Technical Article

History of Alginate Chemistry

Introduction

Alginate is a structural polymer that is found in the cell walls of brown marine algae, more commonly known as seaweeds and is also produced by some bacteria. Alginate is a linear co-polymer of β-D-Mannuronic acid and α-L-Guluronic acid.

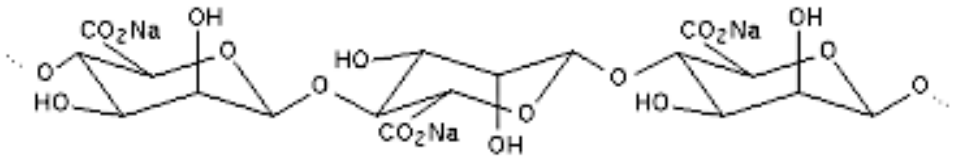
Alginate is widely used as a viscosifying, gelling & stabilizing agent in food, also in pharmaceuticals and for stabilizing & sizing in textiles, paper & printing. Currently, the global usage of alginate in food alone is estimated to be around 26,000 tonnes at a value of US$705 million (IMR 2023).

This article describes how alginate was discovered, how the structure was elucidated (starting with Stanford in the 1880's) and early commercial development in the 1930's. Initially, the technology of alginate production was controlled by a small number of western companies. The major players being Kelco, Protan & Fagertun and Mero Rousselot-Satia (Paul, 1986) however, this has changed dramatically in subsequent years.

◀ Harvested stipes of *Laminaria hyperborea*, a commercial source of alginate (Courtesy of C. Hepburn).

Structure – early work

Alginic acid was discovered, first extracted and patented by Stanford in the 1880's (Stanford 1881 and 1885). The patent explains how alginate can be extracted by soaking seaweed in water or dilute acid, extracting with sodium carbonate and then precipitating the alginate out of solution with acid. Stanford described alginate as containing nitrogen, but this can be attributed to protein impurities and is not due to the alginate itself (Krefting, 1896 and 1898; Hoagland and Lieb, 1915).



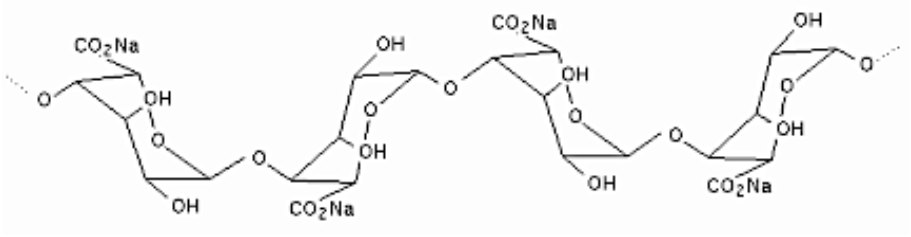
Sodium polymannuronic acid

Krefting (Krefting, 1896 and 1898) was the first to prepare a purified form of alginic acid but it was not until 1930 that the first structure of alginic acid was proposed (Nelson and Cretcher 1929 and 1930). Alginate was heated in acid and the pentoses formed by the loss of carbon dioxide were isolated and identified by osazone formation. The osazone formed could only have come from D-xylose or D-lyxose and consequently the uronic acids possible could only have been D-glucuronic acid, D-iduronic acid, D-Mannuronic acid and L-guluronic acid. Ironically the presence of guluronic or iduronic acid was excluded on the basis that they had never been found in nature before. Glucuronic acid was ruled out experimentally. The structure proposed was that alginate consisted of a polymeric uronic acid consisting largely of D-mannuronic acid residues. This was established by comparing the diamide and diphenyl hydrazide derivatives of the uronic acid isolated from the polymer with analogous derivatives prepared from D-mannurono-6,3-lactone. They also showed that, on heating in acid, carbon dioxide was released from the polysaccharide and that the weight loss was consistent with alginate being a polyuronate structure.

The methylation work of Hirst and his co-workers showed the polymer to be essentially made up of 1:4 linked β-D-mannuronic acid residues (Hirst 1939). It wasn't until 1955 that, by paper chromatography, L-guluronic acid was also detected as a major component of alginate (Fischer and Darfel, 1955). They also demonstrated that the ratio of of L-guluronic acid to D-mannuronic acid was varied depending on the species of seaweed used. Fractional precipitation with potassium chloride (Haug, 1959), manganese salts (McDowell R H, 1958) or a combination of magnesium and calcium salts (Haug and Smidsrod, 1965) leads to a concentration of the two uronides into separate fractions. By partial acid hydrolysis and isolation of the oligomers it was shown that mannosyl gulose was present in the hydrolysis products of reduced alginic acid and hence at least some molecules of alginate contained both uronic acids (Vincent 1960; Hirst, Percival and Wold 1964).

Structure – M/G ratios

The usual method for the determination of the ratio of the two uronic acid types is by hydrolysis of the chain followed by analysis of the sugar components. This has inherent problems in that under acid hydrolysis conditions the monomers are subject to degradation via decarboxylation and the rates of degradation for the two uronic acids are not equal; guluronic acid degrading faster than mannuronic acid (Knutson and Jeanes, 1968). Also, the rates of hydrolysis depends on the actual distribution of the residues along the chain (Grasdalen, 1979). Once hydrolysed the monomers were separated on an ion exchange column with gradient elution of increasing acetic acid concentration and the residues were subsequently detected using a standard assay such as the carbazole assay (Haug and Larsen, 1962). There have been several modifications to this procedure; the column eluent has been changed to tris-acetate buffer (Spiro, 1977), sodium acetate (Mopper, 1978) and a mixed borate / phosphate buffer (Simatupang, 1979). Alternatives to the assay procedure have also been investigated (Blumenkrantz and Asboe-Hansen, 1973; Wardi, 1974 and Mopper 1978).



Sodium polyguluronic acid

A semi quantitative determination of the ratios of the two residues is possible by comparing the peak areas in the infrared spectrum at 808cm^{-1} for β-D-mannuronic acid and 887cm^{-1} for α-L-guluronic acid (Mackie, 1971). A polarimetric method involves the hydrolysis of alginate followed by measuring the optical rotation of the brucine (10,11-dimethoxystrychnine) salt of the free acids, once again this method suffers from the degradation of the monomers during hydrolysis (Siddiqui, 1978). Electrophoresis has been used for the separation of hydrolysed alginates using free boundary methods (Haug, 1967), polyacrylamide gels (Bucke, 1974) and agarose gels (Vreeland, 1978). Another method uses partial hydrolysis, esterification with EDC followed by sodium borohydride reduction and further acid hydrolysis to give D-mannose and L-gulose. Further reduction gives D-mannitol and D-glucitol which are then assayed as their n-butyl boronic esters (Vadas, 1981).

HPLC has proved very popular in the analysis of uronic acid ratios in alginate largely due to its convenience and rapidity of use (Cheetham and Sirimanne, 1983; Honda, 1983). One method involved the methanolysis of alginic acid followed by analysis on an HPLC column with methanolysed D-mannurono-6,3-lactone as a standard. The samples were analysed by NMR (Grasdalen, 1979) but the method appears to overestimate the mannuronic acid content, probably due to incomplete methanolysis, the mannuronic acid residues reacting preferentially to the guluronic acid residues (Annison, 1983). Underivatised alginate hydrolysate has been analysed on an ion exchange column with UV detection (Gaseca, 1983).

Structure – block structure

The presence of three different types of polymer segment has been shown by mild acid hydrolysis (Haug et al 1966, 1967a, 1967b). one type of segment consists entirely of guluronic acid residues, one consists entirely of mannuronic acid residues and the third consists of an alternating sequence of mannuronic and guluronic acid residues. because the mild acid hydrolysis is not very selective it is difficult to say how long the segments are, but it appears that the average segment could be up to eighty residues long. the ratios of the three different types of segments for the various types of alginate have been determined using the partial hydrolysis method. recently it has been suggested, from studies on the enzymatic hydrolysis of alginate, that some deviation exists from the idealised, regular and alternating sequence (Morris et al 1978; Boyd 1975).

NMR has been used to provide information, not only on the ratio of the two polyuronates, but also on the sequential distribution of the residues within the chainand their configuration. The shape of the NMR peaks can be used to provide information on the coupling constants and the relaxation times which subsequently provides information about the chemical environment of the atom under examination. The peaks can be used to provide statistical information on the sequences found with an alginate sample (Casu 1982; Grasdalen et al 1977, 1979a, 1979b, 1981, 1983).

By initially partially hydrolysing a sample of alginate and then analysing the proton NMR, ratios for the three blocks within the co-polymer could be obtained (Penman and Sanderson 1972). The observations about the configuration of the alginate residues corroborates the findings of Atkin's X-ray work (Atkins 1970). High resolution proton NMR on a partially hydrolysed sample has been used to provide information on the L-guluronic to D-mannuronic acid ratios. The frequencies for the four diads were calculated (MM, MG, GM, GG) by using a sequence dependant affect upon the H-5 of the guluronic acid residues (Grasdalen et al, 1979a).

◀ ***Ascophyllum nodosum*** **a source of alginate**

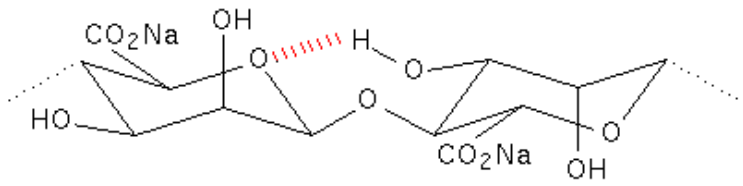
This was later extended to include all the triad groups centred on L-guluronic acid (Grasdalen 1983). Similar work has been carried out using ^{13}C NMR in place of proton NMR (Grasdalen 1979b and 1981). This work gives an idea of the ratios of the three blocks within different samples. Different seaweed sources seem to provide widely differing alginates. Laminaria digitata seems to consist of large amounts of poly β-D-mannuronic acid blocks and poly α-L-Guluronic acid blocks whereas the alginate from macrocystis pyrifera appears to contain a large portion of both poly α-L-guluronic acid blocks and alternating blocks (Grasdalen 1983; Grasdalen et al 1981).

2-dimensional COSY proton NMR has been used to provide block structure information on bacterial alginates as well as information on the level of acetylation and the position of the acetyl groups within the polymer (Skjak-Braek et al 1986). Sodium-23 NMR has been used to provide information on the rate of exchange of Na^+ for polymer bound sodium ions using the relaxation times of the sodium NMR (Grasdalen & Kvam, 1986).

Structure – Poly M

Further fine structure information has been obtained from the action of certain alginate lyases upon the alginate chain. An extracellular poly α-L-guluronate lyase was used to degrade the α-L-guluronic acid and random segments and subsequently measure the chain length of the poly β-D-mannuronic acid blocks. Average chain lengths of about 24 residues were obtained initially (Turvey & Boyd, 1978) but later work indicated shorter block lengths (Turvey, 1983). Products from the action of L-guluronic acid lyase and from the action of D-mannuronic acid lyase were separated by HPLC and examined by high resolution proton NMR. This work suggested an average block length of around seven residues. Seven residues is not long enough for a guluronic acid block to cationically bind to another, fifteen being the calculated minimum (Kohn, 1975). Hence the proposed binding mechanism of chains in a calcium alginate gel needed to be modified to include two separate poly α-L-guluronic acid blocks separated by one β-D-mannuronic acid residue. The study also showed that the mixed block segments were not strictly alternating (Boyd & Turvey, 1978; Kashiwabara et al 1969; Davidson et al 1977; Madgewick et al, 1973; Nakada & Sweeney, 1967; Nisizawa et al, 1968).

An alginate lyase has been extracted from a marine bacterium and shown to cleave endolytically (1-4)- β-D-mannuronic acid polymers to oligomers containing a 4,5 unsaturated terminal residue. The overall effect of the enzyme is to reduce poly mannuronic acid to its trimer, the depolymerisation stops at this point (romeo & Preston; 1986).



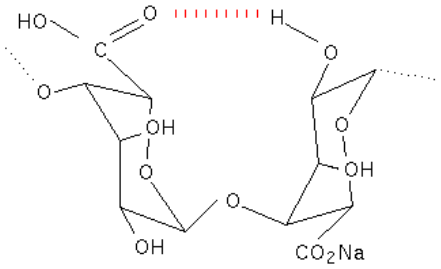
The glycosidic linkage is $^4C_1$ di-equatorial in mannuronic acid

Poly α-L-guluronic acid blocks and poly β-D-mannuronic acid blocks give very different circular dichroism spectra. A recorded spectrum can be fitted to a computer matched combination of the three individual block's spectra. The correlation of results is very good compared to other techniques but the resolution of the spectra for samples with large portions of heteropolymeric blocks was found to be poor (Morris et al, 1973, 1975, 1980a; Craigie, 1984).

The long chain structure of alginate was confirmed by X-ray diffraction analysis on alginate fibres (Astbury, 1945). It was also proposed that the unit cell of the alginate structure consisted of four mannuronic acid residues and perhaps four water molecules. On drying the x-ray diffraction pattern deteriorates but the change is completely reversible on re-exposure to the atmosphere. The availability of the almost pure polyguluronic acid and polymannuronic acid segments has allowed the structure of the two segments to be analysed by X-ray fibre diffraction and infrared spectroscopy of oriented films. The repeat distance along the alginate chain can be measured and a model conformation built to fit these experimental distances, this has shed light on the structure of both the polyguluronic acid and the polymannuronic acid segments. The structure of the poly mannuronic acid segments are very similar to that of cellulose and other β-1,4 linked polysaccharides. The mannuronic acid residues are in the $^4C_1$ conformation and consequently its diequatorially linked. The linkages give the polymer segments containing polymannuronic acid a flattened, ribbon like structure. It has been proposed that this structure is further stabilised by the formation of hydrogen bonds between the proton on the hydroxyl at carbon 3 in one ring with the ring oxygen of an adjacent residue. Another form of hydrogen, between the carboxyl groups hydroxyl and the oxygen atom attached to C3 of a parallel chain causes the poly mannuronic acid chains to bond into sheets of antiparallel residues.

Structure – Poly G

The polyguluronic acid segments are very different to the polymannuronic acid segments. The guluronic acid residues are in the $^1C_4$ conformation and are therefore diaxially linked along the polymer chain. This gives the ribbon structure of the polymer a buckled, as opposed to flat, conformation. This structure is stabilised by a different set of hydrogen bonds; the hydroxyl group on C2 of one residue hydrogenbonds with the carboxyl residue in an adjacent residue. The interchain bonding involves water molecules and is considerably more complex than that seen for the poly mannuronic acid residues. This study also gave the first evidence for the L-configuration of the guluronic acid residues. (Atkins et al, 1970; 1971, 1973a, 1973b).



Proton NMR has helped to substantiate the X-ray work and has also been used to determine the ratios of the three different types of polymer segment in commercial samples of alginate (Penman & Sanderson, 1972). The two different conformations found in polyguluronic acid and polymannuronic acid oligomers could also explain the differences in their ultraviolet dichroism traces (Morris, 1973).

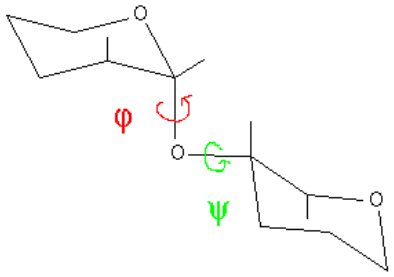
◀ The glycosidic linkage is 1a – 4a di-axial in guluronic acid

The difference in the block copolymer structure of various types of seaweeds explains why alginate with differing properties is extracted from different seaweeds (Haug, 1967; Fisher and Darfel, 1955). It has been shown that the conditions of growth of the weed affects the block composition as does the part of the weed from which the alginate is extracted from (Haug et al, 1968).

Structure – Tertiary structure

The tertiary structure of alginate has been comprehensively reviewed (Morris, 1986). Alginate adopts an extended, ribbon like conformation, in the solid state, being a twofold structure for the free acid form (Atkins, 1973) and a threefold structure in the salt forms so far studied (Mackie, 1973). Alginate is biosynthesised as a polymer of β-D-mannuronic acid, a portion of which is subsequently epimerised at the C-5 position enzymatically to give α-L-guluronic acid (Lin 1966). The D-mannuronic acid residues have been shown to be in the $^4C_1$ conformation and the L-guluronic acid residues in the $^1C_4$ conformation (Rendleman, 1978). Recently a fully unified mechanism for the action of lyases and epimerases in the biosynthesis of alginate has been proposed (Gacesa, 1987).

Molecular modelling has played its part in the structural analysis of alginate. A semi empirical technique was used which assumes that the total conformational energy of a polymer can be broken down into various contributions from bond length and angles superimposed onto he contributions from favourable non-bonding interactions. Each of these contributions can be computer modelled and a contour map calculated representing the energy requirements for the various possible angles of the two C-O bonds joining a dimer together. Hence the probability of a dimer being in a given conformation can be calculated, this can then be extrapolated to give an average structure for a polymer chain. The average structure of the polymer was then related to the viscosity of the polymer solution, alginate was noted as having a very stiff chain in comparison to carrageenan (Brant & Buliga, 1984). The comment on chain stiffness is interesting when compared with the direct electron microscopy studies on the tetra-methyl-ammonium salt of alginate that described alginate as a “linear, unbranched and flexible structure” (Stokke et al, 1987). However, no absolute comparisons where possible.



The use of electron microscopy could well prove useful in the future study of polymer tertiary structure. However, at present the effect the treatment of the sample has on the structure of the polymer prior to examination is unknown. These problems are partly overcome with the newer technique of scanning tunnelling microscopy

◀ Phi and Psi angles for a disaccharide

Alginate production

Alginate, as mentioned before, is found in all types of Phaeophyceae and apart from being a structural polysaccharide and providing rigidity to the algae it also helps prevent the desiccation of those plants expose to air at low tide. Alginate occurs in brown seaweeds in the intercellular mucilage and algal cell wall as an insoluble mixed salt. Not all brown seaweeds are useful as commercial sources of alginate.

The main sources are species of *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis, Saccharina & Sargassum. Saccharina japonica* and species of *Sargassum* are cultivated but the other species are mostly wild harvested. Alginate production is mostly centred in Europe (France & Norway), China, Japan and Chile – close to the natural resources although most manufacturers also import different seaweed biomass. Most species are harvested by purpose built boats and specialised cutting or gathering equipment.

***Sargassum* - commercial source of alginate ▶**

Until recently, alginate processing technology has remained essentially the same since the first factories were built in the 1930s. Raw seaweed is delivered either dried or fresh to the factory. Locally sourced seaweed is often a cheap source because it can be landed from the boats directly into the factory without incurring a drying cost, seaweed from further afield arrives dried and chopped. The weed is milled to a coarse granular consistency. After milling the weed is treated with a preservative and mixed with hot water to form a paste, the paste is transferred to a maturation vat where hot water and sodium carbonate are added. During the alkali treatment, which is usually about three hours in duration, the seaweed swells up, loses its structure and forms a viscous solution. The solution is diluted and pumped to a slurry vat where the slurry is stirred for one to one and half hours after which it is pumped to a settling tank where a polyelectrolyte is added to aid the flocculation of the insoluble residues. After 18-24 hours the alginate containing liquid fraction is decanted off from the residue, chlorine is added as a bleach and the liquor is treated with calcium chloride to form precipitate of calcium alginate. The precipitate is made to float by blowing air through the solution, the calcium alginate fibre can then be skimmed off. The solid is then pressed to remove a large percentage of the water present. The pressed alginate is leached with hydrochloric acid followed by sulphuric acid to leave free alginic acid fibre. The free alginic acid fibre is usually pressed again to remove enough water to give 30-40% solids content. This can then be neutralised with an appropriate base to form a salt which is dried and milled to the required specification for sale

(Kelco Undated, Thornley 1931, Clark 1936, Green 1936, Griffiths 1984). A modified preparation consists of precipitating the alginic acid directly from the filtered, dissolved seaweed by the addition of sulphuric acid and alcohol (le Gloahec 1938).

See our article Introduction to Alginate for more information on production.

Bacterial alginate

Alginate can be produced by a microbial fermentation using bacteria such as *Azobacter vinelandii* and *Pseudomonas eeruginosa* (Linker and Jones 1964, Gorin and Spencer 1966). These bacteria produce a polysaccharide with a structure resembling alginate, differing only in that there are acetyl groups on a portion of the C2 and C3 hydroxyls. It is believed that the acetate groups are associated mainly with the D-mannuronic acid residues (Davidson 1977, Sutherland 1983, Paul 1986). The level of acetylation is variable as is the mannuronic and guluronic acid content. However, the level of guluronic acid in the final polymer can be controlled to some extent by altering the level of calcium in the fermentation broth (Haug and Larsen 1971). The sequence structures and acetylation patterns of bacterial alginate, from different sources, have been studied with 2D COSY proton NMR techniques. The acetyl residues were found to be exclusively associated with the mannuronic acid residues with degrees of acetylation varying from 4-57%. one other interesting point noted was that alginate isolated from four different pseudomonas bacteria all showed a complete lack of consecutive guluronic acid segments, a necessary structural feature for the formation of calcium gels (Skjak-braek 1986).

One of the reasons for the interest in bacterial alginate was the potential development of products with a wide range of molecular weights and properties to compete against algal alginates. However, the technology has never been commercialised and is unlikely to ever be competitive against seaweed based alginate production. It is believed that Tate and Lyle developed a bacterial alginate process up to pilot plant scale and then sold the process to Kelco (Paul 1986, Lawson and Sutherland, 1978). Propylene glycol alginate is produced by taking an alginic acid sample that has been pressed to contain between 30-40% solids and partially neutralise the acid with sodium carbonate to between 5-40% neutralised. The solids are then washed in acetone or propan-2-ol and centrifuged to remove the excess liquid. Finally, a fibre with 60-87% solids is obtained. The fibre is then treated with 1,2 epoxypropane to form an ester. The levels of substitution of over 90% can be produced. (Uniroyal, 1978; Kelco Undated).