

Polysaccharides of Lower Plants

Algal Polysaccharides

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1. INTRODUCTION

One of the characteristic elements of the various phyla that comprise the algae is that they include, next to unicellular organisms, multicellular organisms that form complex thalluses; in other words cell aggregates that are often barely differentiated, flexible, and devoid of lignin. With rare exceptions, the matrix that encloses algal cells is osidic, and the constituent polysaccharides are polymers capable of forming gels: adaptation to the marine environment demands more flexibility than rigidity, since gravity does not exert its effects on these plants as it does on land plants.

The three great classes of algae to which the species currently used belong each have their characteristic polysaccharides: alginic acid and fucans of Phaeophyceae, sulfated galactans (carrageenans and agar) of Rhodophyceae, and complex polysaccharides, often sulfated, of green algae. Other polymers are also present in algae, including cellulose of green algae, mannans (*Codium, Acetabularia*), xylans, hemi-celluloses, and pectin-like materials. In addition to these structural polysaccharides

algae also contain reserve polysaccharides: starch of green algae, starch specific to red algae, and the laminaran (a β -(1 \rightarrow 3)-glucan) of brown algae. In the Phaeophyceae, the main monosaccharides are frequently polyalcohols: D-mannitol, D-sorbitol.

The use of seaweeds in human diet is a common and ancient practice in the Far East: *nori* (leaves and flakes from *Porphyra*), *kombu* (of dried *Laminaria*), or *wakame* (slated or dried *Undaria*) are extensively consumed in Japan. The market (several tens of thousands of metric tons) is supplied by seaweed culture.

Because seaweeds are relatively low in lipids, high (30-50%) in nondigestible polysaccharides—there is a general consensus that they are like dietary fibers—and rich in vitamins and minerals (alkali and alkaline-earth cations, iodine, iron), they have aroused a growing interest in Western countries; their consumption, although still marginal, is rapidly increasing in several countries of the European Union. In the particular case of France, a standard has been put in place in 1990 for 11 species of seaweed that are authorized as occasional vegetables or as condiments, and fall in the following categories: 1. brown seaweeds: *Fucus vesiculosus* L., *Ascophyllum nodosum* (L.) Le Jolis, *Himantalia elongata* (L.) S. Gray, *Undaria pinnatifida* (Harvey) Suringar; 2. green seaweeds, namely different species from the genera *Enteromorpha* and *Ulva* (e.g., *U. lactuca* L.); 3. red seaweeds: *Porphyra umbilicalis* (L.) Kützting, *Palmaria palmata* (L.) Kuntze, *Chondrus crispus* Lingby; 4. *Spirulina* sp. Such commercialized seaweeds must pass toxicological criteria (maximum levels of iodine [≤ 5 g/kg] and of toxic minerals [arsenic ≤ 3 mg/kg, cadmium ≤ 0.5 mg/kg, tin and lead ≤ 5 mg/kg, mercury ≤ 0.1 mg/kg], and dried seaweeds must pass the following microbiological criteria (per g): fecal *E. coli* ≤ 10 , anaerobic microorganisms ≤ 100 , aerobic microorganisms $\leq 10^4$, *Clostridium* ≤ 1 . (Text n° 1705, *Bulletin du Ministère des Affaires Sociales* [1990] Novembre 28th, p. 103). Seaweeds are seldom used in Europe, where they are recognized as foods in only a few countries (e.g., Ireland, Denmark); in the United States, their use as condiments is authorized by the FDA, which requires that they meet quality standards similar to those that apply in France.

Economic Interest of Seaweeds

The main economic interest of seaweeds is that they are an important source of polysaccharides with thickening and gelling properties: the world colloid industry produced, in the late 1980s, 55,000 metric tons* of alginates, carrageenans, and agar mostly for use in food technology.

Although pharmacy is interested primarily in the rheological properties of the gels obtained from seaweed colloids, it is also attentive to the therapeutic potential

of secondary metabolites from marine plants (terpenoids, halogenated polyphenols, miscellaneous nitrogen-containing substances, and more), which are, like those of terrestrial plants, studied systematically by phytochemists and pharmacologists.

Other markets are also available for seaweeds: after having long been a source of potassium and iodide salts, they are employed in the composition of liquid fertilizers for gardens, greenhouses, and some agricultural uses*, of meals and other products for the cosmetic industry and for use in marine spa therapy: seaweed "filtrates" (e.g., *Undaria*, *Fucus*, *Palmaria*, *Ulva*), "concentrates", extracts (in water, oil, glycol), and purified fractions (e.g., polypeptides from *Aosax*). These "miracle" products claim properties that are not always clearly substantiated (e.g., "for the restructuring of aging tissue" or "firming"). Other applications for colloids include the potential for immobilizing cells that produce monoclonal antibodies or the fermentation of champagne in the bottle.

2. ALGINIC ACID, ALGINATES

According to the 3rd edition of the European Pharmacopoeia, alginic acid is "a mixture of polyuronic acids [...] obtained mainly from algae belonging to the Phaeophyceae [...], it contains not less than 19.0 % and not more than 25.0 % of carboxyl groups (COOH), calculated with reference to the dried substance." The European Pharmacopoeia also includes a monograph on sodium alginate.

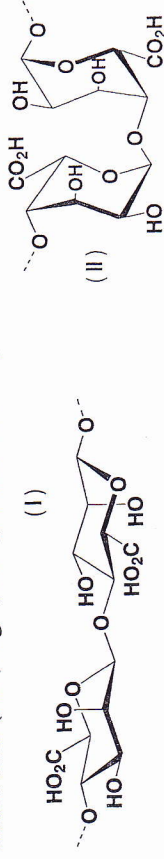
Sources of Alginic Acid. Alginic acid is a virtually constant constituent of Phaeophyceae. This class groups benthic or pelagic algae with the following features: their chloroplasts contain chlorophyll A and chlorophyll C, β -carotene and specific xanthophylls (fucoxanthin), their intercellular matrix is essentially composed of alginates and fucans, and in their cellular vacuoles laminarans and mannitol occur alongside phloroglucinol derivatives. Laminaria and kelp (brown algae) are the principal genera currently used for the industrial preparation of alginic acid and alginates. These polysaccharides represent, depending on the species, the origin, and the season, 15-40% of the dry weight. Other Phaeophyceae may also be used to produce alginic acid: they belong to the genera *Ascophyllum*, *Ecklonia*, *Nereocystis*, and *Durvillea*.

Alginic acid is also produced by some microorganisms. In time, and if these biotechnological approaches become cost effective, it may be possible to use these organisms (e.g., *Azotobacter*, *Pseudomonas*) to secrete exopolysaccharides with modified and uniform structural characteristics.

* Large quantities of "maërl" — a sand, shell, and seaweed mixture for soil improvement — are still used in agriculture. *Maërl* is prepared from red seaweed species which accumulate calcium carbonate, for example *Phymatolithon calcareum* (Pallas) Adey & McKibbin or *Lithothamnion corallioides* P. Crouan & H. Crouan (Corallinaceae), harvested in Ireland. In

* Reference: Mabeau, S., Vallat, O. and Brault, D. (1990). Le charme discret des macroalgues. De l'Orient à l'Occident: les principaux marchés, *Biofutur*, (03), 24-39. Other data: agars, 10,161 t; carrageenans, 25,403 t; alginates, > 25,000 t. M.D. Guiry.

under the form of homogeneous poly-M or poly-G blocks separated by regions where they may alternate (G-M-G-M...). In the native state, alginates occur as mixed salts (Na^+ , Mg^{2+} , Ca^{2+}), which must in part be linked to fucans.



Alginic acid: conformation of mannuronic (I) and guluronic (II) blocks

The relative proportions of the two acids vary with the botanical origin: thus the mannuronic:guluronic ratio is 1.56 in *M. pyrifera*, 1.85 in *Ascophyllum nodosum* Le Jolis, and 0.45 in the stipes of *L. hyperborea*. The length of the blocks, their proportions, and their sequence are also determined by the botanical identity of the sample under consideration and by many other factors: harvest date and position in the seaweed (frond, stipe, receptacles...). As an example, poly-M segments represent about 40% of alginic acid from *M. pyrifera* and poly-G segments 60% of that prepared from *L. hyperborea*.

Preparation of Alginic Acid and Alginates. Because of a marked polyanionic character, alginic acid is insoluble in water and can form salts: soluble sodium, potassium or ammonium salts, and insoluble calcium salts.

Extraction of the fragmented or crushed thalluses generally begins with a deionized acidified water wash which eliminates soluble mineral salts and sugars. It continues by maceration and stirring of the thallus fragments in hot aqueous alkaline (50°C, sodium carbonate) which solubilizes alginic acid. After filtration and elimination of residues, calcium alginate is prepared by adding a calcium chloride solution to the filtrate: the decolorized and deodorized precipitate is recovered and can be purified by redissolution and precipitation as alginic acid.

Alginic acid can also be isolated directly by acidifying the alkaline solution: the polymer loses solubility and the carbon dioxide that is formed carries it to the surface. In the two variations of the procedure, different salts are then prepared (sodium, potassium, ammonium, or calcium), or alternatively an ester (propylene glycol alginate). The principal producers are the United Kingdom (Scotland), Norway, China, and the United States; other producers are Canada, Japan, France, Chile, and Spain.

Properties. Alginates of monovalent cations, and of magnesium, dissolve in water, forming viscous colloidal solutions with pseudoplastic behavior at low concentrations. Progressive addition of divalent cations (calcium) causes the thermally irreversible formation of an elastic gel: the guluronic units with pleated conformation retain calcium ions by coordination, in cooperation with a parallel

- **LAMINARIAS, *Laminaria* spp., Laminariaceae**
mainly *L. digitata* Lamouroux, and *L. hyperborea* (Gunnerus) Fosli

These Laminariales are large perennial seaweeds of tough consistency, with a cylindrical or conical stipe affixed to rocks by a ramified holdfast. The stipe widens at the top into a wide blade sometimes divided into palmated strips (*L. digitata*), or else it is lanceolate, whole and extremely undulate with a textured surface. Laminarias are abundant on the coast of the English Channel where they occupy the subtidal zone, between the low tide zone and a depth of about twenty meters. They are harvested mechanically on the coast of Brittany and constitute (in France) the bulk of the raw material consumed by the colloid industry.

These two species of *Laminaria* provide sterile surgery laminaria (French Pharmacopoeia, 10th edition): the cleaned, scraped, and cut stipes are shaped on a lathe; their diameter varies from 2 to 9 mm.

- **CALIFORNIA GIANT KELP, *Macrocystis pyrifera* Agarth., Lessoniaceae**

These giant seaweeds (50 to 100 m) of the Pacific Ocean have a blade divided into unilateral folioles, swollen at the base into a hollow vesicle that ensures surface flotation; the division of the blade continues into the stipe, giving the latter a ramified aspect. *Macrocystis* are particularly abundant off the California coast, where 120,000 (wet) metric tons are harvested annually, and in the southern seas.

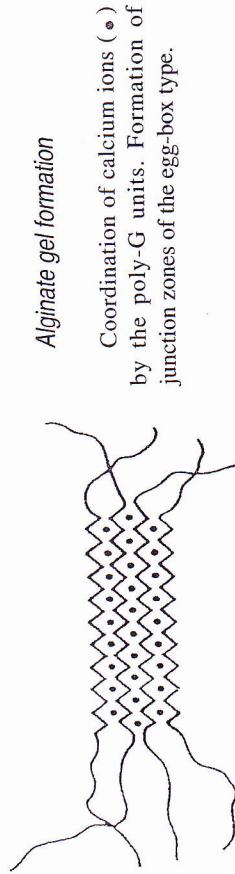
- **KELP, *Fucus serratus* L., Fucaceae**

These perennial seaweeds are abundant on the coasts of temperate and cold seas of the northern hemisphere. In the English Channel, they colonize the intertidal zone, which extends from the highest wave-splashed rocks down to levels uncovered only by the lowest tides. Affixed to rocks by adhesive discs, they form tufts of ribbon-like, membranous, dichotomous strips.

In *F. vesiculosus*, a dioecious species, spermatocysts or oogoniums are grouped in the terminal conceptacles, and on each side of the median "rib", there are thick-walled, isolated or paired pneumatocysts. The dried thallus of these two species is listed in the 10th edition of the French Pharmacopoeia: it consists of blackish-brown to greenish-brown fragments sometimes covered with whitish efflorescences, with horn-like consistency, salty flavor, and a characteristic marine smell.

Alginic Acid Structure. Alginic acid is a linear polymer constructed from two uronic acids, D-mannuronic acid (= M) and L-guluronic acid (= G). The linkage

chain. This regular sequence of the egg-box type occurs periodically: a three-dimensional array is formed with organized zones linked by poly-M or poly (M-G) units. Thus the structure of the polymer is the determining factor of the rheological behavior of alginate gels: the proportion of the poly-G blocks and their length control the formation and the strength of gels obtained in the presence of calcium*.



In practice, gel texture and quality are modulated by fine tuning the calcium ion concentration of the medium (e.g., using calcium salts of different solubility, adding sequestering agents). Alginates have few incompatibilities (quaternary ammonium salts, metal ions).

Tests. The identity of alginic acid is demonstrated by the ability of an alginate solution to remain viscous upon addition of magnesium chloride, or to form a gel upon addition of calcium chloride. Of note is the color reaction that occurs upon warming in the presence of 1,3-hydroxynaphthalene and hydrochloric acid. The assay also includes measuring chlorides (<1%), searching for heavy metals in the residue from calcination in the presence of magnesium oxide, and measuring carboxyl groups by back titration. Alginic acid must pass tests for *Escherichia coli* and *Salmonella*. The total viable aerobic count is not more than 10^3 microorganisms per gram.

Uses of Alginates

- in pharmacy

- Alginates and alginic acid are used in digestive pathology. As a general rule they are combined with sodium bicarbonate and with aluminum hydroxide and taken after meals. Gastric acidity frees alginic acid which forms a foamy gel (carbon dioxide is released from the bicarbonate) and places a floating barrier over the gastric content. Reflux is limited, if it should occur and the gel protects the mucosa of the esophagus against aggression by the gastric juice. Accordingly, these polysaccharides are incorporated into preparations (gastric antacids) for the symptomatic treatment of disturbances due to pathogenic acidity: reflux and other esophagitis, hiatal hernias, and pyrosis.

The sodium salt of β -poly-D-mannuronic acid is proposed as an adjunct in restrictive diets for the treatment of obesity.

- Calcium alginate is also commercialized in the form of hemostatic wool or gauze: upon contact with blood and exudates, alginate forms a fibrillar gel, thus causing a rapid hemostasis. This alginate wool or gauze is very commonly used for extensive superficial bleeding of wet wounds or epistaxis as well as in stomatology (tooth extraction: hemostatic dressing). Calcium alginate is also available in compresses and as a powder (spray). As a micronized powder it is a dressing powder for abrasions (adjunctive treatment of cutaneous ulcers).

- In pharmaceutical technology, alginates are valued for their thickening, binding (stabilization of emulsions, suspensions, and more), and disintegrating properties (tablet formulations); they are also used for slow-release formulations (tablets with hydrophilic matrix) and formulations resistant to gastric acidity (capsules with enteric coating). The cosmetics industry takes advantage of their film-forming, emollient, and hydrating properties, and of their ability to form preparations that spread well onto the skin and are pleasant to the touch.

- other utilizations

Alginic acid and alginates are recognized as devoid of short- and long-term toxicity and therefore authorized as food additives. Their European identification codes are E400, E401, E402, E403, E404, and E405 for alginic acid, and sodium, potassium, ammonium, calcium, and propylene glycol alginate, respectively. The agricultural food industry uses them as gel-forming agents, thickeners, emulsifiers, and as water-retaining agents. The textile industry is also a large consumer of alginates (dye thickener).

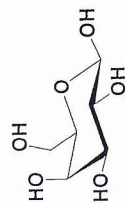
Official *Fucus*. In France, the thallus of *Fucus* may be used as an ingredient of plant-based medicines with the following indication: "traditionally used" as an adjunct in weight loss treatments [French Expl. Note, 1998]. This "traditional" indication is apparently based on an unverified hypothesis which links iodine intake with hypersecretion of thyroid hormones, therefore with increased fat catabolism. The same Note specifies that the thallus of *Fucus* is a bulk laxative, like the thalluses of *Laminaria*, *Chondrus*, and *Ascophyllum*. Thus, these thalluses have approved indications for the symptomatic treatment of constipation, but, in the case of three brown algae, a concentration limit must be proposed for the active constituent in the abridged application dossier for a marketing authorization from the French government or *dossier abrégé d'AMM*. For example, the WHO recommends an iodine intake of 100 to 140 $\mu\text{g/day}$ and an upper limit of 1 mg (17 $\mu\text{g/kg}$); the French Explanatory note of 1998 (IV, p. 37) specifies that the daily intake of iodine must not exceed 120 μg for adults.

The German Commission E 1990 monograph specifies that *Fucus* preparations

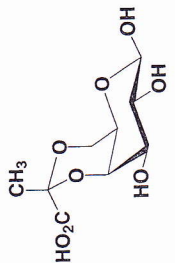
doses smaller than 150 µg/day has not been substantiated for these indications, and doses larger than 150 µg are dangerous, because they may induce or aggravate hyperthyroidism, the Commission does not recommend the use of this drug.

The risk of iodine overdose with seaweed-based products is not negligible: since normal daily intake already exceeds the needs, long-term supplementation, even in small amounts, can cause thyroidal symptoms in susceptible subjects. It is best for pregnant women not to use these products and the same is true for women who breast-feed, because the iodine goes into their milk. Various authors advise against using *Fucus* in children. In general, iodine-containing products should not be used without the advice of a physician.

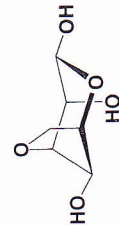
Because of their potential for concentrating metalloids and heavy metals, seaweeds deserve the greatest vigilance regarding their source and the implementation of relevant quality control. This is precisely what is reflected in the monograph recently included in the French Pharmacopoeia: limit test for heavy metals and arsenic, and quantitation of cadmium by atomic absorption spectrophotometry (maximum 5 ppm). French official *Fucus* must have a total iodine level between 0.03 and 0.2%. The iodine concentration of seaweed-based products varies widely and decreases rapidly during storage.



β-D-Galactose



R = H: α-D-Galactose-6-sulfate

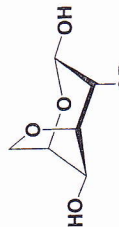
R = SO₃: α-D-Galactose-2,6-disulfate

3,6-Anhydro-α-L-galactose

R = H: 3,6-Anhydro-α-D-galactose

R = SO₃:

3,6-Anhydro-α-D-galactose-2-sulfate



4,6-Carboxyethylidene-β-D-galactose

3. CARRAGEENANS

Carrageenans, often referred to as carrageenates, are, according to the Fr. Ph., 10th Ed.: "obtained from various Rhodophyceae seaweeds, from the Gigartinales, Solieraceae, Hypneaceae, and Furcellariaceae families, after treatment with hot water and precipitation by ethanol, methanol, 2-propanol or potassium chloride [...] they

Sources of Carrageenans. Industrial sources of carrageenans consist of different species of the Rhodophyceae. These algae are characterized by the presence of starch external to plastids (starch specific to red algae, with amylopectin-type structure), of esters of glycerol, of chlorophyll A and D, and of specific pigments that absorb blue-green and green radiations (phycoerythrin: phycoerythrin or equivalent compounds). The needs for carrageenans are covered in major part by extraction from *Chondrus crispus*, particularly in Canada (Nova Scotia), but also by extraction from other Rhodophyceae (e.g., *Eucheuma*, *Gymnogongrus*, *Ahnfeltia*, *Gigartina*), mostly in Denmark and in the United States.

● CHONDRUS, *Chondrus crispus* Lingby, Gigartinales

This seaweed, also known as Irish moss, is a small species, with a ramified thallus. It grows affixed to rocks on the coasts of the Atlantic Ocean and of the English Channel, where it can be harvested manually. This species can also be cultivated in basins.

Structure of Carrageenans. Carrageenans are galactans or polymers of D-galactose, are heavily sulfated, and are anions with multiple electrolytes of molecular weight ranging from 10⁵ to 10⁶. All carrageenans have a linear structure of the (AB)_n type, with alternating 1→3 and 1→4 bonds, where A and B are galactopyranosyl residues:



The A and B units are always sulfated, in the 2- or the 4-position for unit A, and in the 2- or in the 6-position or both for unit B. The B unit can be D-galactose or its internal ether: 3,6-anhydro-D-galactose.

Classically, seven types of carrageenans are distinguished as a function of the nature of the sequence. The structure of these repeating units (ι, κ, λ, μ, ν, θ, ξ) is summarized in the table below:

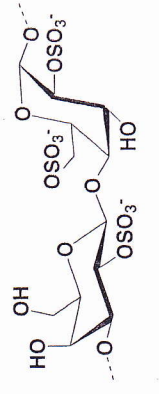
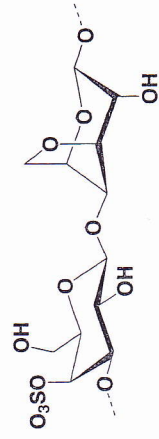
A units	B units	carrageenan
D-galactose 4-sulfate	D-galactose 6-sulfate	μ
	D-galactose 2,6-disulfate	ν
	3,6-anhydro-D-galactose	κ
	3,6-anhydro-D-galactose 2-sulfate	ι
D-galactose 2-sulfate	D-galactose 2-sulfate	ξ
	D-galactose 2,6-disulfate	λ
	3,6-anhydro-D-galactose 2-sulfate	θ

The structural variability is linked to the source species and to numerous factors such as—at least among *Chondrus crispus*—alternating generations:

- the internal ether formation in the B units (3,6-anhydro-D-galactose) and the formation of sulfuric half-esters involving the 4-hydroxyl groups of the A units are specific to the haploid gametophyte;
- sulfation in the 2-position of residue A only occurs in the diploid tetra-sporophyte.

The chief carrageenan of sporophytes is generally λ-carrageenan, whereas κ-carrageenan often dominates in the gametophyte.

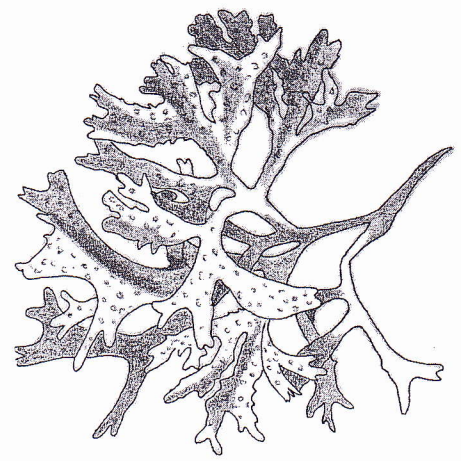
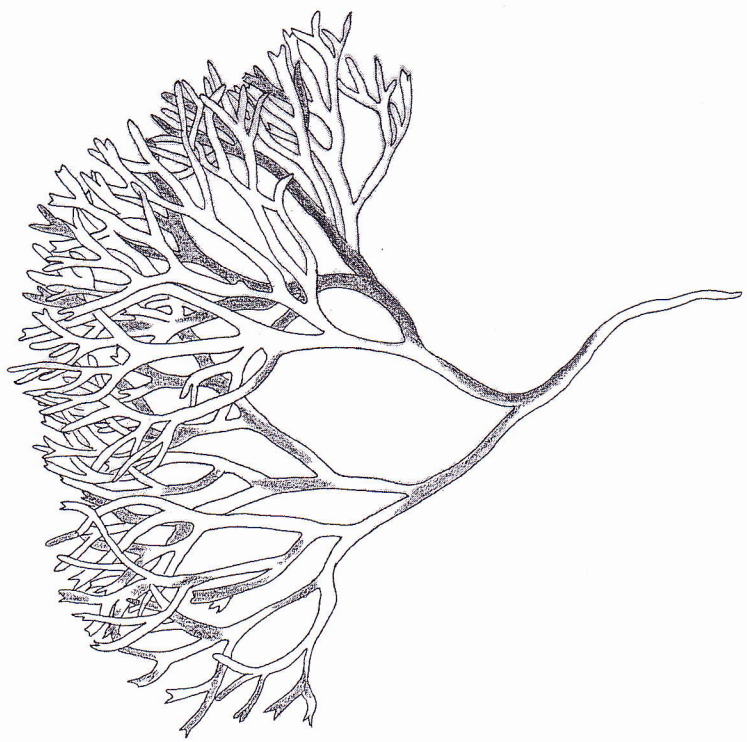
The variations in 3,6-anhydro-D-galactose content observed in gametophyte carrageenan appear to be linked to a varying degree of conversion between the structures of the μ and ν type (considered to be precursors) and the desulfated structures, respectively κ and λ. It is as if the (enzymatic) conversion rate were influenced by environmental conditions. Note that the proportion of sporophytes and gametophytes in a given population depends on the depth... and recall the variability linked to the geographical origin and the species. In fact, carrageenans are hybrids of the extreme polymers that are possible: there do not seem to exist carrageenans formed by the repetition of a unique disaccharide pattern.



Properties of Carrageenans. Gel-forming capability and the properties of the resulting gels depend on the structure of the carrageenan.

- κ- and λ-carrageenans dissolve readily in warm water: the macromolecular chain distributes itself statistically (random coil). At normal temperatures, the regular portions of the molecules associate in double helices stabilized by weak inter-chain bonds: a thermally reversible gel results. The occurrence of irregularities within the polymer creates kinks that force each chain to associate with several neighboring chains, into a three-dimensional array responsible for the cohesion of the gel. In the case of λ-carrageenans, the sulfate groups located on the outside of the double helix prevent—by electrostatic repulsion—the double helices from associating: the gel is elastic and does not contract. κ-carrageenans, on the other hand, form double helices which, in the absence of sulfuric half-esters in the 2-position of the B unit, may aggregate: the resulting gels are rigid, brittle, and tend themselves to syneresis.

- λ-carrageenan solutions do not gel: the half-ester groups in the 2- and 6-position of the B units prevent the formation of the helical structures; soluble in cold water, they only yield very viscous solutions.



CHONDRUS CRISPUS Lingby and GIGARTINA sp.

Carrageenans interact with galactomannans which reinforce gel cohesion. They also interact with proteins, particularly those of milk with which they form specific ionic interactions. They have few incompatibilities (gelatin in acidic medium, quaternary ammonium salts) and their gel stability is good.

Preparation of Carrageenans. Although the principle of the extraction is simple, its technological implementation demands substantial know-how. After a wash that eliminates debris and minerals, the seaweeds are extracted by slightly alkaline warm water. The residual thalluses, filtered under pressure, are discarded. The supernatant is partially concentrated and an alcohol is added (for example 2-propanol) to precipitate the polysaccharide. Carrageenans are wrung, dried, and milled. If necessary, carrageenan can be fractionated (in the laboratory) by selective precipitation of κ -carrageenan by potassium chloride, leaving the λ fraction in solution.

Tests. Among the numerous qualitative and quantitative determinations requested by the French Pharmacopoeia are the following:

- characterization of galactose (by TLC) after sulfuric acid hydrolysis of the polymer;
- estimation of the apparent viscosity of a 15 g/L solution at 75 °C;
- limit test for heavy metals;
- residual level in methanol and 2-propanol (by GC: <0.1%);
- quantitation of sulfates by barium perchlorate after mineralization in oxygen.

Uses of Carrageenans. The pharmaceutical industry takes advantage of the properties of the gels for applications in pharmaceutical technology (e.g., formulations of pastes, creams and emulsions), as well as for therapeutic or diabetic applications: symptomatic treatment of constipation (increasing the bulk of feces), protection of mucosae in proctology, use as an adjunct in restrictive diets (sensation of satiety). Carrageenans also enter in the formulation of hygiene and cosmetic products: toothpastes, shampoos, ointments, creams, gels, lotions, and so forth.

Additional current uses of carrageenans are essentially all in the food domain. These polymers (κ - and λ -carrageenans), which are not absorbed, not digestible, and non toxic (Eur. id. code E407), are incorporated at low concentrations:

- as gel-forming agents, stabilizers, inhibitors of ice cream crystallization, and so on, in dairy products (taking advantage of the interaction with milk proteins), and also in aqueous products (e.g., glazes)
- as emulsion stabilizers or thickeners (λ -carrageenans).

Semi-refined Carrageenans. Semi-refined carrageenans first appeared in the late 1970s in the Philippines. They consist of *Eucheuma* that have been washed, crushed, dried, and pressed. They are approved for use in the United States and in the European Union (carrageenan refined by the alternate method, E407a). They are

4. AGAR (GELOSE)

According to the 3rd edition of the European Pharmacopoeia, agar "consists of the polysaccharides from various species of Rhodophyceae mainly belonging to the genus *Gelidium*. It is prepared by treating the algae with boiling water; the extract is filtered whilst hot, concentrated and dried".

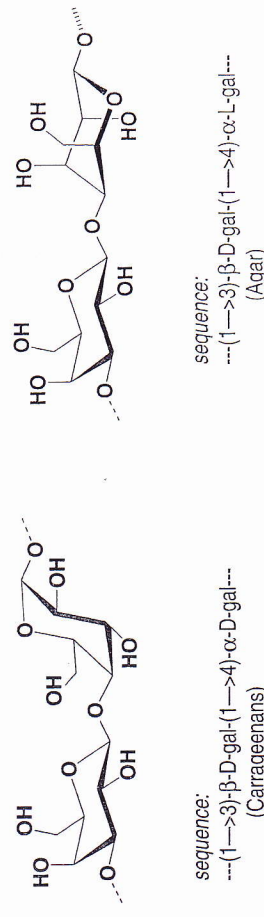
Sources of Agar. Like carrageenans, agar is extracted from thalluses of various Rhodophyceae, especially red algae. Among the numerous species that may be used, note the *Gelidium*, seaweeds of temperate and warm seas with pinnately ramified fronds (*G. corneum* [Hudson] Lamouroux, *G. amansii* Lamouroux). Note also various *Gracilaria* (*G. confervoides* Greville of the north Atlantic coast of France and *G. lichenoides* Agardh of Java), as well as a few species of the genera *Gelidiella* or *Pterocladia*.

All of these seaweeds are small and grow clinging to rocks. Agar production, initially Japanese, is now carried out by many countries, including Korea, Spain, Portugal, Morocco, Chile, Mexico, the United States, Australia, and New Zealand.

The seaweeds, harvested from their natural sites or cultivated on artificial supports, are traditionally sun-dried prior to treatment. They are first washed with fresh water then extracted with hot water. After filtering and discarding the residue, the major part of the supernatant water is eliminated by freezing which causes phase separation. Finally, the product is washed, decolorized, dried, and milled.

Structure of Agar. This polysaccharide is a complex galactan, formerly considered to be a mixture of two fractions, agarose and agaropectin. In fact, it is a variable mixture of forms that are intermediate between three extreme forms: agarose, pyruvyl agarose, and a form highly sulfated, but poor in internal ethers.

Agarose is a barely sulfated linear polymer, constructed as a linear structure of the (AB) n -type with alternate 1 \rightarrow 3 and 1 \rightarrow 4 bonds, where the A units are partially methylated D-galactoses and the B units are L enantiomers of galactose, almost always of the 3,6-anhydro-L-galactose type.



Pyruvyl-agarose is also barely sulfated, it contains a large proportion of internal anhydrides (3,6) and a small part of its A units are 4,6-O-(1-carboxyethylidene)-D-

in a cyclic ketal formed by reaction with pyruvic acid). The proportions of the different forms vary greatly with the source species.

Characteristics, Tests, Properties, and Uses. Agar consists of colorless to pale yellow, translucent and resilient ribbons or flakes. It can be characterized by a fleeting color reaction in the presence of iodine, and by precipitation of sulfate ions after heating in the presence of acid. Soluble in hot water (1% solution), agar forms a gel around 30–35°C, which liquefies only above 80°C. The assay for agar includes the determination of the swelling index (>10 and within 10% of the value printed on the label), total ashes (<5%), loss on drying (<20%), verification of the absence of gelatin (absence of turbidity in the presence of picric acid), and an estimate of matter insoluble in water in the presence of acid (<1%). It must pass tests for *Escherichia coli* and *Salmonella*. The total viable aerobic count allowed is not more than 10⁵ micro-organisms per gram.

Agar dissolves in hot water and forms thick gels upon cooling: agarose forms double helical structures that aggregate into a three-dimensional network able to retain water molecules. It cannot be assimilated, will not ferment, and is non toxic, thus it is a mechanical laxative because it increases the bulk and hydration of feces, and makes transit regular. It can also be used in the formulation of gastrointestinal protective agents.

A classic culture medium in bacteriology, agar can be used for the *in vitro* production of plants. For chemists and biochemists, it forms gels of great resilience with multiple uses: alone or in combination with polyacrylamide, it is a stationary phase for size exclusion chromatography, and after substitution with various substances, for affinity chromatography; it is also a medium for electrophoresis and immunological techniques.

Like other hydrocolloids of vegetable origin, agar is listed in the category of authorized texture agents (Eur. id. code E406) and used as such in food technology.

5. OTHER POLYMERS

Furcellaran

This polymer with a structure close to that of κ -carrageenan, is isolated from *Furcellaria fastigiata* (L.) Lamouroux, a small red alga abundant in cold seas (Denmark, Sweden). The rheological properties of its solutions make it useful in animal feed. It is sometimes advocated by phytotherapists (danish agar).

Fucans

Fucans are sulfated polysaccharides present in the intercellular matrices of Phaeophyceae. They form a group of heterogeneous and nondispersed polymers,

in the native state as proteoglycans. Some authors classically distinguish three categories of structures:

- fucoidans, polymers of L-fucose-4-sulfate with α -(1→2) bonds, more or less substituted by neutral monosaccharides, uronic acids, or other sulfated moieties;
- ascophyllans, that are xylofucuronans, most often poly β -(1→4)-D-mannurans substituted laterally by short sulfated chains;
- sargassans or glucuronofucoglucans, (1→4)-linked linear chains of glucose or galactose substituted at C-5 by fucosyl-3-sulfate residues or by uronic acid.

These polysaccharides have an anticoagulant effect as does another sulfated containing polysaccharide—heparin—and this is in spite of marked structural differences (e.g., absence of nitrogen, different linkages, higher molecular weight, and weaker polydispersity). The proposed mechanism of action is also different.

Several fucans have also shown interesting antitumor potential in some experimental models. Their action seems linked to an activation of the non specific immune response.

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