Cah. Biol. Mar. (2001) 42 : 137-157

Chemistry and physico-chemistry of phycocolloids

Marc LAHAYE
Institut National de la Recherche Agronomique,
Unité de Recherche sur les Polysaccharides, leurs Organisations et Interactions,
Rue de la Géraudière, B.P. 71627, 44316 Nantes cedex 03, France.
e-mail: lahaye@nantes.inra.fr

Abstract: A review on the chemistry, physico-chemistry and some gel characteristics of major phycocolloids is presented. These concern mainly agar, gelling carrageenans from red seaweeds, alginate from brown seaweeds and ulvans from green seaweeds. Based on the available data, future research areas are proposed to better define the relationships between the different structural levels of these polysaccharides and their use as texturing agents.

Résumé : Une revue de la chimie, de la physico-chimie et des caractéristiques des gels des principaux phycocolloides est presentée. Elle concerne principalement les agars et les carragénanes gélifiants des algues rouges, les alginates des algues brunes et les ulvanes des algues vertes. A partir des données disponibles, des domaines de recherche future sont proposés pour mieux définir les relations entre les différents niveaux de structure de ces polysaccharides et leurs utilisations comme agents texturants.

Keywords: seaweed, polysaccharides, chemistry, physico-chemistry.

Introduction

More than 3.5 million tons of seaweeds are harvested annually in the world and the main use of this biomass is for food consumption (~60 %) and for phycocolloid productions (~40 %, Jensen, 1993). Seaweeds are not consumed as staple foods but rather for their particular organoleptic and functional properties arising for a good part from their cell-wall polysaccharides. The ability of these polymers to texture aqueous solutions has been used for many years and is at the basis of an industry using seaweeds as source of gelling, thickening and stabilizing agents. The purpose of this review is to summarize the chemical and physico-chemical basis of the textural properties of phycocolloids. The relationships between the biology of seaweeds and the chemistry of the polysaccharides (biosynthesis, ecophysiological effects…) will not be covered in details and informations can be found in other recent reviews (Craigie, 1990, Cosson et al., 1995). The phycocolloids considered are the industrially used agar, carrageenan and alginate from red and brown seaweeds and some informations will also be presented on ulvans from green seaweeds.

General aspects on the chemistry and physico-chemistry of texturing polysaccharides

The basis of phycocolloids properties relies on the nature and extent of intermolecular associations in ordered

assemblies forming hydrated gel networks or on their interactions by entanglement of random coil polysaccharides, leading to viscous solutions. The aim of this section is to summarize general principles relating structure to function of polysaccharides.

Pyranose (five carbons and one oxygen) sugar rings (primary structure, Fig. 1) are the main building units of phycocolloids and these rings adopt in general the most energetically favourable chair conformations noted $^4C_1$ or $^1C_4$ depending on the positions of C-4 and C-1 with respect to the plane O-5, C-2, C-3 and C-5, the C-6 being outside the ring. On these carbons, hydrogen and hydroxyl groups are linked either perpendicularly to the plane and are then refer to be in axial configuration or in the plane, in which case they are said to be equatorial. Linkages between sugars in polysaccharides occur through the hydroxyl group on C-1 (anomeric carbon) with any of the other free hydroxyl present on another sugar ring. The glycosidic linkage formed can either be α if the OH on C-1 is on the opposite side of C-6 on the ring or β if they both are on the same side (Fig. 1). This linkage gives torsional dihedral angles φ and ψ (secondary structure, Fig. 2A) which are responsible for the shape of the polysaccharide (tertiary structure). Angles of similar value all along the polysaccharide chain induce an ordered conformation of the macromolecule, otherwise, it will be a random coil. Methyl ether, sulphate hemiester, pyruvate acetal, and glycosides can substitute other hydroxyl groups. The methylene group at C-6 can also be replaced by a carboxylic acid group and in that case the sugar is a uronic acid (Fig. 1).

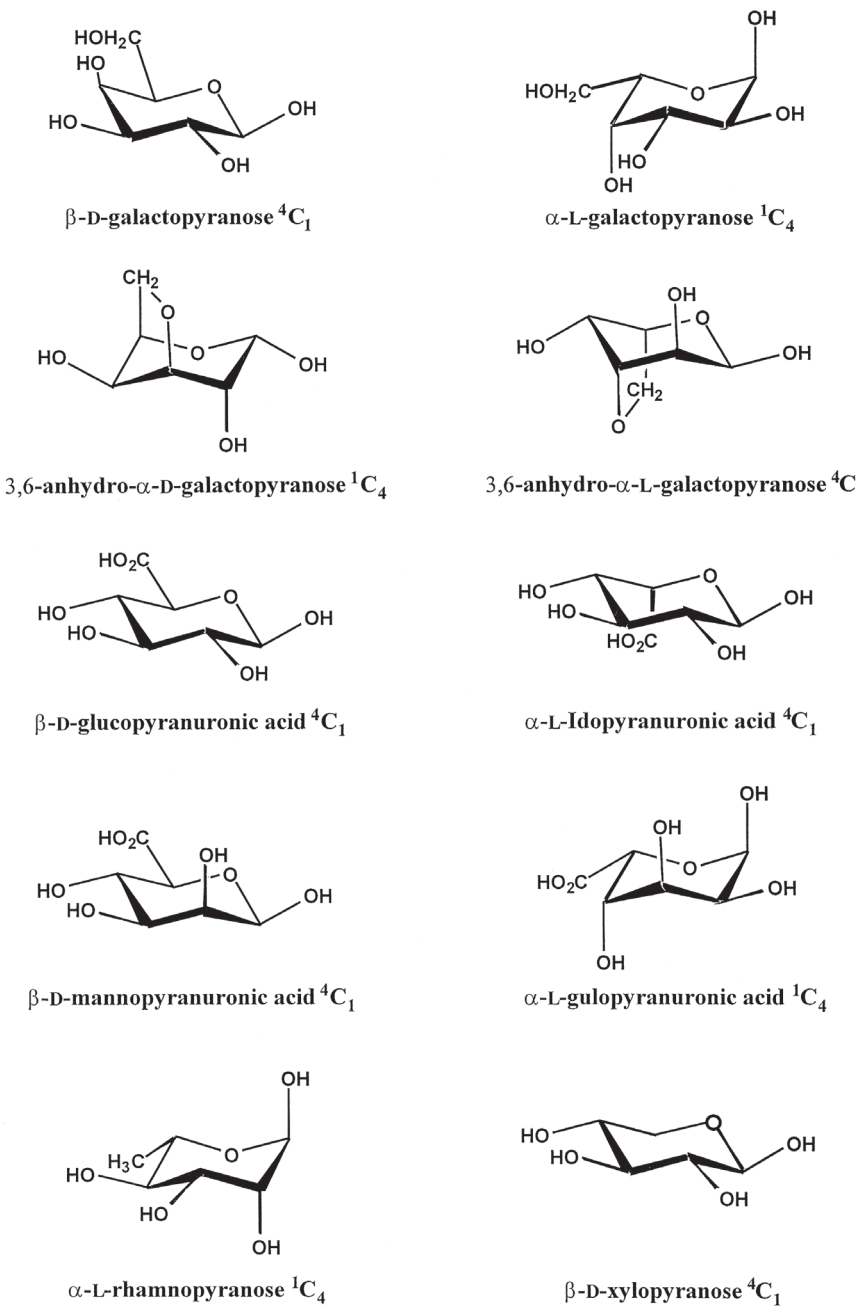

Figure 1. Chemical structure of the principal constitutive sugars of phycocolloids.
Figure 1. Structure chimique des principaux oses constituants des phycocolloïdes.

The gelling ability of phycocolloids depends on the overall shape given by the glycosidic linkage angles but also on the ability of the polysaccharides to form intra- and inter-molecular physical linkages (hydrogen bonds, electrostatic and Van der Waals interactions, Rees, 1982). These associations are affected by negative charges originating from carboxylic acid and/or sulphate hemiester groups which tend to expend the polysaccharides in water through mutual electrostatic repulsions, and/or by steric hindrance due to other groups (methyl, glycosylation…) substituting for hydroxyls involved in intra-molecular or inter-molecular hydrogen bonds. Co-solutes such as salts modify the solvating ability of water and shift the equilibrium from polymer-solvent interactions to polymer-self-associations

and lead to gel formation or precipitation (carrageenan, alginates, ulvans). The loss in mobility of the macromolecule through the gel formation or precipitate is compensated by the energetically favourable non-covalent bonds formation. Many of these bonds are in themselves weak and easily broken, but, acting co-operatively, they stabilize the mesostructure. Thus, a minimum polysaccharide chain length is required for the establishment of stable ordered self-associated structures. It is therefore clear that the primary and secondary structures including the molar weight reflecting the degree of polymerization of the polysaccharides are basic parameters for their overall properties. Irregular composition, glycosidic linkages or substitutions contribute to increase the solubility of the polysaccharides. Other physico-chemical conditions, such as temperature, ionic strength and pH also affect the formation and melting of the ordered structure in breaking or preventing the formation of the co-operative weak bonds.

The type of ordered structure formed by polysaccharides depends more on the glycosidic linkage geometry than on the nature of the building units (Rees et al., 1982). β-1,4 diequatorial glycosidic linkages such as those found in cellulose, mannan, chitin are parallel and about co-linear with the sugar residues. They lead to flat ribbons able to pack into fibrillar assemblies (Fig. 2B). Diaxial parallel linkages as found in alginate, allow cavities that can accommodate and stabilize ions ("egg-box" structures) and form ordered structures such as highly buckled ribbons (Fig. 2C). Glycosidic linkages not parallel or diagonally opposite introduce a regular "twist" to the polysaccharide chain resulting in an helix usually stabilized by co-axial packing such as that found with agar and carrageenans (Fig. 2D). The consequences of such associations are at the basis of the gelling properties of phycocolloids. The supramolecular organizations of the polysaccharides giving rise to the 3-dimensionality of the gel network (Fig. 3A, Rees et al., 1982) are stabilized by "junction zones" segments interspersed with disordered or "solubilizing" sequences. The latter arise from structural inhomogeneities disrupting the phycocolloids regular geometry. In case of carrageenan helices, "super-helical fibre bundles" packings have also been proposed where "solubilizing sequences" would be absent or very short (Viebke et al., 1994, Fig. 3B). When ordering of the polysaccharides is not achieved, the volume occupied by polysaccharide coils in the solvent leads to an increase in the solution viscosity (Morris, 1992). This volume, predominantly dependent on the molar size of the polysaccharide, is measured as the fractional increase in viscosity per unit concentration under conditions of extreme dilution (without inter-chain interactions). This "intrinsic viscosity" [η] is related to the molecular weight (M) of the polysaccharide by the Mark-Houwink relationship:

$$[\eta] = KM^a$$

where K and a values reflect the polysaccharides shape in the solvent. In case anionic polysaccharides such as carrageenans, alginate and ulvan, polysaccharides coil dimensions are affected by ionic strength, electrostatic repulsions of chains in absence of added electrolytes increase the coil volume. For such polysaccharides, the intrinsic viscosity decreases linearly with $1/I^{1/2}$ and extrapolates at infinite ionic strength to the intrinsic viscosity of the equivalent neutral polysaccharide of the same primary structure and degree of polymerization (Morris, 1992). The properties of polysaccharides in

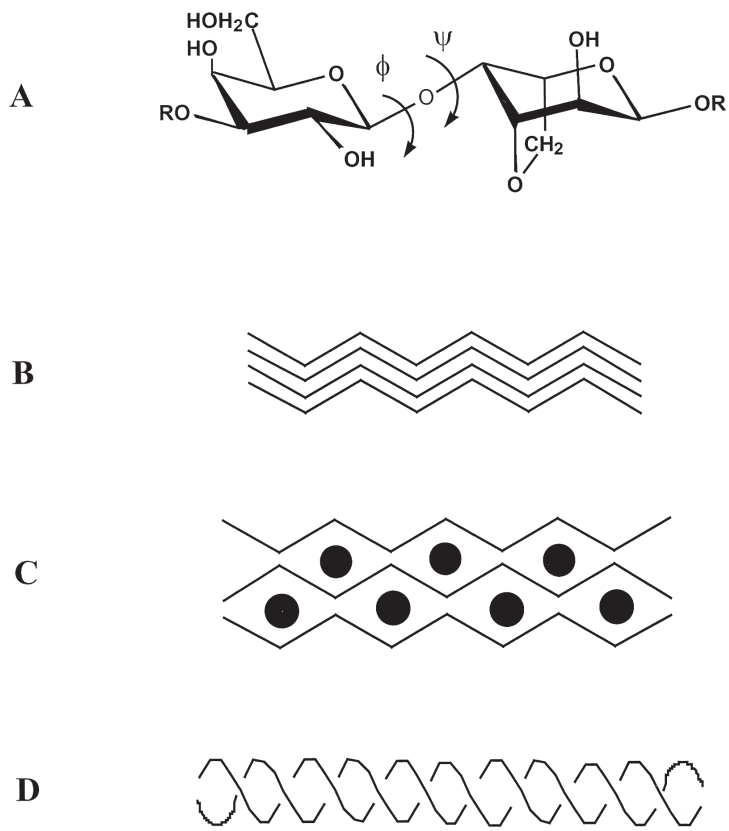

Figure 2. Glycosidic linkage angles **A**, and the overall flat ribbon **B**, buckled ribbon **C**, and helical **D** shapes induced by the regular repetition of these angles along polysaccharides.

Figure 2. Angles de la liaison glycosidique **A** et conformations en ruban plat **B**, ruban torsadé **C**, et hélice **D** induites par la répétition régulière de ces angles le long de la chaine du polysaccharide.

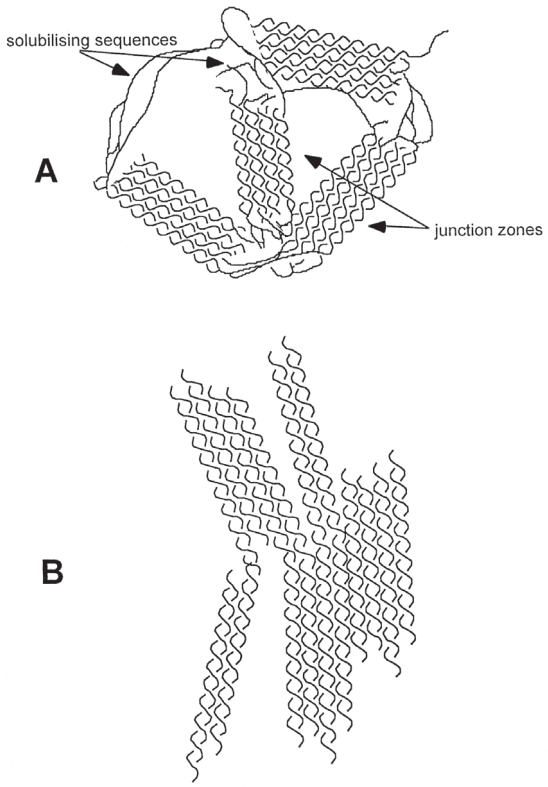

Figure 3. Schematic representations of "junction zones" and "solubilizing sequences" in three-dimensional gel networks according to the model of Rees et al., (1982) **A**, and to the "super-helical bundles" of Viebke et al., (1994) **B**.

Figure 3. Représentation schématique des "zones de jonction" et des "séquences solubilisantes" dans le réseau tri-dimensionnel du modèle de Rees et al., (1982) **A**, et des "super-hélices" de Viebke et al., (1994) **B**.

solution also depend on concentration. Above a critical concentration c*, the polysaccharides interact together by physical entanglement. Below c*, the polysaccharides can move freely with little interference with each other and the viscosity is independent of shear rate ("Newtonian behaviour"). Above c*, shear rates will physically affect the entangled chains by more or less favouring their separation ("reptation") from one another. At low shear rate, the rate of dissociation and re-association of the interactions will equilibrate and the viscosity will be the highest. At higher shear rate, the viscosity decrease ("shear-thinning") as the rate of entanglement formation is lower than the rate of de-association. The fractional increase in viscosity linked to polysaccharide concentrations below c* is increased by a factor of about 2.5, consecutive to a doubling of the polysaccharide concentration; above c* it increases by a factor of about 10 (Morris, 1992).

Chemical structure and physico-chemical properties of phycocolloids

With the above introductory remarks aimed at defining the different important factors involved in the physico-chemical properties of phycocolloids, the main chemical and physico-chemical characteristics of water-soluble gelling and/or thickening algal polysaccharides will now be reviewed.

1. Galactans

Sources and extraction

Red seaweeds are well known sources of industrial gelling and thickening cell-wall galactans, referred to as agar and carrageenans. *Gelidium*, *Pterocladia*, *Gelidiella* and *Gracilaria* species constitute the main seaweed raw materials used for the production of agar and agarose and, now-a-days, most of the carrageenan productions are from farmed *Eucheuma* species (Stanley, 1987, Armisen & Galatas, 1987). These polysaccharides are extracted from dried algae by more or less hot alkaline solutions (100-120°C). After filtration, agar solutions are allowed to gel, then de-watered by pressing, dried and ground (Armisen, 1987) whereas carrageenan in the filtered extract are generally precipitated by alcohol (Stanley, 1987). Carrageenan solutions (kappa-carrageenan) can also be gelled by addition of potassium ions, de-watered by pressing, dried and ground (Stanley, 1987). Semi-refined carrageenans are also produced by an alkaline treatment of the seaweeds followed by thorough water rinsing. These treatments improve the gelling characteristics of the carrageenan preparation and remove most of the proteins, pigments and small metabolites. However, due to the absence of a true carrageenan extraction and of a liquid-solid separation, such preparations contain other polymers such as cellulosic materials (Hoffman et al., 1995).

Nomenclature and chemistry

The naming of gelling algal galactans has recently been under consideration by several authors (Craigie 1990, Knudsen et al., 1994, Miller 1997). Originally, agar was defined as polysaccharides with a basic backbone structure based on the repetition of alternating 1,3-linked β-D-galactose and 1,4-linked 3,6-anhydro-α-L-galactose. This disaccharide repeating unit was named agarobiose or neoagarobiose (Fig. 4A, Araki 1966). Very early on, chemical modifications of this structure by sulphate hemiesters, pyruvate acetal and/or methyl ethers were identified and the extent of substitution, particularly by sulphate esters, led to the distinction of gelling agarose and non-gelling agaropectin molecules (Araki, 1966). It was latter shown to be an oversimplistic view of the distribution of the agar molecules with regard to the existing continuum

between neutral and highly charged galactans (Duckworth & Yaphe, 1971) and between low to highly methyl substituted molecules (Lahaye et al., 1986). Thus, agar-type polysaccharides were shown to have an important intra- and inter-molecular hybrid nature, explaining in part the variability of gelling properties observed between different agar extracts or fractions. For that reason, Craigie (1990) proposed to refer to the agar-type polysaccharides as the agarocolloids and then to distinguish among them the gelling agar and non-gelling agaroids. The term agarose, referring to the highest gelling fraction of agar used for biotechnological purposes (Renn, 1984), has a precise chemical definition that is basically and ideally composed solely of unsubstituted agarobiose repeating units (Fig. 4A). The basic common feature in agarcolloids is their construction on the repetition of the 3-linked β-D-galactose alternating with the 4-linked α-L-galactose to which Knutsen et al. (1994) has proposed the name "agaran". The agar fraction is enriched in agarobiose repeating units; that is, it contains more 3,6-anhydo-α-L-galactose. The closely related galactan, carrageenan, based on the carrabiose or neocarrabiose repeating structure (Fig. 4B), differs from agarocolloids by the 4-linked α-D-galactose replacing for the 4-linked α-L-galactose and in being usually more substituted by sulphate groups. In fact, the number and position of the latter and the presence of 4-linked 3,6-anhydro-α-D-galactose are at the basis of the 15 different disaccharide structures among the 42 theoretical ones (Stortz & Cerezo, 1992) and to which Greek letters have been given (Table 1). This nomenclature followed the original naming of the carrageenan from *Chondrus crispus* extracts that precipitate on addition of potassium chloride (kappa-carrageenan) from that remaining soluble having a low concentration of 3,6-anhydrogalactose (lambda-carrageenan). Latter on, a third carrageenan type, gelling with calcium ions was defined as iota-carrageenan and groupings of the different carrageenan disaccharides repeating structures by families according to the sulphation patterns were proposed (Craigie, 1990, Table 1). However, such namings cannot account for the highly complex hybrid nature of the polysaccharides since generally more than one type of repeating carrabiose structure is found in the polysaccharides. With respect to the difficulty of properly

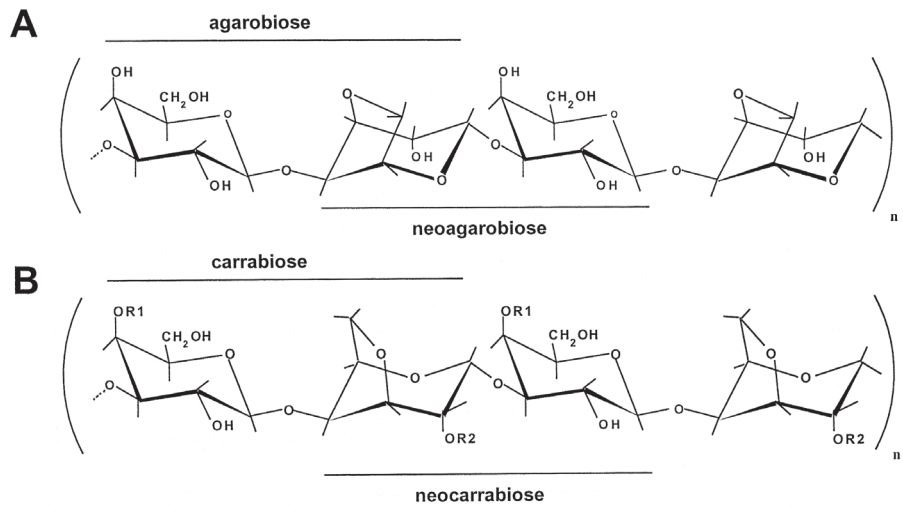

Figure 4. Chemical structure and names of the repeating disaccharide units in agarose **A** and carrageenan **B**. R1= sulphate and R2 = hydroxyl: kappa-carrageenan; R1 = R2 = sulphate: iota-carrageenan.

Figure 4. Structure chimique et noms des unités disaccharidiques de répétition dans l'agarose **A** et le carraghénane **B**. R1= sulfate et R2 = hydroxyl : kappa-carraghénane ; R1 = R2 = sulfate : iota-carraghénane.

name these polysaccharides and as a counterpart to the new terms proposed for agarcolloids, Knutsen et al. (1994) introduced the names "carrageenan" and "carrageenose". These refer to polysaccharides based on the repetition of 4-linked α -D-galactose alternating with 3-linked β-D-galactose and to those where the 4-linked sugar is a 3,6-anhydro-α-D-galactose, respectively. As for "agaran" these new terms are probably increasing the confusion around these galactans, but in fact reflect the complexity of properly defining these polysaccharides in a way to conceal both chemical characteristics and functional properties.

In this text, the term defined by Craigie (1990) will be used for agar-type polysaccharides and the old Greek symbol system for the carrageenan polysaccharides. Knutsen et al. (1994) proposed very useful shorthand notations to describe the different agar- and carrageenan-types repeating structures (Table 1). In these notations, the **D** and **L** letters refer to the 4-linked α-D- and α -L-galactose, respectively, **DA** and **LA** to the corresponding anhydrogalactose derivatives, **G** to the 3-linked β-D-galactose. Substituent groups are defined as **M** for methyl ether, **P** for pyruvate, **S** for sulphate ester, the position of which on the sugar being given by the carbon number. These new notations are particularly helpful for describing hybrid galactans having both agar and carrageenan-type backbone characteristics. Such structures are found, for example, in some extracts of *Gigartina skottsbergii*, a carrageenophyte having a galactan fraction with L-galactose (Ciancia et al., 1993; 1997), and in galactans produced by

Table 1. Disaccharide repeating structures of carrageenans[a].
Tableau 1. Structure des unités disaccharidiques de répétition des carragénanes[a].

1,3-linked[b]	1,4-linked[b]	Greek symbol	
G4S[c]	**DA**	κ (kappa)	kappa-family
G4S	**DA2S**	ι (iota)	
G4S	**D6S**	μ (mu)	
G4S	**D2S,6S**	υ (nu)	
G4S	**D2S**	ο (omicron)	
G	**DA**	ß (beta)	beta-family
G	**D6S**	γ (gamma)	
G6S	**DA**	ω (omega)	
G6S	**D6S**	ψ (psi)	
G	**D2S,6S**	δ (delta)	lambda-family
G	**DA2S**	α (alpha)	
G2S	**D2S,6S**	λ (lambda)	
G2S	**DA2S**	θ (theta)	
G2S	**DAS**	ξ (xi)	
GP,2S	**D2S**	π (pi)	

[a] adapted from Craigie (1990) and Knutsen et al. (1994), [b] refer to 1,3- and 1,4- linked galactose residues, [c] letters: G ß-D-galactopyranosyl; D α-D-galactopyranosyl; DA 3,6-anhydro-α-D-galactopyranosyl; Lα-L-galactopyranosyl; LA 3,6-anhydro-α-L- galactopyranosyl; S sulphate; P pyruvate acetal; numbers correspond to the carbon atom number on which the substitution is found.

Gloiopeltis, *Lomentaria*, *Grateloupia* or *Anatheca* (Nunn et al., 1971; Takano et al., 1994; 1998; Usov & Barbakadze, 1978). For the description of agar and carrageenan-type oligosaccharides, **nr** is added after the sugar symbol positioned at the non-reducing end and **nr'** for the sugar next to it. For the sugar at the reducing end, its letter symbol **r** is extended in **r** α**,** β and that of the sugar associated with it in the disaccharide repeating structure is extended by **r'**. For example kappa-neocarratetraose oligosaccharide is symbolized as **DAnr-G4Snr'-DAr'-G4Sr**α**,**β. This nomenclature has been further extended to take into account proportions of different substitutions in galactans (Miller, 1997). With these new notations and proposed namings in mind, the commercial polysaccharides sold as agarose, kappa-carrageenan, iota-carrageenan are hybrid structures mainly composed of agarobiose (**G-LA**), carrageenose 4'-sulfate (**G4S-DA**) and carrageenose 2,4'-disulfate (**G4S-DA2S**) repeating structures. The industrial lambda-carrageenan is usually a mixture of different galactans of the carrageenan-type unable to gel.

The basic chemistry of agar and carrageenan-type polysaccharides has been the subject of several recent reviews (Cosson et al., 1995; Craigie, 1990; Lahaye & Rochas, 1991; Miller, 1997; Murano, 1995; Piculell, 1995; Stanley, 1995, Usov, 1998) and the following section will briefly summarize some recent methods used for the analysis of the primary and secondary structures of these galactans.

Sugar compositional analysis of red algal galactans has recently been markedly facilitated by introducing the acid-stable reductant 4-methylmorpholine-borane during acid hydrolysis and by optimizing methanolysis conditions. These two depolymerization methods stabilize the acid-labile 3,6-anhydrogalactose and derivatives into 3,6-anhydrogalactitol or 3,6-anhydogalactose dimethyl acetal (Quemener & Lahaye, 1998; Stevenson & Furneaux, 1991; Usov, 1993) which until then could only be quantified by colorimetry (Yaphe & Arsenault, 1965). It is now possible to identify and quantify all the sugar components of algal galactans by HPLC techniques or by GC, after further chemical derivations (Quemener & Lahaye, 1998; Stevenson & Furneaux, 1991; Usov, 1993). The reductive hydrolysis can also be used on unextracted algae, thus facilitating chemotaxonomic studies of unknown seaweeds (Usov & Klochkova, 1992). Under mild conditions, both reductive hydrolysis and methanolysis methods can release the basic disaccharide repeat units which can then be identified as of agarobiose or carrabiose type by HPLC or

GC (Falshaw & Furneaux, 1995; Quemener et al., 1995; Usov, 1998). Once the sugars have been identified, it can be necessary to determine the absolute sugar configuration (i.e. D or L enantiomer). This can be determined by conversion of the monosaccharides into glycosides of chiral alcohols (see Usov, 1998; Takano et al., 1993), by reductive amination with chiral amines (Cases et al., 1995) or by oxidative hydrolysis followed by acetylation and conversion into *sec*-butyl esters (Errea et al., 1998) prior to GC or GC-mass spectrometry analysis. It can also simply be achieved by D-galactose oxidase enzymatic kits on acid hydrolysate. Sulphate and pyruvic acid substituent groups are usually quantified by enzymatic, chemical or spectroscopic methods (Caceres et al., 1997; Chopin & Whalen, 1993; Craigie & Leigh, 1978; Matsuhiro & Rivas, 1993; Rochas et al., 1986a).

Linkage analysis and substituting group locations have been facilitated by improvements brought in the chemical methylation methods of sulphated galactans (Stevenson & Furneaux, 1991). The combination of both methylation and reductive hydrolysis with GC-MS analysis can rapidly give informations on the substitution pattern of repeating disaccharides (Falshaw & Furneaux, 1995). IR spectroscopy is a convenient mean to determine sulphate location and quantity in algal galactans (Caceres et al., 1997; Chopin & Whalen, 1993; Matsuhiro & Rivas, 1993; Rochas et al., 1986a; Sekkal & Legrand, 1993) and coupled to microscopy, it can provide informations on the location of different sulphated polysaccharides in seaweeds (Sekkal et al., 1993; Fournet et al., 1997). However, today the most powerful mean to characterize the chemical structure of regular algal galactans and their oligosaccharides remains nuclear magnetic resonance spectroscopy (Usov, 1984). Several carrageenan and agar-type disaccharide structures are now easily identified from their characteristic sets of ^{13}C signals (Greer et al., 1985; Lahaye et al., 1985; 1989; Rochas et al., 1986b; Usov et al., 1980; 1983; Usov & Shashkov; 1985). With the improvements in the instruments and the development of two-dimensional experiments, NMR spectroscopy is being increasingly used and the database of both characteristics ^{13}C and ^{1}H signal chemical shifts of particular repeating structures is extending (Chiovitti et al., 1996; 1997; Falshaw et al., 1996; Falshaw & Furneaux, 1998; 1994; Knutsen & Grasdalen 1992; Stortz et al., 1994). Methods using computer simulations have also been proposed for the prediction or as an help in the identification of chemical shifts of agar and carrageenan-type repeating structures (Stortz & Cerezo, 1992; Miller, 1998). Solid state ^{13}C NMR spectroscopy has also been used to characterize agars and carrageenans extracts, or in situ in algae (Rochas & Lahaye, 1989a; Saitô et al., 1990), but this technique is still waiting for improvements to yield higher resolution spectra.

The galactan structures is often complex owing in part to their heterogeneous nature. Fractionations can facilitate structural analysis, but in many cases, the complexity is due to the intra-molecular hybrid nature of the polysaccharides. Several chemical approaches can be used to isolate particular repeating sequences to ease their chemical structure elucidation and to sequence their distribution in these galactans (Usov, 1998). However, by their high substrate specificity, enzymes represent tools of choice and several agarases & carrageenases have been isolated (Potin et al., 1999) and used successfully to demonstrate such hybrid nature of the algal galactans (Greer et al., 1984, Greer & Yaphe, 1984a;b;c; Morrice et al., 1983; Usov & Ivanova, 1987). In particular, the use of kappa- and iota-carrageenases revealed that commercial kappa- and iota-carrageenans were containing small amounts of the iota- or kappa-carrageenan-type repeating structures either in blocks or as separate chains (Bellion et al., 1981; Rochas & Heyraud, 1981).

To complete the primary and secondary structure analyses of polysaccharides, it is important to determine the mean number of repeating units, that is, their average molecular weight and the distribution of the molecules with regard to their weight average ($<M>_W$) and number average ($<M>_N$) molecular weight (polydispersity). Such values correspond to $<M>_N = \Sigma_i N_i M_i / \Sigma_i N_i$ and $<M>_W = \Sigma_i w_i M_i / \Sigma_i w_i = \Sigma_i N_i M_i^2 / \Sigma_i N_i M_i$, where N_i is the number of molecules and w_i the weight of molecules having a specific molecular weight M_i. As stated earlier, the molecular weight of the galactan is related with the gel mechanical properties (Rochas et al., 1990; Stanley, 1995; Tashiro et al., 1997a). These last years have seen the development of chromatographic technics coupled to multiple or low angles laser light scattering detectors making it possible to determine more easily the molecular weight and molecular weight distribution of phycocolloids. The average molecular weight of commercial agars is between 80 000 and 140 000 g mol^{-1} with polydispersity lower than 1.7 (Rochas & Lahaye, 1989b) and from 176 000 to 420 000 g mol^{-1} in *Gracilaria* agars with polydispersity values between 1.9 to 3.3 (Murano, 1995). These values agree with those obtained by sedimentation experiments (Hickson & Polson, 1968; Tashiro et al., 1997b). Furthermore, the Mark-Houwink relationship for agarose molecular weight determination by viscosity measurement was established $[\eta] = 0.07\ M^{1.72}$ at 37 °C in 0.75 M NaSCN (Rochas & Lahaye, 1989b). The molecular weight determined by HPSEC and laser light scattering detection of carrageenans demonstrate a rather high polydispersity (Mw/Mn between 2.3 and 5.1) and a weight average molecular weight between 300 000 and 600 000 g mol^{-1} (particularly for λ-carrageenan) (Lecacheux et al., 1985; Singh & Jacobson, 1994; Slootmaekers et al., 1991; Vannest

et al., 1996; Viebke et al., 1995) which are also in good agreement with values obtained by sedimentation determination (Harding et al., 1997; Slootmaekers et al., 1991). A Mark-Houwink relationship relating intrinsic viscosity to molecular weight was given for κ-carrageenan in 0.1 M NaCl at 25°C (Rochas et al., 1990) $[\eta] = 3.11 \times 10^{-3} M^{0.95}$.

Chemical and biological factors affecting the chemistry of agar and carrageenans

Deviations from ideal repeating structures form agarobiose, carrabiose 4-sulphate or carrabiose 2,4-disulphate in commercial agarose, kappa- and iota-carrageenan modify the geometry of the galactan molecules and thus affect their gelling properties by introducing "solubilizing" sequences (Rees et al., 1982). Among these repeating structures, those containing 1,4-linked galactose 6-sulphate instead of 3,6-anhydrogalactose are often referred to as the "biological precursor" since enzymes have been isolated from algae (Rees, 1961; Wong & Craigie, 1978; Zinoun et al., 1997) responsible for the conversion of 1,4-linked galactose 6-sulphate to 3,6-anhydrogalactose. Thus, these enzymes are responsible for the conversion of "solubilizing" sequences to "gelling" sequences. The expression of such sulphate eliminases has recently been followed by mass spectrometry and ^{13}C nmr spectroscopy through the fate of ^{13}C label incorporated in agarcolloids (Hemmingson et al., 1996a;b; Hemmingson & Furneaux, 1997). The NMR work clearly showed that the newly synthesized *Gracilaria chilensis* agarocolloid is enriched in **L6S** and then converted in vivo to an agar mainly composed of agarobiose repeating units. These biosynthetic studies also stressed that agar biosynthesis is linked to starch metabolism, the **L6S** conversion to **LA** is incomplete, indicating some potential physiological function of this sugar sulphate in the alga. They also demonstrated the early synthesis of **G6M** containing disaccharidic structures although further or different methyl ether substitutions may also occur at some latter stages in older tissues as inferred from the work of Craigie & Wen (1984) on *Gracilaria tickvahiae*. A strong alkali treatment of agarcolloids and carrageenans also converts the **L6S** and **D6S** into **LA** and **DA** sugar residues (Percival & McDowell, 1967) and such a treatment is classically used in laboratories (Craigie & Leigh, 1978) and industries (Armisen & Galatas, 1987; Stanley, 1987) to desulphate and improve the gelling properties of algal galactans. The kinetics of alkaline cyclisation has been studied in different galactans demonstrating higher rates of conversion when neither the 4-linked nor the 3-linked galactoses are sulphated on O-2 (Ciancia et al., 1993; Noseda & Cerezo, 1995).

Besides the growth status of the algae with regard to polysaccharide biosynthesis, seaweeds belonging to the Gigartinales demonstrate marked variations in the galactans synthesized according to their life cycle (Craigie, 1990). Thus haploid gametophytes synthesize carrageenans belonging to the kappa-family (kappa-, iota-, mu-, nu-carrageenans; **G4SDA**, **G4SDA2S**, **G4SD6S**, **G4SD2,6S**) whereas the diploid tetrasporophyte produce carrageenans belonging to the lambda-family (delta-, lambda-, epsilon-, alpha-, theta-carrageenan; **GD2,6S**, **G2SD2,6S**, **G2SD2S**, **GDA2S**, **G2SDA2S**). No such ploidy effect has been observed for agarophytes.

Conformation, physico-chemical properties and gel formation

The above review of the chemistry of the agar and carrageenan-type polysaccharides showed that chemical substitutions can affect in many ways the regularity of the ordered structure of the galactans and thus their self-associations and properties in solution. The following part summarise present data on the conformation, the solution and some gel properties of more ideal polysaccharides such as agarose, and commercial kappa- and iota-carrageenans.

Agarose

Conformation

The molecular shape of agarose has been unravelled by X-ray diffraction studies of films and fibres (Arnott et al., 1974a; Chandrasekaran, 1998). It is an oriented non-crystalline, double, half-staggered, left-handed helix of pitch 1.9 nm (length of the repeating unit h = 0.633 nm) with a threefold symmetry and made only of sugars in $^4C_1$ conformation. The inner and outer diameter dimensions are 0.42 and 1.36 nm and the inner cavity can accommodate water molecules which mediate interchain hydrogen bonds with the oxygen O-2 from the galactose residue and O-5 of the anhydrogalactose positioned toward the interior. There is no internal hydrogen bound stabilizing the double helix. In agreement with these data are the results obtained by the optical rotation studies of Shafer & Stevens (1995). It is important to stress that in the double helical conformation only the O-2 of the galactose, facing the inside of the helices is required for the stabilization of the system. Therefore, substitution by any chemical group at that position would prevent gelation. Other X-ray diffraction patterns have been obtained from films dried at 100 °C and favour extended single helices conformations of pitch (h) ranging from 0.89 to 0.97 nm (Foord & Atkins, 1989). These interpretations are consistent with the conformation of single strand agarose deduced by molecular modelling studies using agarobiose and neoagarobiose repeating units as models (Jimenez-Barbero et al., 1989) or the UV circular dichroic data of agarose solutions and dried solutions (Arndt & Stevens, 1994). Other data confirming the existence of single rigid agarose extended helices in the sol-state came

from the low angle neutron scattering measurements (Guenet et al., 1993). This apparent discrepancy in the conformation of agarose may be explained by the observation of the polysaccharide in two different states i.e. the sol- and the gel-state. In solution, agarose may have extended helical shapes which contract to a wider diameter double helices as it gels. Further insight of the conformation of agarose in the gel state and particularly to confirm or in validate the double intertwined helical structure versus the helical dimer is still waiting for better resolved X-ray patterns.

Gel formation and some properties

Agarose gelation involves the conversion of a fluctuating extended helical conformation in solution to a rigid, ordered double helical structure forming the junction zones of the gel network (Rees et al., 1982). The final gel formation occurs as a two steps mechanism: 1) contraction and doubling of the helices formally referred to as the coil-helix transition and 2) aggregation of helices. The gel setting occurs through the demixion of the solution into polymer-rich and solvent-rich regions and the extension of gelation by agglomeration of the polymer-rich domains along preferential pathways of higher polymer concentration. Such behaviours are observed only at polysaccharide concentration below 2% in solution. At higher concentration, gelation is thought to happen directly from the homogeneous solution (San Biagio et al., 1996). The large difference between gel-setting and gel-fusion, also called thermal hysteresis, is thought to arise from the formation of large aggregates which remain stable at temperature well above that of individual helices formation on cooling. The extent of hysteresis decreases with increasing content of substituent groups (Guiseley, 1970; Arnott et al., 1974a) which is consistent with the inhibition of helices aggregation. The onset of gel formation on cooling as determined by mechanical measurement occurs at a lower temperature than the appearance of the conformational transition measured by optical rotation. Such behaviour reflects the requirement for a certain amount of helices to create a continuous network. Conversely, the gel fusion determined by mechanical measurements occurs at a slightly lower temperature than that of the total helix-coil transition (Mohamed et al., 1998). This temperature is particularly affected by the way the gel was first obtained. With rapid quenching of an agarose solution from 45 °C to 5 °C, the gel-melting temperature is about 10 °C lower than that from the gel formed at a cooling-rate of 1°C min^{-1} (Mohamed et al., 1998). Furthermore, the storage modulus (G') which is an expression of the gel mechanical properties, is also decreased in fast quenched gels (about 50% lower). These behaviours reflect the better assembly of helices aggregation formed at lower cooling rate of the agarose solution and is associated with a higher gel turbidity compared to that obtained by fast quenching. This particular behaviour can be of practical interest in facilitating, for example, the incorporation of agar in food processings. The gel-setting temperature and the final storage modulus of the gel also depend on the concentration of agarose in solution. Once the conformational change has occurred (observed by optical rotation), the gel develops with time. The temperature at which the conformational change begins depends on the agarose concentration and the rate of gel formation depends on both temperature and polysaccharide concentration (Mohamed et al., 1998).

The gelling properties are related to the primary and secondary structures of the polysaccharides. Natural methyl ether substitutions increase the gel setting temperature (Guiseley, 1970) and the methyl on position O-6 of the 3-linked β-D-galactose is particularly responsible for this behaviour (Falshaw et al., 1998). In contrast, chemical methylation lowers the gel-setting temperature as the results of substitution at O-2 of the 3-linked β-D-galactose residues thought to be involved in the stabilization of the double helices through water-hydrogen bonds (Miller et al., 1994). Double methylation with methyl ether substitutions at position O-2 of the 4-linked 3,6-anhydro-α-L-galactose and O-6 of the 3-linked β-D-galactose significantly increase the gel fusion temperature (112-113 °C for *Curdiea coriacea* and 120-121 °C for *C. obesa* which are nearly completely doubly methylated agars; Falshaw et al., 1998). Agarose with high gelling temperatures or agaroses that have been chemically modified to lower their gelling and melting temperatures are of a particular interest for specific biotechnological applications (Kirkpatrick et al., 1993).

Details on agarose gel junction zones have been investigated by various technics (microscopy, scattering methods such as small angle light scattering, small angle X-ray scattering and small angle neutron scattering). By small angle neutron scattering (SANS), Krueger et al. (1994) observed that the junctional nets are more swollen at low agarose concentrations, which is coherent with an increase in pore size in the gel as the concentration is lowered (Maaloun et al., 1998). A minimal junction zone thickness of about 3.5-4.0 nm has been reported and is close to a fibre diameter of 5.0 nm determined by chromatographic methods (Laurent, 1967) or observed by microscopy (Dormoy & Candau, 1991; Whytock & Finch, 1991). Melting of the gel obviously markedly affect the SANS pattern, suggesting the presence of a wide distribution of particles of relatively small size which is in accordance with the model of extended rods described by Guenet et al., (1993). If a fast quenching is used to gel the agarose solution, then the nature of the intra-junctional strand associations are affected but not really the large-scale structure. From this behaviour, Krueger et al., (1994) infer

that agarose gel represents a random distribution of compact self-similar junctions of arbitrary length but differing in thickness. Within this gel, agarose fibres of varying thickness are present as randomly distributed clusters of net-like appearances, structured in 3D webs of dimension between one nm (thickness of individual strands) to several hundred nm. Such fibre bundle heterogeneity was suggested by Djabourov et al. (1989) by small angle X-ray scattering who subdivided the populations of fibre bundles arbitrarily to fit the data into two populations, one of 3.0 nm and the other of 9.0 nm. The heterogeneous distribution of fibre bundles was also deduced from the birefringence of agarose gels either when an electric field is applied or not (Stellwagen & Stellwagen, 1995). This optical property reflects the orientation of agarose fibre bundles or microgels fractions within the macroscopic gel. These objects can have sizes up to 10 micrometers depending on the electric field strength and the orientation changes can be irreversible if the applied field is too high or for a too long period of time. The intrinsic birefringence of agarose gel, which has also been observed for carrageenan gels, is inhomogeneous. It varies from gel to gel but appears inherent to physical gels and not to chemical gels such as polyacrylamide. In agarose, the orientation of the birefringent objects follows a macroscopical sinusoidal pattern. These objects have length scales of millimetres. The fact that the orientation of smaller parts of the macroscopic gels can be reversibly affected by electric fields is indicative of the existence of a hierarchy of loosely connected substructures held together by metastable hydrogen bonds (Stellwagen & Stellwagen, 1995). The distribution and organization of the fibre bundles are important with regard to application of agarose as a chromatographic and electrophoretic matrix since they condition the gel porosity. Pore sizes ranging from about 200 nm to about 2000 nm and their proportion where recently measured by atomic force microscopy (AFM, Pernodet et al., 1997; Maaloun et al., 1998). This non-invasive method does not require special preparation of the gel and therefore gives a picture of the "unperturbed" gel allowing the direct measurement of pore size distribution by image analysis. It represents a recent alternative to indirect methods such as electron microscopy requiring sample preparations such as dehydration (Attwood et al., 1988; Griess et al., 1993). Using AFM, a direct relationship of agarose pore size was found with polymer concentration and with ionic strength of the casting buffer. An empirical law relating pore size (a) and agarose concentration (C) was deduced ($a \sim C^{-0.6}$). A relationship was also observed between ionic strength of the casting buffer and pore size, the lower the ionic strength, the lower the pore diameter (Maaloun et al., 1998).

Electroendosmosis is one important practical property of agarose used as an electrophoresis matrix. It is related to the presence of minor amounts of charged groups (sulphate, pyruvate) and is due to the migration of their counter ions (cations) under the application of an electric field. This migration creates a flow of water molecule from the anode toward the cathode as a consequence of which, neutral molecules that would not migrate are pulled toward the cathode.

Agar gels loose water on ageing, a phenomenon called syneresis. This water exclusion is attributed to the contraction of the polymer network by a slow aggregation of helices resulting in the decrease in interstitial space available for holding water. Syneresis is lower for agars containing charged groups and is roughly inversely related to the square of the agar concentration (Stanley, 1995).

Carrageenans

Conformation

Similarly to agarose, kappa-, iota-carrageenan and partly desulphated kappa-carrageenan (furcellaran) oriented fibers yield X-ray diffraction patterns interpreted as representative of threefold, right-handed double helices (Chandrasekaran, 1998; Picullel, 1995; Millane et al., 1989). The calcium form of the iota-carrageenan double helices are half-staggered with a pitch of 2.6 nm and the **DA2S** and **G4S** residues are respectively in the $^1C_4$ and $^4C_1$ conformations (Arnott et al., 1974b). The two sulphate groups are oriented toward the outside and that of the **DA2S** residue of two adjacent helices are thought to be involved in iota-carrageenan gelation by calcium ions, forming ionic interactions. The **G4S** residues are connected by six interchain hydrogen bonds between O-6 and O-2 per helix turn. The inner and outer diameters without hydrogen atoms are 0.34 and 1.42 nm.

The fibers from the potassium form of kappa-carrageenan are less oriented, less crystalline and are less laterally organized (Millane et al., 1988). The most reasonable fit of the X-ray data corresponds to a parallel double helix of pitch 2.5 nm (h = 0.833 nm) offset by a translation of 0.11 nm along and a rotation of 28° from the half-staggered position. There are only 3 hydrogen bonds between the **G4S** residues and the overall diameters of the helix are 0.1 nm larger than those of the iota-carrageenan. Cairns et al., (1991) re-investigated the X-ray diffraction pattern of kappa-carrageenan and partly desulphated kappa-carrageenan (furcellaran) in the presence of glucomannan or various galactomannans. The authors did not observe interaction between the different polysaccharides although computer modelling suggested mutual polysaccharide conformational change to optimise van der Waals and electrostatic interactions (Tvaroska et al., 1992). Such mixed gel systems however provided clearer X-ray patterns for kappa-carrageenan which interpretation contradicts the Millane et

al., (1988) model. Cairns et al., (1991) proposed a loosely packed nematic liquid crystalline array of double helices of which component chains rotate without an axial translation from the half-staggered position.

Lambda carrageenan would form threefold helices of pitch 2.52 nm. Indications of left-handed or right-handed helices stabilized by hydrogen bonds between O-2 and O-3 across the (1->4) linkage were obtained from molecular modelling studies and the replacement of the **DA** residue by **D6S** or **D2,6S** in the $^4C_1$ conformation instead of the $^1C_4$ or the presence of **G2S** would render the double-helix formation impossible (Millane et al., 1989).

Computer modelling of kappa-, iota- and lambda-carrageenan starting form crystallographic data or calculated data for sulphated monosaccharides, yielded models predicting a more flexible character for kappa- and iota-carrageenans than for lambda-carrageenan. In all cases the computed structures were in good agreement with the above proposed from X-ray diffraction of fibers (Urbani et al., 1993, Le Questel et al., 1995). At any rate, as for agarose conformation, there is still a debate on the fundamental tertiary and quaternary structures conformation of carrageenans and particularly that of kappa-carrageenan. Recent results from high performance size exclusion chromatography coupled to laser light scattering measurements show the doubling of kappa- and iota-carrageenan molecular weight on cooling the chromatographic solvent temperature or on increasing its ionic strength (Viebke et al., 1995; Ueda et al., 1998; Hjerde et al., 1998). Whether these are duplex of single helices or double intertwined helices await for further refinement of X-ray diffractions or other physical data.

Gel formation

As for agarose, carrageenan gelation is associated with a conformational transition going from random coils in solution to double-helices on cooling and/or adding cations (Piculell, 1995). Single helices are also obtained with certain salts such as NaI (Smidsrød & Grasdalen, 1984a), or at very low salt concentrations (Ueda et al., 1998). In case of kappa-carrageenan a minimum of four disaccharide repeat units was determined as a minimum to observe a conformational transition by optical rotation (Rochas et al., 1983). The transition of the chain ordering proceeds cooperatively and starts from an initiation site and then extends along the chains. Such transition depends on the molecular weight and the concentration (Piculell, 1995) and is kinetically affected by salt contents and temperature (Norton et al., 1983a,b, Austen et al., 1985, 1988). There is a striking selectivity for cations in kappa-carrageenan conformational transition which however is not always associated to gel formation (Piculell, 1995). Rochas & Rinaudo (1980) clearly demonstrated that the temperature of transition at which the concentration of helices and coils are equal (Tm) is inversely and linearly related to the logarithm of the total cation concentration (CT, including the sulphate counterions Cc, with $CT \equiv Cs + \gamma Cc$ and Cs the concentration of added cations and γ the activity coefficient of the cation). They distinguished three groups of cations with respect to their helix-promoting efficiencies : non-specific monovalent cations (Li^+, Na^+ and $(CH_3)_4N^+$), non-specific divalent cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Co^{2+} and Zn^{2+}) and the specific monovalent cations (NH_4^+, K^+, Cs^+ and Rb^+). No evidence has yet been obtained to demonstrate specific binding of the cations to the polysaccharide in the coil state but, in contrast, several studies have shown their specific binding to the helices even in non-aggregating conditions although the site of fixation is still unknown (Piculell, 1995). Anions also affect the conformational transition of kappa-carrageenan in the order $F^- < Cl^- < NO_3^- < SCN^- < I^-$ which are in reverse order from the so-called Hofmeister or lyotropic series promoting denaturation (Norton et al., 1984).

Furcellaran and partially desulphated kappa-carrageenan behave like kappa-carrageenan (Tanner et al., 1990, Zhang et al., 1991, 1994) except that the temperature of transition Tm is higher. The salt dependency of the iota-carrageenan has been also well documented and shows a marked helix-stabilizing effect of Ca^{2+} (Piculell, 1995). The reported small specificities to some monovalent cations is now interpreted as arising from the small kappa-carrageenan proportions in iota-carrageenan (Piculell et al., 1987) which also affect the rheological properties of the gel (Piculell et al., 1992) and thus, there would be no cation specificity for the iota-carrageenan coil-helix transition.

As indicated above, there is still a debate on the conformation of carrageenans in the gel state but most of the informations indicate the rapid formation of double helices (Piculell, 1995). The minimum degree of polymerization for both iota- and kappa-carrageenans necessary to stabilize double helices is around 100 as determined by mechanical measurements (Hjerde et al., 1998). Once the double helices are formed, they aggregate (Viebke et al., 1994) as demonstrated by the thermal hysteresis for kappa-carrageenan. Such difference in gelling and melting temperatures reflect, as for agarose, the temperature of coil-helix formation and that of the denaturation of helical aggregates, respectively. Such hysteresis does not exist for iota-carrageenan (Piculell et al., 1992) and for kappa-carrageenan in NaI (Chronakis et al., 1996), a salt known to promote kappa-carrageenan single helices that do not aggregate at low concentrations (Smidsrød & Grasdalen, 1984a). These two behaviours indicate the existence of possible different gelling mechanisms for kappa- and iota-carrageenans. As for agarose, the observed turbidity and birefringence of kappa-carrageenan and furcellaran gels are

evidences for the formation of organized, oriented bundles. The hysteresis disappears at low salt concentrations for kappa-carrageenan evidencing the existence of conditions were conformational transition occurs without aggregation even with specific ions such as potassium (Rinaudo & Rochas, 1981). By choosing appropriate salts types and concentrations, different kappa-carrageenan helices associations can thus be obtained : from liquid nematic crystals using segments of kappa-carrageenan in NaI/CsI to double helical-rods and super-helical rods in gels (Piculell et al., 1997). Iota-carrageenan is unable to form liquid crystals (Borgström et al., 1998) conforting differences in molecular association behaviours between kappa and iota-carrageenan. Kappa-carrageenan gel appears by electron microscopy as a network of fibre bundles (Hermansson, 1989, Hermansson et al., 1991, Sugiyama et al., 1994, Borgström et al., 1996). In contrast, iota-carrageenan under salt free conditions and in presence of LiI appears as linear extended or macrocyclic structures (Stokke et al., 1993). To date, no information is available on the pores size and distribution formed in carrageenan gels.

Smidsrød & Grasdalen (1984b) and Rochas et al., (1990) demonstrated a dependancy of kappa-carrageenan storage modulus (G') and the Young's modulus (E) on molecular weight. G' and E increase up to an average molecular weight of about 200 000 and then plateau, while the yield stress still increase (Smidsrød & Grasdalen, 1984b, Rochas et al., 1990). The critical concentration of kappa-carrageenan to form a gel (c0) is in the order of the concentration of the polysaccharide overlap (c*) in solution (Rochas et al., 1990). As for agarose, the way the kappa-carrageenan gel is formed has incidence on the storage modulus G' (Hermansson et al., 1991). G' is three times higher for a gel obtained by cooling at 0.5 °C min^{-1} than at 1.5 °C min^{-1}.

2. Polyuronanes

Alginate

Alginate are the major matricial cell wall polysaccharides of brown seaweeds (Kloareg & Quatrano, 1988) also found in coralline red algae (Okazaki et al., 1982, Usov et al., 1995) and produced by some bacteria (Gacesa, 1988). It is widely used as a food gelling, thickening and stabilizing agent but has various other technical uses, particularly in the textile industry (McHugh, 1987). The industrial alginate production relies on the harvest of wild populations of *Macrocystis pyrifera*, *Laminaria digitata*, *L. hyperborea*, *L. japonica* and *Ascophyllum nodosum*, although farmed *L. japonica* is being increasingly exploited. The mode of alginate extraction starts with the removal of undesirable small compounds by acid washes and the transformation of alginate in the cell wall into alginic acid. The alginic acid is then extracted, while converted into the soluble sodium form by sodium carbonate or sodium hydroxide neutralization. The insoluble algal residues are removed by filtration, flotation or centrifugation and the soluble alginate is precipitated either by conversion into alginic acid or calcium alginate. The alginic acid is then converted into the required counter ion by neutralisation with the appropriate hydroxide; the calcium alginate is converted to alginic acid and then neutralized as above. The difference in the alginate recovery process depends on the source and thus the chemistry of the alginate (McHugh, 1987).

Chemistry

Alginate consists in a family of linear polysaccharides based on 1,4-linked β-D-mannuronic acid (M) and its C-5 epimer 1,4-linked α-L-guluronic acid (G) of varying proportions and distributions. Partial acid hydrolysis and enzymatic degradation by alginate-lyases have demonstrated that alginate contains homogeneous blocks of mannuronic acid and guluronic acid with regions where both acids alternate more or less regularly (Fig. 5). The latter region can contain some defective repetition in having MMG or GGM structures interspersed. The chemical composition and

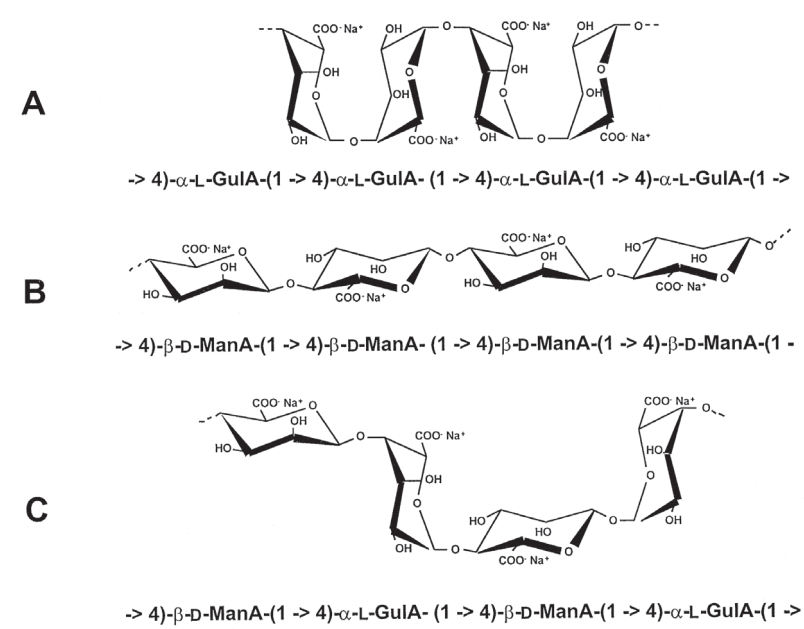

Figure 5. Chemical structure of poly-guluronate **A**, poly-mannuronate **B** and alternating mannuronate-guluronate **C** block sequences.

Figure 5. Structure chimique des séquences de blocs de poly-guluronate **A**, poly-mannuronate **B** et mannuronate-guluronate alternés **C**

structure of alginate has always been difficult to determined owing to the fact that the uronic acid linkages are particularly resistant to acid hydrolysis. Most methods for compositional analysis rely on colorimetric measurements although several other methods have been proposed: HPLC, IR, circular dichroism, GLC, NMR, enzymes (Moe et al., 1995). ^{13}C and ^{1}H nmr spectroscopy is a powerful method for determining the sequence of the two uronic acids in alginate (Grasdalen et al., 1981, Grasdalen, 1983). From the area of specific signals the proportion of mannuronic and guluronic acid (M, G), the frequencies of diads MM, MG/GM, GG, as well as the triad GGG, MMM, GGM, GMG, MMG and MGM can be measured. Average block length can then be calculated $<N>_G = F_G/F_{MG}$ and $<N>_M = F_M/F_{MG}$ or by excluding single G and M units in average to take into account irregularities in the alternating sequence distribution $<N>_{G>1} = (F_G - F_{MGM}) / F_{GGM}$ and $<N>_{M>1} = (F_M - F_{GMG}) / F_{MMG}$.

The composition and the distribution of alginate differ among species, as well as between tissues. Old tissue and stipes are generally richer in polyG blocks which most likely confer a high mechanical resistance of the algae to environmental stresses. The guluronic acid content and distribution is under the control of activity of the enzyme mannuronan C-5 epimerase (Hagen Rødde & Larsen, 1997; Skjåk-Bræk et al., 1986). The different enzymes and genes involved in the alginate biosynthesis are well studied, using alginate producing bacteria (Gacesa, 1998).

Alginate average molecular weight $<M>_W$ vary between 170 000 to 1640 000 g mol^{-1} depending on the source and the mode of extraction (Martinsen et al., 1991; Moe et al., 1995). Generally the polydispersity index ($<M>_W / <M>_N$) is between 1.4 - 2.6 for commercial preparation and can reach 5.6 for lab prepared samples (Moe et al., 1995). Mark-Houwink constants have been determined to measure the molecular weight of alginate from their intrinsic viscosity in 0.1 M NaCl at 25 °C (Martinsen et al., 1991). The K and a values differ from 7.3×10^{-5} to 6.9×10^{-6} and 0.92 to 1.13 for M- and G-rich alginate, respectively and reflect the extended shape of G-rich alginate.

Conformation, physico-chemical properties and gel formation

The conformation of alginate has been determined from X-ray diffraction patterns observed from polyG and polyM blocks of fibre bundles (Chandrasekaran, 1998). PolyM fibres are extended ribbon-like twofold helices (h = 0.52 nm) stabilized by hydrogen bonds between O-3 and O-5 (0.27 nm) across each glycosidic bridge oxygen atom. The mannuronic acid is in the $^{4}C_1$ conformation. In packing two helices, a hydrogen bond is formed between one of the carboxylate oxygens to O-3 of a neighbouring chain of the same polarity. Antiparallel chains are connected by H-bonds between O-2 and O-5. PolyG fibres form twofold helices (h = 0.435 nm) with a 0.87 nm pitch. The guluronic acid is in the $^{1}C_4$ conformation and because of the 1,4-diaxial linkages, the helix is a buckled ribbon stabilized by H-bonds between O-2 and one of the carboxylate oxygens across every glycosidic linkages. When packed with another chain, water connects the guluronate residues via O-2 and O-3 and via O-5 and O-3. The pairwise association of helices is thought to be mediated by calcium ions in the middle resembling an “egg-box” (Grant et al., 1973).

Alginate is well known for its ion binding and demonstrates an affinity for alkaline earth metals in the order Mg << Ca < Sr < Ba. This selectivity is due to the guluronic acid blocks as the polyM and alternating sequences do not demonstrate selectivity. The selectivity is also dependent on the ionic composition of the alginate gel as specific affinities show a strong cooperativity (Moe et al., 1995).

Alginate is insoluble at pH below the mean pKa of their constitutive uronic acids (3.4-4.3) and its solubility also depends on its composition, molecular weight and on the ionic strength of the solution (Moe et al., 1995). If the pH of an alginate solution is slowly lowered below this value, an acid gel is obtained. On fast acidification of the solution, the alginate precipitates. Alginate in solution is more stable at pH around 6-9; acidic pH lead to hydrolysis while high pH promote β-elimination reactions leading to the formation of unsaturated non-reducing ends. Furthermore, alginate stability is markedly affected by reducing agents such as polyphenolics contaminants which also promote β-elimination reactions. This is one of the reasons formaldehyde is used during the industrial extraction of alginate. This compound precipitates proteins and polyphenols in the algal residues.

Alginate forms very viscous solutions depending on the concentration, molecular weight, composition and sequence of the polysaccharide and on the ionic strength of the solvent. Temperature has little effect on viscosity. A gel or precipitate is formed when a gel promoting divalent cation is introduced to an alginate solution. The reaction is instantaneous and leads to the formation of strong junction zones in the gel and consequently, it is difficult to obtain an homogeneous gel. A dialysis and an internal gelation methods are available to form homogeneous gels under controlled kinetics. In the dialysis method, the gelling ion is allowed to diffuse through the alginate solution. The gradient of gelling ions in the gel is controlled by the molecular weight of the alginate and the concentration of gelling and non-gelling ions (Skjåk-Bræk et al., 1989) but cannot totally be avoided. Calcium ion concentration in alginate beads of various composition made in different salt conditions was nevertheless shown to be higher from the outside to the inside of beads (Thu et al., 1997). The internal

gelation method uses the property of the slow degradation of D-glucono-δ-lactone (GDL) with calcium carbonate or Ca-EDTA complex. The slow degradation of GDL acidifies the solvent which allows the release of Ca from the carbonate or the EDTA (Draget et al., 1990). The gelling kinetic can also be modulated by the ionic-form of the alginate (Draget et al., 1998). Sodium ion demonstrates a higher selective binding than potassium ion to alginate and thus can somehow retards gelation by competing with calcium. Once formed, the gel will show tendencies to syneresis, that is water will be excluded and this is related to the content of added gelling ions, the uronic acid composition and sequence of the alginate (Draget et al., 1990, Moe et al., 1995). Calcium alginate gel in solutions of monovalent ions swells consequently to the displacement of Ca by non-gelling ions and even the polyG region demonstrate Ca-monovalent exchange in high monovalent salt concentration (Wang & Spencer, 1998).

Alginate gel forms fibre bundles of thickness between 13-26 nm composed of guluronic acid rich sequences and pores ranging in size from 5 to 150 nm (Andresen et al., 1977, Veluraja & Atkins, 1989).

The gel mechanical properties rely on the concentration of alginate, its content of guluronic acid and polyG sequences and the gelling ion used (Skjåk-Bræk et al., 1986, Smidsrød & Grasdalen, 1984b). The storage modulus of gel made by dialysis is dependent on molecular weight of alginate up to 80 000 g mol^{-1} (Moe et al., 1995, Smidsrød & Grasdalen, 1984b).

Ulvan

Although green seaweeds belonging to Ulvales are not industrially used as source of phycocolloids, they synthesize water-soluble polysaccharides that are able to form weak gels in presence of divalent cations, boric acid and at a lightly basic pH (Haug, 1976; Lahaye & Axelos, 1993; Lahaye et al., 1996). Ulvan extracted from several different species are composed of rhamnose, xylose, glucose, glucuronic and iduronic acid and sulphate (Percival & McDowell, 1967; Quemener et al., 1997; Ray & Lahaye, 1995a; Yamamoto et al., 1980). As for alginate, the presence of acid stable uronic acid linkages leads to difficulties in the quantitative determination of the component sugars. Two types of linkages are particularly refractory to acid hydrolysis in ulvan, these are the aldobiuronic acid i.e. the uronic acid-aldose linkage of the glucuronic acid-rhamnose and the linkage between contiguous glucuronic acid. The former can be cleaved by the use of a glucuronidase after a mild acid hydrolysis (Quemener et al., 1997; Lahaye et al., 1999) but a quantitative degradation method for glucuronan is still lacking. Furthermore, ulvan contains also the acid labile iduronic acid for which an easily available standard is not available to quantitatively determine its concentration. In several ulvan, rhamnose is sulphated on O-3 and some xylose residues are sulphated on O-2 (Lahaye et al., 1998; Ray & Lahaye, 1995b). Four repeat units have been identified in ulvan (Fig. 6). Two aldobiuronic acids called ulvanobiuronic acid 3-sulphate type A and type B and noted **A3s** and **B3s** in short. These are 1,4-linked β-D-glucuronic acid or α-L-iduronic acid linked to 1,4-linked α-L-rhamnose 3-sulphate, respectively. Two other disaccharides called ulvanobioses were also identified where the uronic acids of **A3s** and **B3s** are replaced by

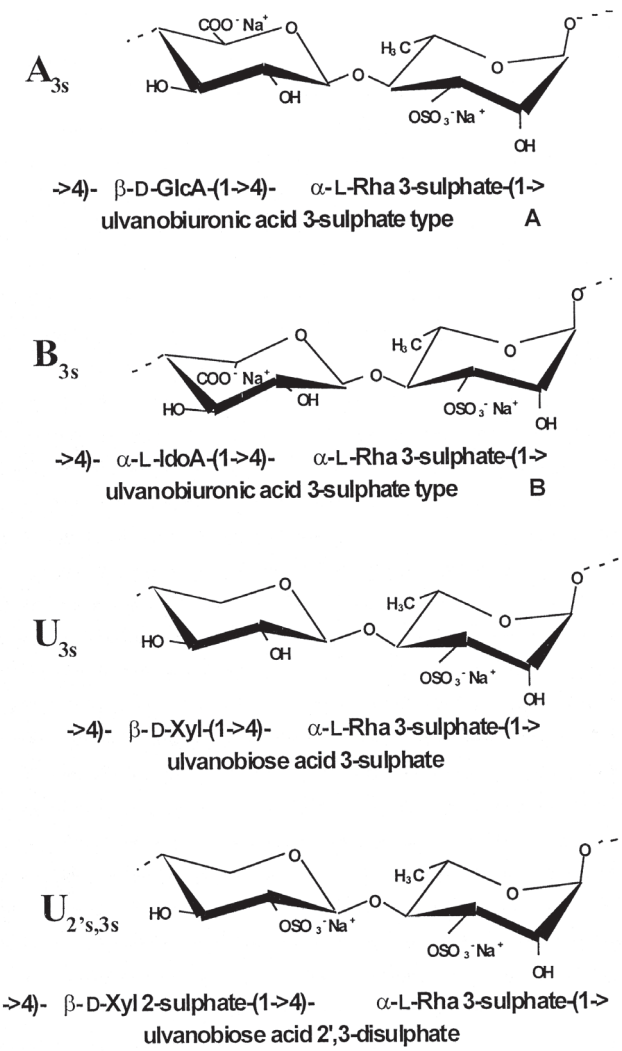

Figure 6. Chemical structure, names and shorthand notations of repeating disaccharides found in ulvan.

Figure 6. Structure chimique, noms et notations abrégées des unités de répétition de l'ulvane.

1,4-linked β-D-xylose or xylose 2-sulphate (**U3s** and **U2s'3s**, Lahaye et al., 1998, Lahaye, 1998). Other structures containing branching of single glucuronic acid residue to O-2 of rhamnose 3-sulphate in **A3s** (**A2g3s**) or contiguous 1,4-linked β-D-glucuronic acids where also observed (Lahaye & Ray, 1996; Lahaye et al., 1997; 1999). As for other phycocolloids, the isolation of an enzyme, an ulvan-lyase, has greatly helped in the fine structural elucidation of ulvan. In particular, it allowed to observed the different proportions and sequence patterns existing for the repeating disaccharides (Lahaye et al., 1997; Lahaye, 1998). Molecular weights of ulvan from Japanese *Ulva pertusa*, *U. conglobata* and *Enteromorpha prolifera* were determined by sedimentation equilibrium to be 91 000, 820 000 and 320 000 g.mol^{-1} (Yamamoto et al., 1980; Yamamoto, 1980). The molecular weight of *E. intestinalis* ulvan determined by light scattering experiments, ranged between 189 000 to 502 000 g.mol^{-1} depending on the extraction conditions (De Reviers & Leproux, 1993). Besides gelling properties, ulvans from *Ulva* sp. and *Enteromorpha* sp. develop low viscous solutions : the intrinsic viscosities at 20 °C in 0.1M citrate buffer ranged between 51, 155 ml g^{-1} for *U. pertusa*, *U. conglobata* and *E. prolifera* (Yamamoto et al., 1980) and were 175 and 36 ml g^{-1} for ulvan from *U. lactuca* and *E. compressa* at 37 °C in 0.150 M NaCl (Lahaye & Jegou, 1993).

Conclusions and perspectives

There are now good informations on the primary structure but still partial data on the secondary and tertiary structures of the major texturing algal polysaccharides. Further developments could focus of the distribution of the building structures in these hybrid polysaccharides. Other efforts could be placed on the biosynthesis of these polysaccharides and particularly on the relationships between ecophysiological growth conditions and the expression of key enzymes controlling the polysaccharide conformations, such as sulphate eliminase in agarophytes and carrageenophytes, or mannuronate C5-epimerase in alginate modifying the texturing properties of these phycocolloids. Additional works on the different levels of ulvan structures with regard to its gelling or thickening properties will also help in proposing new applications for this largely under-used complex polysaccharide. Moreover, the secondary, tertiary and quaternary structures of all of these polysaccharides are still matter of debate as there is yet no absolute methods available for obtaining unambiguous data. Informations relating the chemistry and the physic of the polysaccharides "junction zones" to the gel mechanical properties would be of prime interest in the design of polysaccharides with specific functions, either by a better control of their biosynthesis, or by controlled modifications during their extraction and processing.

References

Andresen I.L., Skipnes O., Smidsrød O., Østgaard K. & Hemmer P.C. 1977. Some biological functions of matrix components in benthic algae in relation to their chemistry and the composition of seawater. *ACS Symposium Series*, **48**: 361-XXX

Araki C. 1966. Some recent studies on the polysaccharides of agarophytes. *Proceedings of the International Seaweed Symposium*, **5**: 3-17.

Armisen R. & Galatas F. 1987. Production and uses of agar. In: *Production and Utilization of Products from Commercial Seaweeds* McHugh D.J. (Ed). *FAO Fisheries Technical Paper*, **288**: 1-57.

Arndt E. R. & Steven E. S. 1994. A conformational study of agarose by vacuum UV CD. *Biopolymers,* **34**: 1527-1534.

Arnott S., Fulmer A., Scott W.E., Dea I.C.M., Moorhouse R. & Rees D.A. 1974a. The agarose double helix and its function in agarose gel structure. *Journal of Molecular Biology*, **90**: 269-284.

Arnott S., Scott W.E., Rees D.A. & McNab C.G.A. 1974b. ι-Carrageenan: Molecular structure and packing of polysaccharide double helices in oriented *fiber*s of divalent cation salts. *Journal of Molecular Biology*, **90**: 253-267.

Attwood T.K., Nelmes B.J. & Sellen D.B. 1988. Electron microscopy of beaded agarose gels. *Biopolymers*, **27**: 201-212.

Austen K.R., Goodall D.M. & Norton I.T. 1985. Anion-independent conformational ordering in iota-carrageenan: disorder-order equilibria and dynamics. *Carbohydrate Research*, **140**: 251-262.

Austen K.R., Goodall D.M. & Norton I.T. 1988. Anion effects on equilibria and kinetics of the disorder-order transition of κ-carrageenan. *Biopolymers*, **27**: 139-155.

Bellion C., Hamer G.K. & Yaphe W. 1981. Analysis of kappa-iota hybrid carrageenans with kappa-carrageenase, iota-carrageenase and ^{13}C NMR. *Proceedings International Seaweed Symposium*, **10**: 379-384.

Borgström J., Egermayer M., Sparrman T., Quist P.O. & Piculell L 1998. Liquid crystallinity versus gelation of k-carrageenan in mixed salts: effects of molecular weight, salt composition, and ionic strength. *Langmuir*, **14**: 4935-4944.

Borgström J., Piculell L., Viebke C. & Talmon Y. 1996. On the structure of aggregated kappa-carrageenan helices. A study by cryo-TEM, optical rotation and viscometry. *International Journal of Biological Macromolecules*, **18**: 223-229.

Caceres P.J., Faudez C.A, Matsuhiro B. & Vasquez J.A. 1997. Carrageenophyte identification by second-derivative Fourier transform infrared spectroscopy. *Journal of applied Phycology*, **8**: 523-527.

Cairns P., Atkins E.D., Miles M.J. & Morris V.J. 1991. Molecular transforms of kappa carrageenan and furcellaran from mixed gel systems. *International Journal of Biological Macromolecules*, **13**: 65-68.

Cases M.R., Cerezo A.S. & Stortz C.A. 1995. Separation and quantitation of enantiomeric galactoses and their mono-O-methylethers as their diastereomeric acetylated 1-deoxy-1-(2-hydroxypropylamino) alditols. *Carbohydrate Research*, **269**: 333-341.

Chandrasekaran, R. 1998. X-ray diffraction of food polysaccharides. *Advances in Food Nutrition Research*, **42**: 131-210.

Chiovitti A., Bacic A., Craik D.J., Munro S.L.A., Kraft G.T. & Liao M.L. 1997. Cell-wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): novel, highly pyruvated carrageenans from the genus *Callophycus*. *Carbohydrate Research* , **299**: 229-243.

Chiovitti A., Liao M.L., Kraft G.T., Munro, S.L.A., Craik D.J. & Bacic A. 1996. Cell wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): Highly methylated carrageenans from the genus *Rhabdonia*. *Botanica marina*, **39**: 47-59.

Chopin T. & Whalen E. 1993. A new rapid method for carrageenan identification by FT IR diffuse reflectance spectroscopy directly on dried, ground algal material. *Carbohydrate Research*, **246**: 51-59.

Chronakis I.S., Piculell L. & Borgström J. 1996. Rheology of kappa-carrageenan in mixtures of sodium and cesium iodide: two types of gels. *Carbohydrate Polymers* , **31**: 215-225.

Ciancia M., Matulewicz M.C. & Cerezo A.S. 1993. L-galactose containing galactans from the carrageenophyte *Gigartina skottsbergii*. *Phytochemistry*, **34**: 1541-1543.

Ciancia M., Matulewicz M.C. & Cerezo A.S. 1997. A L-galactose-containing carrageenan from cystocarpic *Gigartina skottsbergii*. *Phytochemistry* , **45**: 1009-1013.

Ciancia M., Noseda M.D., Matulewicz M.C., Cerezo A.S. 1993. Alkali-modification of carrageenans: mechanism and kinetics in the kappa/iota-, mu/nu- and lambda-series. *Carbohydrate Polymers*, **20**: 95-98.

Cosson J., Deslandes E., Zinoun M., Mouradi-Givernaud A. 1995. Carrageenans and agars, red algal polysaccharides. *Progress in phycological Research*, **11**: 269-324.

Craigie J.S. 1990. Cell walls. In: *Biology of the red algae*. (Cole KM, Sheath RG eds), pp.221-257. Cambridge University Press, Cambridge.

Craigie J.S. & Leigh C. 1978. Carrageenans and agars. In: *Handbook of Phycological Methods: Physiological and Biochemical Methods*. (Hellebust JA, Craigie JS eds), pp. 109-131. Cambridge University Press, Cambridge.

Craigie J.S. & Wen Z.C. 1984. Effects of temperature and tissue age on gel strength and composition of agar from *Gracilaria tikvahiae* (Rhodophyceae). *Canadian Journal of Botany*, **62**: 1665-1670.

De Reviers B. & Leproux A. 1993. Characterization of polysaccharides from *Enteromorpha intestinalis* (L.) Link, Chlorophyta. *Carbohydrate Polymers*, **22**: 253-259.

Djabourov M., Clark A.H., Rowlands D.W. & Ross-Murphy S.B. 1989. Small-angle X-ray scattering characterization of agarose sols and gels. *Macromolecules*, **22**: 180-188.

Dormoy Y. & Candau S. 1991. Transient electric birefringence study of highly dilute agarose solutions. *Biopolymers*, **31**: 109-117.

Draget K.I., Østgaard K. & Smidsrød O. 1990. Homogeneous alginate gels: a technical approach. *Carbohydrate Polymers*, **14**: 159-178.

Draget K.I., Steinsvåg K., Onsøyen E. & Smidsrød O. 1998. Na- and K-alginate; effect on Ca^{2+}-gelation. *Carbohydrate Polymers*, **35**: 1-6.

Duckworth M. & Yaphe W. 1971. The structure of agar. Part I : Fractionation of a complex mixture of polysaccharides. *Carbohydrate Research*, **16**: 189-197.

Errea M., Ciancia M., Matulewicz M. & Cerezo A. 1998. Separation and quantitation of enantiomeric 3,6-anhydrogalactoses by conversion to the corresponding diastereomeric acetylated *sec*-butyl 3,6-anhydrogalactonates. *Carbohydrate Research,* **311**: 235-238.

Falshaw R. & Furneaux R.H. 1994. Carrageenan from the tetrasporic stage of *Gigartina decipiens* (Gigartinaceae, Rhodophyta). *Carbohydrate Research,* **252**: 171-182.

Falshaw R. & Furneaux R.H. 1995. The structural analysis of disaccharides form red algal galactans by methylation and reductive partial-hydrolysis. *Carbohydrate Research,* **269**: 183-189.

Falshaw R. & Furneaux R.H. 1998. Structural analysis of carrageenans from tetrasporic stages of the red algae, *Gigartina lanceata* and *Gigartina chapmanii* (Gigartinaceae, Rhodophyta). *Carbohydrate Research,* **307**: 325-331.

Falshaw R., Furneaux R.H. & Stevenson D.E. 1998. Agars from nine species of red seaweeds in the genus *Curdiea* (Gracilariaceae, Rhodophyta). *Carbohydrate Research,* **308**: 107-115.

Falshaw R., Furneaux R.H., Wong H., Liao M.L., Bacic A. & Chandrkrachang S. 1996. Structural analysis of carrageenans from Burmese and Thai samples of *Catenella nipae* Zanardini. *Carbohydrate Research*, **285**: 81-98.

Foord SA. & Atkins E.D.T. 1989. New X-ray diffraction results from agarose: extended single helix structures and implications for gelation mechanism. *Biopolymers*, **28**: 1345-1365.

Fournet I., ArGall E., Deslandes E., Huvenne J.-P., Sombret B. & Floc'h J.-Y. 1997. In situ measurements of cell wall components in the red alga *Solieria chordalis* (Solieriaceae, Rhodophyta) by FTIR microspectrometry. *Botanica marina*, **40**: 45-48.

Gacesa P. 1988. Alginates. *Carbohydrate Polymers*, **8**: 161-182.

Gacesa P. 1998. Bacterial alginate biosynthesis - recent progress and future prospects. *Microbiology*, **144**: 1133-1143.

Grant G.T., Morris E.R., Rees D.A., Smith P. J. C. & Thom, D. 1973. Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Letters*, **32**: 195-198.

Grasdalen H. 1983. High-field ^{1}H-n.m.r. spectroscopy of alginate: sequential structure and linkage conformation. *Carbohydrate Research*, **118**: 255-260.

Grasdalen H., Larsen B. & Smidsrød O. 1981. ^{13}C nmr studies of monomeric composition and sequence in alginate. *Carbohydrate Research*, **89**: 179-191.

Greer C.W., Rochas C. & Yaphe W. 1985. Iota-carrageenan oligosaccharides as model compounds for structural analysis of iota-carrageenan by ^{13}C-NMR spectroscopy. *Botanica marina*, **28**: 9-14.

Greer C.W., Shomer I., Goldstein M.E. & Yaphe W. 1984**.** Analysis of carrageenan from *Hypnea musciformis* by using κ- and ι-carrageenases and ^{13}C-N.M.R. spectroscopy. *Carbohydrate Research*, **129**: 189-196.

Greer C.W. & Yaphe W. 1984a. Characterization of hybrid (beta-kappa-gamma) carrageenan from *Eucheuma gelatinae* J. Agardh (Rhodophyta, Solieraceae) using carrageenases, infrared and ^{13}C-nuclear magnetic resonance spectroscopy. *Botanica marina*, **27**: 473-478.

Greer C.W. & Yaphe W. 1984b. Enzymatic analysis of carrageenans: structure of carrageenan from *Eucheuma nudum*. *Hydrobiologia*, **116/117**: 563-567.

Greer C.W. & Yaphe W. 1984c. Hybrid (iota-nu-kappa) carrageenan from *Eucheuma nudum* (Rhodophyta, Solieriaceae), identified using iota- and kappa-carrageenases and ^{13}C-nuclear magnetic resonance spectroscopy. *Botanica marina*, **27**: 479-484.

Griess G.A., Guiseley K.B. & Serwer P. 1993. The relationship of agarose gel structure to the sieving of spheres during agarose gel electrophoresis. *Biophysical Journal*, **65**:138-148.

Guenet J.-M., Rochas C. & Brûlet A. 1993. Molecular structures in biopolymers sols and gels. *Journal de Physique*, **3**: 99-102.

Guiseley K.B. 1970. The relationship between methoxyl content and gelling temperature of agarose. *Carbohydrate Research*, **13**: 247-256.

Hagen Rødde R.S. & Larsen B. 1997. Protoplasts of *Laminaria digitata* and *Laminaria saccharina* (Phaeophyta) - Cultivation and biosynthesis of alginate. *Botanica marina*, **40**: 391-395.

Harding S.E., Day K., Dhami R. & Lowe P.M. **1997.** Further observations on the size, shape and hydration of kappa-carrageenan in dilute solution. *Carbohydrate Polymers*, **32**: 81-97.

Haug A. 1976. The influence of borate and calcium on the gel formation of a sulfated polysaccharide from *Ulva lactuca*. *Acta Chemica Scandinavica*, B30: 562-566.

Hemmingson J.A. & Furneaux R.H. 1997. Biosynthetic activity and galactan composition in different regions of the thallus of *Gracilaria chilensis* Bird, McLachlan et Oliveira. *Botanica marina*, **40**: 351-357.

Hemmingson J.A., Furneaux R.H. & Murray-Brown V.H. 1996a. Biosynthesis of agar polysaccharides in *Gracilaria chilensis* Bird, McLachlan et Oliveira. *Carbohydrate Research*, **287**: 101-115.

Hemmingson J.A., Furneaux R.H. & Wong H. 1996b. In vivo conversion of 6-O-sulfo-L-galactopyranosyl residues into 3,6-anhydro-L-galactopyranosyl residues in *Gracilaria chilensis* Bird, McLachlan et Oliveira. *Carbohydrate Research*, **296**: 285-292.

Hermansson A.M. 1989. Rheological and microstructural evidence for transient states during gelation of kappa-carrageenan in the presence of potassium. *Carbohydrate Polymers*, **10**: 163-181.

Hermansson A.M., Eriksson E. & Jordansson E. 1991. Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, **16**: 297-320.

Hickson T.G.L. & Polson A. 1968. Some physical characteristics of the agarose molecule. *Biochimica et Biophysica Acta*, **195**: 43-56.

Hjerde T., Smidsrød O., Stokke B.T. & Christensen B.E. 1998. Acid hydrolysis of k- and i-carrageenan in the disordered and ordered conformations: characterization of partially hydrolyzed samples and single-stranded oligomers released from the ordered structures. *Macromolecules*, **31**: 1842-1851.

Hoffmann R.A., Gidley M.J., Cooke D. & Frith W.J. 1995. Effect of isolation procedures on the molecular composition and physical properties of *Eucheuma cottonii* carrageenan. *Food Hydrocolloids*, **9**: 281-289.

Jensen A. **1993**. Present and future needs for algae and algal products. *Hydrobiologia*, **260/261**: 15-23.

Jimenez-Barbero J., Bouffar-Roupe C., Rochas C. & Perez S. 1989. Modelling studies of solvent effects on the conformational stability of agarobiose and neoagarobiose and their relationship to agarose. *International Journal of Biological Macromolecules*, **11**: 265-272.

Kirkpatrick F.H., Dumais M.M., White H.W. & Guiseley K.B. **1993.** Influence of the agarose matrix in pulsed-field electrophoresis. *Electrophoresis*, **14**: 349-354.

Kloareg B. & Quatrano R.S. 1988. Structure of the cell-wall of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanography and Marine Biology Annual Review*, **26**: 259-315.

Knudsen S.H. & Grasdalen H. 1992. The use of neocarrabiose oligosaccharides with different length and sulphate substitution as model compounds for ^{1}H-NMR spectroscopy. *Carbohydrate Research*, **229**: 233-244.

Knudsen S.H., Myslabodski D.E., Larsen B. & Usov A.I. 1994. A modified system of nomenclature for red algal galactans. *Botanica marina*, **37**: 163-169.

Krueger S., Ploplis-Andrews A. & Nossal R. 1994. Small angle neutron scattering studies of structural characteristics of agarose gels. *Biophysical Chemistry*, **53**: 85-94.

Lahaye M. 1998. NMR spectroscopic characterisation of oligosaccharides from two *Ulva rigida* ulvan samples (Ulvales, Chlorophyta) degraded by a lyase. *Carbohydrate Research*, **314**: 1-12.

Lahaye M., Alvarez-Cabal Cimadevilla E., Kuhlenkamp R., Quemener B., Lognoné V. & Dion P. 1999. Chemical composition and ^{13}C NMR spectroscopic characterization of ulvans from *Ulva* (Ulvales, Chlorophyta). *Journal of Applied Phycology*, **11**: 1-7.

Lahaye M. & Axelos M.A.V. 1993. Gelling properties of water-soluble polysaccharides from proliferating marine green seaweeds (*Ulva* spp.). *Carbohydrate Polymers*, **22**: 261-265.

Lahaye M., Brunel M. & Bonnin E. 1997. Fine chemical structure analysis of oligosaccharides produced by an ulvan-lyase degradation of the water-soluble cell-wall polysaccharides from *Ulva* sp. (Ulvales, Chlorophyta). *Carbohydrate Research*, **304**: 325-333.

Lahaye M., Inizan F. & Vigouroux J. 1998. NMR analysis of the chemical structure of ulvan and of ulvan-boron complex formation. *Carbohydrate Polymers*, **36**: 239-249.

Lahaye M. & Jegou D. **1993.** Chemical and physico-chemical characteristics of dietary fibres from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev. *Journal of Applied Phycology*, **5**: 195-200.

Lahaye M. & Ray B. 1996. Cell-wall polysaccharides from the marine green alga *Ulva 'rigida'* (Ulvales, Chlorophyta). NMR analysis of ulvan oligosaccharides. *Carbohydrate Research*, **283**: 161-173

Lahaye M., Ray B., Baumberger S., Quemener B. & Axelos M.A.V. 1996. Chemical characterisation and gelling properties of cell wall polysaccharides from species of *Ulva* (Ulvales, Chlorophyta) *Hydrobiologia*, **326/327**: 473-480.

Lahaye M. & Rochas C. 1991. Chemical structure and physico-chemical properties of agar. *Hydrobiologia*, **221**: 137-148.

Lahaye M., Rochas C. & Yaphe W. 1986. A new procedure for determining the heterogeneity of agar polymers in the cell walls of *Gracilaria* spp. (Gracilariaceae, Rhodophyta). *Canadian Journal of Botany*, **64**: 579-585.

Lahaye M., Yaphe W. & Rochas C. 1985. ^{13}C-N.m.r. spectral analysis of sulfated and desulfated polysaccharides of the agar type. *Carbohydrate Research*, **143**: 240-245.

Lahaye M., Yaphe W., Viet M.T.P., Rochas C. 1989. ^{13}C-N.M.R. spectroscopic investigation of methylated and charged agarose oligosaccharides and polysaccharides. *Carbohydrate Research*, **190**: 249-265.

Laurent T.C. 1967. Determination of the structure of agarose gels by gel chromatography. *Biochimica et Biophysica Acta*, **136**: 199-205.

Le Questel J.-Y., Cros S., Mackie W. & Pérez S. 1995. Computer modelling of sulfated carbohydrates: applications to carrageenans. *International Journal of Biological Macromolecules*, **17**: 161-175.

Lecacheux D., Panaras R., Brigand G. & Martin G. 1985. Molecular weight distribution of carrageenans by size exclusion chromatography and low angle laser light scattering. *Carbohydrate Polymers*, **5**: 423-440.

Maaloun M., Pernodet N. & Tinland B. 1998. Agarose gel structure using atomic force microscopy: gel concentration and ionic strength effects. *Electrophoresis*, **19**: 1606-1610.

Martinsen A., Skjåk-Bræk G. & Smisdrød O. 1991. Comparison of different methods for determination of molecular weight and molecular weight distribution of alginates. *Carbohydrate Polymers*, **15**: 171-193.

Matsuhiro B. & Rivas P. 1993. Second-derivative Fourier transfrom infrared spectra of seaweed galactans. *Journal of Applied Phycology*, **5**: 45-51.

McHugh D.J. 1987. Production, properties and uses of alginates. In: *Production and Utilization of Products from Commercial Seaweeds*. (McHugh D.J. Ed*), FAO Fisheries Technical Paper*, **288**: 58-115

Millane R.P., Chandrasekaran R., Arnott S. & Dea I.C.M. 1988. The molecular structure of kappa-carrageenan and comparison with iota-carrageenan. *Carbohydrate Research*, **182**: 1-17.

Millane R.P., Nzewi E.U. & Arnott S. 1989. Molecular structures of carrageenans as determined by X-ray fibre diffraction. In: *Frontiers in Carbohydrate Research - 1: Food Applications*. (Millane RP, BeMiller JN, Chandrasekaran R eds), pp. 105-131. Elsevier Applied Science, London.

Miller I.J. 1997. The chemotaxonomic significance of the water-soluble red algal polysaccharides. *Recent Research in Development and Phytochemistry*, **1**: 531-565.

Miller I.J. 1998. The structure of a pyruvylated carrageenan extracted from *Stenogramme interrupta* as determined by ^{13}C NMR spectroscopy. *Botanica marina*, **41**: 305-315.

Miller I.J., Falshaw R., Furneaux R.H. 1994. Chemical methylation of agaroid hydroxyl-groups. *Carbohydrate Research*, **262**: 127-135.

Moe S.T., Draget K.I., Skjåk-Bræk G. & Smidsrød O. 1995. Alginates. In: *Food Polysaccharides and Their Applications* (Stephen A.M. ed), pp: 245-286. Marcel Dekker Inc., New York.

Mohamed Z.H., Hember M.W.N., Richardson R.K. & Morris E.R. 1998. Kinetic and equilibrium processes in the formation and melting of agarose gels. *Carbohydrate Polymers*, **36**: 15-26.

Morris E.R. 1992. Physico-chemical properties of food polysaccharides. In*: Dietary Fibre - A component of food*. (Schweitzer TF and Edwards CA eds), pp. 41-56. Springer-Verlag, Berlin.

Morrice L.M., McLean M.W., Long W.F. & Williamson F.B. 1983. Porphyran primary structure. An investigation using β-agarase I from *Pseudomonas atlantica* and ^{13}C-NMR spectroscopy. *European Journal of Biochemistry*, **133**: 673-684.

Murano E. 1995. Chemical structure and quality of agars from *Gracilaria*. *Journal of Applied Phycology*, **7**: 245-254.

Norton I.T., Goodall D.M., Morris E.R. & Rees D.A. 1983a. Equilibrium and dynamic studies of the disorder-order transition of kappa carrageenan. *Journal of Chemical Society Faraday Transactions*, 1 **79**: 2489-2500.

Norton I.T., Goodall D.M., Morris E.R. & Rees D.A. 1983b. Dynamics of cation-induced conformational ordering in solutions of segmented iota carrageenan. *Journal of Chemical Society Faraday Transactions* 1, 79: 2501-2515.

Norton I.T., Morris E.R. & Rees D.A. 1984. Lyotropic effects of simple anions on the conformation and interactions of kappa-carrageenan. *Carbohydrate Research*, **134**: 89-101.

Noseda M.D. & Cerezo A.S. 1995. Alkali modification of carrageenans — II. The cyclization of model compounds containing non-sulfated α-D-galactose units. *Carbohydrate Polymers*, **26**: 1-3.

Nunn J.R., Parolis H. & Russell I. 1971. Sulphated polysaccharides of the Solieriaceae family. Part I. A polysaccharide from *Anatheca dentata*. *Carbohydrate Research*, **20**: 205-215.

Okazaki M., Furuya K., Tsukayama K. & Nisizawa K. 1982. Isolation and identification of alginic acid from calcareous red alga, *Joculator maximus*. *Journal of Biochemistry*, **61**: 497-53.

Painter T.J. 1983. Algal polysaccharides. In: *The Polysaccharides (Vol 2)*, (Aspinall GO ed), pp. 195-285. Academic Press, New York.

Percival E. & McDowell R.H. 1967. *Chemistry and Enzymology of Marine Algal Polysaccharides*. London, Academic Press.

Pernodet N., Maaloum M. & Tinland B. 1997. Pore size of agarose gels by atomic force microscopy. *Electrophoresis*, **18**: 55-58.

Picullel L. 1995. Gelling carrageenans. In: *Food Polysaccharides and Their Applications* (Stephen AM ed), pp. 205-244. Marcel Dekker Inc., New York.

Piculell L., Borgström J., Chronakis I.S., Quist P.O., Viebke C. 1997. Organisation and association of k-carrageenan helices under different salt conditions. *International Journal of Biological Macromolecules*, **21**: 141-153.

Picullel L., Håkansson C. & Nilsson S. 1987. Cation specificity of the order-disorder transition in iota carrageenan: effects of kappa carrageenan impurities. *International Journal of Biological Macromolecules*, **9**: 297-301.

Piculell L., Nilsson S. & Muhrbeck P. 1992. Effects of small amounts of kappa-carrageenan on the rheology of aqueous iota-carrageenan. *Carbohydrate Polymers*, **18**: 199-208.

Potin P., Bouarab K., Küpper F. & Kloareg B. 1999. Oligosaccharide recognition signals and defence reactions in marine plant-microbe interactions. *Current Opinion in Microbiology*, **2**: 276-283.

Quemener B. & Lahaye M. 1998. Comparative analysis of sulfated galactans from red algae by reductive hydrolysis and mild methanolysis coupled to two different HPLC techniques. *Journal of Applied Phycology*, **10**: 75-81.

Quemener B., Lahaye M. & Bobin-Dubigeon C. 1997. Sugar determination in ulvans by a chemical-enzymatic method coupled to high performance anion exchange chromatography. *Journal of Applied Phycology*, **9**: 179-188.

Quemener B., Lahaye M. & Metro 1995. Assessment of methanolysis for the determination of composite sugars of gelling carrageenans and agarose by HPLC. *Carbohydrate Research*, **266**: 53-64.

Ray B. & Lahaye M. 1995a. Cell-wall polysaccharides from the marine green alga *Ulva 'rigida'* (Ulvales, Chlorophyta). Extraction and chemical composition. *Carbohydrate Research*, **274**: 251-261.

Ray B. & Lahaye M. 1995b. Cell-wall polysaccharides from the marine green alga *Ulva 'rigida'* (Ulvales, Chlorophyta). Chemical structure of ulvan. *Carbohydrate Research*, **274**: 313-318.

Rees A. 1961. Enzymic synthesis of 3:6-anhydro-L-galactose within porphyran from L-galactose-6-sulphate units. *Biochemical Journal*, **81**: 347-352.

Rees D.A., Morris E.R., Thom D. & Madden J.K. 1982. Shapes and interactions of carbohydrate chains. In: *The Polysaccharides, Vol 1*. (Aspinall GO ed), pp. 195-290. Academic press, New York.

Renn D.W. 1984. Agar and agarose : indispensable partners in biotechnology. *Industrial Engineering and Chemical Products Research Development*, **23**: 17-21.

Rinaudo M. & Rochas C. 1981. Investigations of aqueous solution properties of k-carrageenans, *ACS Symposium Series* **150**: 367-378.

Rochas C. & Heyraud A. 1981. Acid and enzymic hydrolysis of kappa carrageenan. *Polymer Bulletin*, **5**: 81-86.

Rochas C. & Lahaye M. 1989a. Solid state ^{13}C-NMR spectroscopy of red seaweeds, agars and carrageenans. *Carbohydrate Polymers*, **10**: 189-204.

Rochas C. & Lahaye M. 1989b. Average molecular weight and molecular weight distribution of agarose and agarose-type polysaccharides. *Carbohydrate Polymers*, **10**: 289-298.

Rochas C., Lahaye M. & Yaphe W. 1986a. Sulfate content of carrageenan and agar determined by infrared spectroscopy. *Botanica marina*, **29**: 335-340.

Rochas C., Lahaye M., Yaphe W. & Phan Viet M.T. 1986b. ^{13}C NMR-spectroscopic investigation of agarose oligomers. *Carbohydrate Research*, **148**: 199-207.

Rochas C. & Rinaudo M. 1980. Activity coefficients of counterions and conformation in kappa-carrageenan systems. *Biopolymers*, **19**: 1675-1687.

Rochas C., Rinaudo M. & Landry S. 1990. Role of the molecular weight on the mechanical properties of kappa carrageenan gels. *Carbohydrate Polymers*, **12**: 255-266.

Rochas C., Rinaudo M. & Vincendon M. 1983. Spectroscopic characterization and conformation of oligo kappa carrageenans. *International Journal of Biological Macromolecules*, **5**: 111-

Saitô H., Yokoi M. & Yamada J. 1990. Hydration-dehydration induced conformational changes of agarose, and kappa- and iota-carrageenans as studied by high-resolution solid-phase ^{13}C-nuclear magnetic resonance spectroscopy. *Carbohydrate Research*, **199**: 1-10.

San Biagio P.L., Bulone D., Emmanuele A., Palma-Vittorelli M.B & Palma M.U. 1996. Spontaneous symmetry-breaking pathways: time resolves study of agarose gelation. *Food Hydrocolloids* **10**: 91-97.

Schafer S.E. & Stevens E.S. 1995. A reexamination of the double-helix model for agarose gels using optical rotation. *Biopolymers*, **36**: 103-108.

Sekkal M., Huvenne J.-P., Legrand P., Sombret B., Mollet J.-C, Mouradi-Givernaud A. & Verdus M.-C. 1993. Direct structural identification of polysaccharides from red algae by FTIR microscopy I: localization of agar in *Gracilaria verrucosa* sections. *Mikrochimica Acta*, **112**: 1-10.

Sekkal M. & Legrand P. 1993. A spectroscopic investigation of the carrageenans and agar in the 1500-100 cm^{-1} spectral range. *Spectrochimica Acta* , **49A**: 209-221.

Singh SK & Jacobson S.P. 1994. Kinetics of acid hydrolysis of k-carrageenan as determined by molecular weight (SEC-MALLS-RI), gel breaking strength, and viscosity measurements. *Carbohydrate Polymers*, **23**: 89-13.

Skjåk-Bræk G., Grasdalen H. & Smidsrød O. 1989. Inhomogeneous polysaccharide ionic gels. *Carbohydrate Polymers*, **10**: 31-54.

Skjåk-Bræk G., Smidsrød O. & Larsen B. 1986. Tailoring of alginates by enzymatic modification in vitro. *International Journal of Biological Macromolecules*, **8**: 330-336.

SlootmaekersD., van Dijk J.A.P.P., Vakevisser FA, Bloys van Treslong C.J. & Reynaers H. 1991. Molecular characterisation of k- and i-carrageenan by gel permeation chromatography, light scattering, sedimentation analysis and osmometry. *Biophysical Chemistry*, **41**: 51-59.

Smidsrød O. & Grasdalen H. 1984a. Conformations of κ-carrageenan in solution. *Hydrobiologia*, **116/117:** 178-186.

Smidsrød O. & Grasdalen H. 1984b. Polyelectrolytes from seaweeds. *Hydrobiologia*, **116/117**: 19-28.

Stanley N. 1987. Production, properties and uses of carrageenan. In: *Production and Utilization of Products from Commercial Seaweeds*. (McHugh D.J. Ed) *FAO Fisheries Technical Paper*, **288**: 116-146.

Stanley N.F. 1995. Agars. In: *Food Polysaccharides and Their Applications*, (Stephen AM ed), pp. 187-204. Marcel Dekker Inc., New York.

Stellwagen J. & Stellwagen N.C. 1995. Internal structure of agarose gel matrix. *Journal of Physical Chemistry*, **99**: 4247-4251.

Stevenson T.T. & Furneaux R.H. 1991. Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research*, **210**: 277-298.

Stokke B.T., Elgsaeter A. & Kitamura S. 1993. Macrocyclization of polysaccharides visualized by electron microscopy. *International Journal of Biological Macromolecules*, **15**: 63-68.

Stortz CA, Bacon BE, Cherniak R. & Cerezo A.S. 1994. High-field NMR spectroscopy of cystocarpic and tetrasporic carrageenans from *Iridaea undulosa*. *Carbohyrate Research*, **261**: 317-326.

Stortz C.A. & Cerezo A.S. 1992. The carbon-13 NMR spectroscopy of carrageenans: calculation of chemical shifts and computer aided structural determination. *Carbohydrate Polymers*, **19**: 237-242.

Sugiyama J., Rochas C., Turquois T., Taravel F. & Chanzy H. 1994. Direct imaging of polysaccharide aggregates in frozen aqueous dilute systems. *Carbohydrate Polymers*, **23**: 261-264.

Takano R., Iwane-Sakata H., Hayashi K., Hara S. & Hirase S. 1998. Concurrence of agaroid and carrageenan chains in funoran from the red seaweed *Gloiopeltis fucata* Post. et Ruprecht (Cryptonemiales, Rhodophyta). *Carbohydrate Polymers*, **35**: 81-87.

Takano R., Kamei-Hayashi K., Hara S. & Hirase S. 1993. Assignment of the absolute configuration of partially methylated galactoses by combined gas-liquid chromatography-mass spectrometry. *Bioscience Biotechnology and Biochemistry*, **57**: 1195-1197.

Takano R., Nose Y., Hayashi K., Hara S. & Hirase S. 1994. Agarose-carrageenan hybrid polysaccharides from *Lomentaria catenata*. *Phytochemistry*, **37**: 1615-1619.

Tanner S.F., Morris V.J. & Belton P.S. 1990. Multinuclear n.m.r. studies of group I cations in furcellaran gels. *International Journal of Biological Macromolecules*, **12**: 302-304.

Tashiro Y., Ogawa H. & Iso N. 1997a. Effect of the molecular weight distribution of agar on the gel strength. *Fisheries Science*, **63**: 407-408.

Tashiro Y., Ogawa H. & Iso N. 1997b. Sedimentation analysis of agar molecule. *Fisheries Science*, **63**: 281-285.

Thu B., Skjåk-Bræk G., Micali F., Vittur F. & Risso R. 1997. The spatial distribution of calcium in alginate gel beads analysed by synchrotron-radiation induced X-ray emisson (SRIXE) *Carbohydrate Research*, **297**: 101-105.

Tvaroska I., Rochas C., Taravel F.R. & Turquois T. 1992. Computer modelling of polysaccharide-polysaccharide interactions: an approach to the k-carrageenan-mannan case. *Biopolymers*, **32**: 551-560.

Ueda K., Itoh M., Matsuzaki Y., Ochiai H. & Imamura A. 1998. Observation of the molecular weight change during the helix-coil transition of k-carrageenan measured by SEC-MALLS methods. *Macromolecules*, **31**: 675-680.

Urbani R., Di Blas A., Cesaro A. 1993. Conformational features of carrabiose polymers: I. Conformational statistics of k-carrageenan. *International Journal of Biological Macromolecules*, **15**: 24-29.

Usov A.I. 1984. NMR spectroscopy of red seaweed polysaccharides: agars, carrageenans, and xylans. *Botanica marina*, **27**: 189-202.

Usov A.I. 1993. A new chemical tool for characterization and partial depolymerization of red algal galactans. *Hydrobiologia*, **260/261**: 641-645.

Usov A.I. 1998. Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids*, **12**: 301-308.

Usov A.I. & Barbakadze V.V. 1978. Polysaccharides of algae. XXVII Partial acetolysis of the sulfated galactan from the red seaweed *Grateloupia divaricata Okam. Bioorganicheskaya Khimiya*, **4**: 1107-1115.

Usov A.I., Bilan M.I. & Klochkova N.G. 1995. Polysaccharides of algae 48. Polysaccharide composition of several calcareous red algae: isolation of alginate from *Corallina pilulifera* P. et R. (Rhodophyta, Corallinaceae). *Botanica marina,* **38**: 43-51.

Usov A.I. & Ivanova E.G. 1987. Polysaccharides from algae XXVII. Characterization of hybrid structure of substituted agarose from *Polysiphonia morrowii* (Rhodophyta, Rhodomelaceae) using β-agarase and ^{13}C-NMR spectroscopy. *Botanica marina,* **30**: 365-370.

Usov A.I., Ivanova E.G. & Shashkov A.S. 1983. Polysaccharides of algae XXXIII: Isolation and ^{13}C NMR spectral study of some gel-forming polysaccharides from Japan sea red seaweeds. *Botanica marina* **26**: 285-294.

Usov A.I. & Klochkova N.G. 1992. Polysaccharides of algae 45. Polysaccharide composition of red seaweeds from Kamchatka coastal waters (Northwestern Pacific) studies by reductive hydrolysis of biomass. *Botanica marina*, **35**: 371-378.

Usov A.I. & Shashkov A.S. 1985. Polysaccharides of algae. XXIV. Detection of iota-carrageenan in *Phyllophora brodiaei* (Turn.) J. Ag. (Rhodophyta) using ^{13}C-NMR spectroscopy. *Botanica marina*, **38**: 367-373.

Usov A.I., Yarotsky S.V. & Shashkov A.S. 1980. ^{13}C NMR spectroscopy of red algal galactans. *Biopolymers*, **19**: 977-990.

Vannest K., SlootmaekersD. & Reynaers H. 1996. Light scattering studies of the dilute solution behaviour of κ-, ι- and λ-carrageenan. *Food Hydrocolloids*, **10**: 99-107.

Veluraja K. & Atkins E.D.T. 1989. Electron microscopic study of guluronate-rich alginate. *Carbohydrate Research,* **187**: 313-316.

Viebke C., Borgström J. & Piculell L. 1995. Characterisation of kappa- and iota-carrageenan coils and helices by MALLS/GPC. *Carbohydrate Polymers*, **27**: 145-154.

Viebke C., Piculell L. & Nilsson S. 1994. On the mechanism of gelation of helix-forming biopolymers. *Macromolecules*, **27**: 4160-4166.

Wang X. & Spencer H.G. 1998. Calcium alginate gels: formation and stability in the presence of an inert electrolyte. *Polymer* , **39**: 2759-2764.

Whytock S. & Finch J. 1991. The substructure of agarose gels as prepared for electrophoresis. *Biopolymers*, **31**:1025-1028.

Wong K.F. & Craigie J.S. 1978. Sulfohydrolase activity and carrageenan biosynthesis in *Chondrus crispus* (Rhodophyceae) *Plant Physiology*, **61**: 663-666.

Yamamoto M. 1980. Physicochemical studies on sulfated polysaccharides extracted from seaweeds at various temperatures. *Agricultural Biology and Chemistry*, **44**: 589-593.

Yamamoto M., Tadokoro Y., Imai H., Mita K. 1980. Physicochemical characterization of sulfated polysaccharides from green seaweeds: *Ulva pertusa* and *Ulva conglobata*. *Agricultural Biology and Chemistry*, **44**: 723-729.

Yaphe W. & Arsenault G.P. 1965. Improved resorcinol reagent for the determination of fructose, and 3,6-anhydrogalactose in polysaccharides. *Analytical Biochemistry*, **13**: 143-148.

Zhang W., Piculell L. & Nilsson S. 1991. Salt dependence and ion specificity of the coil-helix transition of furcellaran. *Biopolymers*, **31**: 1727-1736.

Zhang W., Piculell L., Nilsson S. & Knudsen S 1994. Cation specificity and cation binding to low sulfated carrageenans. *Carbohydrate Polymers*, **23**: 105-110.

Zinoun M., Diouris M., Potin P., Floc'h J.-Y. & Deslandes E. 1997. Evidence of sulfohydrolase activity in the red alga *Calliblepharis jubata*. *Botanica marina*, **40**: 49-53.