obtained from *S. angustifolium* produced the stable form of DPPH in a dose-dependent manner and additionally were able to reduce ferric to ferrous in a redox linked colorimetric reaction. In the work of Janarthanan and Kumar [22] the antibacterial activity (against *Staphylococcus aureus* and *Escherichia coli*) of the cotton fabric coated with *Sargassum wightii* alginate film was tested. Alginate has good antibacterial activity.

6 Alginates applications in a bio-medical field

The introduction of this review paper contains a description of general applications of alginates. The following section characterizes in details applications of alginates in bioengineering sectors including medical textiles thereby supporting healing wound.

Alginates are increasingly used in a health care industry due to their favorable properties. This compound of brown seaweeds has antimicrobial ability, strong free radical scavenging and antioxidant activity, renoprotective effect, anticancer and immunostimulatory properties [13, 15, 22]. These properties are result of the structure, as well as the presence of bioactive compounds such as amino acid, alkaloids, tannins, flavonoids and phenols [22] which contribute higher healing of skin wound. Beneficial alginates properties give the possibility to build skin graft and deliver medications in a controlled manner. They can be used in a treatment of patients suffering from diabetes mellitus, liver and parathyroid disease, as well as in repair and regeneration of tissues, certain cartilages and organs (e.g., liver) in the case of loss or failure of tissues or organs. It is possible to construct alginate-based scaffolds made for cells growth matrix, but also, for other health cases [22, 23, 51–53].

Low-cost, wetting and visco-elastic properties make a possibility to use alginate in dental area, especially as impression materials [54, 55]. Non-toxicity and non-irritating characterisitic makes them a popular choice to using them in preliminary impressions, for full-arch impressions, partial removable dental prosthesis frameworks, provisional crown-and-bridge impressions and more [55, 56]. Alginate impressions have good qualities of reproduction surface detail and they are easier to remove compared with elastomeric materials. Unfortunately, they have low tear strength, thus they might tear upon removal over deep undercuts [56]. Most of dental applications consist of using alginate as an irreversible hydrocolloid, which are composed of 80% of water, but they are dimensionally unstable on storing because of evaporation, likewise alginate powder are not safe in the presence of humidity and higher temperatures due to its hydrophilic nature. Hydrophilicity of the alginate impression materials allows to be used in the presence of saliva or blood [55, 56, 57, 58].

In tissue engineering, alginates are utilized mainly in the hydrogel form since several decades [59]. Moreover this field is still being developed due to possibilities of future applications, as well as healing properties of alginates and advancement of technology in medicine. These hydrocolloids are utilized in alginates scaffolds as a three-dimensional structure for cells growth matrix, to transport cells or proteins to the desired location, which can control the process of engineering or regeneration of tissues and organs. Furthermore, alginate scaffold can provide a protection of a drug or cell from the biological environment and it is a temporary skeleton supporting the formation of a new tissue, then the biodegradability ensures absorption of alginate scaffold by tissues in the nearby environment [23, 53, 60, 61]. Alginates are applied in tissue engineering due to their structural similarities to the macromolecular-based human tissues. Moreover they are characterized by an easy injectability and they have a hydrophilic nature [11]. Another aspect is the ability to avoid the stimulation of a chronic inflammation or immunological reactions and the ability to biodegrade [23, 30, 53, 62].

Regarding the damage of the external tissues of patients, nowadays, there are many professional medical textiles on different injury and there is no universal wound dressing. Wound healing is a complex process and each wound requires other procedure and a proper care, because this process may be compromised by a numerous factors (e.g., improper oxygenation, infections, inadequate dressing choice to type of wound) [52, 63]. However, wound management material based on alginate has been created to enable faster heal from injuries and thus, has an positive effect on most of the

wounds, especially traumatic, chronic and surgical wounds created to enable faster humans' recovery [14, 19, 52]. For instance, professional medical alginate fibres, biodegradable alginates films, which could control drug delivery on injury place by the slow disappearance of the material or could be used for making skin graft and even alginate agent applied to ordinary gauze [22, 64]. Alginate bandage ensures a beneficial, moist and occlusive microenvironment, what can prevent the wound bed from drying out, accelerates and facilitates the process of wound healing due to minimizing of bacterial infection at injury site [22, 53, 59, 60]. Furthermore they absorb exudate or serous fluid and form a hydrophilic gel by a chemical reaction with it. Alginate wound dressings are able to absorb of 15-20 times their own weight of liquid [19]. These bandages are characterized by a flexibility, thus, they are comfortable in daily using, moreover they are water and air permeable [22, 53].

Alginates bandages are suitable for burn patients due to reduction of pain. They are also painless during their application and removal [52]. Their absorbable properties make them widely used in the treating of wounds with a high exudation, due to the hemostatic properties of calcium ions released during their use, as well as for patients with amputations, lacerations, chronic wound, diabetic and leg ulcers, diabetic foot lesions, pressure sores and many others [14, 19, 20, 65]. Researches confirmed a significantly reduced area of chronic and acute wounds in a faster period after use of alginate bandage when compared with an ordinary gauze. It is also a result of the debridement of the necrotic tissue from a chronic wound [20, 66].

In the case of the application of alginates in the biomedical field it is important to cleanse them before extraction, because this natural polymer can be impure. These contaminations can lead to the development of fibrotic cell overgrowth around alginate microcapsules, what might be a collateral damage on human health. Main alginate contaminants are endotoxins, polyphenols and proteins [4].

Wound management is an intricate process, in particular with regard to acute and chronic wounds. Chronic wounds such as ulcers, diabetes, burns, cancer etc. require recovery time more than 12 weeks [67]. The healing process consists of four phases: (a) coagulation and hemeostasis, (b) inflammation, (c) proliferation and (d) wound remodeling with a scar tissue formation. It is important to choose a proper approach to wound healing, adequate dressing to the type of injury since they may impact on the clinical outcome [68]. The most common available wound dressings are based on alginates, collagen, chitosan, hyaluronic acid and silicon, but also cellulose, gelatin and heparin [69]. Due to the variety of injuries, alginate-based bandages are supplied in different forms such as alginate fibre, hydrocolloids, hydrogels, films, foams and also combination dressings, which contain several ingredients supporting the healing process [60, 70, 71]. Commercially used alginate wound dressings with their purpose of application are presented in Table 3. The particular types of alginate wound dressings are characterized below.

6.1 Fibre

The majority of commercially available bandages are based on calcium alginate fibre. They absorb a large amount of exudate to create a gel-like covering over the wounds, what ensures moist environment and prevents wound from drying wound bed [72]. These dressings don't require frequent changing. Depending on the amount of exudate, wearing time is 2-7 days. They are suitable for wounds with moderate to heavy exudation, partial and full-thickness wounds, surgical wounds, infected wounds, pressure ulcers, diabetic and venous ulcers [73, 74]. Alginate fibres are not recommended for wounds with little or no exudate, due to the possibility of drying the wound bed and separating or sticking fibres on wound bed. Moreover, alginate fibres always require a secondary dressing [75]. During dressing change, wound may have an odor. Examples of the most popular alginate fibre dressings are: AlgiDERM® (Bard), Algosteril® (Johnson & Johnson Medical), Kalginate (DeRoyal), Kendall™ Calcium Alginate Dressing (Covidien), Sorbsan® (Dow B. Hickam, Inc.) [71, 74, 76–79].

6.2 Hydrofibre

Another type of alginate bandage is hydrofibre dressing, which is a non-woven ribbon or pad composed of hydrocolloid fibres – sodium carboxymethylcellulose [80]. Alginate hydrofibre dressings are highly sorptive and form gel in combination with exudate to maintain a moist environment, what is an advantage in a fast wound healing and in a painless removal without harming fragile granulation tissue [80, 81]. These dressings don't require frequent changing - depending on the amount of exudate wearing time is 2-7 days. Hydrofibre dressing, such as AQUACEL Hydrofibre Wound Dressing (Conva Tec), is a proper to acute or chronic wounds with large amount of exudate, partial and full-thickness wounds, donor sites, surgical wounds, pressure ulcers, venous and diabetic

Table 3: Commercially available alginate- based wound dressing.

Alginate dressing	Company	Composition	Applications	Type of material	Reference
Acticoat Absorbent with SILCRYST	Smith & Nephew Healthcare	Calcium alginate with na-nocrystalline silver	Diabetic ulcers, burns, grafts and donor sites, infected wounds, pressure ulcers, surgical wounds, venous ulcers	Alginate fibre with PE film	[77]
Algicell® Ag	Derma sciences	Sodium alginate, 1.4% silver	Aertial ulcers, diabetic foot ulcers, leg ulcers, pressure ulcers, donor sites, and traumatic and surgical wounds, venous ulcers	Alginate fibre	[71]
AlgiDERM®	Bard	Calcium alginate	Highly exudating wounds, partial- or full- thickness wounds	Alginate fibre	[76, 78]
AlgiSite M™	Smith & Nephew	Calcium alginate	Leg ulcers, pressure ulcers, diabetic foot ulcers and surgical wounds, thickeness burns	Alginate fibre	[71, 76, 78, 89]
Algisorb	Calgon- Vestal, St Louis, MO	Calcium/sodium alginate	Pressure ulcers, venous leg ulcers, diabetic foot ulcers, cavity wounds, donor and graft sites, postoperative wounds, skin abrasions and lacerations, thickness burns	Alginate fibre	[78]
Algivon®	KIKgel	Calcium alginate and Manuka honey	It eliminates odour and ideal for necrotic wounds and wounds with odours	Alginate fibre	[71]
Algosteril®	Johnson & Johnson Medical	Calcium alginate	Heavily exuding wounds, surgical and traumatic wounds, chronic wounds, burns, infected wounds	Alginate fibre	[76–78]
AQUACEL Ag Hydrofibre Dressing with Silver	Conva Tec	Sodium carboxymethylcellulose and ionic silver (Ag+)	Second degree burns, infected wounds, diabetic foot ulcers, venous ulcers, aertial ulcers, leg ulcers, pressure ulcers, surgical wounds, traumatic wounds, oncology wounds with exudate (fungoides-cutaneous tumors, fungating carcinoma, cutaneous metastasis Kaposi's sarcoma and angiosarcoma)	Composite dressing with hydrofibre	[82, 86]
AQUACEL Hydrofibre Wound Dressing	Conva Tec	Sodium carboxymethylcellulose	Leg ulcers, pressure ulcers, abrasions, lacerations, incisions, donor sites, oncology wounds, first- and second-degree burns, surgical wounds, traumatic wounds, manage exuding wounds	Hydrofibre	[82]

Continued **Table 3:** Commercially available alginate- based wound dressing.

Alginate dressing	Company	Composition	Applications	Type of material	Reference
Calcium alginate rope and sheet	McKessonMedical-Surgical	Calcium alginate	Acute wounds, burns, cavity wounds, deep wounds, deshisced wounds, diabetic foot, granulating/epithelializing wounds, infected wounds, moderate/highly exudating wounds, palliative wounds, pressure ulcers, sloughy wounds, superficial wounds, surgical wounds, vonous wounds	Alginate fibre	[79]
CarraSorb H®	Carrington Laboratories	Calcium alginate	Highly exudating wounds, pressure ulcers, aertial, venous, diabetic ulces and donor sites, traumatic wounds, infected wounds	Hydrogel	[76–78]
Comfeel Plus™	Coloplast AS	Sodium carboxymethylcellulose and calcium alginate	Ulcers such as venous leg ulcers, pressure ulcers; burns, donor sites, postoperative wounds and necrotic wounds, burns	Hydrocolloid	[71]
Curasorb™	The Kendall Company	Calcium alginate	Highly exudating wounds, pressure ulcers, tunneling wounds, infected wounds, red or yellow wounds	Alginate fibre	[76, 78, 82]
ExcelGinate®	MPM Medical, Inc.	Calcium alginate	Chronic wounds, deep wounds, dehisced wounds, moderate/highly exudating wounds, palliative wounds, pressure ulcers, surgical wounds, vonous ulcers	Alginate fibre	[79]
DermaGinate®	DermaRite Industries,LLC	Calcium alginate	Acute wounds, chronic wounds, deep wounds, moderate/highly exudating wounds, palliative wounds, venous ulcers	Alginate fibre	[79, 82]
Fibracol™ Plus	Acelity	Calcium alginate (10%) and collagen (90%)	Full and partial-thickness wounds, for ulcers such as pressure ulcers, venous ulcers, diabetic ulcers, second-degree burns, traumatic wounds, donor sites	Combination dressing	[71]
FyBron®	B. Braun	Calcium alginate	Highly exudating wounds, pressure and leg ulcers, surgical wounds	Combination dressing	[76]
Gentell® Calcium Alginate Ag Dressing	Gentell	Calcium alginate with silver	Acute and cavity wounds, burns, dehisced wounds, chronic wounds, diabetic foot, infected wounds, pressure ulcers, surgical wounds, venous ulcers, highly exudating wounds	Alginate fibre	[76]

Continued **Table 3:** Commercially available alginate- based wound dressing.

Alginate dressing	Company	Composition	Applications	Type of material	Reference
Guardix-SG®	Genvel	Sodium alginate and ploxamer	To avoid post-operative adhesions in thyroid and spine surgeries	Thermosensitive gel	[71]
Hyalogran®	Anika	An ester of hyaluronic acid and sodium alginate	Used for ulcers, diabetic wounds, pressure sores, ischemic, necrotic wounds	Mikrogranules	[71]
Kalginate®	DeRoyal	Calcium alginate	Acute wounds, chronic wounds, diabetic foot, infected wounds, pressure ulcers, surgical wounds, highly exudating wounds, superficial wounds	Alginate fibre	[76, 78]
Kaltogel®	ConvaTec	Calcium/sodium alginate	First/second degree burns,moderate/high exudate, surgical wounds, donor sites, pressure ulcers, venous ulcers, diabetic ulcers	Gellingfibre	[79]
Kaltostat®	ConvaTec	Sodium alginate	Acute wounds, burns, infected wounds, dehisced wounds, pressure ulcers, venous ulcers, diabetic ulcers, donor sites, and traumatic wounds, surgical wounds	Alginate fibre	[71, 76, 77, 90]
Kendall™ Calcium Alginate Dressing	Covidien	Calcium alginate	Acute wounds, burns, chronic wounds, diabetic foot, moderate/highly exudating wounds, pressure ulcers, venous ulcers	Alginate fibre	[79]
Maxorb®	Medline Industries, Inc.	Calcium alginate	Burns, diabetic foot, highly exudating wounds, pressure ulcers, surgical wounds, venous ulcers	Alginate fibre	[76, 82]
Medihoney®	Derma Science	Calcium alginate and honeycolloid	Moderate/High Exudate, pressure ulcers, surgical wounds, venous leg ulcers, diabetic ulcer, burn, infected wounds, reduce odour	Alginate fibre	[82]
Melgisorb®	Mölnlycke Health Care	Calcium/sodium alginate	Highly exudating wounds, infected wounds, pressure ulcers, diabetic ulcers, venous and aertial ulcers, donor sites, postoperative wounds, fungating exogenous tumours (commonly from late stage breast cancer)	Alginate fibre	[78, 82]
Omniderm®	Omikron Scientific Ltd.	Polyurethane film	Chronic ulcer, high exutates wounds, surgical wounds, thickness burns, skin grafts	Film	[89]
PolyMem®	Ferris Mfg.	Calcium alginate	Acute wounds, burns, diabetic foot ulcers, pressure ulcers, surgical wounds, venous ulcers	Composites	[79]

Continued **Table 3:** Commercially available alginate- based wound dressing.

Alginate dressing	Company	Composition	Applications	Type of material	Reference
Restore® Calcium Alginate	Hollister Woundcare	Calcium alginate	First/second degree burns, moderate/highly exudating wounds, partial and full thickness wouns, pressure ulcers, surgical wounds, trauma wounds	Hydrocolloid	[86]
SeaSorb®	Coloplast, Holtedam, Denmark	Calcium alginate	Good for high exuding wounds e.g., ulcers such as diabetic and leg pressure ulcers	Combination dressing	[71, 78]
SILVERCEL™	Johnson & Johnson	Alginate with high guluronic acid (G), carboxymethylocellulose (CMC) and silver-coated fibres	Aertial ulcers, burns, donor sites, infected wounds, pressure ulcers, surgical wounds	Combination dressing	[79]
Silverlon®	Argentum Medical, LLC	Calcium alginate with silver ions	Acute wounds, chronic wounds, pressure ulcers, venous ulcers, diabetic ulcers, donor and graft sites, traumatic and surgical wounds	Alginate fibre	[79]
Sorbsan®	Dow B. Hickam, Inc.	Calcium alginate	Arterial, venous, and diabetic leg ulcers, pressure ulcers, post-operative wounds, donor and graft sites and traumatic wounds	Alginate fibre	[71, 78]
Suprasorb A (+ Ag)	Lohmann & Rauscher GmbH and Co. KG	calcium alginate with antimicrobial silver	Acute wounds, cavity wounds, chronic wounds, diabetic foot, infected wounds, pressue ulcers, donor sites, surgical wounds, venous ulcers	Alginate fibre	[77]
Tegaderm™	3 M Health Care	Calcium alginate	Pressure ulcer, wound care	Film	[79]
Tegagel®	3 M Health Care	Sodium alginate	Diabetic and infected wounds	Hydrogel	[71, 76]
Tegasorb™	3M Health Care	Calcium alginate	Leg ulcers, donor sites, chronic wounds, donor sites, burns, pressure ulcers, first/second degree burns	Hydrocolloid dressing	[85]
Tromboguard®	Tricomed	Sodium alginate, calcium alginate, chitosan, polyurethane and silver cations	Used to stop bleeding in postoperative wounds, traumatic wounds, gun shots, skin graft donor sites, bleeding from accidents	Combination dressing	[71]
UrgoSorb™	URGO Medical	Calcium alginate and hydrocolloid particles	Highly exuding and bleeding wounds, post-surgical wounds, pressure ulcers, leg ulcers	Hydrocolloid	[52]

Continued **Table 3:** Commercially available alginate- based wound dressing.

Alginate dressing	Company	Composition	Applications	Type of material	Reference
UrgoSorb™ Ag/ Silver	URGO Medical	Calcium alginate, hydrocolloid and silver particles (0.5% ionic silver)	Moderate and heavily exuding wounds at risk of infection, post-operative and traumatic wounds, superficial and partial thickness burns, cavity wounds, pressure ulcers, diabetic foot ulcers, leg ulcers, grafts and donor sites	Hydrocolloid	[88]

ulcers [80, 82]. However, they demand a secondary dressing. These bandages are contraindicated in wounds with low amount of exudate, due to the fact that they may induce drying of wound bed.

6.3 Hydrogels

Hydrogel dressing based on alginate contains 70-90% water, in consequence it provides a moist environment. Furthermore, this promotes a self-cleaning wound process, as the result of liquifying necrotic tissue on the wound surface [81, 83]. The wearing time is 1-3 days [71]. Alginate hydrogels, such as CarraSorb H® (Carrington Laboratories), Tegagel®™ (3 M Health Care), are intended for wound with minimal exudation [71, 76–78]. These dressings are viable for wounds such as minor burns, abrasions, infected wounds, partial and full-thickness wounds, surgical wounds, pressure, diabetic and venous ulcers. In the other hand, hydrogels are not suitable for wounds with large amount of exudate and require a secondary dressing [80, 81, 84].

6.4 Hydrocolloids

Wafer dressings fused adhesive elastomers with absorbent colloidal materials are called hydrocolloids [81]. They create gel together with little to moderate amount of exudate, what can provide a moisture environment to protect wound bed against drying [84]. Hydrocolloids are comfortable in the application and painless removal, more importantly, they do not require a secondary wound dressing [80]. Wear time is up to 7 days. They are waterproof, thus they protect from bacterial contamination. These dressings are oxygen impermeable which can cause unattractive odor during removal, and then they are not recommended for infected wounds [83]. Hydrocolloids based on alginate are the most common applied on granulating and epitheliazing wounds, partial and full-thickness wounds, surgical wounds, necrotic wounds, pressure ulcers, diabetic and venous ulcers [80, 81]. They are not proper for highly exudation wounds, because large amount of fluid may contribute to more frequent change of dressings [80]. The most known hydrocolloids, based on alginate, are Comfeel Plus™ (Coloplast AS), Restore® Calcium Alginate (Hollister Woundcare), Tegasorb™ (3 M Health Care), UrgoSorb™ (URGO Medical) [52, 71, 85, 86].

6.5 Films

Film is a transparent, semi-permeable membrane dressing, which is impermeable to liquid and bacteria contamination from outside, but permeable to water vapor, carbon dioxide and oxygen [71, 81, 87]. It creates a moist environment in conjunction with a small amount of exudate what provides removal of necrotic tissue and enables a cellular migration to promote wound healing [80]. Films based on alginate have some advantages, for example they prevent or reduce friction, wound healing progress may by controlled by the transparent film and they are economical. Dressing like Tegaderm™ (3 M Health Care) is suitable for lightening exudation wounds, superficial wounds, blisters, wounds on heels, elbows, flat surfaces [79, 81]. These bandages are not recommended for wounds with a heavy exudation [80].

6.6 Foams

Foam dressings are made from hydrophilic polyurethane foam and they are proper for highly exudation wounds, due to their highly sorptive properties, what promote autolysis of necrotic tissue [80]. Moreover, they are appropriate for partial and full-thickness wounds, surgical wounds, pressure ulcers, venous and diabetic ulcers [71]. They are comfortable for using and conform to wounds. Wearing time is up to 7 days, in most cases 3-5 days [80]. Alginate based foam dressings are not intended for wounds with

little to no exudate, due to the possibility of drying the wound bed. Most of foam dressings is suitable for infected wounds, when is changed daily [80, 83].

6.7 Antibacterial dressings

Alginate dressings with additives, such as nanoparticles of silver, charcoal or other medical care ingredients have antibacterial, antifungal and antiviral properties and reduce unpleasant odors [80, 81]. They are also able to absorb a large amount of exudate, although they should be used short-term only and often requires a secondary dressing. These alginate dressings maintain a favorable moist wound environment to help to put through autolysis process and to accelerate wound healing [80]. Dressing such as Algicell® Ag (Derma sciences), AQUACEL Ag Hydrofibre Dressing with Silver (Conva Tec), Suprasorb A (+ Ag) (Lohmann & Rauscher GmbH and Co. KG), UrgoSorb™ Ag/Silver (URGO Medical) are proper to chronic nonhealing wounds, wounds with moderate to high exudate, surgical wounds, pressure ulcers, diabetic foot and leg ulcers [71, 77, 82, 86, 88]. It is not recommended for patients, which are sensitive to silver and for use in conjunction with oil based product.

6.8 Combination dressings

Combination dressings are creating by fusing two or more various wound care ingredients (e.g., foam, hydrocolloid, charcoal). An example of combination dressings based on alginate are: PolyMem® (Ferris Mfg.), SeaSorb® (Coloplast), Tromboguard® (Tricomed) [71, 78, 79]. They may by applied for low to moderately exudation wounds, partial and full-thickness wounds, surgical wounds, pressure ulcers, venous and diabetic ulcers. Combination dressings are usually waterproof, what prevent from environmental and bacterial contamination. Furthermore, they are comfortable in using and conformable. Frequency of dressing change is dependent on the amount of exudate, although these dressings are not recommended for wounds with a heavy exudation [83].

7 Properties of alginate hydrogel used in biomedical field

Bioengineering use alginate in different form, such as hydrogels, microspheres, microcapsules, foams, sponges and fibres [60]. Hydrogel is the most common form of this natural polymer used in a medical industry. They are three-dimensionally cross-linked networks constructed of hydrophilic polymers with a high water content [53, 60]. Creation of gels from alginate depends primarily on the inherence of zones rich in GG block [2]. Alginate creates gels in the presence of divalent cations, mainly calcium ions, which bind GG blocks of aligned alginate chains. This process is giving rise gel-network, commonly called “egg-box”, as shown in Figure 3 [4, 12, 13, 60].

Alginates are also found in bioengineering as thermo-responsive and thermo-reversible hydrogels. Thermo-reversible alginate hydrogel has a possibility to form gel in response to variation of at least two physical parameters at the same time (e.g., pH, temperature or ionic strength) with ability to convert to the previous consistency. They can be used as a smart delivery of bioactive agents. Thermo-responsive alginate hydrogel has potential application in tissue engineering as injectable cell scaffold [38, 60].

On the chemical and physical properties of alginates have considerable impact their molecular characteristic, especially uronic acid ratio (M/G), gelation agent concentration (e.g., calcium ions concentration), molecular weight, degree of polymerization and block-structure of alginate backbone. The monomer sequence (M and G) may be individual for different algal species, as well as in various tissues of identical species [2, 4, 13, 19]. Content of guluronic acid from alginate gels decides about their fragility and strength, whereas amount of mannuronic acid determines their flexibility and weaker form of gel [11, 49]. Pre-gel solution viscosity and post-gelling stiffness can be individually regulated by manipulation of the molecular weight and its distribution. Molecular weight is dependent upon extraction method, it can be increased by using cold method. Assessment of alginate molecular weight is determined by viscosity measurement [14, 47, 53]. Increasing alginates molecular weight can improve the physical properties of gels, although excessively high molecular weight in alginates solution can lead to extreme viscosity, which could be unacceptable in some process. Using fusion of a high and low molecular weight alginate polymers creates opportunity to increase elastic modulus and minimally raise the viscosity of the solution [53]. Biopolymers with higher amounts of G can be used to get more resistant gels and find applications in cosmetic and food industries. However, gels more flexible, with higher block M, can be applied in paper industries and can be used for the production of micro- and nanoparticles of drug [27, 47]. The physical properties substantially control the stability of the gels, what can determine applications of alginate, for example in drug release from gels [14].

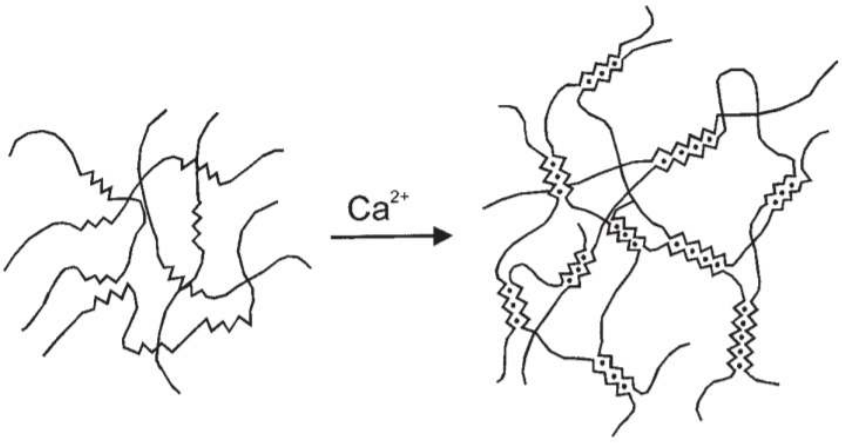

Figure 3: Schematic illustration of alginate gelation induced by calcium ions according to the "egg-box" structural model [24].

The mechanical characteristics of alginate hydrogels depend on, inter alia, gel uniformity, which may be controlled by gelation rate. Slower gelation give a possibility to obtain more uniform structures, thereby better mechanical properties. Important parameter, which has influenced on gelation rate, is temperature of gelation. Slower gelation proceed at lower temperature (reactivity reduction of ionic cross-linkers (e.g., Ca^{2+}) and slower cross-linking) [53]. The mechanical strength of alginate hydrogel is comparable with a soft tissue elasticity, what makes it a proper component for soft tissue reconstruction [62]. In order to improve or change physical and mechanical properties of alginates hydrogels, they can be combined with other biomaterials [60]. Stability of an alginate molecule is highly influenced by the environment conditions (e.g. pH, temperature and amount of contaminants) [11].

8 Conclusions

Alginates are one of the compounds of brown seaweeds, which characterise of favourable properties. There are known many methods to obtain these natural polysaccharides from algae, but quantity and quality of achieved yield depend on many aspects [3]. Algae species, season and place of their harvesting are important, but on the yield rate have an impact also process parameters (temperature, time of extraction, alkali concentration, pre-treatment) and method of extraction (conventional or novel method) [3, 14, 15, 25]. The higher alginate yield was attained by higher temperatures and longer time of extraction. Moreover, using a novel technique of alginate extraction (e.g., ultrasound assisted extraction) allow to reduce the extraction time, but also solvents volume used and to increase the efficiency of the process [10, 42].

These natural polysaccharides are widely applied in everyday life sectors such as food and beverage industry, textile manufacturing, pharmaceutical industry, but also in bioengineering technology with the area of biomedical field [3, 11, 14, 15, 17–23]. Extensive applications are result of alginate rheological properties, but also due to biocompatibility, biodegradability and absence of toxicity [8–11]. Their biological and pharmacological properties, such as antimicrobial ability, antioxidant activity, anticancer and immunostimulatory properties, strong free radical scavenging and renoprotective effect, have contributed to the development of alginate research in the biomedical area [11, 13, 15, 22]. The using of alginates in medicine assists in a faster healing recovery from inside, as a medicine for diabetes mellitus, liver and parathyroid disease, but also as a treatment for external areas of human body, especially in tissue engineering, tissue regeneration, wound dressings. Technological development enabled also the application of alginates in repair and regeneration of certain cartilages and organs, moreover, it enabled to construct alginate-based scaffolds. The biodegradation ability of alginates gives the possibility for the release of medications in a controlled manner [22, 23, 51–53].

In this review paper, in particular management of wound healing and type of wound dressings have been described. Alginates are applied in the wound management material sector due to their therapeutic properties, faster healing and positive effect on most of the wounds, especially traumatic, chronic and surgical wounds, when compared to traditional bandages [14, 19, 52]. Alginates extracted from marine seaweeds have to be purified to achieve medical grade [4]. Moreover, medical

sector requires to obtain alginates in reproducible way to achieve obtain an equal structure. It is important, because chemical modifications may change alginate form and also their mechanical properties [60]. Depending on the purpose for which the alginates are applied, there are different forms of alginate, such as hydrogels, hydrocolloids, fibres, films, foams, microspheres or microcapsules [60]. Continued technical development and researches will widen up perspectives and potential uses in the future.

There is many articles regarding conventional extraction methods of alginates and there are increasing novel techniques of extraction which are using more eco-friendly equipment, improving extraction yield of alginates and reduction time of the extraction. During write this review, it is noted that there is lack of information about combination or composite alginate wound dressings. Using of properties such as biodegradability might contribute to creation of a new generation of drugs or wound dressings enabling slow medicament release in a controlled manner. This is important topic, due to the possibility of connection several medical care ingredients in alginate dressing to accelerate healing wounds. This make perspectives for the future research and innovation.

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