1
Alginates from Algae

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DP	degree of polymerization
EDTA	etylenediamine tetraacetic acid
G	α-L-guluronic acid
GDL	D-glucono-δ-lactone
M	β-D-mannuronic acid (M)
$N_{G>1}$	average G-block length larger than 1
NMR	nuclear magnetic resonance spectroscopy
PGA	propylene glycol alginate
pK_a	dissociation constants for the uronic acid monomers

1

Introduction

Alginates are quite abundant in nature since they occur both as a structural component in marine brown algae (*Phaeophyceae*), comprising up to 40% of the dry matter, and as capsular polysaccharides in soil bacteria (see Chapter 8 on bacterial alginates in Volume 5 of this series). Although present research

and results point toward a possible production by microbial fermentation and also by post-polymerization modification of the alginate molecule, all commercial alginates are at present still extracted from algal sources. The industrial applications of alginates are linked to its ability to retain water, and its gelling, viscosifying, and stabilizing properties. Upcoming biotechnological applications, on the other hand, are based either on specific biological effects of the alginate molecule itself or on its unique, gentle, and almost temperature-independent sol/gel transition in the presence of multivalent cations (e.g., Ca^{2+}), which makes alginate highly suitable as an immobilization matrix for living cells.

Traditional exploitation of alginates in technical applications has been based to a large extent on empirical knowledge. However, since alginates now enter into more knowledge-demanding areas such as pharmacy and biotechnology, new research functions as a locomotive for a detailed further investigation of structure–function relationships. New scientific breakthroughs are made, which in turn may benefit the traditional technical applications.

2
Historical Outline

The British chemist E. C. C. Stanford first described alginate (the preparation of "algic acid" from brown algae) with a patent dated 12 January 1881 (Stanford, 1881). After the patent, his discovery was further discussed in papers from 1883 (Standford, 1883a,b). Stanford believed that alginic acid contained nitrogen and contributed much to the elucidation of its chemical structure.

In 1926, some groups working independently (Atsuki and Tomoda, 1926; Schmidt and Vocke, 1926) discovered that uronic acid was a constituent of alginic acid. The nature of the uronic acids present was investigated by three different groups shortly afterwards (Nelson and Cretcher, 1929, 1930, 1932; Bird and Haas, 1931; Miwa, 1930), which all found D-mannuronic acid in the hydrolysate of alginate. The nature of the bonds between the uronic acid residues in the alginate molecule was determined to be β1,4, as in cellulose (Hirst et al., 1939)

This very simple and satisfactory picture of the constitution of alginic acid was, however, destroyed by the work of Fischer and Dörfel (1955). In a paper chromatographic study of uronic acids and polyuronides, they discovered the presence of a uronic acid different from mannuronic acid in the hydrolysates of alginic acid. This new uronic acid was identified as L-guluronic acid. The quantity of L-guluronic acid was considerable, and a method for quantitative determination of mannuronic and guluronic acid was developed.

Alginate then had to be regarded as a binary copolymer composed of α-L-guluronic and β-D-mannuronic residues. As long as alginic acid was regarded as a polymer containing only D-mannuronic acid linked together with β-1,4 links, it was reasonable to assume that alginates from different raw materials were chemically identical and that any given sample of alginic acid was chemically homogeneous. From a practical and a scientific point of view, the uronic acid composition of alginate from different sources had to be examined, and methods for chemical fractionation of alginates had to be developed. These tasks were undertaken mainly by Haug and coworkers (Haug, 1964), as described in Section 3 below. The discovery of alginate as a block-copolymer, the correlation between physical properties and block structure, and the discovery of a set of epimerases converting mannuronic to guluronic acid in a sequence-dependent manner also are discussed further in later sections.

3
Chemical Structure

Being a family of unbranched binary copolymers, alginates consist of $(1 \rightarrow 4)$ linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (see Figure 1a and b) of widely varying composition and sequence. By partial acid hydrolysis (Haug, 1964; Haug et al., 1966; Haug and Larsen, 1966; Haug et al., 1967a; Haug and Smidsrød, 1965), alginate was separated into three fractions. Two of these contained almost homopolymeric molecules of G and M, respectively, while a third fraction consisted of nearly equal proportions of both monomers and was shown to contain a large number of MG dimer residues. It was concluded that alginate could be regarded as a true block copolymer composed of homopolymeric regions of M and G, termed M- and G-blocks, respectively, interspersed with regions of alternating structure (MG-blocks; see Figure 1c). It was further shown (Painter et al., 1968; Larsen et al., 1970; Smidsrød and Whittington, 1969) that alginates have no regular repeating unit and that the distribution of

the monomers along the polymer chain could not be described by Bernoullian statistics. Knowledge of the monomeric composition is hence not sufficient to determine the sequential structure of alginates. It was suggested (Larsen et al., 1970) that a second-order Markov model would be required for a general approximate description of the monomer sequence in alginates. The main difference at the molecular level between algal and bacterial alginates is the presence of *O*-acetyl groups at C2 and/or C3 in the bacterial alginates (Skjåk-Bræk et al., 1986).

4
Conformation

Knowledge of the monomer ring conformations is necessary to understand the polymer properties of alginates. X-ray diffraction studies of mannuronate-rich and guluronate-rich alginates showed that the guluronate residues in homopoly-meric blocks were in the $^1C_4$ conformation (Atkins et al., 1970), while the mannuronate residues had
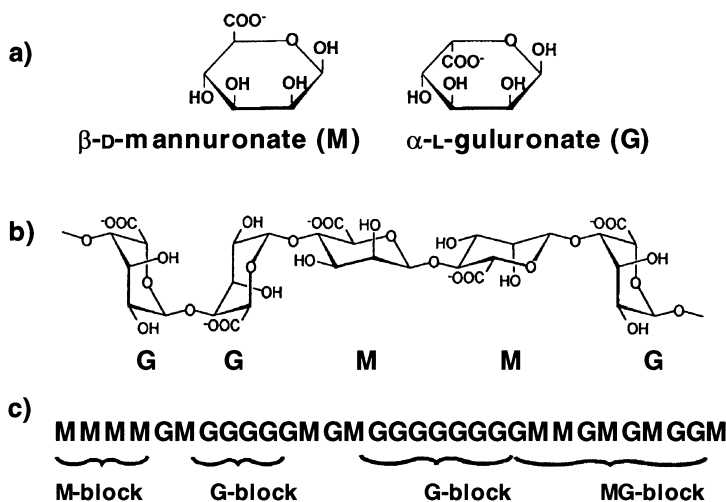

Fig. 1 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution.

the $^4C_1$ conformation (see Figure 1a). Viscosity data of alginate solutions indicated that the stiffness of the chain blocks increased in the order MG < MM < GG. This series could be reproduced only by statistical mechanical calculations when the guluronate residues were set in the $^1C_4$ conformation (Smidsrød et al., 1973) and was later confirmed by ^{13}C-NMR (Grasdalen et al., 1977). Hence, alginate contains all four possible glycosidic linkages: diequatorial (MM), diaxial (GG), equatorial-axial (MG), and axial-equatorial (GM) (see Figure 1b).

The diaxial linkage in G-blocks results in a large, hindered rotation around the glycosidic linkage, which may account for the stiff and extended nature of the alginate chain (Smidsrød et al., 1973). Additionally, taking the polyelectrolyte nature of alginate into consideration, the electrostatic repulsion between the charged groups on the polymer chain also will increase the chain extension and hence the intrinsic viscosity. Extrapolation of dimensions both to infinite ionic strength and to θ-conditions (Smidsrød, 1970) yielded relative dimensions for the neutral, unperturbed alginate chain being much higher than for amylose derivatives and even slightly higher than for some cellulose derivatives.

Another parameter reflecting chain stiffness and extension is the exponent in the Mark-Houwink-Sakurada equation,

$$[\eta] = K \cdot M^a$$

where M is the molecular weight of the polymer, $[\eta]$ is the intrinsic viscosity, and the exponent a generally increases with increasing chain extension. Some measurements on alginates (Martinsen et al., 1991; Smidsrød and Haug, 1968a, Mackie et al., 1980), yielded a-values ranging from 0.73 to 1.31, depending on ionic strength and alginate composition. Low and high a-values are related to large fractions of the flexible MG-blocks and the stiff and extended G-blocks, respectively (Moe et al., 1995).

5

Occurrence and Source Dependence

Commercial alginates are produced mainly from *Laminaria hyperborea, Macrocystis pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Eclonia maxima, Lessonia nigrescens, Durvillea antarctica*, and *Sargassum* spp. Table 1 gives some sequential parameters (determined by high-field NMR-spectroscopy) for samples of these

Tab. 1 Composition and sequence parameters of algal alginates (Smidsrød and Draget 1996)

Source	F_G	F_M	F_{GG}	F_{MM}	$F_{GM,MG}$
Laminaria japonica	0.35	0.65	0.18	0.48	0.17
Laminaria digitata	0.41	0.59	0.25	0.43	0.16
Laminaria hyperborea, blade	0.55	0.45	0.38	0.28	0.17
Laminaria hyperborea, stipe	0.68	0.32	0.56	0.20	0.12
Laminaria hyperborea, outer cortex	0.75	0.25	0.66	0.16	0.09
Lessonia nigrescens[a]	0.38	0.62	0.19	0.43	0.19
Ecklonia maxima	0.45	0.55	0.22	0.32	0.32
Macrocystis pyrifera	0.39	0.61	0.16	0.38	0.23
Durvillea antarctica	0.29	0.71	0.15	0.57	0.14
Ascophyllum nodosum, fruiting body	0.10	0.90	0.04	0.84	0.06
Ascophyllum nodosum, old tissue	0.36	0.64	0.16	0.44	0.20

[a] Data provided by Bjørn Larsen

alginates. The composition and sequential structure may, however, vary according to seasonal and growth conditions (Haug, 1964; Indergaard and Skjåk-Bræk, 1987). High contents of G generally are found in alginates prepared from stipes of old *Laminaria hyperborea* plants, whereas alginates from *A. nodosum, L. japonica*, and *Macrocystis pyrifera* are characterized by low content of G-blocks and low gel strength.

Alginates with more extreme compositions containing up to 100% mannuronate can be isolated from bacteria (Valla et al., 1996). Alginates with a very high content of guluronic acid can be prepared from special algal tissues such as the outer cortex of old stipes of *L. hyperborea* (see Table 1), by chemical fractionation (Haug and Smidsrød, 1965; Rivera-Carro, 1984) or by enzymatic modification *in vitro* using mannuronan C-5 epimerases from *A. vinelandii* (Valla et al., 1996; see Section 9.2). This family of enzymes is able to epimerize M-units into G-units in different patterns from almost strictly alternating to very long G-blocks. The epimerases from *A. vinelandii* have been cloned and expressed, and they represent at present a powerful new tool for the tailoring of alginates. It is also obvious that commercial alginates with less molecular heterogeneity, with respect to chemical composition and sequence, can be obtained by a treatment with one of the C-5 epimerases (Valla et al., 1996).

6
Physiological Function

The biological function of alginate in brown algae generally is believed to be as a structure-forming component. The intercellular alginate gel matrix gives the plants both mechanical strength and flexibility (Andresen et al., 1977). Simply speaking, alginates in marine brown algae may be regarded as having physiological properties similar to those of cellulose in terrestrial plants. This relation between structure and function is reflected in the compositional difference of alginates in different algae or even between different tissues from the same plant (see Table 1). In *L. hyperborea*, an alga that grows in very exposed coastal areas, the stipe and holdfast have a very high content of guluronic acid, giving high mechanical rigidity. The leaves of the same algae, which float in the streaming water, have an alginate characterized by a lower G-content, giving it a more flexible texture. The physiological function of alginates in bacteria will be covered elsewhere in this series.

7
Chemical Analysis and Detection

Since alginates are block copolymers, and because of the fact that their physical properties rely heavily on the sequence of these blocks, it obvious that the development of techniques enabling a sequence quantification is of the utmost importance. Additionally, molecular mass and its distribution (polydispersity) is a significant parameter in some applications.

7.1
Chemical Composition and Sequence

Detailed information about the structure of alginates became available by introduction of high-resolution ^{1}H and ^{13}C NMR-spectroscopy (Grasdalen et al., 1977, 1979; Penman and Sanderson, 1972; Grasdalen, 1983) in the sequential analysis of alginate. These powerful techniques make it possible to determine the monad frequencies F_M and F_G; the four nearest neighboring (diad) frequencies F_{GG}, F_{MG}, F_{GM}, and F_{MM}; and

the eight next nearest neighboring (triad) frequencies. Knowledge of these frequencies enables, for example, the calculation of the average G-block length larger than 1:

$$N_{G>1} = (F_G - F_{MGM}) / F_{GGM}.$$

This value has been shown to correlate well with gelling properties. It is important to realize that in an alginate chain population, neither the composition nor the sequence of each chain will be alike. This results in a composition distribution of a certain width.

7.2
Molecular Mass

Alginates, like polysaccharides in general, are polydisperse with respect to molecular weight. In this aspect they resemble synthetic polymers rather than other biopolymers such as proteins and nucleic acids. Because of this polydispersity, the "molecular weight" of an alginate is an average over the whole distribution of molecular weights.

In a population of molecules where N_i is the number of molecules and w_i is the weight of molecules having a specific molecular weight M_i, the number and the weight average are defined respectively as:

$$\overline{M_n} = \frac{\Sigma_i N_i M_i}{\Sigma_i N_i}$$

$$\overline{M_w} = \frac{\Sigma_i w_i M_i}{\Sigma_i w_i} = \frac{\Sigma_i N w_i M_i^2}{\Sigma_i N_i M_i}$$

For a randomly degraded polymer, we have $\overline{M_w} \approx 2\overline{M_n}$ (Tanford, 1961). The fraction $\overline{M_w}/\overline{M_n}$ is called the polydispersity index. Polydispersity index values between 1.4 and 6.0 have been reported for alginates and have been related to different types of preparation and purification processes (Martinsen et al., 1991; Smidsrød and Haug, 1968a; Mackie et al., 1980; Moe et al., 1995).

The molecular-weight distribution can have implications for the uses of alginates, as low-molecular-weight fragments containing only short G-blocks may not take part in gel-network formation and consequently do not contribute to the gel strength. Furthermore, in some high-tech applications, the leakage of mannuronate-rich fragments from alginate gels may cause problems (Stokke et al., 1991; Otterlei et al., 1991) and a narrow molecular-weight distribution therefore is recommended.

7.3
Detection and Quantification

Detection and quantification of alginates in the presence of other biopolymers, such as proteins, are not straightforward mainly because of interference. Once isolated, a number of colorimetric methods can be applied to quantify alginate. The oldest and most common is the general procedure for carbohydrates, the phenol/sulfuric acid method (Dubois et al., 1956), but there are also two slightly refined formulas specially designed for uronic acids (Blumenkrantz and Asboe-Hansen, 1973; Filisetti-Cozzi and Carpita, 1991).

8
Biosynthesis and Biodegradation

Our knowledge of the alginate biosynthesis mainly comes from studying alginate-producing bacteria. Figure 2 shows the principal enzymes involved in alginate biosynthesis, and the activity of all enzymes (1–7) has been identified in brown algae. During the last decade, the genes responsible for alginate synthesis in *Pseudomonas* and *Azotobacter* have been identified, sequenced, and cloned. For further information on alginate biosynthesis, please see Chapter 8 on bacte-
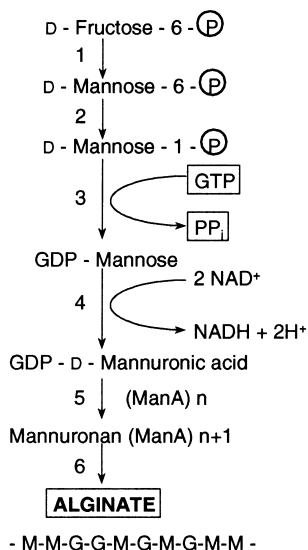
THE BIOSYNTHETIC PATHWAY OF ALGINATE

D - Fructose - 6 - (P)

1

D - Mannose - 6 - (P)

2

D - Mannose - 1 - (P)

3 ⟶ GTP ⟶ PP$_i$

GDP - Mannose

4 ⟶ 2 NAD$^+$ ⟶ NADH + 2H$^+$

GDP - D - Mannuronic acid

5   (ManA) n

Mannuronan (ManA) n+1

6

ALGINATE

- M-M-G-G-M-G-M-G-M-M -

Fig. 2 Biosynthetic pathway of alginates.

rial alginates in Volume 5 of this series. Because of their potential use in alginate modification, the only enzymes we will comment on here are the alginate lyases and the mannuronan C-5 epimerases.

Alginates are not degraded in the human gastric-intestinal tract, and hence do not give metabolic energy. Some lower organisms have, however, developed lyases that degrade alginates down to single components, resulting in alginates that function as a carbon source. Alginate lyases catalyze the depolymerization of alginate by splitting the 1–4 glycosidic linkage in a β-elimination reaction, leaving an unsaturated uronic acid on the non-reducing end of the molecules. Alginate lyases are widely distributed in nature, including in organisms growing on alginate as a carbon source such as marine gastropods, prokaryotic and eukaryotic microorganisms, and bacteriophages. They also are found in the bacterial species producing alginate such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*. All of them are endolyases and may exhibit spe-

cificity to either M or G. Since the aglycon residue will be identical for both M and G, the use of lyases for structural work is limited. Table 2 lists a range of lyases and their specificities.

9

Production: Biotechnological and Traditional

There has been significant progress in the understanding of alginate biosynthesis over the last 10 years. The fact that the alginate molecule enzymatically undergoes a post-polymerization modification with respect to chemical composition and sequence opens up the possibility for *in vitro* modification and tailoring of commercially available alginates.

9.1

Isolation from Natural Sources / Fermentative Production

As already described, all commercial alginates today are produced from marine brown algae (Table 1). Alginates with more extreme compositions can be isolated from the bacterium *Azotobacter vinelandii*, which, in contrast to *Pseudomonas* species, produces polymers containing G-blocks. Production by fermentation therefore is technically possible but is not economically feasible at the moment.

9.2

Molecular Genetics and *in vitro* Modification

Alginate with a high content of guluronic acid can be prepared from special algal tissues by chemical fractionation or by *in vitro* enzymatic modification of the alginate *in vitro* using mannuronan C-5 epimerases from *A. vinelandii* (Ertesvåg et al., 1994, 1995, 1998b; Høydal et al., 1999). These epimerases, which convert M to G in the

Tab. 2 Substrate specificity and biochemical properties of some alginate lyases (Gacesa, 1992; Wong et al., 2000)

Source	Localization	Sequence specifity	Major end-product	pHopt	Mw (kDa)	pI	Reference
K.aerogenes	Extracellular	G↓X	Trimer	7.0	31.4	8.9	Boyd and Turvey, 1978
Enterobacter cloacae	Extracellular	G	–	7.8	32–38	8.9	Nibu et al., 1995
	Intracellular	G↓G	Dimer/pentamer	7.5	31–39	8.9	Shimokawa et al., 1997
P. aeruginosa (AlgL)	Periplasmic	M-X	Trimer	7.0	39	9.0	Boyd and Turvey, 1977
A. vinelandii	Periplasmic (AlgL)	M↓X M_{Ac}↓X	Trimer/tetramer	8.1–8.4	39	5.1	Ertesvåg et al., 1998a
	Extracellular (AlgE7)	G↓X	Tetramer- septamer	6.3–7.3	90.4	–	Ertesvåg et al., 1998a
P. alginovora	Extracellular	G↓G	–	7.5	28	5.5	Boyen et al., 1990a
	Intracellular	M↓M	–	–	24	5.8	Boyen et al., 1990a
Haliotis tuberculata	Hepato-pancreas	M↓X, G↓M	Trimer/ dimer	8	34	–	Boyen et al., 1990b
Sphingomonas sp. ALYI-III	Cytoplasmic	M↓X M_{Ac}↓X	–	5.6–7.8	38	10.16	Murata et al., 1993
Littorina sp.	Hepato-pancreas	M↓M	Trimer	5.6	~40	–	Elyakova and Favorov, 1974
A. vinelandii phage	Extracellular	M↓X M $_{Ac}$↓X	Trimer	7.7	30–35	–	Davidson et al., 1977

polymer chain, recently have allowed for the production of highly programmed alginates with respect to chemical composition and sequence. *A. vinelandii* encodes a family of 7 exocellular isoenzymes with the capacity to epimerize all sorts of alginates and other mannuronate-containing polymers, as shown in Figure 3, where the mode of action of AlgE4 (giving alternating introduction of G) is presented. Although the genes have a high degree of homology, the enzymes they encode exhibit different specificities. Different epimerases may give alginates with different distribution of M and G, and thus alginates with tailored physical and chemical properties can be made as illustrated in Figure 4. None of the enzymatically modified polymers, however, are commercially available at present. Table 3 lists the modular structure of the mannuronan C-5 epimerase family and its specific action.

9.3
Current and Expected World Market and Costs

Industrial production of alginate is roughly 30,000 metric tons annually, which is probably less than 10% of the annually biosynthesized material in the standing macroalgae crops. Because macroalgae also may be cultivated (e.g., in mainland China where 5 to 7 million metric tons of wet *Laminaria japonica* are produced annually) and because production by fermentation is technically possible, the sources for industrial production of alginate may be regarded as unlimited even for a steadily growing industry.

It is expected that future growth in the alginate market most likely will be of a qualitative rather than a quantitative nature. Predictions suggest that manufacturers will move away from commodity alginate production toward more refined products, e.g., for the pharmaceutical industry.
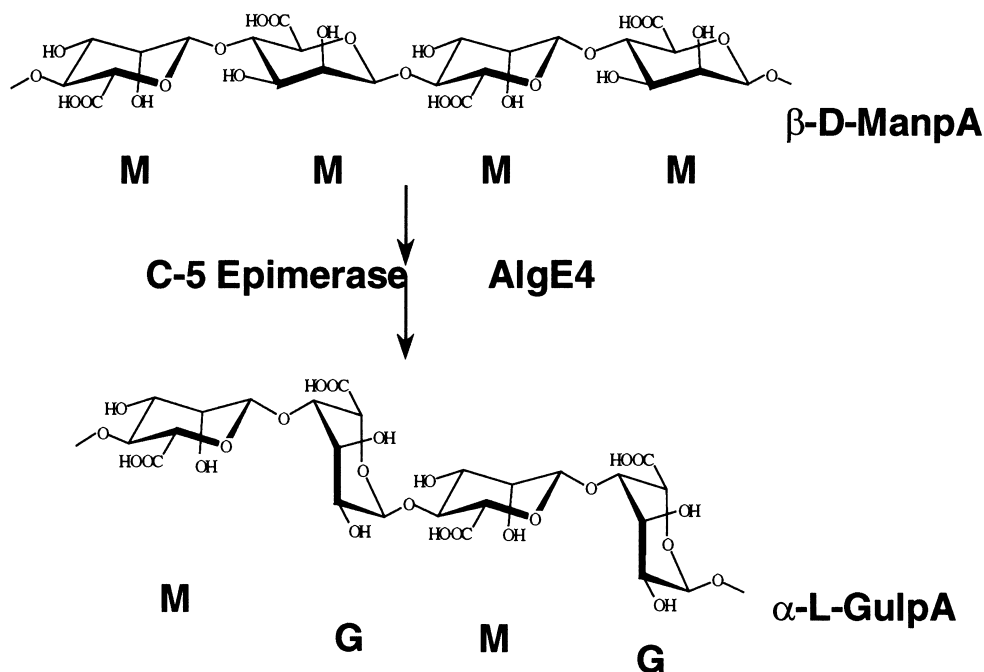
β-D-ManpA

M   M   M   M

C-5 Epimerase   AlgE4

M

G   M

G

α-L-GulpA

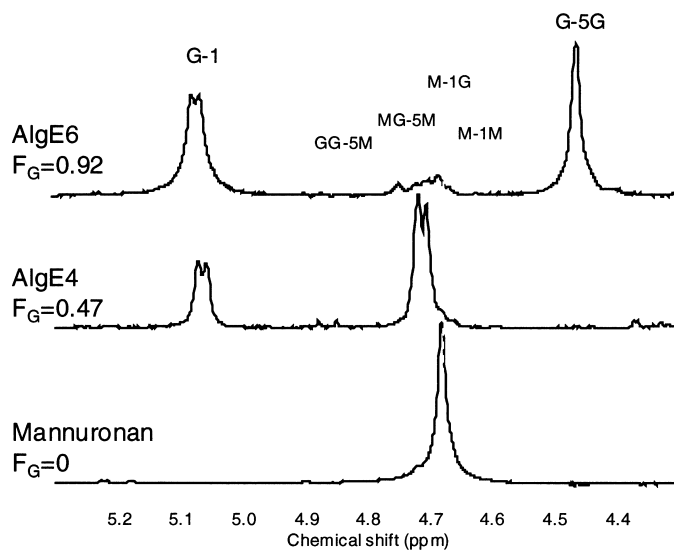**Fig. 3**   Mode of action for the mannuronan C5-epimarase AlgE4.

Fig. 4   Resulting chemical composition and sequence after treating mannuronan with different C5-epimerases.

Tab. 3 The seven AlgE epimerases from *A. vinelandi*[a]

Type	[kDa]	Modular structure	Products
AlgE1	147.2	A1 R1 R2 R3 A2 R4	Bi-functional G-blocks + MG-blocks
AlgE2	103.1	A1 R1 R2 R3 R4	G-blocks (short)
AlgE3	191	A1 R1 R2 R3 A2 R4 R5 R6 R7	Bi-functional G-block + MG-blocks
AlgE4	57.7	A1 R1	MG-blocks
AlgE5	103.7	A1 R1 R2 R3 R4	G-blocks (medium)
AlgE6	90.2	A1 R1 R2 R3	G-blocks (long)
AlgE7	90.4	A1 R1 R2 R3	Lyase activity + G-blocks + MG-blocks

[a] A – 385 amino acids; R – 155 amino acids

The cost of alginates can differ extremely depending on the degree of purity. Technical grade, low-purity alginate (containing a substantial amount of algae debris) can be obtained from around 1 USD per kilogram, and ordinary purified-grade alginate can be obtained from approximately 10 USD per kilo, whereas ultra-pure (low in endotoxins) alginate specially designed for immobilization purposes typically costs around 5 USD per gram.

9.4
Alginate Manufacturers

The alginate producers members list of the Marinalg hydrocolloid association includes six different companies. These are China Seaweed Industrial Association, Danisco Cultor (Denmark), Degussa Texturant Systems (Germany), FMC BioPolymer (USA), ISP Alginates Ltd. (UK), and Kimitsu Chemical Industries Co., Ltd. (Japan). In addition to these, Pronova Biomedical A/S (Norway) now commercially manufactures ultra-pure alginates that are highly compatible with mammalian biological systems following the increased popularity of alginate as an immobilization matrix. These qualities are low in pyrogens and facilitate sterilization of the alginate solution by filtration due to low content of aggregates.

10
Properties

The physical properties of the alginate molecule were revealed mainly in the 1960s and 1970s. The last couple of decades have exposed some new knowledge on alginate gel formation

10.1
Physical Properties

Compared with other gelling polysaccharides, the most striking features of alginate's physical properties are the selective binding of multivalent cations, being the basis for gel formation, and the fact that the sol/gel transition of alginates is not particularly influenced by temperature.

10.1.1
Solubility
There are three essential parameters determining and limiting the solubility of alginates in water. The pH of the solvent is important because it will determine the

presence of electrostatic charges on the uronic acid residues. Total ionic strength of the solute also plays an important role (salting-out effects of non-gelling cations), and, obviously, the content of gelling ions in the solvent limits the solubility. In the latter case, the "hardness" of the water (i.e., the content of Ca^{2+} ions) is most likely to be the main problem.

Potentiometric titration (Haug, 1964) revealed that the dissociation constants for mannuronic and guluronic acid monomers were 3.38 and 3.65, respectively. The pK_a value of the alginate polymer differs only slightly from those of the monomeric residues. An abrupt decrease in pH below the pK_a value causes a precipitation of alginic acid molecules, whereas a slow and controlled release of protons may result in the formation of an "alginic acid gel". Precipitation of alginic acid has been studied extensively (Haug, 1964; Haug and Larsen, 1963; Myklestad and Haug, 1966; Haug et al., 1967c), and addition of acid to an alginate solution leads to a precipitation within a relatively narrow pH range. This range depends not only on the molecular weight of the alginate but also on the chemical composition and sequence. Alginates containing more of the "alternating" structure (MG-blocks) will precipitate at lower pH values compared with the alginates containing more homogeneous block structures (poly-M and poly-G). The presence of homopolymeric blocks seems to favor precipitation by the formation of crystalline regions stabilized by hydrogen bonds. By increasing the degree of alternating "disorder" in the alginate chain, as in alginates isolated from *Ascophyllum nodosum* (see Table 1), the formation of these crystalline regions is not formed as easily. A certain alginate fraction from *A. nodosum* is soluble at a pH as low as 1.4 (Myklestad and Haug, 1966). Because of this relatively limited solubility of alginates at low pH, the esterified propylene glycol alginate (PGA) is applied as a food stabilizer under acidic conditions (see Section 10.3).

Any change of ionic strength in an alginate solution generally will have a profound effect, especially on polymer chain extension and solution viscosity. At high ionic strengths, the solubility also will be affected. Alginate may be precipitated and fractionated to give a precipitate enriched with mannuronate residues by high concentrations of inorganic salts like potassium chloride (Haug and Smidsrød, 1967; Haug, 1959a). Salting-out effects like this exhibit large hysteresis in the sense that less than 0.1 M salt is necessary to slow down the kinetics of the dissolution process and limit the solubility (Haug, 1959b). The gradient in the chemical potential of water between the bulk solvent and the solvent in the alginate particle, resulting from a very high counter-ion concentration in the particle, is most probably the drive of the dissolution process of alginate in water. This drive becomes severely reduced when attempts are made to dissolve alginate in an aqueous solvent already containing ions. If alginates are to be applied at high salt concentrations, the polymer should first be fully hydrated in pure water followed by addition of salt under shear.

For the swelling behavior of dry alginate powder in aqueous media with different concentrations of Ca^{2+}, there seems to be a limit at approximately 3 mM free calcium ions (unpublished results). Alginate can be solubilized at $[Ca^{2+}]$ above 3 mM by the addition of complexing agents, such as polyphosphates or citrate, before addition of the alginate powder.

10.1.2

Selective Ion Binding

The basis for the gelling properties of
alginates is their specific ion-binding char-
acteristics (Haug, 1964; Smidsrød and
Haug, 1968b; Haug and Smidsrød, 1970;
Smidsrød, 1973, 1974). Experiments involv-
ing equilibrium dialysis of alginate have
shown that the selective binding of certain
alkaline earth metals ions (e.g., strong and
cooperative binding of Ca^{2+} relative to Mg^{2+})
increased markedly with increasing content
of α-L-guluronate residues in the chains.
Poly-mannuronate blocks and alternating
blocks were almost without selectivity. This
is illustrated in Figures 5 and 6, where a
marked hysteresis in the binding of Ca^{2+}
ions to G-blocks also is seen.

The high selectivity between similar ions
such as those from the alkaline earth metals
indicates that some chelation caused by
structural features in the G-blocks takes
place. Attempts were made to explain this
phenomenon by the so-called "egg-box"
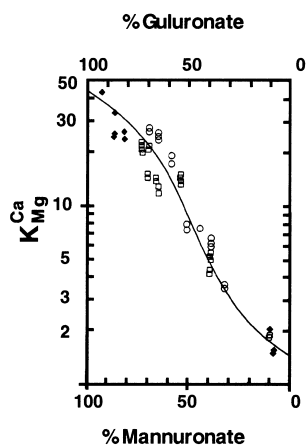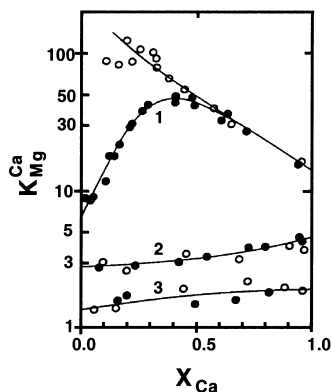model (Grant et al., 1973), based upon the

Fig. 6 Selectivity coefficients, K_{mg}^{Ca} as a function of
ionic composition (X_{Ca}) for different alginate frag-
ments. Curve 1: Fragments with 90% guluronate
residues. Curve 2: Alternating fragment with 38%
guluronate residues. Curve 3: Fragment with 90%
mannuronate residues. ●: Dialysis of the fragments
in their Na^+ form. ○: Dialysis first against 0.2 M
$CaCl_2$, then against mixtures of $CaCl_2$ and $MgCl_2$.

linkage conformations of the guluronate
residues (see Figure 1b). NMR studies
(Kvam et al., 1986) of lanthanide complexes
of related compounds suggested a possible
binding site for Ca^{2+} ions in a single alginate
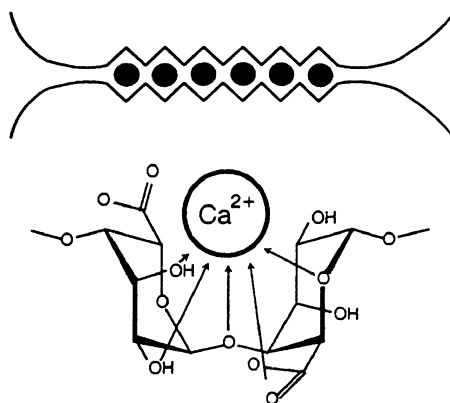chain, as given in Figure 7 (Kvam 1987).

Fig. 5 Selectivity coefficients, K_{mg}^{Ca}, for alginates and
alginate fragments as a function of monomer
composition. The experimental points are obtained
at $X_{Ca} = X_{Mg} = 0.5$. The curve is calculated using
$K_{mg\ guluronate}^{Ca} = 40$ and $K_{mg\ mannuronate}^{Ca} = 1.8$

Fig. 7 The egg-box model for binding of divalent
cations to homopolymeric blocks of α-L-guluronate
residues, and a probably binding site in a GG-
sequence.

Although more accurate steric arrangements have been suggested, as supported by x-ray diffraction (Mackie et al., 1983) and NMR spectroscopy (Steginsky et al., 1992), the simple "egg-box" model still persists, as it is principally correct and gives an intuitive understanding of the characteristic chelate-type of ion-binding properties of alginates. The simple dimerization in the "egg-box" model is at present questionable, as data from small-angle x-ray scattering on alginate gels suggest lateral association far beyond a pure dimerization with increasing $[Ca^{2+}]$ and G-content of the alginate (Stokke et al., 2000). In addition, the fact that isolated and purified G-blocks (totally lacking elastic segments; typically $DP = 20$) are able to act as gelling modulators when mixed with a gelling alginate suggests higher-order junction zones (Draget et al., 1997).

The selectivity of alginates for multivalent cations is also dependent on the ionic composition of the alginate gel, as the affinity toward a specific ion increases with increasing content of the ion in the gel (Skjåk-Bræk et al., 1989b) (see Figure 6). Thus, a Ca-alginate gel has a markedly higher affinity toward Ca^{2+} ions than has the Na-alginate solution. This has been explained theoretically (Smidsrød, 1970; Skjåk-Bræk et al., 1989b) by a near-neighbor, auto-cooperative process (Ising model) and can be explained physically by the entropically unfavorable binding of the first divalent ion between two G-blocks and the more favorable binding of the next ions in the one-dimensional "egg-box" (zipper mechanism).

10.1.3
### Gel Formation and Ionic Cross-linking
A very rapid and irreversible binding reaction of multivalent cations is typical for alginates; a direct mixing of these two components therefore rarely produces homogeneous gels. The result of such mixing

is likely to be a dispersion of gel lumps ("fish-eyes"). The only possible exception is the mixing of a low-molecular-weight alginate with low amounts of cross-linking ion at high shear. The ability to control the introduction of the cross-linking ions hence becomes essential.

A controlled introduction of cross-linking ions is made possible by the two fundamental methods for preparing an alginate gel: the diffusion method and the internal setting method. The diffusion method is characterized by allowing a cross-linking ion (e.g., Ca^{2+}) to diffuse from a large outer reservoir into an alginate solution (Figure 8a). Diffusion setting is characterized by rapid gelling kinetics and is utilized for immobilization purposes where each droplet of alginate solution makes one single gel bead with entrapped (bio-) active agent (Smidsrød and Skjåk-Bræk, 1990). High-speed setting is also beneficial, e.g., in restructuring of foods when a given size and shape of the final product is desirable. The molecular-weight dependence in this system is negligible as long as the weight average molecular weight of the alginate is above 100 kDa (Smidsrød, 1974).

The internal setting method differs from the diffusion method in that the Ca^{2+} ions are released in a controlled fashion from an inert calcium source within the alginate solution (Figure 8b). Controlled release usually is obtained by a change in pH, by a limited solubility of the calcium salt source, and/or the by presence of chelating agents. The main difference between internal and diffusion setting is the gelling kinetics, which is not diffusion-controlled in the former case. With internal setting, the tailor-making of an alginate gelling system toward a given manufacturing process is possible because of the controlled, internal release of cross-linking ions (Draget et al., 1991). Internally set gels generally show a
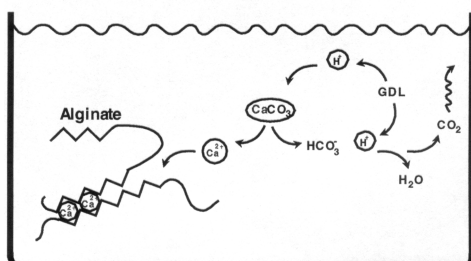
DIFFUSION SETTING
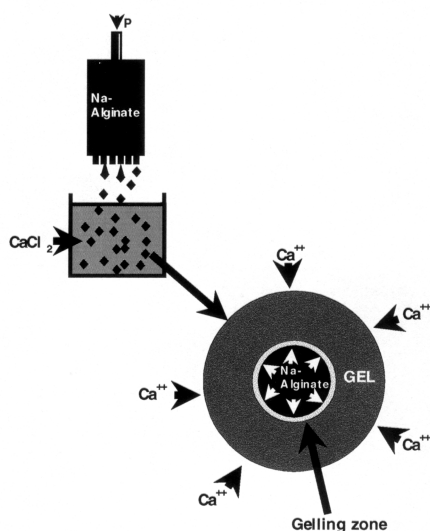
INTERNAL GELATION

Fig. 8 Principal differences between the diffusion method exemplified by the immobilization technique and the internal setting method exemplified by the CaCO₃/GDL technique.

more pronounced molecular weight dependence compared with diffusion set gels. It has been reported that the internally set gels depend on molecular weight even at 300 kDa (Draget et al., 1993). This could be due to the fact that internally set gels are more calcium-limited compared with the gels made by diffusion, implying that the non-elastic fractions (sol and loose ends) at a given molecular weight will be higher in the internally set gels.

10.1.4
Gel Formation and Alginic Acid Gels

It is well known that alginates may form acid gels at pH values below the pK_a values of the uronic residues, but these alginic acid gels traditionally have not been as extensively studied as their ionically cross-linked counterparts. With the exception of some pharmaceutical uses, the number of applications so far is also rather limited. The preparation of an alginic acid gel has to be performed with care. Direct addition of acid to, e.g., a Na-alginate solution leads to an instantaneous precipitation rather than a gel. The pH must therefore be lowered in a controlled fashion, and this is most conveniently carried out by the addition of slowly hydrolyzing lactones like D-glucono-δ-lactone (GDL).

10.2
Material Properties

Since alginates are traditionally used for their gelling, viscosifying, and stabilizing properties, the features of alginate based materials are of utmost importance for a given application. Recently some quite unique biological effects of the alginate molecule itself have been revealed.

10.2.1
Stability

Alginate, being a single-stranded polymer, is susceptible to a variety of depolymerization processes. The glycosidic linkages are cleaved by both acid and alkaline degradation mechanisms and by oxidation with free radicals. As a function of pH, degradation of alginate is at its minimum nearly neutral and increases in both directions (Haug and Larsen, 1963) (Figure 9). The increased instability at pH values less than 5 is explained by a proton-catalyzed hydrolysis, whereas the reaction responsible for the
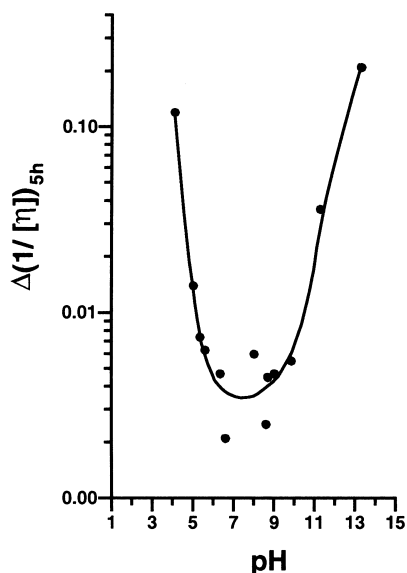
Fig. 9 Degradation of alginate isolated from *Laminaria digitata* measured as the change (Δ) in intrinsic viscosity ([η]) after 5 h at different pH and at 68°C.

degradation at pH 10 and above is the β-alkoxy elimination (Haug et al., 1963, 1967b). Free radicals degrade alginate mainly by oxidative-reductive depolymerization reactions (Smidsrød et al., 1967; Smidsrød et al., 1963a,b) caused by contamination of reducing agents like polyphenols from the brown algae. Since all of these depolymerization reactions increase with temperature, autoclaving generally is not recommended for the sterilization of an alginate solution. Since alginate is soluble in water at room temperature, sterile filtering rather than autoclaving has been recommended as a sterilization method for immobilization purposes in order to reduce polymer breakdown and to maintain the mechanical properties of the final gel (Draget et al., 1988).

Sterilization of dry alginate powder is also troublesome. The effect of γ-irradiation is often disastrous and leads to irreversible damage. It is generally believed that, under these conditions, O_2 is depleted rapidly with formation of the very reactive $OH^{\bullet}$ free radical. A short-term exposure in an electron accelerator could be an alternative to long-term exposure from a traditional ^{60}Co source. It has been shown that sterilization doses applied by ^{60}Co irradiation reduce the molecular weight to the extent that the gelling capacity is almost completely lost (Leo et al., 1990).

10.2.2
Ionically Cross-linked Gels

In contrast to most gelling polysaccharides, alginate gels are cold-setting, implying that alginate gels set more or less independent of temperature. The kinetics of the gelling process, however, can be strongly modified by a change in temperature, but a sol/gel transition will always occur if gelling is favored (e.g., by the presence of cross-linking ions). It is also important to realize that the properties of the final gel most likely will change if gelling occurs at different temperatures. This is due to alginates being non-equilibrium gels and thus being dependent upon the history of formation (Smidsrød, 1973).

Alginate gels can be heated without melting. This is the reason that alginates are used in baking creams. It should be kept in mind that alginates, as described earlier, are subjected to chemical degrading processes. A prolonged heat treatment at low or high pH might thus destabilize the gel because of an increased reaction rate of depolymerizing processes such as proton-catalyzed hydrolysis and the β-elimination reaction (Moe et al., 1995).

Because the selective binding of ions is a prerequisite for alginate gel formation, the alginate monomer composition and sequence also have a profound impact on the final properties of calcium alginate gels. Figure 10 shows gel strength as a function of
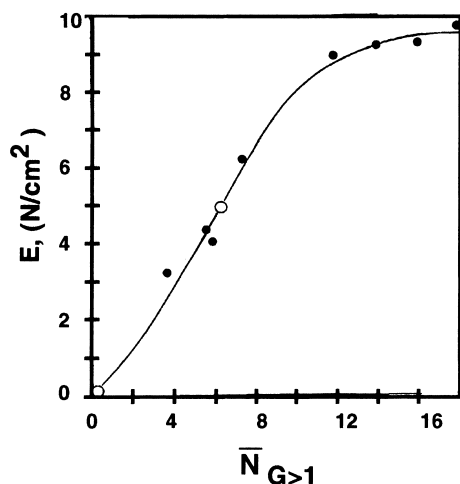
Fig. 10 Elastic properties of alginate gels as function of average G-block length.

the average length of G-blocks larger than one unit ($N_{G>1}$). This empirical correlation shows that there is a profound effect on gel strength when $N_{G>1}$ changes from 5 to 15. This coincides with the range of G-block lengths found in commercial alginates.

The polyelectrolyte nature of the alginate molecule is also important for its function, especially in mixed systems where, under favorable conditions, alginates may interact electrostatically with other charged polymers (e.g., proteins), resulting in a phase transition and altering the rheological behavior. Generally, it can be stated that if the purpose is to avoid such electrostatic interactions, the mixing of alginate and protein should take place at a relatively high pH, where most proteins have a net negative charge (Figure 11). These types of interactions also can be utilized to stabilize mixtures and to increase the gel strength of some restructured foods. In studies involving gelling of bovine serum albumin and alginate in both the sodium and the calcium form, a consid-
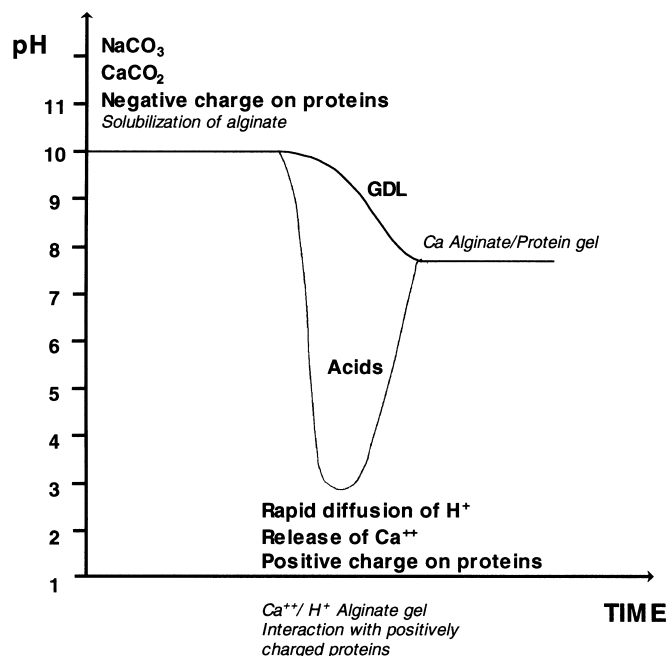

Fig. 11 Alginate/protein mixed gels exemplified by the internal gelation with $CaCO_3$. Release of Ca^{2+} is achieved by either a slow pH-lowering agent (GDL) or by a fast lowering with acids.

erable increase in Young's modulus was found within some range of pH and ionic strength (Neiser et al., 1998, 1999). These results suggest that electrostatic interactions are the main driving force for the observed strengthening effects.

An important feature of gels made by the diffusion-setting method is that the final gel often exhibits an inhomogeneous distribution of alginate, the highest concentration being at the surface and gradually decreasing towards the center of the gel. Extreme alginate distributions have been reported (Skjåk-Bræk et al., 1989a), with a five-fold increase at the surface (as calculated from the concentration in the original alginate solution) and virtually zero concentration in the center (Figure 12). This result has been explained by the fact that the diffusion of gelling ions will create a sharp gelling zone that moves from the surface toward the center of the gel. The activity of alginate (and of the gelling ion) will equal zero in this zone, and alginate molecules will diffuse from the internal, non-gelled part of the gelling body toward the zero-activity region

Fig. 12 Polymer concentration profiles of alginate gel cylinders formed by dialyzing a 2% (w/v) solution of Na-alginate from *Laminaria hyperborea* against 0.05 M CaCl$_2$ in the presence of NaCl. ☐: 0.2 M; ●: 0.05 M; ▲: no NaCl.

(Skjåk-Bræk et al., 1989a, Mikkelsen and Elgsæter, 1995). Inhomogeneous alginate distribution may or may not be beneficial in the final product. It is therefore important to know that the degree of homogeneity can be controlled and to know which parameters govern the final alginate distribution. Maximum inhomogeneity is reached by placing a high-G, low-molecular-weight alginate gel in a solution containing a low concentration of the gelling ion and an absence of non-gelling ions. Maximum homogeneity is reached by gelling a high-molecular-weight alginate with high concentrations of both gelling and non-gelling ions (Skjåk-Bræk et al., 1989b).

The presence of non-gelling ions in alginate-gelling systems also affects the stability of the gels. It has been shown that alginate gels start to swell markedly when the ratio between non-gelling and gelling ions becomes too high and that the observed destabilization increases with decreasing F_G (Martinsen et al., 1989).

Swelling of alginate gels can be increased dramatically by a covalent cross-linking of preformed Ca-alginate gels with epichlorohydrin, followed by subsequent removal of Ca^{2+} ions by etylenediamine tetraacetic acid (EDTA) (Skjåk-Bræk and Moe, 1992). These Na-alginate gels can be dried, and they exhibit unique swelling properties when re-hydrated. The forces affecting the swelling of a polymer network can be split into three terms. Two of these terms favor swelling and can be said to constitute what might be called "swelling pressure": (1) the mixing term (Π_{mix} = the osmotic pressure generated by polymer/solvent mixing) and (2) the ionic term (Π_{ion} = the osmotic effect of an unequal distribution of the polymer counter-ions between the inside and the outside of the gel; the Donnan equilibrium). Of these two terms, the ionic part has been shown to contribute approximately 90% of
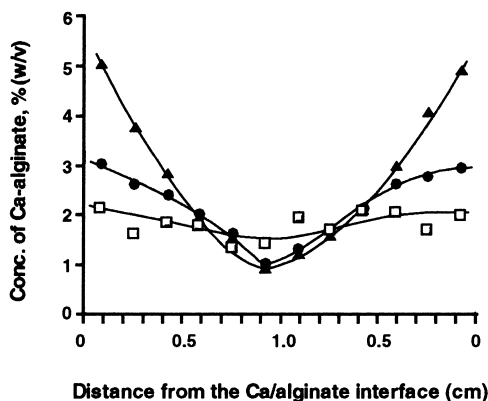
the swelling pressure, even at 1 molar ionic strength, for highly ionic gels like Na-alginate (Moe et al., 1993). The third term (Π_{el} = the reduction in osmotic pressure due to the elastic response of the polymer network) balances the "swelling pressure" so that the total of these three terms equals zero at equilibrium.

These Na-alginate gels would function well as water absorbents in hygiene and pharmaceutical applications. However, Π_{ion} depends upon the ionic strength of the solute; with increasing ionic strength, the difference in chemical potential is reduced because of a more even distribution of the mobile ions between the inside and the outside of the gel. Therefore, reduced swelling will be observed at physiological ionic conditions compared with deionized water,
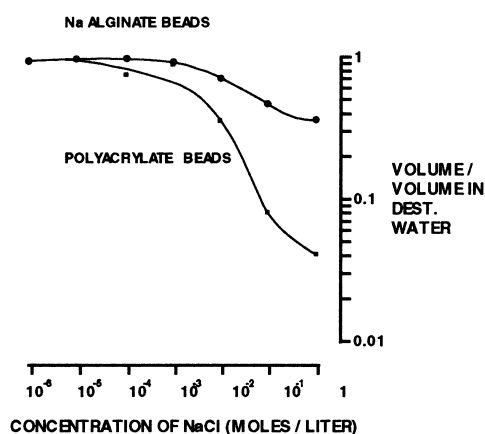
but this reduction will be less pronounced than that for other water-absorbing materials, such as cross-linked acrylates, as a result of the inherent stiffness of the alginate molecule itself (Skjåk-Bræk and Moe, 1992) (Figure 13).

10.2.3
Alginic Acid Gels

It has been shown (Draget et al., 1994) that the gel strength of acid gels becomes independent of pH below a pH of 2.5, which equals 0.8 M GDL in a 1.0% (w/v) solution. Table 4 shows the Young's moduli of acid gels prepared (1) by a direct addition of GDL and (2) by converting an ionic cross-linked gel to the acid form by mineral acid. The modulus seems to be rather independent of the history of formation. Therefore, a most important feature of the acid gels compared with the ionic cross-linked gels seems to be that the former reaches equilibrium in the gel state.

Figure 14 shows the observed elastic moduli of acid gels made from alginates with different chemical composition, together with expected values for ionically cross-linked gels. From these data, it can be concluded that acid gels resemble ionic gels in the sense that high contents of guluronate (long stretches of G-blocks) give the strongest gels. However, it is also seen that poly-mannuronate sequences support alginic acid gel formation, whereas poly-alternating sequences seem to perturb this transition. The obvious demand for homopolymeric sequences in acid gel formation suggests

Fig. 13 Salt tolerance of covalently cross-linked Na-alginate and polyacrylate gel beads measured as swelling at different ionic strengths.

Tab. 4 E_{app} (kPa) for gels made from three different high-G alginates at 2% (w/v) concentration

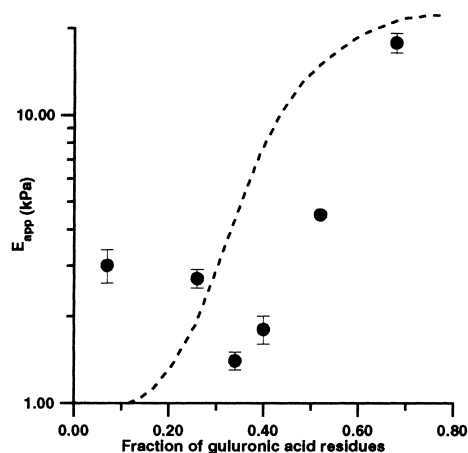| *Ca-alginate gel*	*Ca-gel to alginic acid gel*	*Syneresis correction*	*Direct addition of GDL*
105 ± 4.6	52 ± 4.3	15.5 ± 0.3	15 ± 1.1
116 ± 11	64 ± 8.1	17.1 ± 1.8	17.8 ± 1.4
127 ± 6.4	79 ± 5.8	19.8 ± 1.3	20.4 ± 0.7

Fig. 14 Young's modulus E_{app} of alginic acid gels at apparent equilibrium as function of guluronic acid content. Dashed line refers to expected results for Ca^{2+} cross-linked alginate gels.

that cooperative processes are involved just as in the case of ionic gels (Draget et al., 1994). High molecular weight dependence has been observed, and this dependence becomes more pronounced with increasing content of guluronic acid residues.

A study of the swelling and partial solubilization of alginic acid gels at pH 4 has confirmed the equilibrium properties of the gels (Draget et al., 1996). By comparing the chemical composition and molecular weight of the alginate material leaching out from the acid gels with the same data for the whole alginate, an enrichment in mannuronic acid residues and a reduction in the average length of G-blocks were found together with a lowering of the molecular weight.

10.3
"Biological" Properties

Through a series of papers, it has been established that the alginate molecule itself has different effects on biological systems. This is more or less to be expected because of the large variety of possible chemical compositions and molecular weights of alginate preparations. A biological effect of alginate initially was hinted at in the first animal transplantation trials of encapsulated Langerhans islets for diabetes control. Overgrowth of alginate capsules by phagocytes and fibroblasts, resembling a foreign body/inflammatory reaction, was reported (Soon-Shiong et al., 1991). In bioassays, induction of tumor necrosis factor and interleukin 1 showed that the inducibility depended upon the content of mannuronate in the alginate sample (Soon-Shiong et al., 1993). This result directly explains the observed capsule overgrowth; mannuronate-rich fragments, which do not take part in the gel network, will leach out of the capsules and directly trigger an immune response (Stokke et al., 1993). This observed immunologic response can be linked in part to $(1\rightarrow4)$ glycosidic linkages, as other homopolymeric di-equatorial polyuronates, like D-glucuronic acid (C6-oxidised cellulose), also exhibit this feature (Espevik and Skjåk-Bræk, 1996). The immunologic potential of polymannuronates have now been observed in *in vivo* animal models in such diverse areas as for protection against lethal bacterial infections and irradiation and for increasing non-specific immunity (Espevik and Skjåk-Bræk, 1996).

11
Applications

Given the large number of different applications, alginates must be regarded as one of the most versatile polysaccharides. These applications span from traditional technical utilization, to foods, to biomedicine.

11.1
Technical Utilization

The quantitatively most important technical application of alginates is as a shear-thinning viscosifyer in textile printing, in which alginate has gained a high popularity because of the resulting color yield, brightness, and print levelness. Alginates also are used for paper coating to obtain surface uniformity and as binding agents in the production of welding rods. In the latter case, alginate gives stability in the wet stage and functions as a plasticizer during the extrusion process. As a last example of technical applications, ammonium alginate frequently is used for can sealing. The ammonium form is used because of its very low ash content (Onsøyen, 1996).

11.2
Medicine and Pharmacy

Alginates have been used for decades as helping agents in various human-health applications. Some examples include use in traditional wound dressings, in dental impression material, and in some formulations preventing gastric reflux. Alginate's increasing popularity as an immobilization matrix in various biotechnological processes, however, demonstrates that alginate will move into other and more advanced technical domains in addition to its traditional applications. Entrapment of cells within Ca-alginate spheres has become the most widely used technique for the immobilization of living cells (Smidsrød and Skjåk-Bræk, 1990). This immobilization procedure can be carried out in a single-step process under very mild conditions and is therefore compatible with most cells. The cell suspension is mixed with a sodium alginate solution, and the mixture is dripped into a solution containing multivalent cations (usually Ca^{2+}). The droplets then instantaneously form gel-spheres entrapping the cells in a three-dimensional lattice of ionically cross-linked alginate. The possible uses for such systems in industry, medicine, and agriculture are numerous, ranging from production of ethanol by yeast, to production of monoclonal antibodies by hybridoma cells, to mass production of artificial seed by entrapment of plant embryos (Smidsrød and Skjåk-Bræk, 1990).

Perhaps the most exciting prospect for alginate gel immobilized cells is their potential use in cell transplantation. Here, the main purpose of the gel is to act as a barrier between the transplant and the immune system of the host. Different cells have been suggested for gel immobilization, including parathyroid cells for treatment of hypocalcemia and dopamine-producing adrenal chromaffin cells for treatment of Parkinson's disease (Aebisher et al., 1993). However, major interest has been focused on insulin-producing cells for the treatment of Type I diabetes. Alginate/poly-L-lysine capsules containing pancreatic Langerhans islets have been shown to reverse diabetes in large animals and currently are being clinically tested in humans (Soon-Shiong et al., 1993, 1994). Table 5 lists some biomedical applications of alginate-encapsulated cells.

Tab. 5 Some potential biomedical application of alginate-encapsulated cells

Cell type	Treatment of
Adrenal chromaffin cells	Parkinson's disease[a]
Hepatocytes	Liver failure[a]
Paratyroid cells	Hypocalcemia[a]
Langerhans islets (β-cells)	Diabetes[b]
Genetically altered cells	Cancer[c]

[a] Aebischer et al., 1993 [b] Soon-Shiong et al., 1993, 1994 [c] Read et al., 2000

11.3
Foods

Alginates are used as food additives to improve, modify, and stabilize the texture of foods. This is valid for such properties as viscosity enhancement; gel-forming ability; and stabilization of aqueous mixtures, dispersions, and emulsions. Some of these properties stem from the inherent physical properties of alginates themselves, as outlined above, but they also may result from interactions with other components of the food product, e.g., proteins, fat, or fibers. As an example, alginates interact readily with positively charged amino acid residues of denatured proteins, which are utilized in pet foods and reformed meat. Cottrell and Kovacs (1980), Sime (1990), and Littlecott (1982) have given numerous descriptions and formulations on alginates in food applications. A general review on this topic is given by McHugh (1987).

Special focus perhaps should be placed on restructured food based on Ca-alginate gels because of its simplicity (gelling being independent upon temperature) and because it is a steadily growing alginate application. Restructuring of foods is based on binding together a flaked, sectioned, chunked, or milled foodstuff to make it resemble the original. Many alginate-based restructured products are already on the market (see Figure 15), as is exemplified by meat products (both for human consumption and as pet food), onion rings, pimento olive fillings, crabsticks, and cocktail berries.

For applications in jams, jellies, fruit fillings, etc., the synergetic gelling between alginates high in guluronate and highly esterified pectins may be utilized (Toft et al., 1986). The alginate/pectin system can give thermoreversible gels in contrast to the purely ionically cross-linked alginate gels. This gel structure is almost independent of

Fig. 15 An example of alginate used in the restructuring of foods: the pimiento fillings of olives. (picture kindly provided by FMC BioPolymer).

sugar content, in contrast to pectin gels, and therefore may be used in low calorie products.

The only alginate derivative used in food is propylene glycol alginate (PGA). Steiner (1947) first prepared PGA, and Steiner and McNeely improved the process (1950). PGA is produced by a partial esterification of the carboxylic groups on the uronic acid residues by reaction with propylene oxide. The main product gives stable solutions under acidic conditions where the unmodified alginate would precipitate. It is now used to stabilize acid emulsions (such as in French dressings), acid fruit drinks, and juices. PGA also is used to stabilize beer foam.

As for the regulatory status, the safety of alginic acid and its ammonium, calcium, potassium, and sodium salts was last evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 39th meeting in 1992. An ADI "not specified" was allocated. JECFA allocated an ADI of 0 to 25 mg/kg bw to propylene glycol alginate at its 17th meeting.

In the U.S, ammonium, calcium, potassium, and sodium alginate are included in a list of stabilizers that are generally recognized as safe (GRAS). Propylene glycol

alginate is approved as a food additive (used as an emulsifier, stabilizer, or thickener) and in several industrial applications (used as a coating for fresh citrus fruit, as an inert pesticide adjuvant, and as a component of paper and paperboard in contact with aqueous and fatty foods). In Europe, alginic acid and its salts and propylene glycol are all listed as EC-approved additives other than colors and sweeteners.

Alginates are inscribed in Annex I of the Directive 95/2 of 1995, and as such can be used in all foodstuffs (except those cited in Annex II and those described in Part II of the Directive) under the Quantum Satis principle of the EU.

12
Relevant Patents

A search in one of the international databases for patents and patent applications yielded well above 2000 hits on alginate. It is outside the scope of this chapter, not to mention beyond the capabilities of its authors, to systematically discuss all this literature, which covers inventions for improved utilization of alginates in the technical, pharmaceutical, food, and agricultural areas. We have therefore limited this section to only a handful of *prior art* inventions (Table 6), with the present authors as co-inventors, that point toward a production of alginate and alginate fractions with novel structures and some new biomedical applications based on the physical and biological properties of certain types of alginates with specified chemical structures.

US Patent 5,459,054 may represent a large number of patents dealing with the immobilization of living cells in immuno-protective alginate capsules for implantation purposes as discussed in Section 10.2. US Patents 5,169,840 and 6,087,342 cover the

use of alginates enriched with mannuronate for the stimulation of cytokine production in monocytes, which could be of future importance in the treatment of microbial infections, cancer, and immune deficiency and autoimmune diseases. This stimulating effect has been discussed and connected to the use of alginate fibers in wound-healing dressings. A closer look at these specific effects is presented in Section 9.3.

When the calcium ions in alginate gels are exchanged by covalent cross links, the resulting gel with monovalent cations as counter-ions has the ability to swell several hundred times its own weight in water or salt solutions at low ionic strength, as shown in US Patent 5,144,016. This super-absorbent system, further elaborated in Section 9.2.2, still has not found any commercial uses, mainly because of competition from similar materials based on starch and cellulose derivatives, but certain biomedical applications may be foreseen.

A patent on the genes encoding the different C5-epimerases (US Patent 5,939,289) points to the possibility of producing alginates with a large number of different predetermined compositions and sequences and opens up the possibility for the tailor-making of different alginates, as discussed in Sections 8.2 and 12.

An alternative way of manufacturing alginate fractions with extreme compositions is by using selective extraction techniques, as disclosed in Patent WO 98/51710. One possible use of such fractions as gelling modifiers is revealed in Patent WO 98/02488, where it is suggested that these purified low-molecular-weight guluronate blocks give a gel enforcement at high concentrations of calcium ions by connecting and shortening elastic segments that otherwise would be topologically restricted. In conclusion, it may be argued that this relative high rate of patent filing suggests

Tab. 6 Summary of prior art patents on alginates pointing toward specialty applications

Patent number	Holder	Inventors	Patent title	Public date
U.S. 5,459,054	Neocrine	G. Skjåk-Bræk O. Smidsrød T. Espevik M. Otterlei P. Soon-Shiong	Cells encapsulated in alginate containing a high content of α-L-guluropnic acid	2 July 1993
U.S. 5,169,840	Nobipol, Protan Biopolymer	M. Otterlei T. Espevik O. Smidsrød G. Skjåk-Bræk	Diequatorially bound β-1,4-polyuronates and use of same for cytokine stimulation	27 March 1991
U.S. 6,087,342	FMC BioPolymer	T. Espevik G. Skjåk-Bræk	Substrates having bound polysaccharides and bacterial nucleic acids	15 May 1998
U.S. 5,144,016	Protan Biopolymer	G. Skjåk-Bræk S. Moe	Alginate gels	29 May 1991
U.S. 5,939,289	Pronova Biopolymer, Nobipol	H. Ertesvåg S. Valla G. Skjåk-Bræk B. Larsen	DNA compounds comprising sequences encoding mannuroran C-5-epimerase	9 May 1995
WO 98/51710	FMC BioPolymer	M. K. Simensen O. Smidsrød K. I. Draget F. Hjelland	Procedure for producing uronic acid blocks from alginate	11 November 1998
WO 98/02488	FMC BioPolymer	M. K. Simensen K. I. Draget E. Onsøyen O. Smidsrød T. Fjæreide	Use of G-block polysaccharides	22 January 1998

that new alginate-based products are being developed and that there is continuous stable demand for alginates and their products.

13
Outlook and Perspectives

From a chemical point of view, the alginate molecule may look very simple, as it contains only the two monomer units M and G linked by the same 1,4 linkages. This simplification of its chemical structure may lead potential commercial users of alginate to treat it as a commodity like many of the cellulose derivatives. In this chapter, we have shown that alginate represents a very high diversity with respect to chemical composition and monomer sequence, giving the alginate family of molecules a large variety of physical and biological properties. This may represent a challenge to the unskilled users of alginate, but it may be an advantage for the producers and new users of alginate who are interested in developing research-based, high-value applications. When microbial alginate and epimerase-modified alginate enter into the marked in the future, the possibility of alginate being tailor-made to diverse applications will be increased even further.

We therefore see a future trend, which has already started, in which the exploitation of alginate gradually shifts from low-tech applications with increasing competition from cheap alternatives to more advanced, knowledge-based applications in the food, pharmaceutical, and biomedical areas. We then foresee continuous, high research activity in industry and academia to describe, understand, and utilize alginate-containing products to the benefit of society.

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