BIOSYNTH®

Polysaccharides Toolbox

Handbook for

Polymeric Sugars



Global Reach

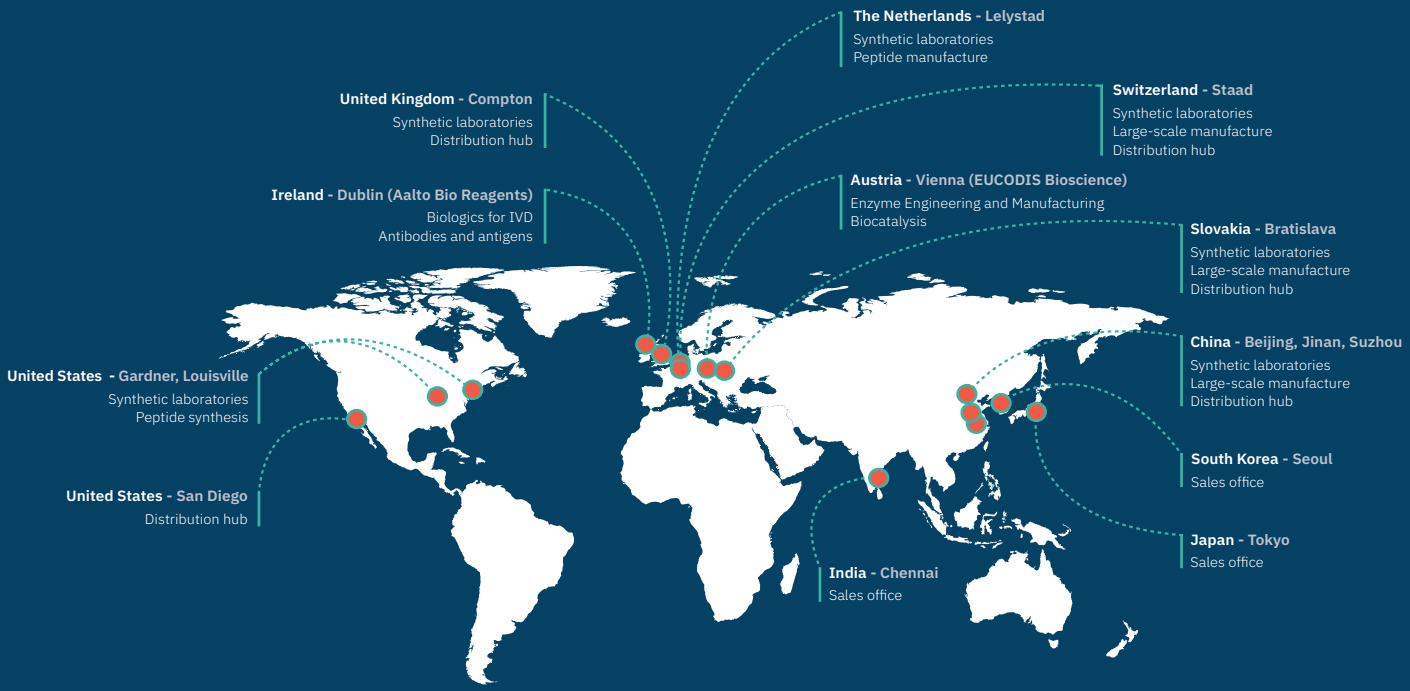


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Section 1 & 2 Introduction and Classification

About Biosynth

Biosynth is an innovative life sciences reagents, custom synthesis and manufacturing services company. We are by scientists, for scientists, securing supply chains with consistent quality, across the globe. We manufacture and source a vast range of chemical and biochemical products, and take pride in delivering products that others cannot. We are experts in complex chemistry, peptides and key biological raw materials. We provide a full range of products and services to support life science research and development, with more than half a million products in our research catalog and hundreds of complex manufacturing service projects. Our complex chemistry specialties include enzyme substrates, carbohydrate and nucleoside chemistry, with manufacturing services from the first idea to the finished product, from route scouting to GMP or ton scale production. For peptides, we also have a full end-to-end offering, from lead discovery and optimization, library production, through to GMP NCE or Neoantigen projects.

Biosynth's mission is to be the leading life sciences platform for scientists developing revolutionary medicines and diagnostics.

The trusted supplier, manufacturer and partner for the pharmaceutical, life science and diagnostic sectors, along with customers across food, agrochemistry and cosmetics, we have facilities across three continents and a rapid global distribution network. Our main chemical research and manufacturing laboratories are in Switzerland, the United Kingdom, Slovakia and China, with peptide production in the USA and the Netherlands. Enzyme projects are based in Austria and biological IVD reagents in Ireland. Our R&D resources and production facilities are modern and versatile, allowing us to produce chemicals on the milligram to ton scale, and at ISO 9001 and GMP, with peptides at mg to multikilogram scale.

Four Areas of Focus

Research Products	Complex Chemicals	Quality Peptides	Key Biologics
• Over 500,000 Products • Small Scale • Rapid Distribution • Retail Chemicals	• Custom Synthesis • Optimization • Large Scale Production • GMP Manufacture	• Custom Synthesis • Neoantigen Peptides • GMP Production • End to End Services	• Custom Antibodies • Reagents for IVD • Enzyme Development • Epitope Mapping

Development of Innovative New Products

Chemical Manufacturing Capabilities

- Custom Synthesis
- Large-Scale Manufacturing
- GMP Manufacturing
- Quality Control and Quality Assurance
- CDMO Services
- Custom Filling and Packaging
- Logistics and Warehousing
- Sourcing

Biosynth History

Biosynth was founded in 1966 by Hans Spitz, and has grown to a global products and services business. Biosynth merged with Carbosynth in 2019 to form Biosynth Carbosynth, creating a world leader in carbohydrates, nuclesosides and enzyme substrates. In 2022 Biosynth Group acquired both vivitide and Pepscan, which strengthened the offering in peptide manufacturing services and catalog products. Also in 2022 Biosynth Group acquired Aalto Bio Reagents, expanding the range of biological products and key raw materials for clinical diagnotics including antigens and antibodies, as well as EUCODIS Bioscience, experts in Enzyme development. Following these acquisitons, the company returned to the Biosynth brand. Biosynth is owned by KKR, Ampersand and senior management.

Ordering

You can conveniently order products online at www.biosynth.com

You can also place an order or make a product inquiry at sales@biosynth.com

For more information, please visit www.biosynth.com

1 Introduction

Welcome to the Biosynth® handbook and catalogue of polysaccharides and related biopolymers. These important products have a huge range of uses that exploit their unique functionalities across the food, pharmaceutical and industrial sectors. However, due to their complexity and diverse attributes, other laboratory suppliers have included them in general biochemical listings. In recognition of this, Biosynth® has assembled these products in a dedicated handbook and catalogue to provide comprehensive support for your research, development and application requirements.

Biosynth® has recently acquired the speciality polysaccharide business Glycomix and the combined strengths of products, applications, analysis and process development will provide you with a fully integrated range of products and services.

This new toolbox and catalogue will guide you through the complexities of these important carbohydrates in some detail including sources, properties, applications and the relationship between their primary, secondary & tertiary structures and functionality. The most important analytical procedures for structural determination will also be discussed.

As an additional tool to provide background detail, a review section has been included covering the product range. Each polysaccharide has been examined in some detail outlining the source, covalent structure, important functionalities and key references. Finally, the catalogue section contains the comprehensive range of polysaccharides that Biosynth® now offers for research and development.



2 Classification

Polysaccharides have been recognized and exploited by man for centuries. As far as is known, they occur as energy reserves and structural materials in the tissues of all living things. In animals their structural role is performed in connective tissue, in plants in the cell walls, and in microorganisms as cell-wall materials and extracellular capsules. A large number of polysaccharides obtained from plants, animals and microorganisms including seeds, algae, fruits, plant cell walls, animal tissues, bacteria and fungi have been developed into commercially important products known collectively as industrial gums (Whistler, 1993) and, in Table 1, examples of these are listed and categorized according to source and type.

In addition, a large number of carbohydrate polymers have been developed as reaction products of the natural polysaccharides listed in Table 1. Examples of these are the ethers, esters and sulphated derivatives of cellulose, starch, dextran, chitin and curdlan. Other derivatives are the creation of fluorescent derivatives that are used as markers in medical scanning procedures.

Other products are the result of random polymerisation of sugar residues through the treatment of starch with acid under conditions of low water activity. Polydextrose is an example of this type of product.

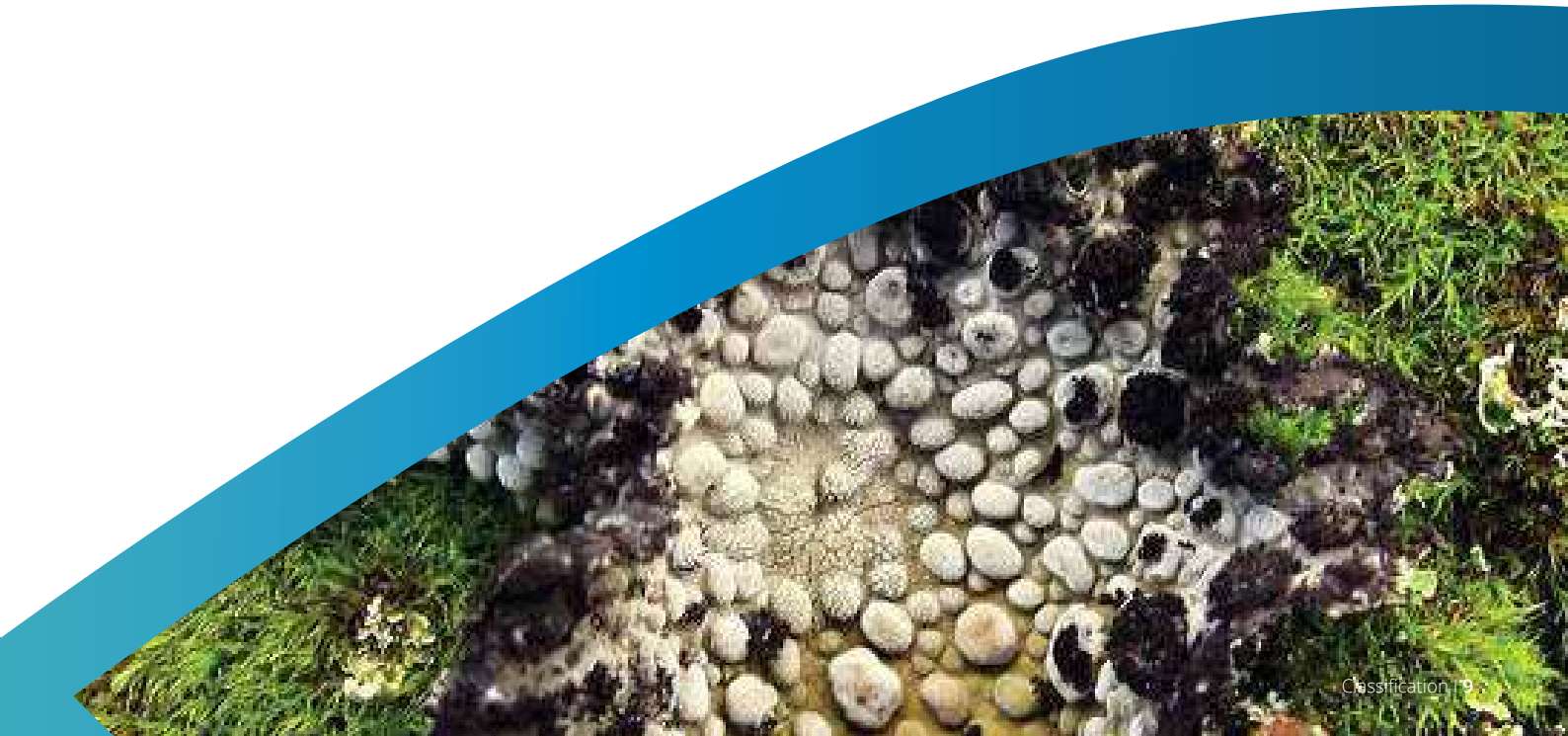


Table 1 **Classification of polysaccharides**

Origin →	Source →	Type →	Example
Higher plants	Trees	Celluloses	Cotton
		Hemicelluloses	Xylans, arabinogalactans
		Exudate gums	Arabic, tragacanth
	Seeds	Galactomannans	Guar, locust bean gum
		Mannans	Ivory nut
		Glucomannans	Konjac
	Cereals	Amylose	Rice, wheat
		Amylopectin	Maize (waxy maize)
		β-glucans	Oat glucan, barley glucan
	Fruits (citrus)	Pectins	Lime, sugar beet
	Tubers	Starches	Potato, yam
Algae	Red (Rhodophyceae)	Sulphated galactans	Carrageenan, porphyran
	Brown (Phaeophyceae)	Uronides, fucans	Alginates, fucoidans
	Green (Chlorophyceae)	Complex sulphated	Ulvan
Lichens	Lichens	Lichenans	Complex glucans
Bacteria	Gram –ve, *(Leuconostoc)*	Branched glucan	Dextran
	Gram +ve *(Micrococcus)*	Ac., mannurono, gluco repeat	Teichuronic acid
Fungi	Micro *(Pullularia)*	α-glucan	Pullulan
	Macro *(Schizophyllum)*	Branched β-glucan	Schizophyllan
Animal tissues	Cartilage (shark)	Glycosaminoglycan	Chondroitin sulphate
	Liver	Glycogen	Complex amylopectin lookalike
	Crustacea shells	β-Glucosamine, N-acetyl glucosamine	Chitosan, chitin

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Section 3
Properties and Applications

3 Properties and Applications

The commercial usefulness of gums is based upon their functionality. In the food and health food industries (Table 2) they are exploited because of their ability to modify texture and as suspending agents. They do this by performing two broadly interconnected functions, namely by gelling aqueous solutions or by modifying their flow characteristics, often producing marked non-Newtonian behavior such as pseudoplasticity or thixotropy particularly at very low concentrations and in synergistic combinations (Fig 1).



Fig 1a A selection of gums in food and dietary fibre products



Fig 1b A selection of gums in food and dietary fibre products

Table 2 **Food and health food applications**
(Whistler, 1993; Blanshard, 1997)

Physical property	Gum	Application
Cold set, clear, gel formation with divalent cations	Alginate	Reformed fruit pieces
Gel formation with sucrose or acid	Pectin	Jams, jellies, dietary fibre
Slow metabolic uptake, low viscosity at high solids	Pectin, inulin	Dietary fibre
Heat reversible gel formation	Agar, gellan	Synthetic meat gels, dietetic jellies
Heat reversible gel formation in the presence of potassium ions	Kappa & iota-carrageenan	Synthetic meat gels, instant desserts, chocolate drinks
Smooth 'short' texture	Fully pregelatinised corn starch	Proprietary desserts, puddings
Cold set soft gel formation with water or milk	Pregelatinised and/or oxidized potato or tapioca starch	Instant desserts, puddings
Pseudoplastic behavior under conditions of high shear	Xanthan gum	Instant desserts, low calorie products
Synergistic binary component gel formation, high viscosity	Xanthan gum, galactomannans	Synthetic meat gels, slimming foods
Retardation of sugar crystallisation at low moisture contents	Gum arabic	In solid confectionery, soft sweets, pastilles
Complex formation with milk protein	Lambda carrageenan	Chocolate milk drinks
Stability at low pH-good viscosity	Propylene glycol alginate, xanthan gum	French dressings, salad dressings
Ice-crystal size retardation	Propylene glycol alginate	Ice creams and lollipop, popsicles
Gel formation with heat	Curdlan	Jelly sweets
Synergistic ternary component very high viscosity at low concentration	Xanthan, konjac glucomannan, alginate (polyglycoplex)	Low calorie, reduced blood glucose, reduced cholesterol products

In the pharmaceutical and cosmetic industries (Table 3) they perform similar functions to those used in foods but in addition, a number of polysaccharides are used directly as drugs with specific therapeutic activities (Fig 2).



Fig 2 Examples of polysaccharides in pharmaceuticals

Table 3 **Cosmetic and pharmaceutical applications**
(Dumitriu, 1996)

Physical property	Gum	Application
Moisture retention	Hyaluronic acid	Skin creams
Blood anticoagulant	Heparin	Open heart surgery
Thermoreversible gelation	Gellan	Agar substitute in microbiology
Gel formation with calcium	Alginate	Antacid proprietory products e.g. Gaviscon
Blood anticoagulant	Fucoidan	Surgery (Heparin substitute)
High shear pseudoplasticity	Xanthan gum	High solids medicines and syrups
Stability at low pH-high viscosity	Xanthan gum	Fluoride dental gels
Anti-inflammatory properties	Chondroitin sulphate	Treatment of inflammation e.g. osteoarthritis
Protection for damaged tissues e.g. urinary tract	Pentosan polysulphate	Interstitial cystitis
Water retention	Psyllium seed gum	Laxative
Inert plasma thickener	Dextran	Blood plasma extender

Industrial (Table 4), polysaccharides have many applications in oil exploration and recovery, textile printing and building materials to name a few (Fig 3).



Fig 3a Industrial polysaccharide example



Fig 3b Polysaccharide usage in oil exploration

Table 4 **Industrial applications**
(Whistler, 1993)

Physical Property	Gum	Application
Pseudoplastic behavior under conditions of high shear	Xanthan gum	Oil drilling muds
Compatibility with reactive dyestuffs (Procion)	Sodium alginate	Cotton textile printing, carpets
Stability, high viscosity	Guar gum	Fracking
Controlled dispersion, viscosity	Na+ carboxymethyl cellulose	Wallpaper paste
High solids compatability, viscosity	Na+ carboxymethyl cellulose	Cement setting retardation
Film formation	Starch derivatives	Paper coating
Water soluble films	Pullulan	Water soluble seed coatings
Gel formation	Xanthan, locust bean gum	Explosive gels
Mineral suspension	Xanthan, guar	Mineral flotation aids
Metal chelation	Chitin	Heavy metal separation
Ion exchange support	DEAE dextran	Chromatography
High viscosity, non-newtonian, gel forming	Methyl cellulose	Adhesives, paints, cements
Viscous, compatability with organic solvents	Hydroxyethyl cellulose	Latex paints
Film formation	Chitosan	Textile finishing

B

Section 4 & 5

Polysaccharide Isolation, Purification and Structure Determination

4 Polysaccharide Isolation and Purification

4.1 Isolation Techniques

Polysaccharides occur in a wide variety of environments as exocellular bacterial slimes and capsules, plant cell wall components, covalently linked to proteins in animal tissues, as storage components in plant seeds and tubers and in the exoskeletons of crustacians and insects. Also, they often occur in mixtures as in brown algae (alginate + laminarin + fucoidan).

Thus, in an ideal world, the isolation of a polysaccharide must be approached by a consideration of all the circumstances likely to influence the production of pure and undegraded material for further study. It is very difficult to generalize and few books on the chemistry of polysaccharides cover all the available options although two older publications (Smith, 1959; Whistler, 1965) provide chapters on many of the techniques that are still relevant today. The reader who wishes to embark on the isolation and purification of representative polysaccharide material should also undertake a thorough survey of the current literature.

A selection of the techniques that are commonly used in polysaccharide isolation are:

- Homogenization of plant tissue
- Enzymatic digestion of unwanted components such as proteins and polysaccharides
- Water extraction
- Solvent extraction
- Cell wall disruption by either chemical or mechanical processes, for example with urea or by mechanical grinding
- Winnowing of seed husks
- Centrifugation to remove bacterial cells
- Precipitation with anions, cations or quaternary ammonium compounds

4.2 Purification Techniques

Commonly used purification techniques include:

- Dialysis to remove low MW materials and inorganic ions
- Lyophilization to remove water and solvents (Fig 4)
- Various forms of chromatography
- Solvent extraction
- Precipitation with solvents such as ethanol, isopropanol
- Electrophoresis
- Enzyme treatment



Fig 4 Laboratory Freeze Drier (Lyophilization)

5 Polysaccharide Structure Determination

5.1 Basic Structural Features

In describing the structure of a polysaccharide, various methods are employed to characterize (i) the basic organic and inorganic features of structure, (ii) the monomeric sugar units in the polymer and any other organic and inorganic structural features that these carry, (iii) the architecture of the polymer including the positions and relative attachment (anomeric configuration) of monomer linkages and details of chain branching, (iv) the molecular size and dispersion of the polymer chains and (v) any inter or intramolecular interaction of the polysaccharide chains or interaction with other components such as proteins and lipids.

5.1.1 Basic Structural Features

The basics of structure that are required to start with are:

(i) The moisture content which can be measured in many ways but examples include loss on drying to constant weight and Karl Fischer analysis for water content (Fig 5).

(ii) The total carbohydrate content of the material which is often determined using the phenol-sulphuric acid analysis for carbohydrates (Fig 6) (Dubois, 1956).

(iii) The protein content of the product can be determined by the Kjeldahl method.

(iv) The fat content by lipid analysis after solvent extraction.



Fig 5 Karl Fischer apparatus for moisture determination



Fig 6 Use of the phenol-sulphuric test for carbohydrates (sensitivity ~ 10 µg/ml)

5.1.2 Monomeric Structural Units and Substituents

In order to release monomeric sugars from the polysaccharide matrix, it is necessary to employ reagents that can selectively attack (protonate) the glycosidic oxygen atom between each sugar residue. The selection of suitable reagents requires a degree of insight, trial and error too involved for discussion here, but useful candidates include acids (acetic, hydrochloric, trifluoroacetic, methanolic HCl) and enzymes (hydrolases). Some polysaccharides have glycosidic bonds which are resistant to hydrolysis e.g. polyuronides (containing carboxyl groups) when much stronger conditions will be required to break these but do not destroy the sugar residues themselves (Churms, 1982).

The method used to separate and show monosaccharide components released from polysaccharides is analytical chromatography, a technique that has been used for many years (White, 1991).

The earliest methods that were developed were based on the elution of components on paper and visualization by spraying or dipping the papers in a wide variety of reagents (Fig 7).

Both analytical and preparative methods were developed using this technique but were superseded by gas chromatography, a method that was difficult due to the need for volatile derivatives, not liked by carbohydrate analysts (Fig 8 - see page 14).

Fluorophore-assisted carbohydrate electrophoresis (FACE) was also used successfully on carbohydrates often using fluorophores such as 8-aminonapthaline-1,3,6, trisulphonic acid disodium salt (ANTS) as a charged visualization agent. (Fig 9 - see page 14).

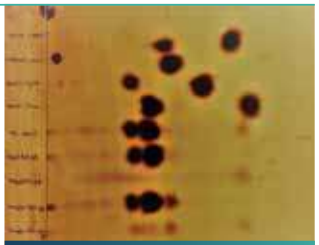


Fig 7 Paper chromatogram of monosaccharides in a polysaccharide

In recent years, high performance liquid chromatography (HPLC), a method that does not require derivatization, was developed and is now used in virtually all analytical laboratories worldwide. Methods of detection have also improved markedly and can now cope with gradient elution to enhance the separations of components.

For the analysis of mono- and oligosaccharides the chromatographic method of choice is often Dionex ion chromatography, a technique which has proved particularly successful for carbohydrates (Fig 10).

As an example of a fairly simple polysaccharide, the hydrolysis of dextran will release only glucose using either trifluoroacetic acid or the enzyme dextranase (Fig 11).

In the case of a polysaccharide mixture, for example konjac glucomannan (Fig 12), xanthan gum (Fig 13) and sodium alginate (Fig 14 - see page 15), the expected monosaccharides would be glucose and mannose (from the konjac), glucose, mannose and glucuronic acid (from the xanthan) and mannuronic acid and guluronic acid (from the alginate).

The ion chromatogram of the hydrolysis products of this mixture shown (Fig 15 - see page 15) is of both the neutral and charged monosaccharides. The difficulty is the presence of the three uronic acids (glucuronic, mannuronic, guluronic) in the mixture which requires one strength of acid to release the neutral sugars (glucose, mannose) and stronger conditions for the uronic acids.

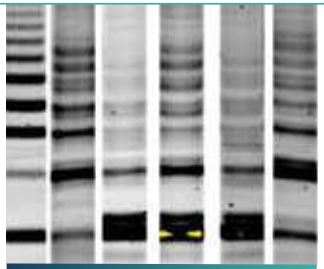


Fig 9 Electrochromatogram of glucose syrup at various stages of a beer fermentation (G4 is the tetrasaccharide marker in the ladder standard)



Fig 10 Dionex chromatography of carbohydrates

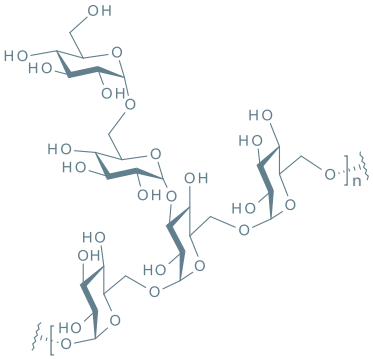


Fig 11 Dextran

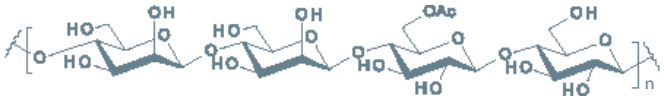


Fig 12 Konjac glucomannan

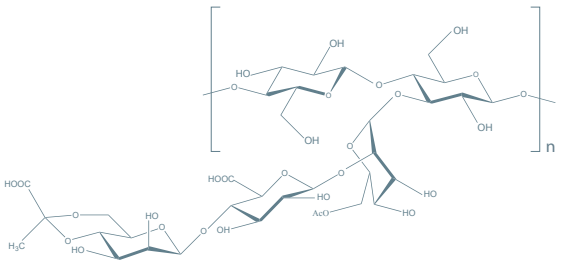


Fig 13 Xanthan gum

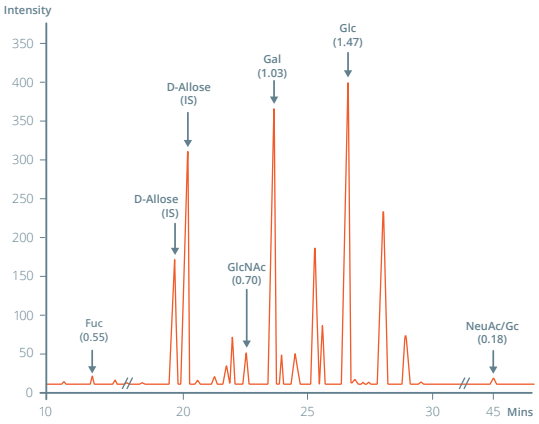


Fig 8 Gas chromatogram of monosaccharide trimethyl silyl ethers

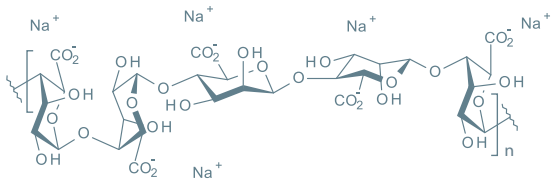


Fig 14 Sodium alginate

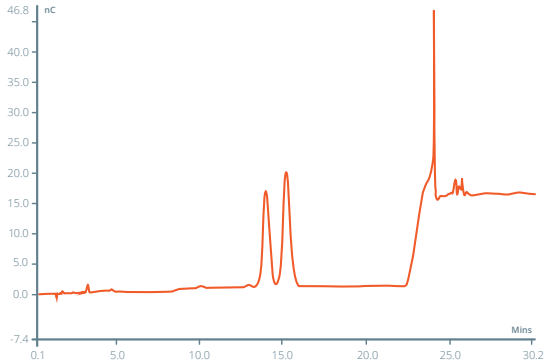
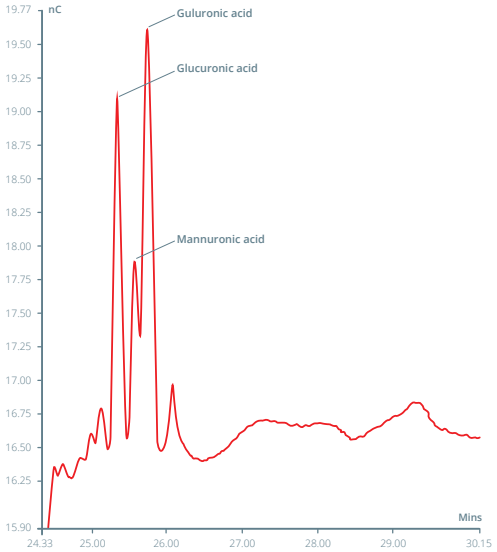


Fig 15a An example of ion chromatography in the separation of neutral monosaccharides and uronic acids



Uronic acid expansion showing glucuronic, guluronic and mannuronic acids

Fig 15b An example of ion chromatography in the separation of neutral monosaccharides and uronic acids

5.2 Linkage Positions, Branching & Anomeric Configuration

There are several ways of identifying the position and configuration of the linkages that join sugar residues and these together with their identity provide the main key to polysaccharide covalent structure. The four methodologies used most commonly are (i) methylation analysis, (ii) retro analysis based on partial degradation, (iii) methods based on spectroscopy and (iv) the use of enzymes.

It should be emphasized that the highlighted analytical techniques described will always require assistance from one or more of the others so, for example, methylation will require the use of mass spectroscopy. Analysis by nuclear magnetic resonance spectroscopy on the other hand will need careful chemical or enzymatic degradation to provide molecules small enough for analysis but large enough to be representative of the whole structure.

5.2.1 Methylation Analysis



Fig 16 Mass spectroscopy

The polysaccharide is treated with reagents that selectively convert the free hydroxyl groups to methyl ethers. Methyl ethers are very stable and the methylated polysaccharide can then be hydrolyzed to release monomers that are characteristic of the original polymer, having free hydroxyl groups where the linkages had previously been in the intact polymer. In the experimental protocol the released methylated monomers are reduced to alditols and acetylated and these are characterized as partially methylated alditol acetates by a combination of gas chromatography and mass spectroscopy (GC/MS) (Fig 16).

This method has become the standard for polysaccharide linkage analysis in most laboratories throughout the world (Bouveng, 1960; Hakomori, 1964; Bjorndal, 1970).

Very briefly, as an example, in a polysaccharide having a single sugar building block (e.g. starch or dextran) linked through positions 1 and 4 then unbranched sugars in the chain would release sugars methylated at positions 2,3 and 6. A branch point at, for instance, position 3 would release sugars methylated at positions 2 and 6 and an end group sugar with position 4 free would release sugars methylated at positions 2,3,4 and 6 (Fig 17).

The drawback with methylation analysis is that it does not give information about the configuration of the anomeric link between sugar building blocks and is not very useful in providing information about non-sugar attachments in the polysaccharide such as O-acetylation, pyruvylation, succinylation etc.

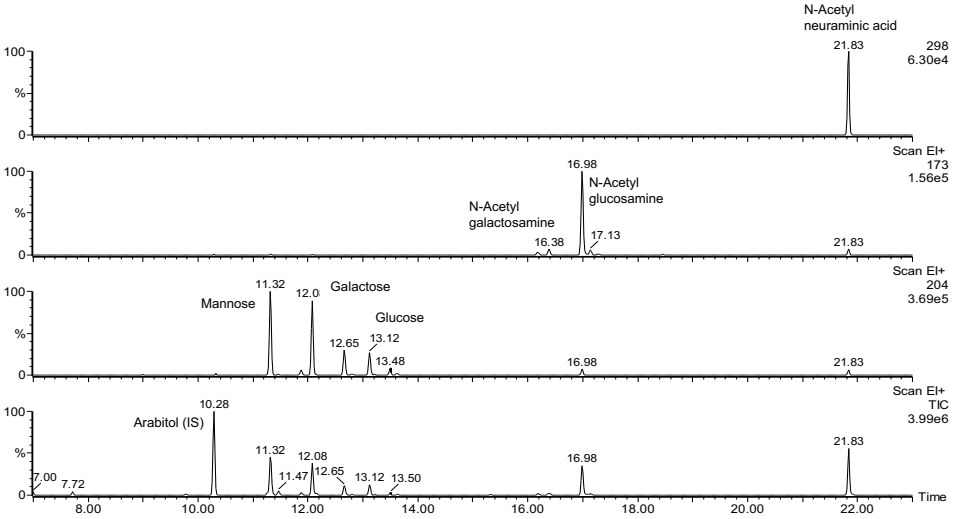


Fig 17 Mass spectrum of partially methylated alditol acetates

5.2.2 Retro Analysis by Partial Degradation

In this method, the polysaccharide is treated with reagents (Lindberg, 1975) or enzymes (McCleary, 1987) that break it down into smaller fragments that can then be characterized and reassembled into the intact polymer unit. In this procedure the chemicals that are used are normally acidic in character and examples are acetic, hydrochloric, sulphuric and trifluoroacetic acids at low concentration. Another useful reagent is methanolic hydrochloric acid. Enzymes that are employed have to be chosen with care as they will be specific to particular links in the polymer chain and so some knowledge of the polysaccharide will be important (source, monomer units etc).

Examples of the use of degradation were in (i) the analysis of xanthan gum by partial acid hydrolysis (Rees, 1976), (ii) treatment with a combination of acetic acid, sulphuric acid and acetic anhydride to produce di- and trisaccharides (Fig 18) that were recombined to form the pentasaccharide repeating residue (Lawson, 1977) and (iii) in the analysis of starch where a number of enzymes isolated and characterized by Whelan in the 1950s were elegantly employed to reveal the intricate and very different molecular architecture of the branching from starches from plants ranging through corn, potatoes, rice and tapioca (Drummond, 1970).

One technique that is very important in this method is preparative chromatography, as in order to obtain structural information it is critical to isolate, purify and identify the fragments that are released by the respective degradative methods (Hicks, 1987). The fragments so obtained can then be used to give useful information about the anomeric linkage positions of the monosaccharides using chiral enzymes (McCleary, 1987) or spectroscopic methods (section 5.2.3).

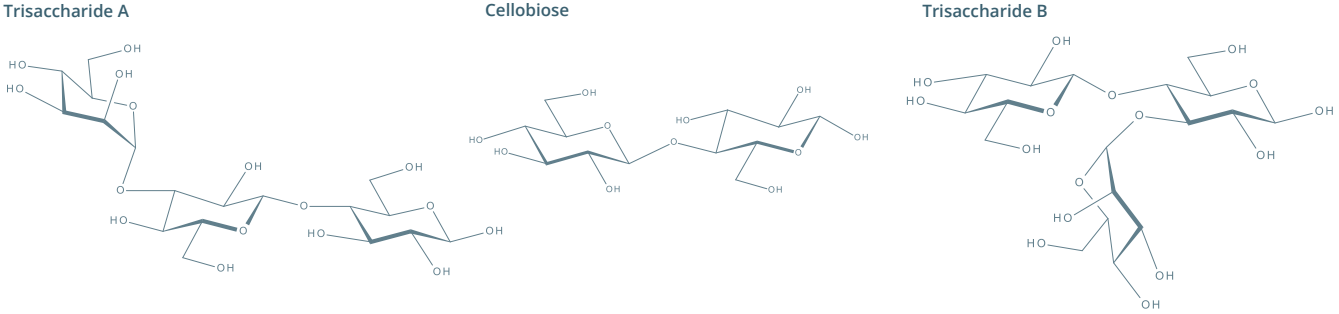


Fig 18 Cellobiose and trisaccharides A&B from the acetolysis of xanthan gum

5.2.3 Spectroscopic Methods of Analysis

There are many methods of analysis that can be loosely classed as being based on spectroscopy, namely the use of irradiation energy of various sorts to excite structural features in the molecule of interest that will then give back useful information. These methods include infrared spectroscopy, ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, and more recently, Raman spectroscopy and mass spectroscopy.

5.2.3.1 Nuclear Magnetic Resonance (NMR)

NMR spectroscopy (Fig 19) can reveal many detailed structural features in organic molecules such as how the links between the atoms in an organic molecule are organized, coupled and linked together. Couplings between atoms are used to determine the chirality of optically active molecules, a key feature in the development of drugs where one isomer can be active and another inactive or have different activity (e.g. thalidomide). A very useful feature of NMR is that substituents such as methyl, acetyl and pyruvyl groups can be identified in the NMR spectrum, something that can be difficult using other techniques (Robyt, 1998).



Fig 19 NMR instrumentation

Figure 20 is an example of a 1H NMR spectrum of an oligosaccharide released by the partial degradation of a polysaccharide showing the anomeric and ring protons crucial to the identity of the chiral nature of the structure.

However, intact polysaccharides often produce poor spectra due to problems with increased proton relaxation times caused by the molecular weights of these large molecules. It is often necessary therefore to slightly degrade samples with care in order to obtain meaningful spectra (Fig 21).

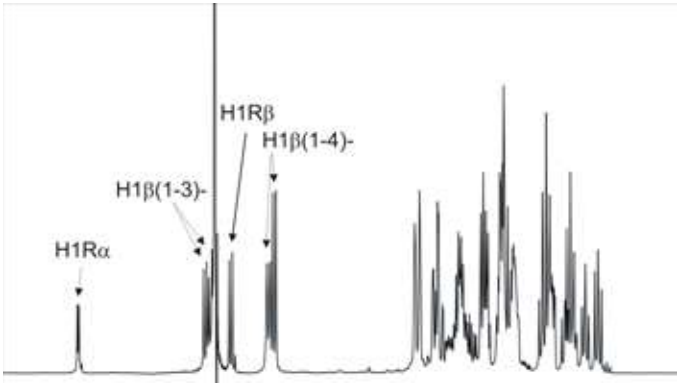


Fig 20 700 MHz 1H NMR spectrum of a tetrasaccharide produced by partial hydrolysis of a polysaccharide

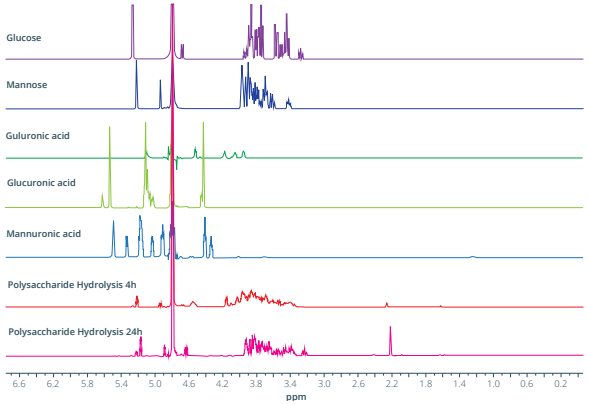


Fig 21 400 MHz ^{1}H NMR spectra of reduced polysaccharide structures

5.2.3.2 Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a very useful analytical technique which can highlight structural features not shown up using other techniques (Kacurakova, 2001). An example is the semi-quantitative analysis of the mannuronic to guluronic acid ratio in alginate by comparing the resonances at 808 & 787 cm^{-1} (Fig 22) (Mackie, 1971).

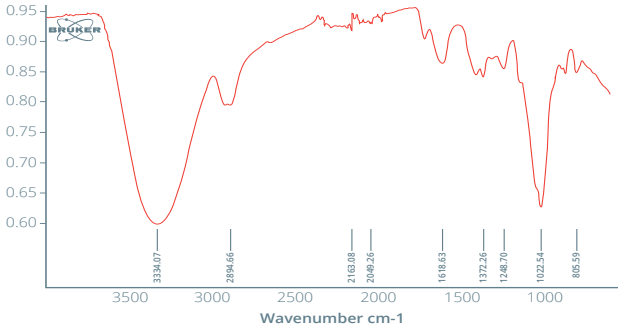


Fig 22 Infrared spectrum of sodium alginate

5.2.3.3 Raman Mapping

Raman mapping spectroscopy has rapidly gained acceptance as an invaluable tool for detecting, quantifying, and analyzing the chemical composition of materials across a broad range of industries. Raman spectroscopy, is inherently nondestructive, noninvasive, works with trace amounts of any substance in nearly any lab or field environment, and requires no sample preparation (Szymanska-Chargot, 2016).

Fig 23 shows a Raman spectrum in the fingerprint range of a mixture of xanthan gum, sodium alginate and konjac glucomannan where distinct differences can be observed between them (Szymanska-Chargot, 2016).

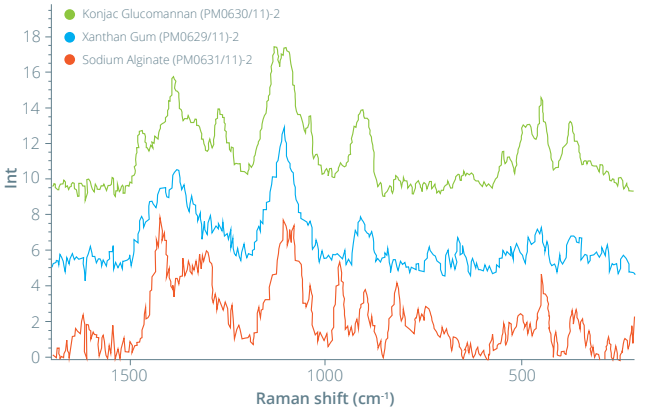


Fig 23 Raman spectrum comparing xanthan gum, sodium alginate and konjac glucomannan

5.2.3.4 Mass Spectroscopy

As has been described in section 5.2.1, mass spectroscopy is a key analytical tool in the analysis of partially methylated alditol acetates in methylation studies. However, in recent years mass spectroscopy has become an invaluable tool for analysis of large oligosaccharides and polysaccharides through the development of methods such as matrix assisted laser desorption/ionization (MALDI) techniques (Stephens 2004). Mass Spectroscopy is also a key analytical technique in the structural analysis of both N- and O- linked glycans attached to glycoproteins (North, 2009).

Methodologies have recently been developed as rapid screening and analytical tools for glucose-containing polysaccharides using a designed 'glycome array' in conjunction with mass spectroscopy (Palma, 2015).

5.3 Molecular Weight Determination (White, 1991)

5.3.1 Light Scattering

When a polysaccharide solution is illuminated with a beam of light at wavelength λ the polymer chains will scatter light in direct proportion to their weight-average molecular mass (MW) and the angular dependence of the scattered light at low angles can be related directly to the z-average of the 'radius of gyration' (Rg). This is the only technique that can be used to measure the dimensions of the molecules without any assumptions about the shape of the molecule.

Light scattering is also an absolute method for determining these molecular parameters, as opposed to viscometry and gel permeation chromatography, both of which require calibration. The method is the major technique for determining MW of polysaccharides. However, experimental difficulty in the method is the demand for high quality solutions free of dust and particulate matter (Fee, 2003; Hokputsa, 2003).

5.3.2 Size Exclusion Chromatography

Size exclusion chromatography coupled with multi-angle laser light scattering (SEC-MALS) is a valuable analytical tool for characterisation of polysaccharides (Fig 24).

Adding multi-angle light scattering (MALS) to SEC allows absolute molecular weight (MW) to be determined without the need for a reference standard. In this technique, the molecular weight of a succession of analytically separated slices is determined which then allows the molecular weight distribution to be built up. Multi-angle, static light scattering (MALS) decouples molar mass analysis from retention time and allows the determination of molar mass. In addition, with SEC-MALS, the determination of polysaccharide properties that cannot be measured using UV detection with SEC is possible (Fee, 2003; Hokputsa, 2003).



Fig 24 Size exclusion chromatography with multi-angle light scattering equipment

5.3.3 Ultracentrifugation (Harding, 2015)

Analytical ultracentrifugation is a technique widely used to characterize the sizes and shapes of polysaccharides and other carbohydrate biopolymers.

The analytical ultracentrifuge (Fig 25) works on the principle that at high rotational speeds small particles like polysaccharides in solution will move under the influence of the centrifugal field, leaving clear solvent behind. The solutions are placed in precision ultracentrifugal cells with transparent end windows. These cells contain two compartments, one for the solution and one for the reference solvent. Light rays from a laser are passed through the solution as the sedimentation process takes place - the signal is then picked up by a CCD camera and the data processed. In this way the rate of change in the concentration distribution of the dissolved macromolecule with time can be followed during the period of sedimentation (usually several hours), and from the change with time of the concentration pattern, the data is processed to yield an estimate of the distribution of sizes of polysaccharide chains in the solution, in terms of the distribution of sedimentation coefficient which gives a good idea of sample purity and heterogeneity (Harding, 2005).

The sedimentation coefficient is a function of shape as well as size (MW). At lower speeds however the sedimentation force is balanced by diffusive forces backwards and the final steady state equilibrium pattern depends only on molecular weight. Although not as resolving (because of the lower speeds), the average molecular weight (generally the weight average) can be combined with the average sedimentation coefficient from sedimentation velocity to convert the sedimentation coefficient distribution into a molecular weight distribution.



Fig 25 Analytical Ultracentrifuge

5.3.4 Viscosity

Viscosity is a molecular weight sensitive method that has been widely used because it is relatively simple to undertake and does not require expensive instrumentation. Although the measurable quantities are easy to obtain, converting them to molecular weights and comparison with other samples is not always a straightforward task, and in many cases the use of intrinsic viscosity, [η], as a hydrodynamic property is used without conversion to molecular weights.

The basis for using viscosity as a molecular weight sensitive method is the relation between the intrinsic viscosity (limiting viscosity number) and an average of the molecular weight. The dependence of the intrinsic viscosity of a homologous series of polysaccharides on the molecular weight is classically described in terms of the Mark-Houwink equation: $[\eta]=K'M^{a}$ where K' and a are empirical parameters constant at a given temperature, for a specified solvent within each polysaccharide for a limited range of molecular weights. The Mark-Houwink equation forms the basis for using viscosity to determine molecular weight. From a series of calibration standards, determination of molecular weight and intrinsic viscosity yields estimates of K' and a for the given polysaccharide-solvent system (Harding, 1997).

5.4 Polysaccharide Physical Properties

In this section, the measurement of the unique physical properties of polysaccharides is explored. These range in a continuum from non-reversible hard gels, characterized by the alginates to high solids Newtonian solutions as exhibited by some of the acacia gums. Between these two extremes the carrageenans and agar form thermally reversible gels, pectins form gels with sucrose, binary combinations of xanthan and galactomannans form highly viscous quasi-gels, xanthan alone shows marked shear-dependent pseudoplastic viscosity characteristics, high molecular weight sodium alginate forms viscous solutions at low concentration and ternary mixtures of several polysaccharides (e.g. xanthan, konjac, alginate) form exceptionally high viscosities at very low concentration.

In addition to the viscosity and gelling properties described above, there are a number of lipophilic functionalities shown by polysaccharides that have been exploited. These properties include emulsion stabilization, film formation, compatibility with non-aqueous solvents, surface tension reduction and reduction of interfacial tension.

The detailed measurement and description of these fundamental characteristics is extremely complex and cannot be fully described here so the following discussion is designed to provide an insight into the key properties that have been discussed above and how they may be measured, with references for further reading.

5.4.1 Rheology

Rheology is the quantification of viscous flow and deformation of polysaccharide solutions under applied shear forces which can be applied and measured using suitable rheometers. The type of rheometer selected for measuring these properties depends on the relevant shear rates and temperatures as well as available sample size. Instruments in current use range from glass capillary viscometers (e.g. Ubbelohde), rotational viscometers (e.g. Brookfield) (Fig 26) to complex rheometers with many configurations such as cone and plate, bob and vane geometries (e.g. TA instruments, Bohlin, etc.) (Fig 27).



Fig 26 Brookfield viscometer



Fig 27 TA instruments rheometer

Rheological properties impact at all stages of polysaccharide use from formulation development and stability to processing and product performance. Examples of rheological measurements include:

- Measurement of the viscosity of non-Newtonian shear-dependant polysaccharides which can then be used to model real-life applications or processing conditions.
- The determination of viscoelastic characteristics to determine the extent of solid or liquid-like behavior.
- Optimising and assessing dispersion stability.
- Comparing polysaccharide products used in food and cosmetic products for their ability to flow, coat and suspend.
- Understanding how carbohydrate polymers can improve dispersion, coatings and emulsion stability in drug formulations.

To illustrate the value of rheological measurements, a plot of xanthan viscosity against shear rate is shown in Fig 28. Xanthan is very shear sensitive and this depends on concentration as shown. This property is used to suspend particles, for example in food sauces, and the use of the plot is to allow the correct concentration to be calculated.

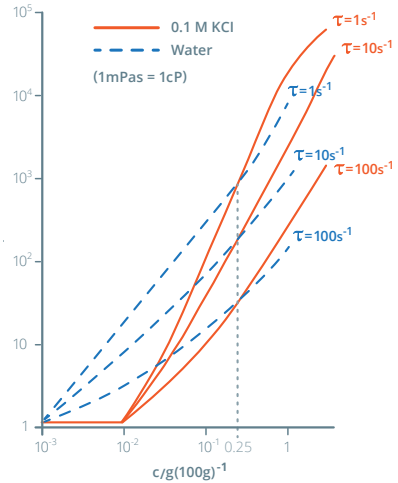


Fig 28 Relationship between xanthan viscosity, shear rate and concentration

5.4.2 Gelation (Harris, 1990; Blanshard, 1979)



Fig 29 Instron model 4201 tensile compression tester

Polysaccharide gels can be defined as cross-linked systems, which exhibit no flow when in a steady-state. By weight, gels are mostly water but behave like solids due to a three-dimensional cross-linked network of polysaccharide chains that give the gel its structure.

Gels can show many different physical textural attributes from a soft fruit gel dessert to a hard pharmaceutical capsule. Texture profile analysis (TPA) permits routine measurement of gel texture and for example, an Instron 4201 tester can be used for compressing the gel, with appropriate software for processing the data (Fig 29).

The textural parameters measured are shown on the idealized force-deformation curve in Fig 30.

Several criteria are used to define the gel properties namely:

- **The modulus** is the initial slope of the force-deformation curve. This is a measure of how the sample behaves when compressed a small amount. The modulus usually correlates very closely with a sensory perception of the sample's firmness.
- **Hardness** is defined as the maximum force that occurs at any time during the first cycle compression. In most cases, the hardness is correlated to the rupture strength of the material.
- **Brittleness** is the point of first fracture or cracking of the sample.
- **Elasticity** is a measure of how much the original structure of the sample was broken down by the initial compression. In sensory terms, it can be thought of as how 'rubbery' the sample will feel in the mouth.
- **Cohesiveness** is measured by taking the total work done on the sample during a second compression cycle and dividing it by the work done during the first cycle. Work is measured as the area under the respective curves. Samples that are very cohesive will have high values and will be perceived as tough and difficult to break up in the mouth.

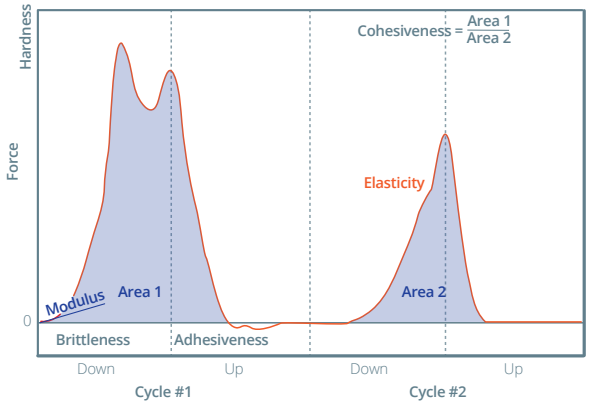


Fig 30 Idealized force-deformation curve for textural parameters

Unsubstituted polysaccharides such as amylose and pullulan show very little, if any lipophilic properties although they form good films. However, when the polysaccharide contains deoxy sugars such as fucose, the polysaccharide will to a greater or lesser extent show surface activity. Examples of polysaccharides with natural surface activity are Xanthan gum, a polysaccharide that acts as a secondary emulsion stabilizer. Sugar beet pectin (Dea, 1986) and Indican (Symes, 1982) have a higher degree of lipophilic substitution (methyl esterification and acetylation) and behave much more like true surfactants reducing for example, interfacial and surface tension and showing compatibility (gelling) with solvents such as glycols and alcohols In addition, a number of the synthetic polysaccharide derivatives including ethyl cellulose, ethyl (hydroxyethyl) cellulose and hydroxypropyl methyl cellulose (hypromellose) show surface properties.(Fig 31).

Techniques often used for measuring surface and interfacial tension are the Wilhelmy plate and De Nouy ring (Fig 32). Methods and instruments used for these measurements are known as tensiometers (Fig 33).

In the laboratory, the ability of polysaccharides to stabilize emulsions are tested by homogenizing the polysaccharide with an appropriate solvent (Fig 34).

Gels can also be formed with solvents as shown in (Fig 35).

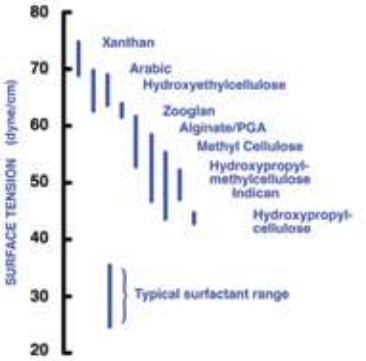


Fig 31 Comparison of surface active polysaccharides

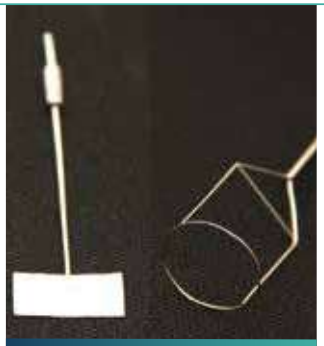


Fig 32 Wilhelmy plate & De Nouy ring



Fig 33 Kruss tensiometer



Fig 34 Polysaccharide emulsion with parrafin



Fig 35 Polysaccharide gel formed with ethylene glycol as solvent



B

Section 6
Secondary/Tertiary Structure and Function

6 Secondary/Tertiary Structure and Function

6.1 Introduction

Polysaccharides have amazing physical properties and these are determined by the way in which they occupy their biological environments. Secondary structure can be defined as the general three dimensional form of a polysaccharide (e.g. open chain, helix etc) and tertiary structure as how the chains occupy three dimensional space.

They can lock up very large amounts of water in gel-like structures that appear to be solid but contain only 0.5% solid material and 99.5% water. They can form bundles called microfibrils that hold up massive trees and can occupy interstitial space between growing cells in plants and animals, forming a matrix in which organisms can grow and cells divide. These properties have been the subject of much scientific effort to work out how polysaccharides do this and in section 6.2, some of the most important structures and how they were elucidated will be discussed.

Polysaccharide primary structural information, discussed in section 4, gives very little information about the way in which the polysaccharide molecule works in three dimensional space. For this reason it has been necessary to turn to other methods of analysis to determine the secondary and tertiary structures of polysaccharides that define functionality.

A key group of biopolymers that provided insights into how the three dimensional structures of polysaccharides could be studied was found in protein chemistry where X-ray crystallography, NMR spectroscopy and electron microscopy coupled with model building (using high powered computers) allowed scientists to elucidate the structures of proteins such as lysosyme and insulin. These methods have been used to study polysaccharides.

6.2 Examples of Secondary & Tertiary Polysaccharide Structures

6.2.1 Cellulose

The polysaccharide that has attracted the most attention has been cellulose as it provides the crucial close-packed microfibrils essential to the strength of the growing plant. As can be seen in Fig 36, the glucose chains can pack together with hydrogen bonds holding them in close-packed bundles.

These bundles then arrange themselves to form the skeleton of the growing plant (Fig 37).

The secondary and tertiary structure of cellulose was determined by a combination of X-ray crystallography and electron microscopy.



Fig 36 X-Ray diffraction diagram of cellulose

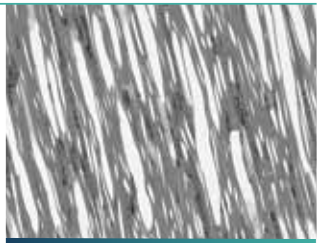


Fig 37 Electron micrograph of cellulose microfibrils

6.2.2 Carrageenan (Whistler, 1993)

Carrageenan forms thermally reversible gels and this has been exploited by the food industry in many applications such a desserts, milk drinks and for its very good freeze-thaw stability. Following extensive studies using X-ray crystallography and spectroscopy, it was found that Carrageenan had a helical structure (Fig 38) and that the helices could combine in a hydrogen bonded double helix arrangement in a similar manner to DNA (Fig 39). The individual double helices then form links with other helical chains to form networks resulting in a solid gel (Fig 40). Carrageenan gels can be melted and reformed due to the breaking and reforming of the hydrogen bonds between chains (Rees, 1970) (Fig 41).

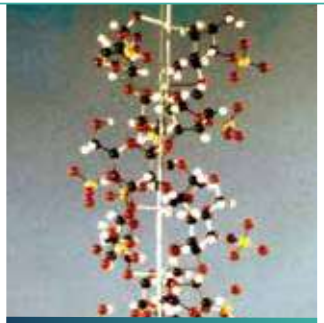


Fig 38 Ball and stick model of carrageenan helical structure



Fig 39 Space-filling model of carrageenan double helix

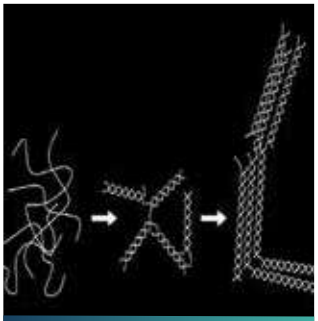


Fig 40 Representation of carrageenan gelation mechanism



Fig 41 Carrageenan gel

6.2.3 Alginate (Harris, 1990)

When sodium alginate is treated with divalent calcium ions, the calcium forms links between carboxyl groups in neighboring chains (most strongly with the guluronate residues) forming a gel matrix known as the 'egg box' due to its regular parallel arrangement (Fig 42).

These gels are not thermally reversible as the bonds are ionic in nature (Rees, 1969). Alginate gels are exploited in such applications as reformed meat and fruit pieces, glazes, dressings and to stabilize the foam on beer.

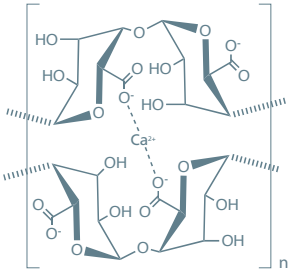


Fig 42 The alginate egg box mechanism

6.2.4 Xanthan Gum (Sandford, 1977)

Xanthan gum combines high viscosity with a remarkable ability to suspend solids under conditions of low shear. These properties arise from the ability of xanthan chains to form loosely associated double helical links with other chains that break, when sheared, and reform when shear is removed. The structural nature of these links has been the subject of much investigation and it has been concluded that the behavior of xanthan is consistent with part gel and part solution. The polymer chains form weak non-covalent bonds that break when sheared but reform when the shear is removed (Fig 43). This has found great application in oil well drilling where the lubricating polymer is required to flow under conditions of high shear but then to thicken in order to bring the drilled-out particles to the surface. Other key uses are in the food industry as a suspending agent with very good texturizing and mouthfeel properties.

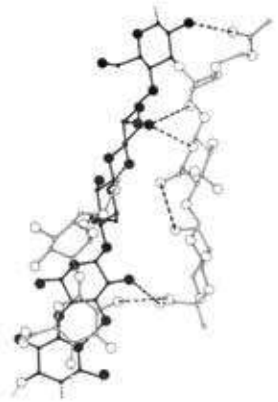


Fig 43 Xanthan gum helix with associated hydrogen bonding

6.2.5 Pectin (Whistler, 1993)

In many ways pectin is similar to alginate, being largely composed of galacturonic acid some of which is methyl esterified. Pectins that have a high level of methoxy groups (HM Pectin) form gels in the presence of sucrose and for this reason are used in jams and in combination with other fruits. Pectins with a low level of methoxy groups (LM Pectin) gel with calcium ions and are used in other food applications such as pastries and recipes designed not to be as sweet. Low methoxy pectin can be used as a fat substitute in baked goods and to stabilize acidic protein drinks such as drinking yogurt. The 'Egg Box' model similar to the alginate concept has also been used for Pectin (Fig 44).

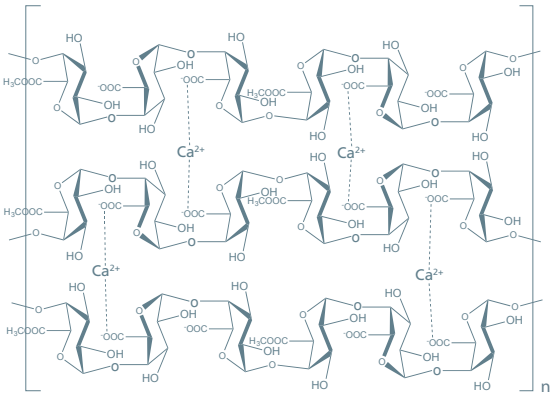


Fig 44 Pectin 'egg box' model of gelation

6.2.6 Konjac Glucomannan

By the use of electron microscopy and X-ray crystallography on konjac fibres, it was shown that konjac glucomannan was an extended semi-flexible linear chain without branches in the form of a double helix stabilised by hydrogen bonds. It is a traditional food in Japan where it is exploited for its ability to form stiff gels and viscous solutions. It is used in gravies, sauces, glazes, soups, stews and casseroles. It is also a thickener in pies, puddings, custards and cake fillings. Being gluten-free, it is used as a substitute in cooking and baking for flour and other wheat products (Ying-quing, 2005; Vipul, 1997).

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Section 7
Binary & Ternary Interactions Between Polysaccharides

7 Binary & Ternary Interactions Between Polysaccharides

7.1 Binary Interactions (Harris, 1990)

In the 1980s scientists working on polysaccharide food ingredients at The Unilever research laboratory at Colworth House in the UK observed a new and as yet unreported phenomenon, namely that a number of binary combinations of some polysaccharides could interact in a synergistic manner (Ross-Murphy, 1995). This interaction appeared to enhance the rheological and gel-forming properties over and above those of each of the components and the property has been used in many applications where high functionality coupled with low concentration is desired.

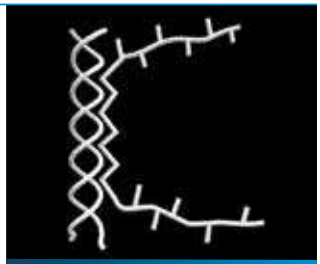


Fig 45 Postulated binary synergistic interaction between polysaccharides

Not all polysaccharides exhibit this behavior and the first synergistic complex formation was observed between xanthan gum and locust bean gum. Other examples were then discovered including a more general interaction between xanthan and galactomannans and glucomannans (xanthan-guar, xanthan-konjac glucomannan), carrageenan-guar and galactomannans and some starches (Dea, 1977).

Very little information has been published on the structure of these binary complexes with most studies concentrating on the rheological and gelation physical properties as will be discussed in the next chapter. The possible interaction of polymer chains has been illustrated as shown in Fig 45.

7.2 Ternary Interactions

More recently, synergistic interactions between three polysaccharides have been reported. The polysaccharides in question are xanthan, konjac glucomannan and sodium alginate and the observed behavior has been an extremely high viscosity at low concentration (Harding, 2011). A published study of this phenome non has concluded that the interactions are non-covalent and that in the ultracentrifuge a shift to a higher molecular weight from the individual components can be observed (Fig 46).

This interaction has been exploited in a product known as PGX (Polyglycoplex). When PGX is taken with meals, or added to a drink, it expands in the stomach over a 30-minute (or longer) timeframe and creates a feeling of fullness (satiety) by absorbing water and filling the stomach while slowing digestion. This keeps blood sugar from rising too high after meals thereby curbing the body's hunger cravings throughout the day (Smith 2014). Other benefits such as reducing blood sugar and cholesterol have been observed (Brand-Miller, 2010; 2012).

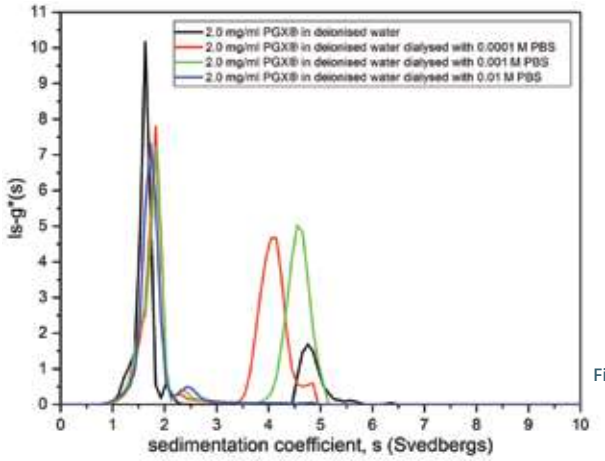


Fig 46 Shift to high MW from starting components in a ternary mixture of alginate, xanthan and konjac glucomannan

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Section 8
Polysaccharide Review

8 Introduction

This review covers all the polysaccharide categories that are now offered through the combined portfolios of Glycomix and Biosynth®. We have grouped and categorized the collection with respect to origin, source and product and have included both the natural polysaccharides and the chemical derivatives that are so useful in food, pharmaceuticals and industry. Each entry contains a representation of the polysaccharide covalent structure, a description of the source and a short description of the features that are key to the functional behavior shown.

8.1 Polysaccharides from Higher Plants

8.1.1 Energy Storage Polysaccharides

8.1.1.1 Starch (Radley, 1968; Robyt, 1998; Whistler, 1993)

Starch is an energy storing polysaccharide containing only α-1,4 and α-1,6-linked glucose residues. Starch is produced by most higher plants and some algae and is the most common carbohydrate consumed by humans. It is contained in large amounts in staple foods such as potatoes, wheat, maize (corn), rice, cassava and tapioca.

Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of polysaccharide: the linear and helical amylose (α-1,4-linked glucose) (Fig1R) and the branched amylopectin (α-1,4 and α-1,6-linked glucose) (Fig 2R). Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. Glycogen, the glucose store in animal tissues, is a more branched version of amylopectin. A variant of starch known as Waxy Maize starch contains very high levels of amylopectin.

In industry, starch is converted into glucose and glucooligosaccharides, for example by malting, and fermented to produce ethanol in the manufacture of beer, whisky and biofuel. It is processed to produce many of the sugars used in processed foods. Dissolving starch in warm water gives wheatpaste which can be used as a thickening, stiffening or gluing agent. The biggest industrial non-food use of starch is as an adhesive in the papermaking process. Starch can be applied to parts of some garments before ironing, to stiffen them. Waxy starch is used mainly in food products, but also in the textile, adhesive, corrugating and paper industry (BeMiller, 2009).

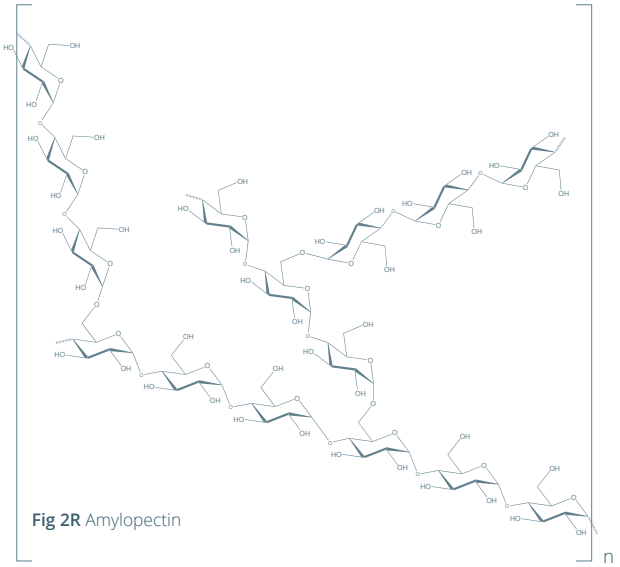


Fig 2R Amylopectin

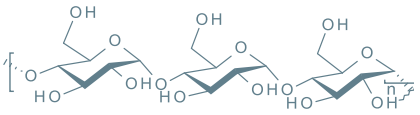


Fig 1R Amylose

8.1.1.2 Inulin

Inulin is a polysaccharide found in many vegetables such as chicory and jerusalem artichoke (Fig 3R). It is composed of β-1,2-linked fructopyranoside units with up to 60 fructose residues per chain (Fig 4R). Uses for inulin are as a diagnostic aid for kidney infections and as a low-calorie dietary fibre (Roberfroid 1993; Barclay 2010).



Fig 3R Jerusalem artichoke

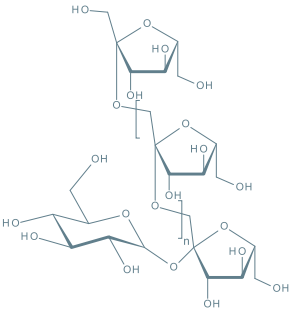


Fig 4R Inulin covalent structure

8.1.2 Structural Polysaccharides

8.1.2.1 Cellulose

Cellulose is probably the most abundant organic compound on the Earth and is the major structural component of the cell wall of higher plants. It occurs in a very pure form in the cotton boll (Fig 5R) and is also a major component of flax (80%), jute (60-70%), and wood (40-50%). Pure cellulose is also elaborated by some bacteria such as *Acetobacter xylinum* (Fig 6R) and related species (Ross, 1991).

Grasses such as papyrus and bamboo are also important sources of cellulose, and cellulosic pulps can be obtained from many agricultural by-products such as sugarcane, sorghum bagasse, corn stalks, and straws of rye, wheat, oats, and rice (Ward, 1983).

Celluloses from all sources are high molecular weight linear polysaccharides of D-glucopyranose residues linked β-1,4. No evidence has been found for branching (Fig 7R).

The conformation of this β-1,4-linked structure gives chains that have every other glucose residue rotated 180°, providing a high propensity to form intermolecular hydrogen bonds. This results in large aggregates of parallel cellulose chains that have crystalline properties and give X-ray diffraction patterns (Marchessault, 1983)

The tertiary structure of parallel-running intermolecular hydrogen-bonded cellulose chains further associate by hydrogen bonds and Van der Waals forces to produce three-dimensional microfibrils. The microfibrils give an X-ray diffraction pattern that indicates a regular, repeating crystalline structure interspersed by less-ordered paracrystalline regions (Brett, 2000).



Fig 5R Cotton

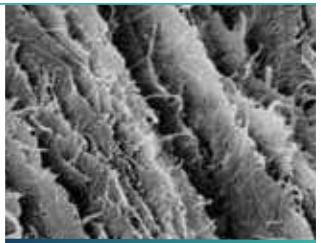


Fig 6R Electron micrograph of cellulose from *Acetobacter xylinum*

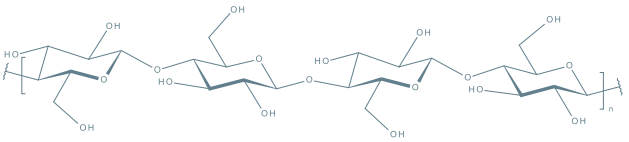


Fig 7R Cellulose

8.1.2.2 Cereal β–Glucans

Cereal β-glucans from oat, barley, wheat, and rye induce a variety of physiological effects that positively impact health. Barley and oat β-glucans (fig 8R) have been studied for their effects on blood glucose regulation in test subjects with hypercholesterolemia.

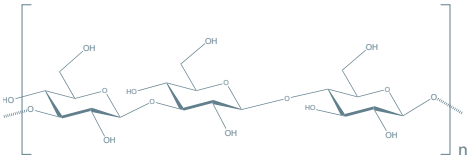


Fig 8R β-Glucan

Oat and barley glucans contain both β-1,3 and β-1,4 linkages but differ in the ratio of trimer and tetramer 1,4 linkages. Barley has more 1,4 linkages with a degree of polymerization higher than 4. However, the majority of barley blocks remain trimers and tetramers. In oats, β-glucan is found mainly in the endosperm of the oat kernel, especially in the outer layers of that endosperm (Keogh, 2003). Oat β-glucans are water-soluble β-glucans (Henry, 1987) derived from the endosperm of oat kernels known for their cholesterol lowering and hypoglycemic properties, as well as their use in various cosmetic applications. Recent research has shown their potential application in immunomodulation and wound healing.

8.1.2.3 Hemicelluloses and Related Polysaccharides

The hemicelluloses are a heterogeneous group of polysaccharides that vary from plant to plant and from one plant part to another. There are four basic types of hemicelluloses: D-xyloglucans, composed of D-xylopyranose attached to a cellulose chain; D-xylans, composed of D-xylose; O-mannans, composed of D-mannose; and D-galactans, composed of D-galactose. These polysaccharides are similar to cellulose in having their main chains linked β-1,4. Most of the hemicelluloses are, however heteropolysaccharides with one to three monosaccharide residues linked to the main monosaccharide chain.

8.1.2.3.1 Xylans

Xylan is a generic term used to describe a wide variety of highly complex polysaccharides that are found in plant cell walls and some algae (Percival, 1967).

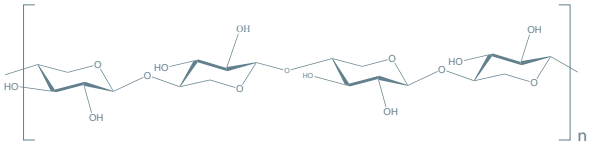


Fig 9R Linear xylan

Xylan is the most abundant noncellulosic polysaccharide present in both hardwoods and annual plants, and accounts for 20–35% of the total dry weight. In softwoods, xylans are less abundant and may comprise about 8% of the total dry weight. Xylan is found mainly in the secondary cell wall as part of the hemicellulose fraction and is considered to form an interface between lignin and other polysaccharides. It is likely that xylan sequences covalently link with lignin phenolic residues, and also interact with other polysaccharides, such as pectin and starch. In their simplest forms, xylans are linear homopolymers of β-1,4-xylose residues (Fig 9R).

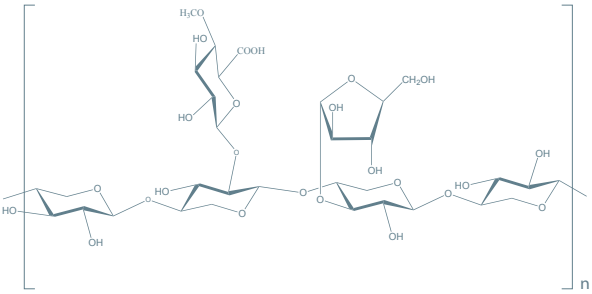


Fig 10R Corn Cob Xylan

In nature, they are partially substituted by acetyl, 4-O-methyl-D-glucuronosyl and α-1,3 L-arabinofuranosyl residues, forming complex heterogenous and polydispersed glycans. An example of this is in the L-Arabino (methyl-D-glucurono) xylan from corn cob (Fig 10R). Many structural aspects of xylans are unclear because of the difficulties associated with their isolation from the biomass complex without significant alteration or loss of the original structure (Ebringerova, 2000).

Xylans are also found in the cell walls of a number of green algae (Embryophyta), especially macrophytic siphonous genera, where they replace cellulose. Similarly, they replace the inner fibrillar cell-wall layer of cellulose in some red algae (Rhodophyta) (Percival, 1967; Usov, 1981; Usov, 1991).

8.1.2.3.2 Spruce Galactoglucomannan

This galactoglucomannan is a water-soluble hemicellulose consisting of galactose, glucose and mannose. Many softwood species, e.g. Norway spruce (Fig 11R) are rich in galactoglucomannans and may contain up to 10-20%. The polysaccharide is a linear backbone of randomly distributed 1,4-linked mannose and glucose with (1,6)-linked galactose units attached to the mannose residues (Fig 12R). The hydroxyl groups at C2 and C3 of the mannose residues are partially acetylated.



Fig 11R Norway spruce

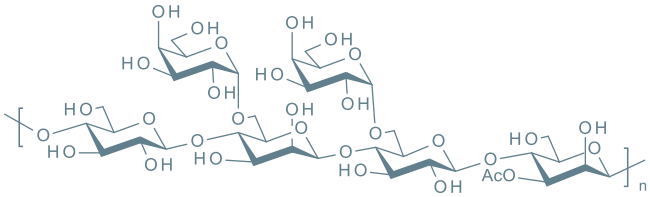


Fig 12R Spruce galactomannan

8.1.2.3.3 Larch Arabinogalactan (Whistler, 1993)

Larch arabinogalactan is extracted from the heartwood of the western larch *Larix occidentalis* (Fig 13R). These arabinogalactans have a backbone of 1,3-linked β -D-galactopyranosyl units each of which contains a side chain at position C-6. Most of these side chains are galacrobiosyl units containing α-1,6-β-D-linkages. Other side chain types that occur are monomeric L-arabinofuranose units or dimeric 3-O-(β-L-arabinopyranosyl)-α -L-arabinofuranosyl units (Fig 14R). Applications for this arabinogalactan are as emulsifiers, stabilizers and binders in the food, pharmaceutical and cosmetic industries. More recently, there has been interest in the use of this polysaccharide as a low viscosity dietary fibre and as a probiotic.



Fig 13R *Larix occidentalis* (western Latch)

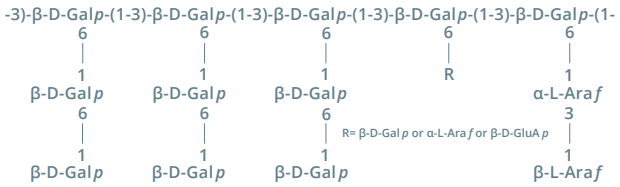


Fig 14R Covalent structure of larch arabinogalactan

8.1.2.3.4 Galactans

The characterisation of β-1,4-galactan as a distinct polysaccharide in the seeds of *Lupinus* albus was demonstrated by Hirst in 1947 (Hirst, 1947). Al-Kaisey and Wilkie (Al-Kaisey, 1992) demonstrated the presence of two types of galactans in lupin seeds namely, an α-1,3-linked galactan with some 4-linkages and an α-1,4 galactan substituted by L- arabinofuranose. Other linear β-1,4-galactans have been isolated from lemon peel (Labavitch, 1976), potato tubers (Wood, 1972), Norwegian spruce compression wood (Bouveng, 1959) and from acacia gums (Fig 15R).

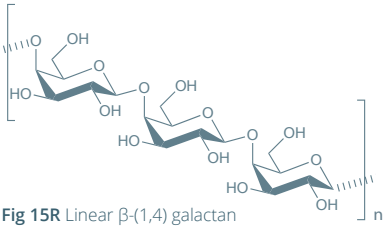


Fig 15R Linear β-(1,4) galactan

8.1.2.4 Pectin & Pectin-related Polysaccharides

Pectins are complex polysaccharides that occur in all plants, primarily in the cell wall, in low amounts of 1-5%. They are, however, particularly prevalent in fruits, where the amounts are much higher. For example, apple pulp contains 10-15% (w/w) pectin, orange, lemon and lime peel contain 20-30% (w/w). Pectins act as an intercellular cementing material that gives body to fruits and helps them keep their shape. When fruit becomes overripe, the pectin is broken down into its constituent monosaccharide sugars. As a result, the fruit becomes soft and loses its firmness.

Commercial pectins are extracted from citrus fruits such as limes and lemons and are 'stripped down' from the complex structures that exist in nature to contain a high percentage of polygalacturonic acid as described below.

The very complex structures that occur in nature have been the subject of research for many years, but are still not fully described. Later in this section, a number of the most important related polysaccharides such as apiogalacturonan, sugar beet arabinan, the pectic arabinogalactans and rhamnogalacturonans I and II will be discussed.

8.1.2.4.1 Commercial Pectin (Whistler, 1993; Harris, 1990)

Commercial pectins extracted mainly from citrus fruits are regarded as linear chains of α-1,4-linked D-galacturonic acid, a homogalacturonan (pectic acid) (Fig 16R). The polyuronide is partly methyl esterified and the free acid groups may be partly or fully neutralised with sodium, potassium or ammonium ions. In the products of commerce, the degree of methylation - termed the degree of esterification (DE) - has a vital influence on the properties of pectin, especially the solubility and gel-forming characteristics. In nature, around 80 percent of the galacturonic acid residues are methyl esterified and this proportion is decreased to a varying degree during pectin extraction but can be increased by treatment with methanol. Pectins are classified as high or low-methoxy pectins (HM-pectin and LM-pectin) and for example in jams and marmalades that contain above 60% sugar and soluble fruit solids, high-ester pectins are used. With low-ester and amidated pectins (treatment of pectin with ammonia) less sugar is needed, so that dietetic products can be made. The mechanism for gel formation with calcium ions is known as the 'egg box' (Fig 17R).

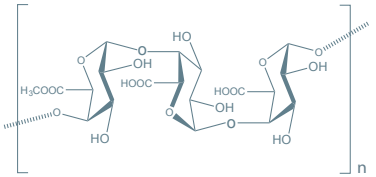


Fig 16R Covalent structure of pectic acid

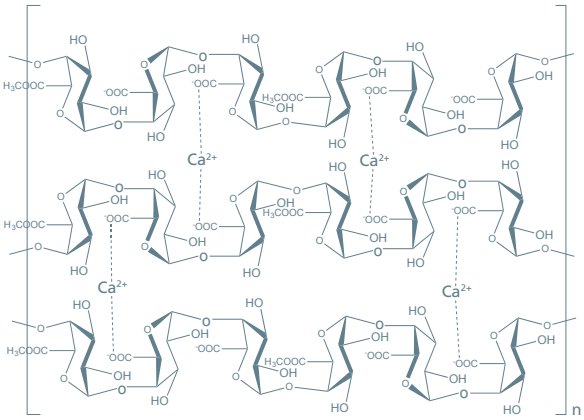


Fig 17R The 'egg box' model for pectin gelation

Rhamnogalacturonan I

Rhamnogalacturonan I (RG-I) contains a backbone of the repeating disaccharide: 4)-α-D-galacturonic acid-(1,2)-α-L-rhamnose-(1. The galacturonic acid residues are not normally substituted, but many of the rhamnose residues carry sidechains of various neutral sugars including D-galactose, L-arabinose and D-xylose. The types and proportions of neutral sugars vary with the origin of pectin (Yapo, 2011).

Rhamnogalacturonan II

Rhamnogalacturonan (RG-II) was first described in 1978 and its structure has subsequently been shown to be conserved in the primary walls of dicotyledenous and monocotyledenous plants and gymnosperms. RG-II is also likely to be present in the walls of some lower plants (ferns, horsetails, and lycopods). RG-II is a low molecular mass (5-10 kDa) pectic polysaccharide that is solubilized by treating a cell wall with endopolygalacturonase. RG-II contains eleven different glycosyl residues.

The backbone of RG-II contains at least eight 1,4-linked α-D-GalpA residues. Two structurally distinct disaccharides (C and D) are attached to C-3 of the backbone and two structurally distinct oligosaccharides (A and B) are attached to C-2 of the backbone (Vidal, 2000; Glushka, 2003) (Fig 18R).

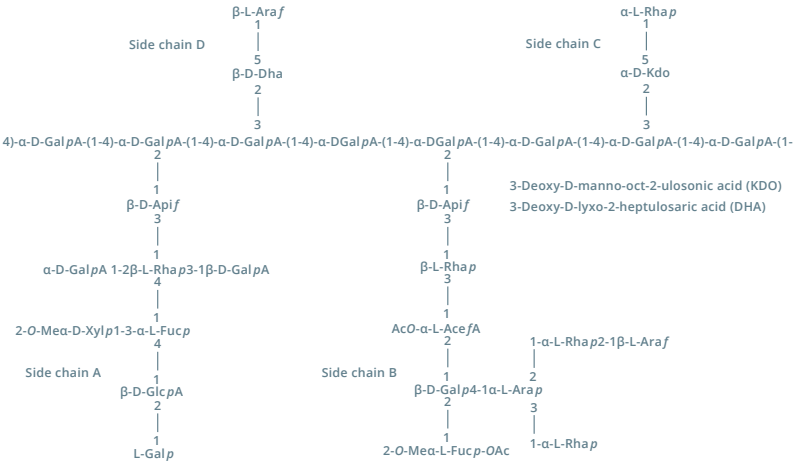


Fig 18R Rhamnogalacturonan II

8.1.2.4.2

Apiogalacturonan

Zosterin, an apiose-rich pectic-like polysaccharide which is found in the sea grass *Zostera marina* (Fig 19R), is typical of a number of similar structures occurring in higher plants. The structure consists of an α-1,4-D-galactopyranosyluronan backbone substituted by 1,2-linked apiofuranose oligosaccharides and single apiose residues (Fig 20R). The average molecular mass of the polysaccharide has been shown to be about 4100 Da with a low polydispersity (Gloaguen, 2010).



Fig 19R *Zostera marina* (©M.D. Guiry)

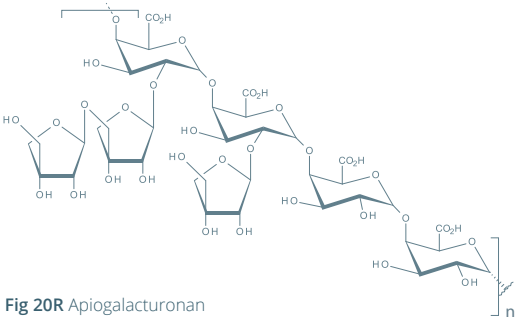


Fig 20R Apiogalacturonan

8.1.2.4.3 Arabinan (Sugar beet)

Arabinans appear in the primary cell walls of different families of plants in seeds, fruits, and roots (e.g. sugar beet) (Fig 21R), usually as pectic polysaccharide side chains or as free polymers unattached to pectic domains. Sugar beet arabinan consists of a 1,5-α-linked backbone to which 1,3-α-linked (and possibly some 1,2-α-linked) L-arabinofuranosyl residues are attached (Fig 22R). Approximately 60% of the main-chain arabinofuranosyl residues are substituted by single 1,3-linked arabinofuranosyl groups. The reducing terminal arabinosyl residue is attached through rhamnose to fragments of the rhamnogalacturonan backbone of the native pectin molecule (Wefers, 2016).



Fig 21R Sugar beet, *Beta vulgaris*

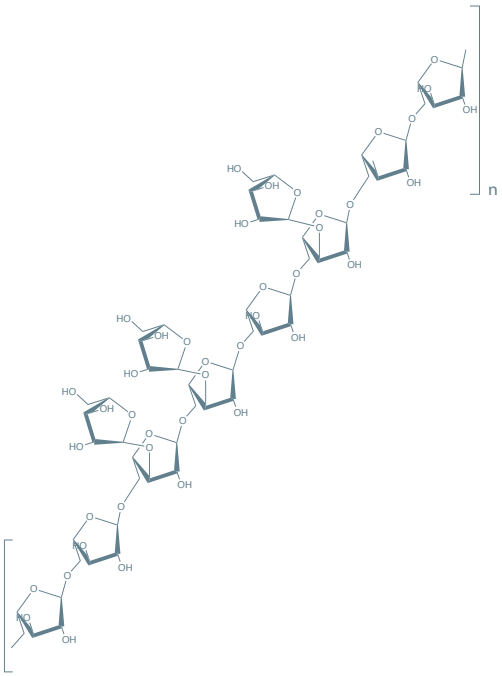


Fig 22R Arabinan

8.1.2.4.4 Pectic Arabinogalactans and Rhamnogalacturonans

Arabinogalactan I

Type I arabinogalactans (AGs) are abundant in primary walls of dicotyledonous plant tissues including many fruits (Perez, 2000). They comprise a main backbone of 1,4-linked β-D-galactose to which short side chains of α-L-arabinose are attached at the O3 position (Fig 23R). Ferulic acid has been found attached to some arabinose and galactose residues in some tissues such as sugar beet (Morris, 2010).

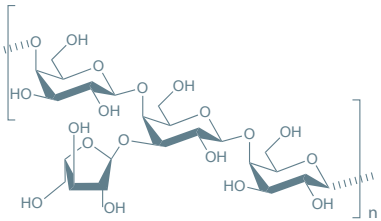


Fig 23R Arabinogalactan I

Arabinogalactan II

Type II Arabinogalactans have been extracted along with pectic polymers with which they were probably covalently linked on rhamnogalacturonan (Vincken, 2003). They exhibit highly complex structures comprising a highly branched galactan core of 1,3- and 1,6-linked β-D-galactose residues. Short side chains of 1,6-linked β-D-Galactose including between one and three residues in length are present. Galactosyl residues in these side chains are often substituted with terminal α-L-Arabinose attached at O3 or O6 (Fig 24R).

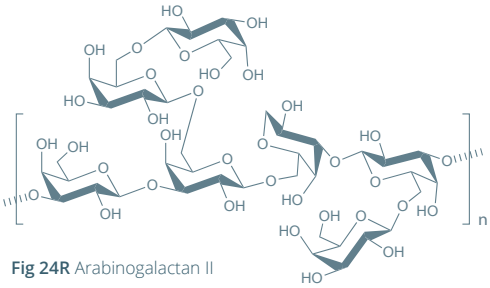


Fig 24R Arabinogalactan II

8.1.3 Polysaccharides from Seeds & Legumes

8.1.3.1 Pysillum Seed Gum (Whistler, 1993)

Psyllium seed gum comes from plants of the *Plantago* genus (Fig 25R) and is cultivated mainly in the Mediterranean and in India. Psyllium has been used for many years in medical applications and more recently the polysaccharide has attracted interest because of its increasing value as a soluble dietary fibre. The proposed structure is of a backbone of D-xylopyranosyl units linked 1,4 and 1,3 with the 4-linked units bearing side chains. The side chains consist of α-L-arabinofuranosyl units linked 1,3 and 1,2 and β-D-xylopyranosyl units linked 1,3 and 1,2 and the α-D-GalAp-1,2-α-L-Rhap aldobiuronic acid units linked 1,2 to the main chain (Fischer, 2004; Gibb, 2015) (Fig 26R).



Fig 25R Plantago

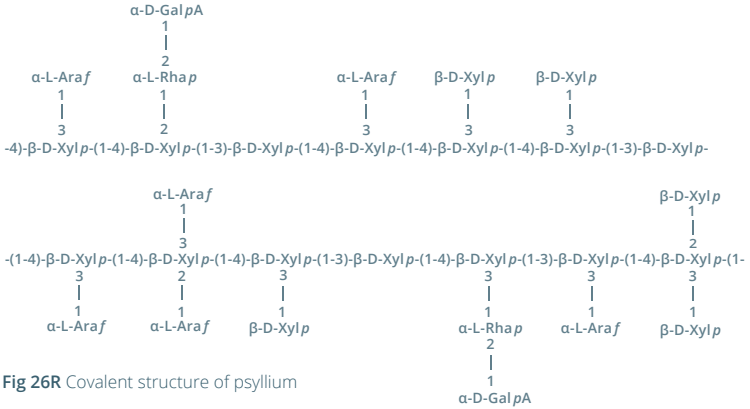


Fig 26R Covalent structure of psyllium

8.1.3.2 Guar Gum (Whistler, 1993)

Guar gum is obtained from the seed of the legume *Cyamopsis tetragonolobus*, (Fig 27R) an annual plant that grows mainly in semi-arid regions of India. The structure of the polysaccharide consists of a main chain of 1,4-linked β-D-mannopyranosyl units with single α-D-galactopyranosyl units linked 1,6 on average to every second main chain residue (Fig 28R). Guar has a high viscosity in aqueous solution, shows marked pseudoplastic behavior and forms synergistic gels in the presence of other gums such as carrageenan and xanthan gum. In the food industry, xanthan gum and guar gum combinations are the most frequently used gums in gluten-free recipes and gluten-free products. Industrial guar gum accounts for about 70% of the total demand. It is used mainly as a proppant (sand-based fracking fluid) in hydraulic fracturing Process.



Fig 27R Seeds of *Cyamopsis tetragonolobus*

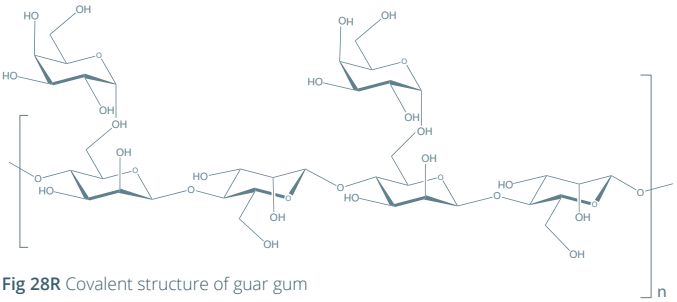


Fig 28R Covalent structure of guar gum

8.1.3.3 Ivory Nut Mannan

Ivory nut mannan occurs in the fruit (nuts) of members of the custard apple, ebony and palm families (ivory nut) (Fig 29R). The polysaccharide contains more than 95% mannose units linked β-1,4 with a few α-D-galactopyranosyl units linked 1-6 (Fig 30R). It is widely used in foods, pharmaceuticals paints and explosives (Timell, 1957).



Fig 29R Ivory nuts

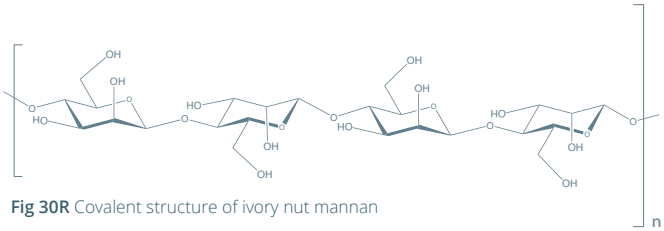


Fig 30R Covalent structure of ivory nut mannan

8.1.3.4 Locust Bean Gum (Whistler, 1993)

Locust bean (carob) gum is the refined endosperm of the seed of the carob tree, an evergreen of the legume family (Ceretonia siliqua) (Fig 31R). The tree grows extensively in Spain and is cultivated in many other Mediterranean countries. Locust bean gum like guar gum is a galactomannan with a backbone of 1,4 β-D-mannopyranosyl units having branches of 1,4-linked α-D-galactopyranosyl units (Fig 32R). However, locust bean gum has substantially fewer side chains than guar gum and these are clustered in blocks leaving longer regions of unsubstituted mannosyl regions. The gum is only partially soluble in water and suspensions require heating before solubility is achieved. As with guar the polysaccharide forms gels with other gums such as carrageenan and xanthan. Applications are in the food industry to enhance texture, in paper making and in the textile industry.



Fig 31R *Ceretonia siliqua* (carob tree)

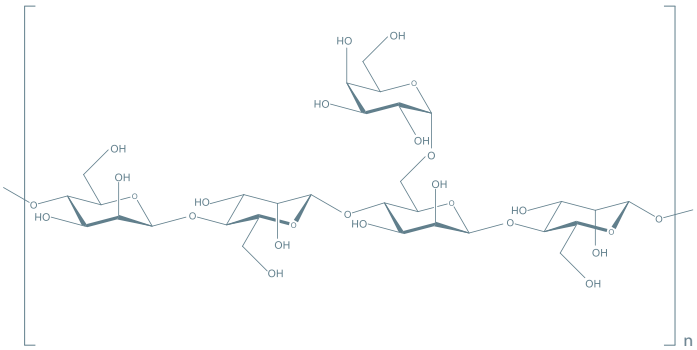


Fig 32R Covalent structure of locust bean gum

8.1.3.5 Tamarind Gum (Whistler, 1993)

Tamarind gum comes from the seed of the tamarind tree *Tamarindus indica* (Fig 33R) and is used in the food industry as a stabiliser and an emulsifier. It has been suggested that the structure is a main chain of cellulose with frequent branching at the O-6 positions with short side chains of one or two D-xylopyranosyl capped with D-xylopyranosyl, D-galactopyranosyl or L-arabinofuranosyl units (Fig 34R). In application tamarind finds use in the sizing of jute and as a stabiliser and thickener in the food industry.



Fig 33R *Tamarindus indica* (tamarind tree)

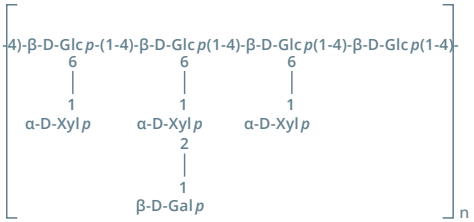


Fig 34R Covalent structure of tamarind gum

8.1.3.6 Tara Gum (Whistler, 1993)

Tara gum is a galactomannan that is obtained from the seeds of the tara shrub *Caesalpinia spinosa* (Fig 35R) which is a native of the northern regions of Africa and South America. The gum is described as having a backbone of 1,4-linked β-D-mannopyranosyl units one third of which possess a single unit side chain of 1,4-linked α-D-galactopyranose (Fig 36R). The gum forms gels with carrageenan and xanthan in a similar manner to guar and locust bean gum. Currently only small quantities of tara gum are sold for use as food thickeners and stabilizers.



Fig 35R *Caesalpinia spinosa* (tara shrub)

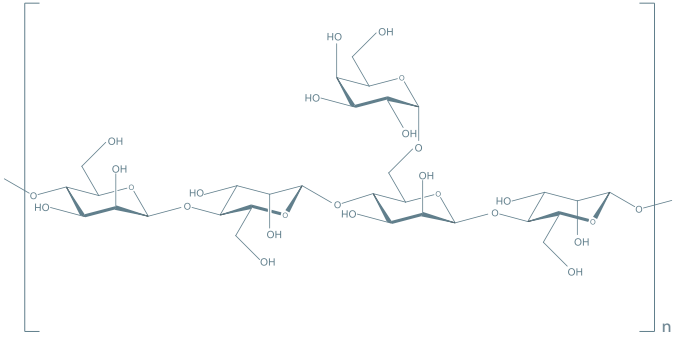


Fig 36R Covalent structure of tara gum

8.1.4 Exudate Gums

Exudate gums are polysaccharides produced by plants to seal wounds in their bark. The plant exudes an aqueous solution of polysaccharide around a break in the bark, covering the injury. The water evaporates, leaving a polysaccharide that hardens and prevents infection and desiccation of the plant.

8.1.4.1 Gum Arabic (Whistler, 1993)

Gum arabic is an exudate gum picked from Acacia trees growing in arid regions (typically *Acacia senegal* (Fig 37R) and *Acacia laetia*). The polysaccharide is branched with a main chain of 1,3 linked β-D-galactopyranosyl units with side chains of 1,3 β-D-galactopyranosyl units joined to it by 1,4 links. The side chains are 2-5 units in length. Both the main chain and the side chains have attached units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units (Fig 38R) (Stephen, 1990).



Fig 37R *Acacia senegal*

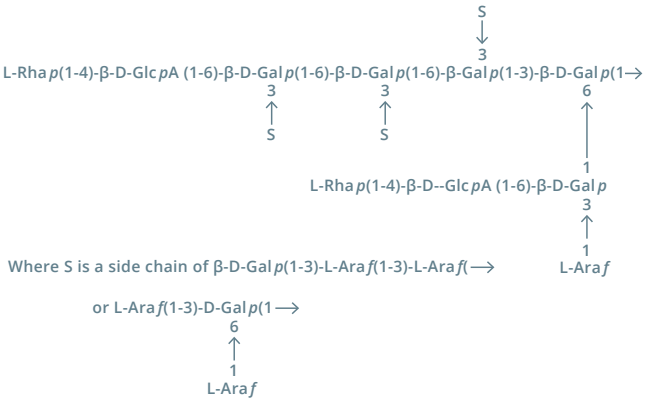


Fig 38R Gum Arabic covalent structure

8.1.4.2 Gum Ghatti (Whistler, 1993)

Gum Ghatti is an exudate gum from the tree *Anogeissus latifolia* (Fig 39R) found in India and Sri Lanka. Applications are similar to gum arabic in the food and pharmaceutical industries where it is used as an emulsifier. The polysaccharide is reported to have an extremely complex structure containing both oligosaccharides and polysaccharide elements. The polysaccharide contains Ara, Gal, Man, GlcA and Rha in a molar ratio of 61:39:6:10:6, a backbone of 1,4-linked β-D-galactopyranosyl units and side chains of L-arabinofuranose units with some 1,4-D-glucopyranosyluronic acid units, some joining 1,2-D-mannopyranosyl units. The oligosaccharides are reported to mimic the side chains and include the structures depicted below (Fig 40R).



Fig 39R *Anogeissus latifolia*

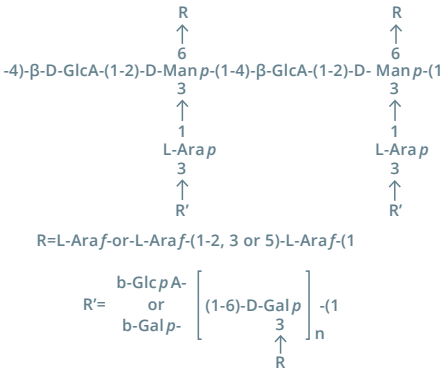


Fig 40R Partial structure of gum ghatti

8.1.4.3 Gum Karaya (Whistler, 1993)

Gum Karaya is exuded from *Sterculia urens* (Fig 41R), a bushy tree found in dry regions of North India. Due to its extensive swelling capacity in water it is used as a laxative and as a denture adhesive.The structure consists of D-galactose, D-glucuronic acid and L-rhamnose but the detailed molecular structure is still incompletely known (Fig 42R) (Aspinall, 1970).

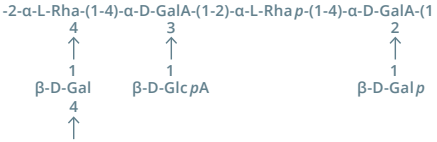


Fig 42R Covalent structure of gum karaya



Fig 41R *Sterculia urens*

8.1.4.4 Gum Tragacanth (Whistler, 1993)

Gum Tragacanth is an exudate gum from species of Astragalus trees (Fig 43R) mainly grown in Iran and Turkey. Tragacanth finds applications as an effective emulsifying and thickening agent in the food and pharmaceutical industries. The gum is a slightly acidic salt consisting of two fractions namely the water-soluble tragacanthin and the bassorin fraction which swells in water to form a gel. Water-soluble tragacanthin is reported as a branched arabiogalactan which is soluble in 70% ethanol. The acidic bassorin has a chain of 1,4-linked α-D-galacturonic acid units some of which are substituted at O-3 with β-D-xylopyranosyl units and some of these being terminated with galactose or fucose (Anderson, 1985) (Fig 44R).

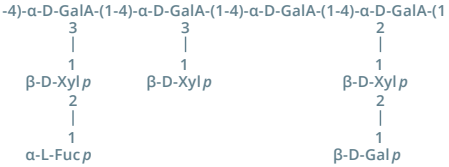


Fig 44R Covalent structure of gum tragacanth



Fig 43R Astralagus tree

8.1.5 Polysaccharides from Tubers

8.1.5.1 Konjac Glucomannan

Konjac glucomannan is an acetylated 1,4-β-D-glucomannan (Fig 45R) obtained from the tubers of Amorphophallus konjac (Fig 46R) or Konnyaku root. It is used in Japan in many food applications as an ingredient and due to its property of swelling in water is seen as a dietary supplement for reducing calorie intake (Vulksan, 1999; Maeda, 1980).

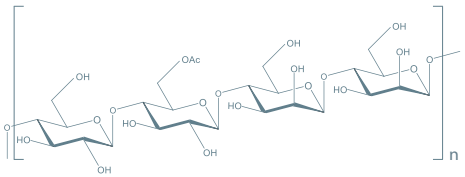


Fig 45R Covalent structure of konjac glucomannan



Fig 46R *Amorphophallus konjac*

8.2.1 Lichenan and Isolichenan



Fig 47R *Cetraria islandica*

Lichenin, also known as lichenan or moss starch, is a complex glucan occurring in certain species of lichens. It can be extracted from *Cetraria islandica* (Iceland moss) (Fig 47R). Chemically, lichenin consists of repeating glucose units linked by β-1,3 and β-1,4 glycosidic bonds (Fig 48R).

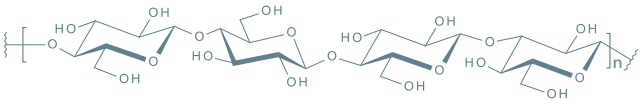


Fig 48R Lichenin

Lichenin, the poly β-D-Glucan of *Cetraria islandica* (Iceland moss), is found by enzymic degradation to differ in fine structure from the poly β-D-glucans of cereal grains. Enzymolysis was with cellulase and laminarinase, the former yielding mainly O-β-D-glucopyranosy-1,3-O-β-D-glucopyranosyl-1,4-α-D-glucose, and the latter mainly O-β-D-glucopyranosyl-1,4-O-β-D-glucopyranosyl-1,3-α-D-glucose. Di and tetra-saccharides are produced in small proportions. Thus, the basic structure of lichenin is a tetrameric unit in which two adjacent 1,4 linkages alternate with an isolated 1,3 linkage; occasionally four consecutive monomers are linked by 1,4 bonds (Perlin, 1962).

Isolichenan is a cold-water soluble 1,3-1,4-α-D-glucan also isolated from *Cetraria islandica* (Fig 47R) and reported to have MW of about 6-8 kDa (Peat, 1961b). A more recent investigation could not confirm the existence of such a small α-glucan in *Cetraria islandica*; instead, a much larger isolichenan with a linkage ratio of 2:1 according to NMR data was found (Fig 49R). The definitions "isolichenan-type" polysaccharide or sometimes only "isolichenans" have been used for α-D-glucans having 1,3-1,4-linkages in their main chain. Lichens produce isolichenan-type polysaccharides with considerable variation in linkage ratios as well as MW, even within the same species. Occasionally these α-glucans can be branched at O2, O3 or O6 (Olafsdottir, 1999). The immunomodulatory activity of isolichenan was tested in *in vitro* phagocytosis and anti-complementary assays, and proved to be active in both cases.

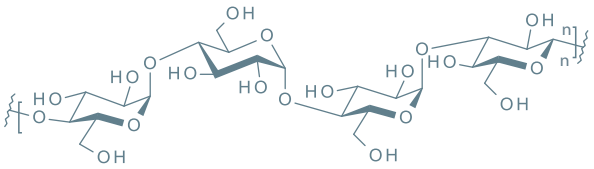


Fig 49R Isolichenin

8.2.2 Pustulan

Pustulan is a β-1,6-glucan (Fig 50R) from *Lasallia pustulata* (Fig 51R), MW (20 kDa). Pustulan is recognized by the membrane bound Dectin-1, a C-type lectin-like pattern recognition receptor. Detection of β-glucans by Dectin-1 receptor leads to the CARD9-dependent activation of NF-κB and MAP kinases. Studies have shown that pustulan can stimulate innate immune responses, inducing heat shock protein expression, eliciting phagocytosis, and the production of pro-inflammatory cytokines (Karunaratne, 2012).



Fig 51R *Lasallia pustulata*

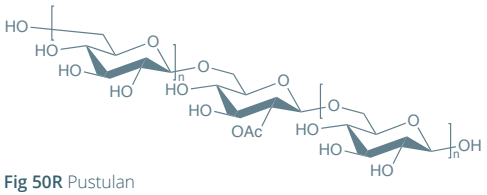


Fig 50R Pustulan

8.3 Polysaccharides from Algae

8.3.1 Polysaccharides from Rhodophyceae (Red algae)

8.3.1.1 Agar (Whistler, 1993)

A sulphated galactan from the red seaweeds (*Gelidium* spp.) (Fig 52R).



Fig 52R *Gelidium latifolium* (©M.D. Guiry)

The major gel forming component agarose consists of a linear chain of sequences of 1,3 linked β-D-galactopyranosyl units and 1,4 linkages to 3,6-anhydro-α-L-galactopyranosyl units (Fig 53R). Gelation is via the formation of double helices as shown below (Fig 54R). Agar is primarily used as a plating gel for microbial cultures (Lahaye, 1991).

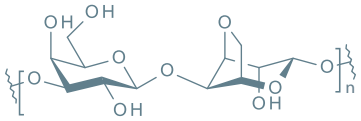


Fig 53R Covalent structure of agarose

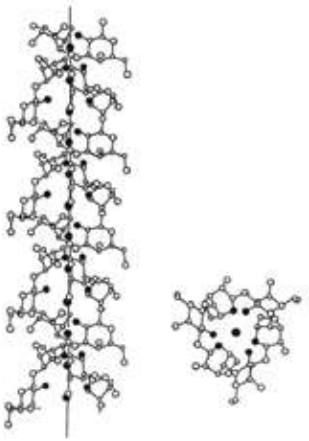


Fig 54R Agarose double helix

The representation of the agarose double helix shown in Fig 54R is viewed normal and parallel to the helix axis. The small filled circles are O2 atoms of galactose and O5 of anhydrogalactose that line the inner cavity of the double helix. The large filled circles on the molecular axis are possible sites for ordered water molecules within the hydrogen-bonding distance of the oxygen atoms lining the cavity (Arnott, 1974).

8.3.1.2 Carrageenan

8.3.1.2.1 λ Carrageenan (Whistler, 1993)

Lambda-Carrageenan is a non-gelling sulphated galactan extracted from red seaweed (typically *Gigartina stellata* and *Chondrus crispus*) (Fig 55R). The structure of all carrageenans consists of a strictly alternating masked repeating unit of 1,3-linked α-D-galactose and 1,4-linked β-D-galactose. λ-Carrageenan has the α-linked unit 2-6-disulphated and the β-linked unit is 2-sulphated (Lawson, 1968) (Fig 56R).

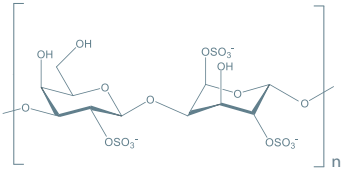


Fig 56R Covalent structure of λ carrageenan



Fig 55R *Chondrus crispus* (©M.D. Guiry)

8.3.1.2.2 K Carrageenan (Whistler, 1993)

Kappa-Carrageenan is a gelling sulphated galactan extracted from red seaweed (typically *Mastocarpus stellata* (Fig 57R) and *Chondrus crispus*). The structure of all carrageenans consists of a strictly alternating masked repeating unit of 1,3-linked α-D-galactose and 1,4-linked β-D-galactose. The α-linked galactose occurs as α-3-6-anhydro unit and the β-linked sugar occurs as the 4-sulphate (Anderson, 1968) (Fig 58R).

Space-filling model of the double helix which is responsible for the gelling behavior of kappa- and iota-carrageenan (Lawson, 1970) (Fig 59R).

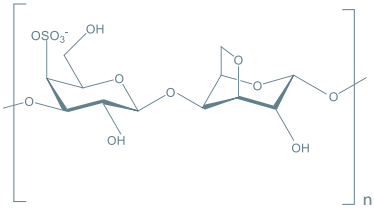


Fig 58R Covalent structure of k carrageenan



Fig 59R Space-filling model of the κ and ι double helix



Fig 57R *Mastocarpus stellata* (©M.D. Guiry)

8.3.1.2.3 ι Carrageenan (Whistler, 1993)

Iota-Carageenan is a gelling sulphated galactan extracted from red algae (typically *Euchuma cottonii* and *Euchuma spinosum*) (Fig 60R). The structure of all carrageenans consists of a strictly alternating masked repeating unit of 1,3 linked α-D-galactose and 1,4 linked β-D-galactose. The α-linked galactose occurs as α-3-6-anhydro-2-sulphate unit and the β-linked sugar occurs as the 4-sulphate (Lawson, 1973) (Fig 61R).

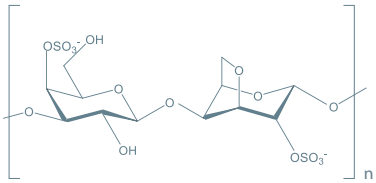


Fig 61R Covalent structure of ι carrageenann



Fig 60R *Euchuma spinosum* (©M.D. Guiry)

8.3.1.3 Porphyran

Porphyran is a highly substituted agarose with a linear backbone consisting of 3 linked β-D-galactosyl units alternating with either 4-linked α-L-galactosyl 6-sulphate or 3-6-anhydro-α-L-galactosyl units. The composition includes 6-O-sulphated L-galactose, 6-O-methylated D-galactose, L-galactose, 3,6-anhydro-L-galactose, 6-O-methyl D-galactose and ester sulphate. Some of the ester is present as 1,4-linked L-galactose 6-sulphate (Rees, 1962; Peat, 1961) (Fig 62R). The precise composition of porphyran shows seasonal and environmental variations. In *Porphyra haitanensis*, the L-residues are mainly composed of alpha-L-galactosyl 6-sulphate units, and the 3,6-anhydro-galactosyl units are minor. In *Porphyra capensis*, the ratio of alpha-L-galactose-6-sulphate and the 3,6-anhydrogalactose is 1.2:1 (Zhang, 2005). Porphyran is not used commercially, but the seaweed, *Porphyra umbilicalis* (Fig 63R) is edible and is consumed in Wales (Laver). It is also made into a delicacy called Laverbread.

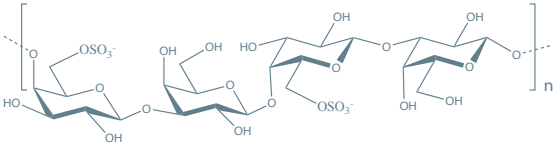


Fig 62R Covalent structure of porphyran



Fig 63R *Porphyra umbilicalis* (©M.D. Guiry)

8.3.1.4 Furcellaran, Danish Agar

Furcellaran (Danish agar) is similar to κ-carrageenan but is less sulphated (50%) (Fig 64R). It has been extracted from *Furcellaria lumbricalis*, which is mainly harvested off the coast of Denmark (Fig 65R).

This species, which is common to most parts of Europe, occurs as a loose form and only reproduces vegetatively.

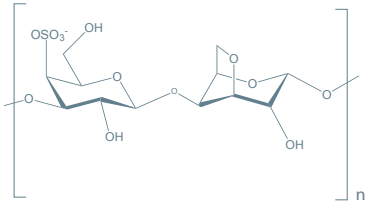


Fig 64R Furcellaran



Fig 65R *Furcellaria lumbricalis* (©M.D. Guiry)

8.3.2 Polysaccharides from Phaeophyceae (Brown algae)

8.3.2.1 Alginate (Whistler, 1993; Percival, 1967)



Fig 66R *Ascophyllum nodosum* (©M.D. Guiry)

A linear polysaccharide obtained from the brown seaweeds (e.g. *Laminaria hyperborea, Fucus vesiculosus, Ascophyllum nodosum*) (Fig 66R). The chemical structure consists of blocks of 1,4 linked-β-D-polymannuronic acid (poly M), 1,4 linked-α-L-polyguluronic acid (poly G) and alternating blocks of the two uronic acids (poly MG) (Fig 67R). The main use for alginate is in textile printing where it is used in the printing of cottons with reactive dyes as a thickener. It is also used as a thickener in the food industry. Alginates form strong gels with divalent metal cations and the 'egg box' model has been used to describe this form of gelation (see below). The propylene glycol ester of alginate is produced by reacting it with with propylene oxide. It is mainly used as a thickener in ice creams.

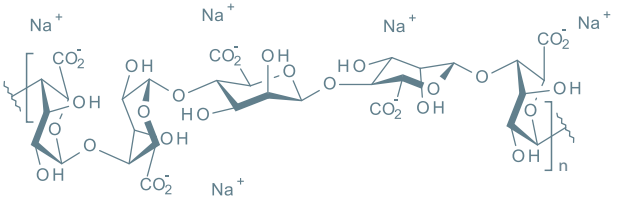


Fig 67R Alginate covalent structure

8.3.2.2 Laminaran



Fig 68R *Laminaria digitata* (©M.D. Guiry)

Laminaran is a polysaccharide that co-occurs with fucoidan and alginate in brown seaweeds such as *Laminaria digitata* (Fig 68R), *Ascophyllum nososum, Eisenia bicyclis* and *Thallus laminariae*. It is a β-1,3-linked glucan (Fig 69R) which it is claimed stimulates the immune system in mammals and fish (Nelson, 1974; Morales-Lange, 2015).

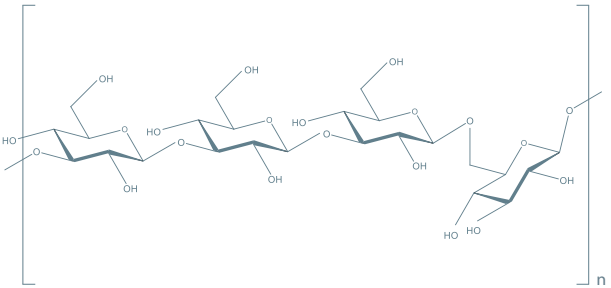


Fig 69R Covalent structure of laminaran

8.3.2.3 Fucoidan (Dumitriu, 1996)

Fucoidan is a fucan sulphate occurring in brown marine algae (Phaeophyta, typically *Fucus serratus* (Fig 70R), *Ascophyllum nodosum, Laminaria digitata* and *Macrocystis pyrifyra*) and has been shown to have anticoagulant activity. The main constituents are α-1,4 and α-1,2 linked L-fucose sulphates although galactose also occurs and there are many variations of the basic structure found in different species of Phaeophyta (Fig 71R).

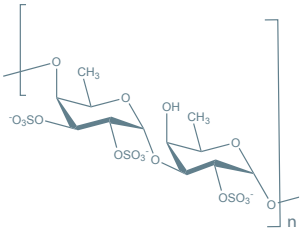


Fig 71R Covalent structure of Ascophyllum Nodosum fucoidan



Fig 70R *Fucus serratus* (©M.D. Guiry)

8.3.2.4 Fucogalactan

Fucogalactan (GFS) is a polysaccharide isolated and purified from the brown seaweed *Undaria pinnatifida* (Fig 72R). The polysaccharide is a sulphated galactose containing fucan (Fig 73R). GFS is currently under investigation for possible therapeutic indications including anti-inflammatory properties, immuno-modulating activities, inhibition of tumor growth, stem cell replentishment, antiviral activity, dementia and ulcer healing (Wang, 2010; Skriptsova, 2010).

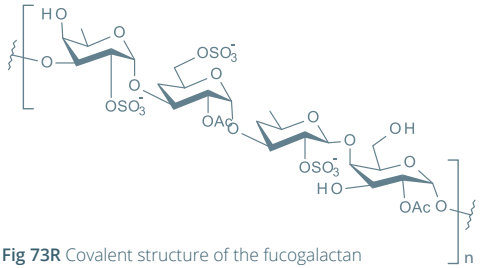


Fig 73R Covalent structure of the fucogalactan from undaria pinnatifada



Fig 72R *Undaria pinnatifida* (©M.D. Guiry)

8.3.3 Polysaccharides from Chlorophyceae (Green algae)

8.3.3.1 Ulvan

Ulvans are structural polysaccharides present in the cell walls of green algae such as *Ulva armoricana* (Fig 74R), *Ulva rotondata, Ulva rigida, Ulva lacterca* and *Ulva pertusa* (Ray 1995a; 1995b).

They are highly sulphated and contain rhamnose 3-sulphate, xylose, xylose 2-sulphate, glucuronic acid and iduronic acid residues (Fig 75R).

Ulvan has several potentially valuable functionalities such as gel formation for agricultural and food applications and possible anticoagulant, antioxidant, antihyperlipidemic and antitumor activities for pharmaceutical applications (Kaeffer, 1999).

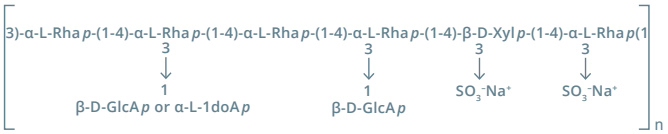


Fig 75R Ulvan from Ulva pertusa



Fig 74R *Ulva amoricana* (©M.D. Guiry)

8.4 Polysaccharides from Microorganisms

8.4.1 Polysaccharides from Gram-negative Bacteria

8.4.1.1 Bacterial Alginate

An alginate-like polysaccharide produced by the fermentation of *Azotobacter vinelandii* (Gorin, 1966; Deavin, 1977) (Fig 76R) or *Pseudomonas mendocina* (Hacking, 1983). The chemical structure consists of blocks of 1,4 linked-β-D-polymannuronic acid (poly M), 1,4 linked-α-L-polyguluronic acid (poly G) and alternating blocks of the two uronic acid (poly MG). Unlike the alginate from seaweed this polysaccharide is partially acetylated.

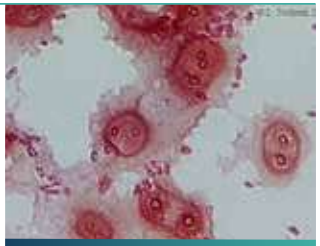


Fig 76R *Azotobacter vinelandii* showing alginate capsules

8.4.1.2 Gellan Gum (Whistler, 1993)

Gellan is a microbial polysaccharide produced by *Pseudomonas elodea* and produces gels having similar properties to agar. Gellan gum is a linear tetrasaccharide of 1,4-β-L-rhamnopyranosyl, 1,3-α-D-glucopyranosyl, 1,4-β-D-glucuronopyranosyl, 1,4-β-D-glucopyranosyl- with O2 L-glyceryl and O(6) acetyl substituents on the 3-linked glucose. Both substituents are located on the same glucose residue, and on average, there is one glycerate per repeat and one acetate per every two repeats (Fig 77R). In low acyl gellan gum, the acyl groups are removed completely. The high acyl form produces soft, elastic, non-brittle gels, whereas the low acyl form produces firm, non-elastic, brittle gels (Fig 78R).



Fig 78R High and low acyl forms of gellan gum

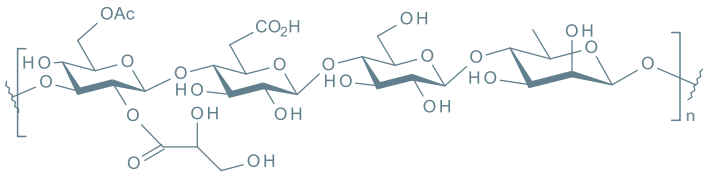


Fig 77R Covalent structure of gellan gum

8.4.1.3 Welan Gum (Whistler, 1993)

Welan gum is a microbial polysaccharide produced by a species of *Alcaligenes* and shows interesting rheological properties of use in the oil and agricultural industries. The structure is similar to gellan based on repeating glucose, rhamnose and glucuronic acid units but with a single side chain of either an α-L-rhamnopyranosyl or an α-L-mannopyranosyl unit linked 1,3 to the 4-O-substituted β-D-glucopyranosyl unit in the backbone (Fig 79R).

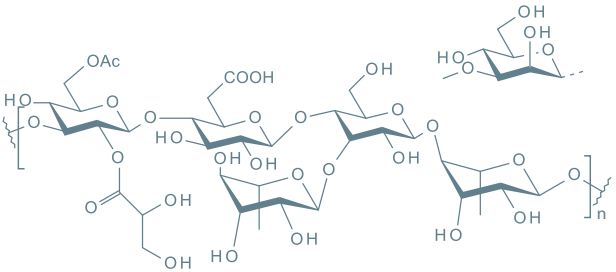


Fig 79R Covalent structure of welan gum

8.4.1.4 Xanthan Gum (Whistler, 1993; Sandford, 1977; Blanshard, 1979)

Xanthan gum is a microbial polysaccharide produced by *Xanthomonas campestris*. It was developed in the 1960s from research conducted at The Northern Regional Research Labs, Peoria, USA and has been produced commercially for many years (Fig 80R). It has unique rheological and gel forming properties and finds many applications particularly in the food and oil industries.



Fig 80R Fermentation plant for xanthan gum

The structure of xanthan is based on a cellulosic backbone of β-1,4-linked glucose units which have a trisaccharide side chain of mannose-glucuronic acid-mannose linked to every second glucose unit in the main chain. Some terminal mannose units are pyruvylated and some of the inner mannose units are acetylated (Rees, 1976; Lawson, 1977) (Fig 81R).

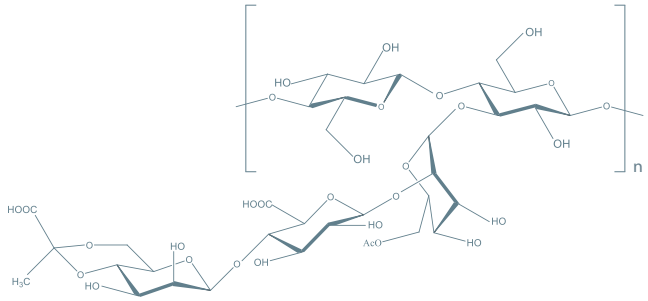


Fig 81R Covalent structure of xanthan gum

8.4.1.5 Dextran (Whistler 1993)

Dextran is α-1,6-linked α-D-glucan with α-1,3-linked glucose branch points (Fig 82R) produced by fermentation of *Leuconostoc mesenteroides* (Fig 83R) via the action of the enzyme dextransucrase on sucrose. The main use for native dextran is as an extender in blood transfusions and products having a range of sharp cut-off molecular weights are produced commercially for this and other applications. A complex of iron with dextran known as iron dextran is used as a source of iron for baby piglets which are often anaemic at birth (Sidebotham, 1974).

Dextran is a huge problem in the cane sugar industry due to the production of dextran in sugar cane following harvesting when the cut ends are exposed and become infected with *Leuconostoc mesenteroides*. This has the result of slowing down the processing of the cane in raw sugar factories and farmers are paid according to how much dextran is detected on delivery of the cane for processing (Ravno, 2005).

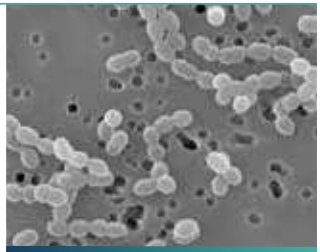


Fig 83R *Leuconostoc mesenteroides*

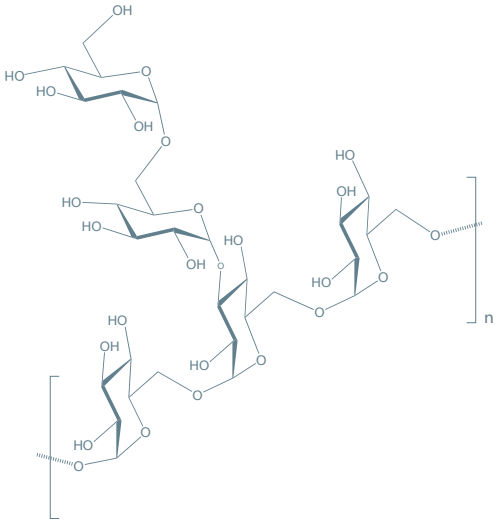


Fig 82R Covalent structure of dextran

8.4.1.6 Arthrobacter viscosus NRRL 1973 Exopolysaccharide

The polysaccharide has a linear structure and consists predominantly of repeating trisaccharide units, -O-(3-D-mannuronic acid-1,4-O-(3-D-glucopyranosyl-1,4-D-galactose. 50% of the hydroxyl groups are acetylated (Sloneker, 1968) (Fig 84R).

The physical properties for both native and deacetylated forms of the polysaccharide include high viscosity in water and in the presence of chlorides, shear and pH stability, and good film forming properties.

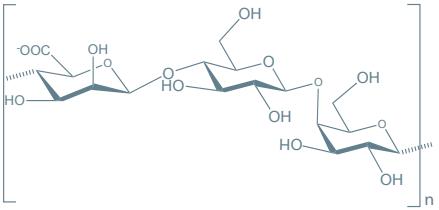


Fig 84R Covalent structure of Arthrobacter viscosus exopolysaccharide

8.4.1.7 Arthrobacter stabilis NRRL B3225 Exopolysaccharide

The extracellular polysaccharide produced by *Arthrobacter stabilis* NRRL B-3225 contains D-glucose, D-galactose, pyruvic acid, O-succinyl, and O-acetyl in the approximate molar ratio of 6:3:1:1:1.5. Succinyl is linked as its half-ester, making it readily removable. The viscosity of aqueous, salt-free solutions of both native and deacylated polymer is relatively low, but atypical of anionic polysaccharides, increases rapidly in the presence of salts, acids, or alkali (Knutson, 1979).

8.4.1.8 Indican

Indican is a polysaccharide comprising 1,3 glucose, 1,4 mannose, 1,4 rhamnose and 1,3 or 4) -0-(1-carboxyethyl) rhamnose units in a molar ratio of about 2:1:1-2:1 respectively; containing 12-15% by weight acetyl units. Indican is soluble to at least 1% by weight in methanol and in ethylene glycol, forms gels with these solvents (Fig 86R), and has an inherent viscosity of about 33.5 dl/g. It has a number of useful thixotropic properties not least being compatible with organic solvents and having surface-active properties (Fig 85R). Indican is produced by fermentation of the gram negative microbe *Beijerinkia indica* (NCIB 8712) (Lawson,1979).

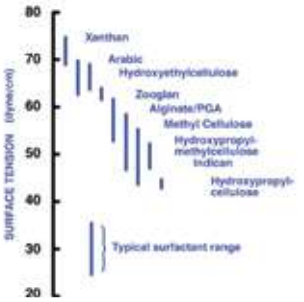


Fig 85R Comparison of Indican Surface Properties with Other Biopolymer



Fig 86R Indican gel formation in ethylene glycol

8.4.1.9 Levan from Erwinia herbicola

Levan is a (2,6)-linked fructan produced by *Erwinia herbicola*. The polysaccharide contains branches every 10-12 fructose residues linked (1,2) and is reported to have a molecular weight in excess of 103 kDa. Potential industrial applications of levan have been proposed as an emulsifier, formulation aid, stabilizer and thickener, surface-finishing agent, encapsulating agent, and carrier for flavor and fragrances. In addition, levan is promising in medicine as a plasma substitute, drug activity prolongator and antihyperlipidemic agent.

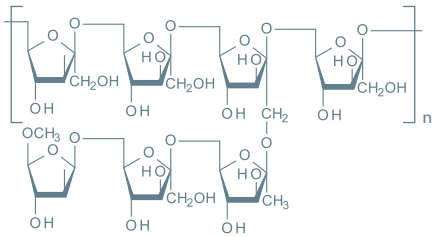


Fig 87R Levan from Erwinia herbicola

8.4.2 Polysaccharides from Gram-positive Bacteria

8.4.2.1 Peptidoglycan from Staphylococcus aureus

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of most bacteria, forming the cell wall. The sugar component consists of alternating residues of β-1,4 linked N-acetylglucosamine and N-acetylmuramic acid (Fig 88R). Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer. Peptidoglycan serves a structural role in the bacterial cell wall, giving structural strength, as well as counteracting the osmotic pressure of the cytoplasm. In *Staphylococcus aureus*, each peptidoglycan is attached to a short (4- to 5-residue) amino acid chain, L-alanine, D-glutamine, L-lysine, and D-alanine with a 5-glycine interbridge between tetrapeptides. Peptidoglycan is one of the most important sources of D-amino acids in nature (Tipper,1970).

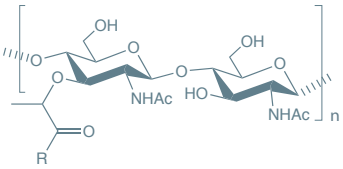


Fig 88R Peptidoglycan from *Staphylococcus aureus*

8.4.2.2 Teichuronic Acid

Teichuronic acid isolated from the cell walls of *Micrococcus luteus* has been examined by natural-abundance 13C NMR spectroscopy. Proton-decoupled and proton-coupled spectra were obtained for native teichuronic acid and also after the teichuronic acid had been oxidized with periodate and reduced with borohydride. The spectra are consistent with the structure [ManNAcUAp-β-1,6-Glcp-α-1,4]n (Fig 89R). Teichuronic acid synthesized *in vitro* from suitable substrates by the particulate enzyme fraction obtained from *Micrococcus luteus* yielded a 13C NMR spectrum which is indistinguishable from that of the native teichuronic acid, indicating a structural identity of the teichuronic acid synthesized *in vitro* with that isolated from cell walls (Johnson, 1981).

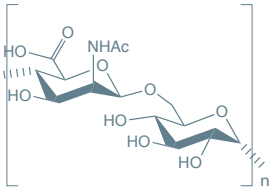


Fig 89R Teichuronic acid from *Micrococcus luteus*

8.5 Polysaccharides from Yeasts & Fungi

The polysaccharides found in Yeasts and Fungi are mostly neutral although at least one example with a glucuronic acid side chain is known. Polysaccharides containing glucose, mannose and xylose have been described but the largest group are glucans that occur both in cell walls (Zymosan, β1,3 β1,6) and as exocellular capsules such as Curdlan (β1,3) and Pullulan (α1,4, α1,6). Typically, the β-glucans form a linear backbone with 1,3 β-glycosidic bonds but vary with respect to molecular weight and branching (often β1,6) (Manners (1973) (Fig 90R). The molecular features of β-glucans appear to be important determinants of their physical properties, such as water solubility, viscosity, and gelation properties and they have found application in nutraceutical and cosmetic products, as texturing agents, and as fibre supplements. (Meena (2013), Izydorczyk (2008)). A number of β-glucans have been used as traditional medicines for many years and more recently have been investigated for their antitumour, immunomodulatory (Han 2020), anti-inflammatory and antioxidative properties. However, the relationship between the molecular structure and function of these polysaccharides is still rather unclear (Li 2019).

8.5.1 Beta Glucans

In addition to the β-glucans from cereals, another group of β-glucans are found in the cell walls of yeast (*Saccharomyces cerevisiae*), bacteria and fungi, with significantly differing physicochemical properties dependent on source. Typically these β-glucans form a linear backbone with 1,3 β-glycosidic bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals (Manners, 1973) (Fig 90R).

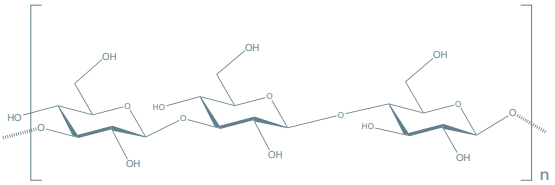


Fig 90R Covalent structure of β-glucan from *Saccharomyces cerevisiae*

The yeast and fungal β-glucans have been investigated for their ability to modulate the immune system. They are also used in various nutraceutical and cosmetic products, as texturing agents, and as fibre supplements. Their detailed molecular structures are key to the physical properties that they exhibit, such as water solubility, viscosity, gelation properties and physiological functions (Izydorczyk, 2008).

8.5.2 Mannan from Saccharomyces cerevisiae

The main cell-wall components of baker's yeast (*Saccharomyces cerevisiae*) as well as β-glucan (Manners, 1973) are mannans with an α-1,6 mannose backbone and α-1,2 and α-1,3 mannose branches (Ballou, 1974) (Fig 91R).

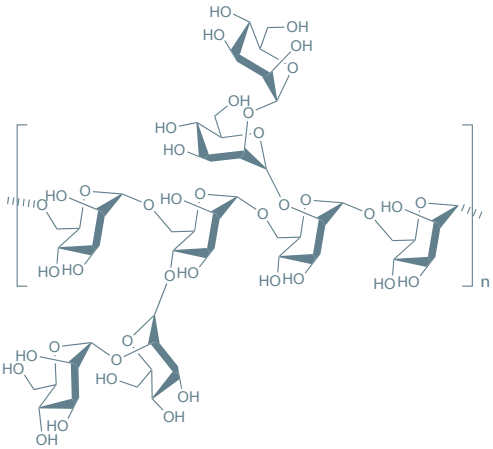


Fig 91R Mannan from *Saccharomyces cerevisiae*

8.5.3 Zymosan

Zymosan is prepared from the cell walls of baker's yeast (*Saccharomyces cerevisiae*) and consists of polysaccharide chains of various molecular weights, containing approximately 73% polysaccharide, 15% protein, 7% lipid and other inorganic components. When injected into animals, it induces inflammation and has been used for many years in inflammation and immunology research. The conditions activated include components of the complement system, prostaglandins and leukotrienes, platelet aggregation factor and lysosomal enzymes.



Fig 92R *Saccharomyces cerevisiae*

The literature on Zymosan reveals few rigorous analytical studies on the detailed structure of this macromolecule due to the wide variation in extraction procedures reported. These include treatment with enzymes such as trypsin followed by ultrafiltration and sonication.

8.5.3 Zymosan (continued)

Zymosan preparations are often insoluble but can be made soluble for separation on DE cellulose or Sepharose to produce more active fractions (Ohno 2007). Other studies report enhanced activity using hypochlorite oxidation (Ohno 2001), acid hydrolysis (Ishimoto 2018) and amination (Venkatachalam 2020).

A hypothetical structure of the carbohydrate part of the molecule is shown below constructed from the available literature. This shows a β1,3 glucan chain with β1,6 branch points.

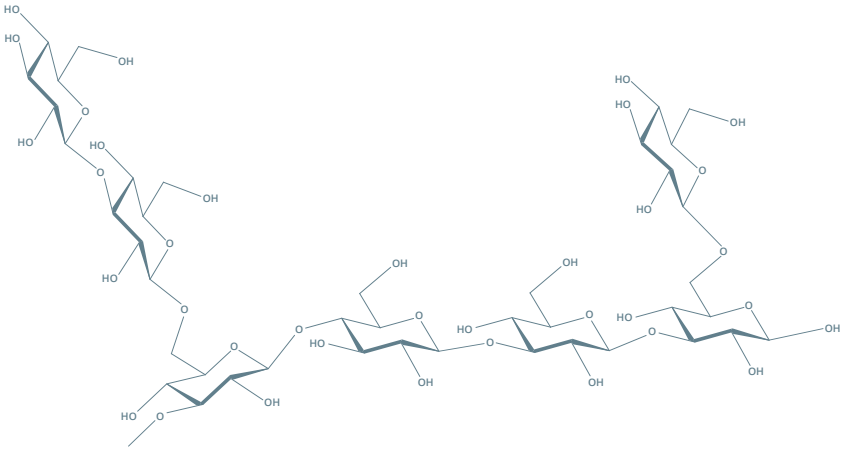


Fig 93R Hypothetical Zymosan structure

8.5.4 Curdlan (Whistler, 1993)

Curdlan is a microbial polysaccharide produced by a mutant strain of *Alcaligenes faecalis* var. *myxogenes* which was first shown to produce succinoglucan prior to mutation. Curdlan is a β-1,3 glucan (Fig 94R) forming clear solutions at about 55 °C which then gels ("low-set") when cooled. Suspensions of curdlan at higher temperatures form firm resilient gels ("high set") that melt at 140 - 160 °C (Fig 95R).



Fig 94R Curdlan gel

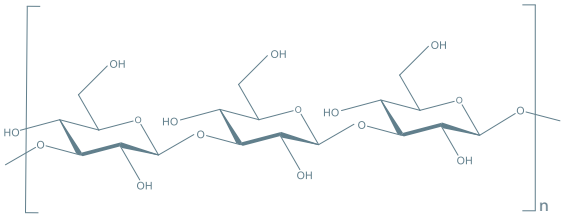


Fig 95R Covalent structure of curdlan

8.5.5 Pullulan (Whistler, 1993)

Pullulan is a glucan elaborated by the fungus *Aureobasidium pullulans* (Fig 96R). The chemical structure is essentially repeating units of maltotriose joined by α-1,6 linkages (Fig 97R). Pullulan dissolves readily in water to form stable, viscous solutions that do not gel. The polysaccharide can be moulded, made into fibres and forms clear soluble films. Applications are in foods as a low calorie ingredient and the polysaccharide forms water soluble films that have been used as seed coatings and to coat fruit. Pullulan can also be used in industrial applications as a binder, film former and in pharmaceutical applications (Catley, 1971).



Fig 96R *Aureobasidium pullulans*

Fig 97R Covalent structure of pullulan

8.5.6 Schizophyllan

Schizophyllan is a neutral extracellular polysaccharide produced by the fungus *Schyizophyllum commune* (Fig 98R). Schizophyllan is a β-1,3 beta glucan with β-1,6 branches (Fig 99R) and a molecular weight of around 450 kDa. It is reported that this polysaccharide can stimulate the immune system, chelate metals, act as an adjuvant in delivering drugs and aid in the production of nanofibres (Saito, 1979).



Fig 98R *Schizophyllum commune*

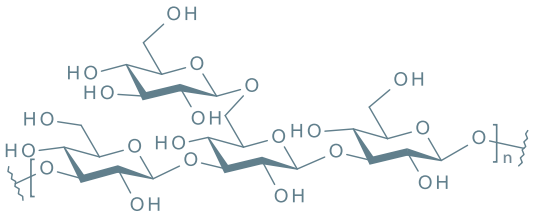


Fig 99R Covalent structure of schizophyllan

8.5.7 Scleroglucan (Whistler, 1993)

Scleroglucan is produced by the fermentation of the fungus *Sclerotium rolfsii* (Fig 100R). It is a glucan with a main chain of 1,3-linked β-D-glucopyranosyl units with every third unit having a single β-D-glucopyranosyl unit linked 1,6 (Fig 101R). Scleroglucan powders disperse in water and give very viscous shear thinning solutions. Applications are in the oil industry in enhanced oil recovery, in agriculture in sprays and in the food and pharmaceutical industries.



Fig 100R *Sclerotium rolfsii*

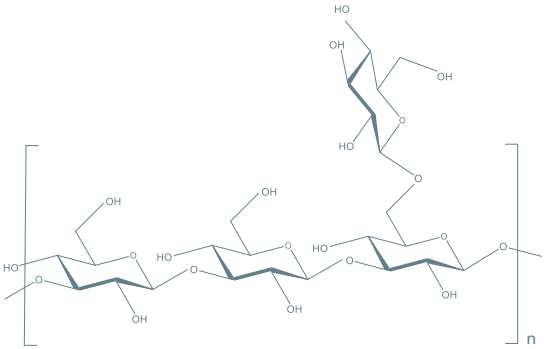


Fig 101R Covalent structure of scleroglucan

8.5.8 Glucuronoxylomannan from Cyptococcus neoformans

The cell envelope of *Cryptococcus neoformans* is composed of a rigid cell wall, composed mainly of glucans and a capsular polysaccharide, glucuronoxylomannan, consisting of mannose, xylose, glucuronic acid, and O-acetyl (Fig 102R). Glucuronoxylomannan is a viscous polysaccharide that constitutes about 88 % of the capsule mass. Glucuronoxylomannan, the antigenic basis for serotype specificity is a set of structurally related capsular polysaccharides (Cherniak, 1994).

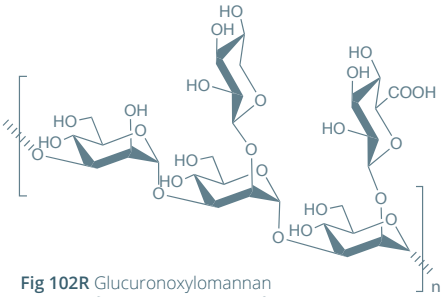


Fig 102R Glucuronoxylomannan from *Cyptococcus neoformans*

8.5.9 Nigeran

Nigeran is a polysaccharide found in the cell wall of lower fungi. In certain *Aspergillus* and *Penicillium* spp., nigeran was first isolated from *Penicillium expansum* and *Aspergillus niger* (Tung, 1967), and has been shown to be synthesized by only a few species of *Aspergillus* and *Penicillium*. The polysaccharide contains unbranched α-D-glucopyranose residues linked 1,3 and 1,4 (Fig 103R) (Barker, 1957).

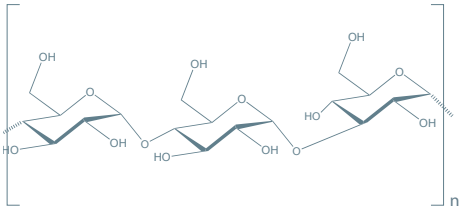


Fig 103R Nigeran

Nigeran is part of the hyphal cell wall, where it can contribute up to 40 % of the cell dry weight. The polysaccharide occupies several domains or location on the hyphal wal and is highly crystalline *in vivo*. Deposition of nigeran is primarily at the outer surface of the hyphal wall.

Little information is available in the literature on the possible uses of nigeran.

8.5.10 Pachyman & Pachymaran

Poria cocos is an edible medicinal fungus known as "Fuling" in Chinese that has been used as a Chinese traditional medicine for more than two thousand years. Polysaccharide material is the most abundant organic substance in the fungus, accounting for about 85% of the total and is a mixture of polysaccharides with the major component being Pachyman, a β-glucan with a 1,3 backbone and 1,6 side chains (Figure 106R). It is reported that Pachyman has a wide range of biological activities including antitumour, immunomodulatory, anti-inflammatory and antioxidative properties (Li, 2019). One problem with Pachyman is that it has poor water solubility and a more soluble derivative was created by removing the side chains which was found to have improved pharmacological activity (Han, 2020). This derivative was called Pachymaran and is regarded as being a linear β-1,3 glucan (Figure 107R).

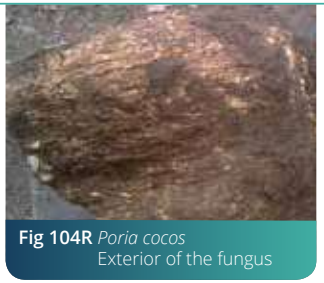


Fig 104R *Poria cocos*
Exterior of the fungus

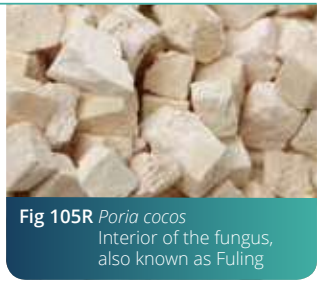


Fig 105R *Poria cocos*
Interior of the fungus, also known as Fuling

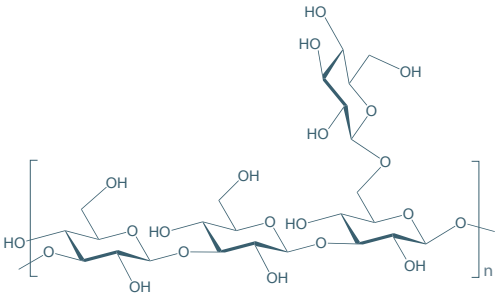


Fig 106R Structure of Pachyman

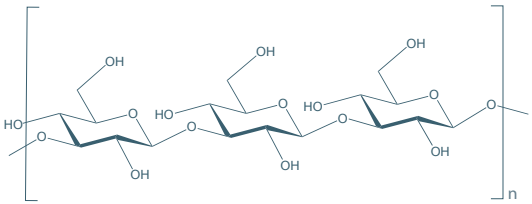


Fig 107R Structure of Pachymaran

8.6 Polysaccharides from Animal or Fish Tissues

8.6.1 Energy Storage Polysaccharides

8.6.1.1 Glycogen

Glycogen is a highly branched polysaccharide of glucose that serves as a form of energy storage in animals and fungi.
It is the main storage form of glucose in the body. In humans, glycogen is made and stored primarily in liver and muscle cells and functions as the second most important energy storage molecule to fat, which is held in adipose tissue. Glycogen is analogous to starch and has a structure similar to amylopectin (Fig 108R), but is more extensively branched and compact than starch (Manners, 1991). It occurs as granules in the cytosol/cytoplasm in many cell types, and plays an important role in the glucose cycle (Geddes, 1985).

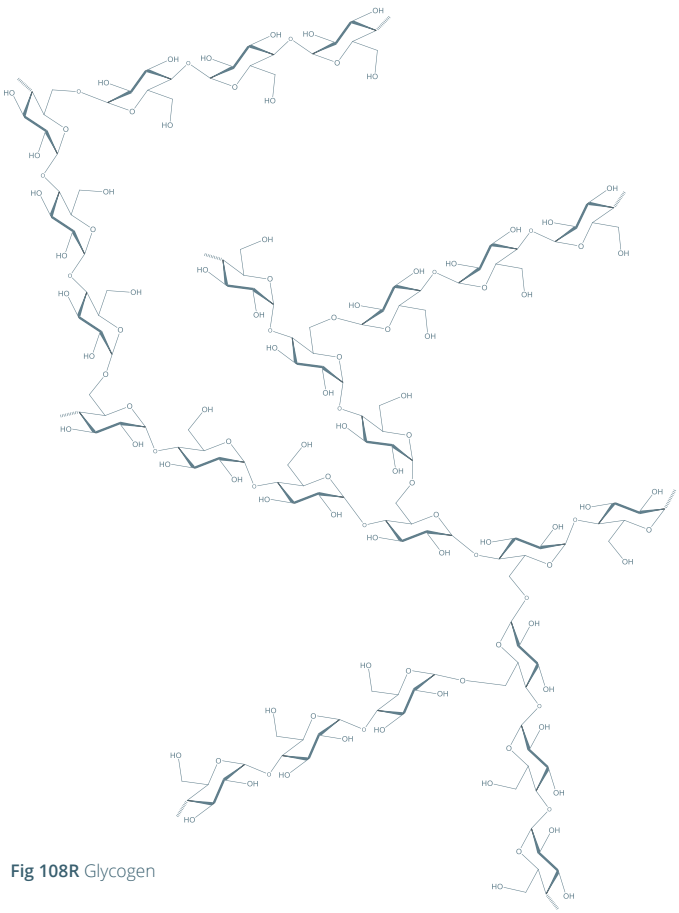


Fig 108R Glycogen

8.6.2 Structural Polysaccharides

8.6.2.1 Chondroitin Sulphate

Chondroitin sulphate is the most abundant glycosaminoglycan in mammalian tissues and occurs both in skeletal and soft connective tissue (typical source-shark cartilage) (Fig 109R). The disaccharide repeat unit consists of N-acetyl galactosamine sulphate linked β-1,4 to glucuronic acid (Zhang, 2009) (Fig 110R). Each monosaccharide may be left unsulphated, sulphated once, or sulphated twice. The most common pattern has the hydroxyls of the 4 and 6 positions of the N-acetyl-galactosamine sulphated, with some chains having the position 2 of the glucuronic acid sulphated.

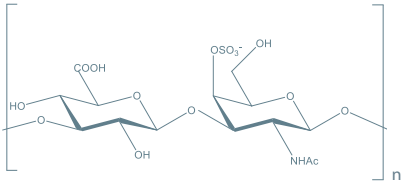


Fig 110R Covalent structure of chondroitin sulphate



Fig 109R Shark cartilage

8.6.2.2 Fucosylated Chondroitin Sulphate

A polysaccharide, isolated from the body wall of the sea cucumber *Ludwigothurea grisea* (Fig 111R), has a backbone like that of mammalian chondroitin sulphate: [4-β-D-GlcA-1,3-β-D-GalNAc-1]n but substituted at the 3-position of the β-D-glucuronic acid residues with sulphated α-L-fucopyranosyl branches (Fig 112R). These sulphated α-L-fucose branches confer anticoagulant activity on the polysaccharide and the specific activity of fucosylated chondroitin sulphate in the activated partial thromboplastin time assay is greater than that of a linear homopolymeric α-L-fucan with about the same level of sulfation (Vieira, 1991).

The antimalarial capacity of heparin-like sulphated polysaccharides (anticoagulant) from the sea cucumbers *Ludwigothurea grisea* and *Isostichopus badionotus*, from the red alga *Botryocladia occidentalis*, and from the marine sponge *Desmapsamma anchorata* has been explored. *In vitro* experiments demonstrated that for most compounds, significant inhibition of *Plasmodium falciparum* growth ocurred at low-anticoagulant concentrations. It was suggested that the retarded invasion mediated by sulphated polysaccharides, and the ensuing prolonged exposure of *Plasmodium* to the immune system, might be explored for the design of new therapeutic approaches against malaria where heparin-related polysaccharides of low anticoagulating activity could play a dual role as drugs and as potentiators of immune responses (Mourao, 1996).



Fig 111R Sea Urchin, *Ludwigothurea grisea*

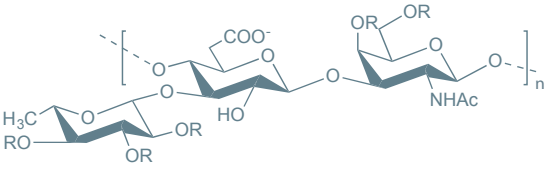


Fig 112R Partial structures of fucosylated chondroitin sulphate

8.6.2.3 Dermatan Sulphate

Dermatan sulphate is a glycosaminoglycan found in skin, blood vessels, heart valves, tendons, aorta, spleen and brain and is usually isolated from pig skin or beef lung tissue. The disaccharide repeat unit is composed of L-iduronic acid and N-acetyl-galactosamine-4-sulphate linked β-1,3 and β-1,4. There are also small amounts of D-glucuronic acid (Zhang, 2009) (Fig 113R).

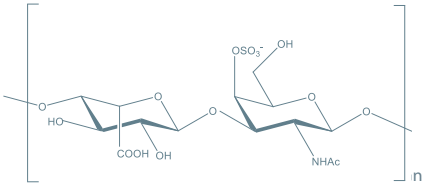


Fig 113R Covalent structure of dermatan sulphate

8.6.2.4 Hyaluronic Acid

Hyaluronic acid is a glycosaminoglycan found in many organs where it functions as a joint lubricant and shock absorber. It is obtained principally from synovial fluid, vitreous humor of the eye, umbilical tissue and cocks comb. The chemical structure of hyaluronic acid is a disaccharide repeat of β-1,3 glucuronic acid and β-1,4 N-acetyl glucosamine (Casu, 1985) (Fig 114R).

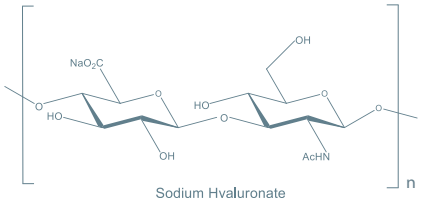


Fig 114R Covalent structure of sodium hyaluronate

8.6.2.5 Chitin (Whistler, 1993)

Chitin is a polysaccharide found widely in nature where it functions in a manner similar to collagen in chordates. It forms the tough fibrous exoskeletons of insects, crustaceans (Fig 115R) and other arthropods, and, in addition to its presence in some fungi it occurs in at least one alga. The structure of chitin is similar to that of cellulose but with glucose replaced with N-acetyl-D-glucosaminyl units linked β-D-1,4 in a linear chain (Fig 116R). It is normally produced from the shells of lobster, crab or shrimp.



Fig 115R Crustaceans

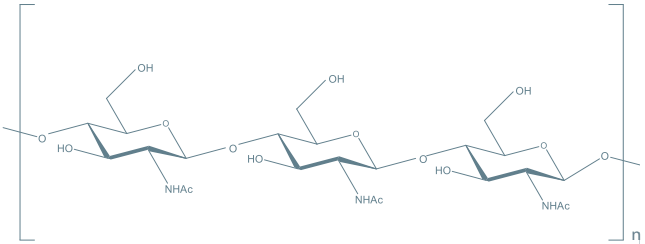


Fig 116R Covalent structure of chitin

8.6.2.6 Chitosan (Dumitriu, 2005)

Chitosan is the deacetylated form of chitin. The polysaccharide is deacetylated in order to render it soluble at pH values of less than 7 (Fig 117R). This then allows the material to be used in a number of industrial applications as a binder and film former. Chitosan has a number of commercial and biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in self-curing polyurethane paint coatings. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

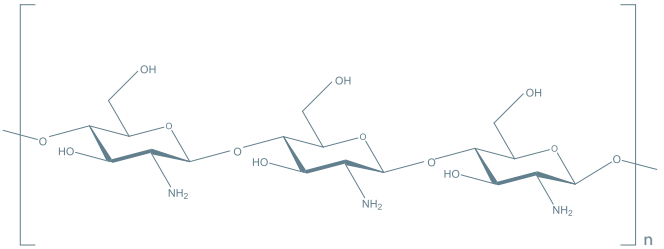


Fig 117R Covalent structure of chitosan

8.6.3 Polysaccharides with Key Physiological Functions

8.6.3.1 Heparin

Heparin is a glycosaminoglycan which occurs in many mammalian tissues, particularly mast cells and has important anticoagulant and thrombolytic properties. The chemical structure is complex and is composed mainly of two disaccharide repeating units A & B. A is L-iduronic acid 2-suplhate linked α-1,4 to 2-deoxy-2-sulfamido-D-galactose 6-sulphate while B is D-glucuronic acid β-1,4 linked to 2-deoxy-2-sulfamido-D-glucose 6-sulphate (Fig 118R).

The three-dimensional structure of heparin is complicated by the fact that iduronic acid may be present in either of two low-energy conformations when internally positioned within an oligosaccharide (Fig 119R) (Casu, 1985).

It has been shown that N-acetyl heparin (Fig 120R) (a derivative of heparin devoid of anticoagulant effects) can protect the heart from injury associated with global ischemia and reperfusion. It was found that N-acetyl heparin protects the heart from subsequent myocardial dysfunction secondary to ischemia/reperfusion (Friedrichs, 1994).

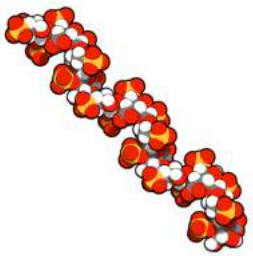


Fig 118R Three dimensional structure of heparin

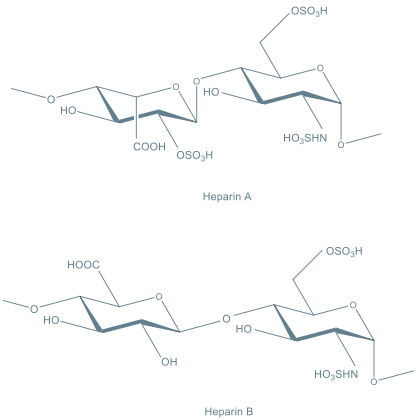


Fig 119R The two disaccharide repeats A&B of heparin

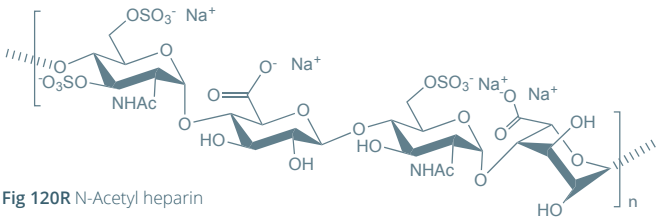


Fig 120R N-Acetyl heparin

8.6.3.2 Heparan Sulphates

Heparan sulphates are linear, anionic polysaccharides in which the basic polymer structure is made up of repeating amino sugar–uronic acid disaccharide units that are commonly modified by sulphation. With the exception of hyaluronic acid, these glycosaminoglycans are normally present in tissues in the form of proteoglycans, the polymer chains being in covalent linkage to various types of protein core that determine the glycosaminoglycan composition and the cellular/extracellular matrix location of the proteoglycan. The protein cores also play active roles in many spheres of cell regulation, particularly in the key areas of cell growth and cell adhesion (Gallagher, 2015).

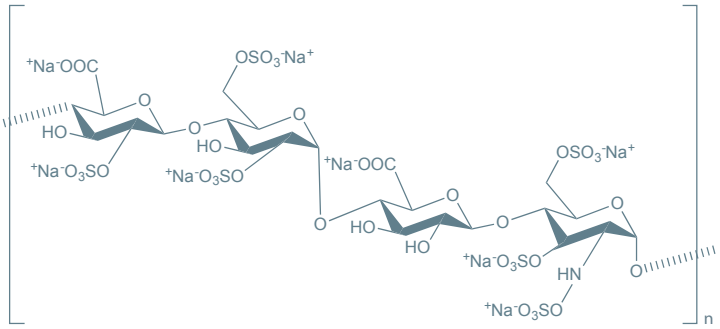


Fig 121R Heparan sulphate

The repeating disaccharide unit in heparan sulphate consists of an α-/β-1,4-linked N-acetyl glucosamine or N-sulphoglucosamine and uronic acid (glucuronic acid, or its C5 epimer iduronic acid) with chain lengths ranging in size from about 50 to 200 disaccharide residues (Fig 121R). The formation of heparan sulphate begins in the cis-Golgi where an heparan sulphate co-polymerase complex synthesizes a De N-sulphated N-acetylated polymer composed of repeating units of -4-β-GlcA 1,4 α-GlcNAc-1 (Fig 122R) (Gallagher, 2015).

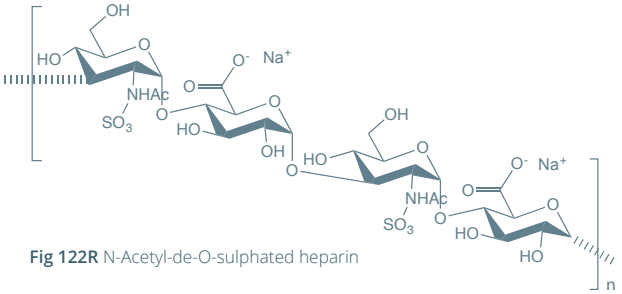


Fig 122R N-Acetyl-de-O-sulphated heparin

8.6.3.3 Colominic Acid or Polysialic Acid

Polysialic acid is an unusual post translational modification carbohydrate that is widely expressed in nature in bacterial capsules, fish, sea urchin eggs, embryonic tissues, amphibians, animal and human brains, and in a variety of cancers. The major carrier of polysialic acids in mammals is the neural cell adhesion molecule (a glycoprotein that belongs to the immunoglobulin superfamily). It is a linear small polysaccharide containing α-2,8-linked sialic acid residues with (n = 8 to >100) of sialic acid residues (Fig 123R). These cell surface glycans are highly expressed during embryonic brain development and modulate cell-cell interactions (mainly during embryonic growth), neural plasticity, and tumor metastasis.

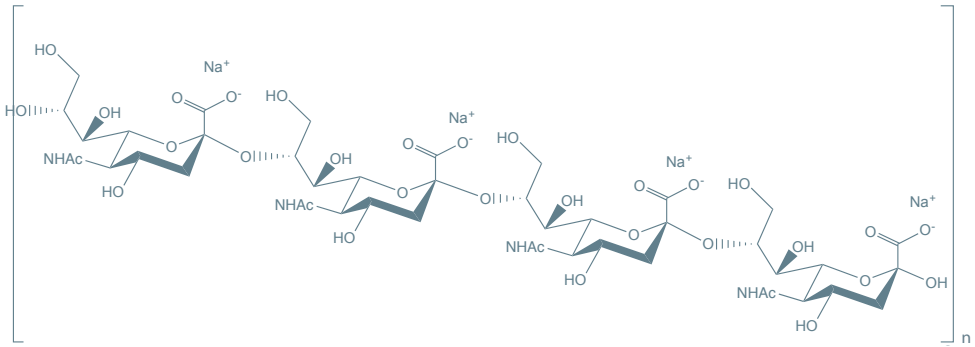


Fig 123R Polysialic acid

This unusual carbohydrate was identified almost three decades ago and has since attracted much interest from biochemists, cell biologists, and neurobiologists. This is not surprising, because the study of polysialic acid has provided key insights into how unusual glycans are synthesized, how carbohydrate conjugation of proteins can be so specific, and how these modifications then function in developmental processes.

The biological roles of polysialylation in the nervous system have been studied almost exclusively in relationship to its major known target, the neuronal cell adhesion molecule (NCAM). The high negativity of colominic acid modifies the surface charge and binding ability of NCAMs. Within the synapse, this negative polysialylation restricts the ability of NCAM to bind to NCAMs on adjacent membranes (Schauer, 2009).

Colominic acid is now available in significant quantities via the metabolic engineering of *E. coli* and has been investigated for the controlled release of drugs and scaffolds in biomedical applications. Polysialic acid has been proposed as the next generation of scaffolds for bioavailable products, augmenting the wide use of polyethylene glycol (Chen, 2015).

8.7 Functionally Modified Polysaccharides

8.7.1 Methyl and Ethyl Cellulose (Whistler, 1993)

Methylcellulose is a water-soluble polymer used as a binder or thickener in pharmaceutical, food, and ceramic processing applications. The methylation of a number of the polysaccharide hydroxyl groups causes hydrogen bond disruption and methylcellulose becomes water-soluble (Fig 124R).

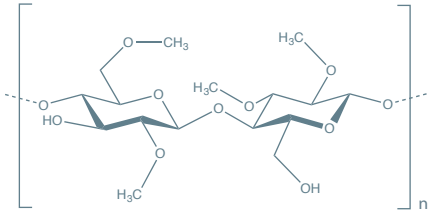


Fig 124R Covaleant structure of methyl (or ethyl) cellulose

Methylcellulose has an unusual lower critical solution temperature (LCST) between 40 °C and 50 °C. At temperatures below the LCST, it is readily soluble in water; above the LCST it is not soluble, which has a paradoxical effect that heating a saturated solution of methylcellulose will turn it solid, because methylcellulose will precipitate out. The temperature at which this occurs depends on DS-value, with higher DS-values giving lower solubility and lower precipitation temperatures because the polar hydroxyl groups are masked (Kobayashi, 1999).

Ethyl cellulose is similar in structure to methyl cellulose with ethyl replacing the methyl groups. It is approved for use in regulated markets such as food and pharmaceuticals. In pharmaceuticals, it can mask the taste of bitter actives, enhance the strength and appearance of tablets and capsules, and enable controlled release formulations. In food products, it functions as a binder, film former and flavor fixative.

8.7.2 Hydroxypropyl Cellulose, Hydroxyethyl Cellulose and Ethyl Hydroxyethyl Cellulose (Whistler, 1993)

Hydroxypropylcellulose (HPC) is an ether of cellulose in which some of the hydroxyl groups have been hydroxypropylated with propylene oxide. Complete substitution would provide a DS of 3 but because each substituted hydroxypropyl group contains a hydroxyl group, these can also be etherified. When this occurs, the number of moles of hydroxypropyl groups per glucose ring (MS), can be higher than 3 (Fig 125R).

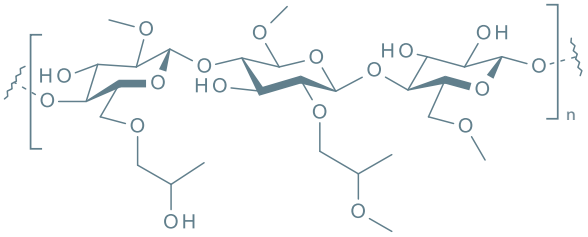


Fig 125R Covalent structure of hydroxypropyl cellulose

Because cellulose is very crystalline, hydroxypropylcellulose must have a DS of about 4 for good water solubility. In water, hydroxypropyl cellulose forms liquid crystals with many mesophases depending on concentration. These mesophases include isotropic, anisotropic, nematic and cholesteric, the latter resulting in many colors such as violet, green and red.

Pharmaceutical applications include treatments for medical conditions such as dry eye syndrome (keratoconjunctivitis sicca), recurrent corneal erosions, decreased corneal sensitivity, exposure and neuroparalytic keratitis. It is also used as a binder in tablets.

Hydroxypropylcellulose is also used as a thickener, a binder and emulsion stabiliser in foods with E number E463. HPC is used as a support matrix for DNA separations by capillary and microchip electrophoresis.

Hydroxyethyl cellulose is useful as a water thickener, rheological control additive, protective colloid, binder, stabilizer, suspending agent and film former. It is used in many industrial applications including latex paints, emulsion polymerization, petroleum, paper, pharmaceuticals, cosmetics and many other applications.

Ethyl (hydroxyethyl) cellulose (EHEC) is a nonionic, water-soluble cellulose derivative produced by introduction of ethyl and ethylene oxide groups to the hydroxyl groups of the cellulose backbone (Fig 126R/127R). It is an important industrial product used mainly in water-based paints and building products as thickener, emulsifier, and dispersing agent (Carlsson, 1986). It is also used as a laxative and has surface active properties.

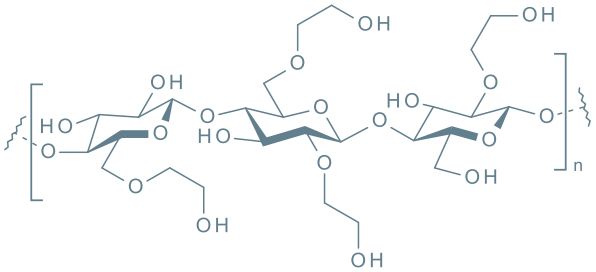


Fig 126R Hydroxyethyl cellulose

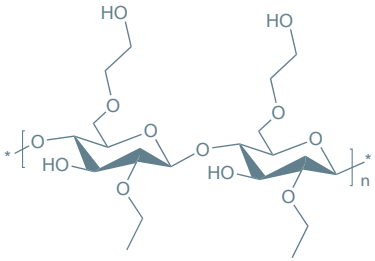


Fig 127R Ethyl hydroxyethyl cellulose

8.7.3 Hydroxypropyl Methyl Cellulose (Whistler, 1993)

Hydroxypropyl methylcellulose (HPMC or Hypromellose) (Fig 128R) is a semisynthetic, inert, viscoelastic polymer used as eye drops, as a semisynthetic substitute for tear-film. When applied, a hypromellose solution acts to swell and absorb water, by increasing the thickness of the tear-film, which results in decreased eye irritation. In addition to its use in ophthalmic liquids, hypromellose has been used as an excipient in oral tablet and capsule formulations, where, depending on the grade, it functions as controlled release agent. It is also used as a binder and as a component of tablet coatings.

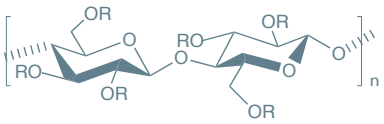


Fig 128R Hydroxypropyl methylcellulose

Hypromellose in aqueous solution, unlike methylcellulose, exhibits thermal gelation properties. Thus, when solutions are heated to the critical temperature, they form a loose gel-like mass. Critical temperatures are inversely related to both the solution concentration of HPMC and the degree of methoxy substitution, ie, the higher the methoxyl concentration, the lower the critical temperature. Also, the texture of the semi-flexible mass is directly related to the degree of methoxyl substitution.

HPMC is approved as a food additive as an emulsifier, thickening and suspending agent, and an alternative to animal gelatin (Codex Alimentarius code (E number) is E464). HPMC has been investigated as a substitute for gluten in making all-oat and other grain breads.

8.7.4 Hydroxypropyl Methylcellulose Phthalate (Whistler, 1993)

Hypromellose phthalate (hydroxypropyl methylcellulose phthalate, or HPMCP) is a phthalic acid ester of hydroxypropyl methylcellulose (Fig 129R). Hypromellose phthalate was introduced in 1971 as a cellulose derivative for enteric coatings, used to protect drugs from degradation by gastric acid or to prevent them from causing side effects in the stomach. HPMCP is also used in sustained-release preparations, in binders and as microcapsule bases (Weiβ, 1995).

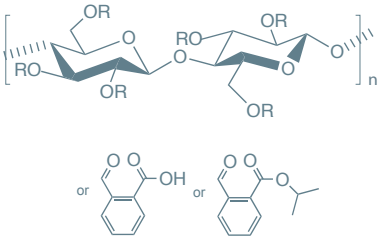


Fig 129R Hydroxypropyl methylcellulose phthalate

8.7.5 Sodium Carboxymethyl Cellulose (Whistler, 1993)

Carboxymethylcellulose (CMC) is synthesized by the alkali-catalyzed reaction of cellulose with chloroacetic acid. The polar carboxyl groups render the cellulose soluble and chemically reactive.

The functional properties of CMC depend on the degree of substitution of the cellulose, the chain length of the cellulose backbone structure and the degree of clustering of the carboxymethyl substituents (Fig 130R).

Carboxymethyl cellulose is used in food under the E number E466 as a viscosity modifier or thickener, and to stabilize emulsions in various products including ice cream. It is also a constituent of many non-food products, such as toothpaste, laxatives, diet pills, water-based paints, detergents, textile sizing, and various paper products. Carboxymethyl cellulose is used extensively in gluten free and reduced fat food products.

In laundry detergents, it is used as a soil suspension polymer designed to deposit onto cotton and other cellulosic fabrics, creating a negatively charged barrier to soils in the wash solution.

CMC is also used in pharmaceuticals as a thickening agent, for example as the lubricant in lubricating eye drops, and in the oil-drilling industry as an ingredient of drilling mud, where it acts as a viscosity modifier and water retention agent.

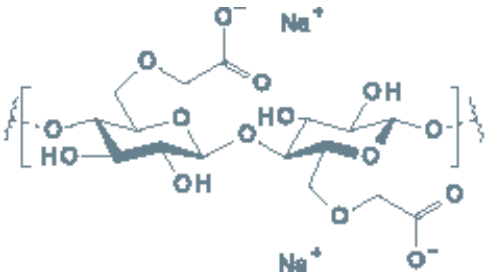


Fig 130R Covalent structure of sodium carboxymethyl cellulose

8.7.6 Cellouronic Acid

The oxidation of the primary hydroxyl groups of cellulose to yield the 6-carboxycellulose derivative, known as cellouronic acid (β-1,4 glucuronide) (Fig 131R), is attracting a substantial interest. As the sodium salt, this water-soluble derivative presents excellent rheological and gel-forming properties and when cast as films, exhibit superior gas barrier properties (Kato, 2005). In addition to these physical characteristics, cellouronic acid is biodegradable (Kato, 2002) and has shown promise in a number of biological and medical applications ranging from wound healing (Dimitrijevich, 1990), to bioabsorbable hemostatic properties (Kato, 2005). Cellouronic acid has also demonstrated activity as a bioactive elicitor for defense response in plants (Lienart, 2001).

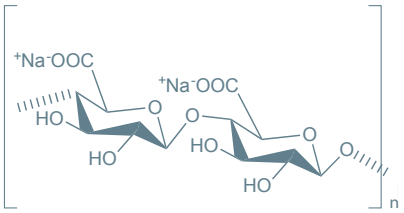


Fig 131R Cellouronic acid

8.7.7 Cellulose Acetate Hydrogen Phthalate

Cellulose acetate phthalate (CAP) (Fig 132R) finds appliction in the formulation of pharmaceuticals, such as the enteric coating of tablets or capsules and for controlled release formulations. It contains about 50 % acetate and 25 % as the phthalate ester with the rest as free hydroxyl groups. The main use of CAP is in pharmaceutical enteric formulations where it is often used with other coating agents such as ethyl cellulose. CAP is commonly plasticized with diethyl phthalate, a hydrophobic compound, or triethyl citrate, a hydrophilic compound; other compatible plasticizers are various other phthalates, triacetin, dibutyl tartrate, glycerol, propylene glycol, tripropionin, triacetin citrate, acetylated monoglycerides, etc.

Enteric coatings based on CAP are resistant to acidic gastric fluids, but easily soluble in the mildly basic medium of the intestine. The pH sensitive solubility of CAP is mainly determined (as are other properties of this mixed ester) by the degree of substitution and by the molar ratio (acetyl and phthaloyl groups) (Delgado, 1998).

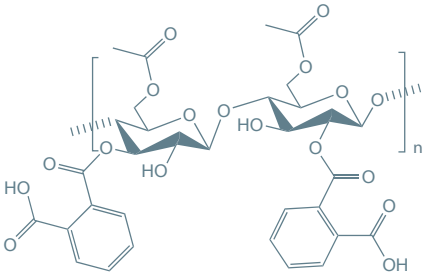


Fig 132R Cellulose acetate hydrogen phthalate

8.7.8 Celluose Acetate

The acetate ester of cellulose, cellulose acetate (Fig 133R) is probably the oldest of the polysaccharide chemical derivatives and was first prepared in 1865. Cellulose acetate is still used as a film base in photography, as a component in some coatings, and as a frame material for eyeglasses. It is also used as a synthetic fiber in the manufacture of cigarette filters and playing cards. In photographic film, cellulose acetate replaced nitrate film in the 1950s, being far less flammable and cheaper to produce but in recent years has been rendered obsolete by the advent of digital cameras.

Cellulose acetate fiber is one of the earliest synthetic fibers and is based on cotton or tree pulp cellulose. These “cellulosic fibers” have been replaced in many applications by cheaper petro-based fibers (nylon and polyester) in recent decades. Acetate shares many similarities with rayon, and was formerly considered as the same textile. Acetate differs from rayon in the employment of acetic acid in production.

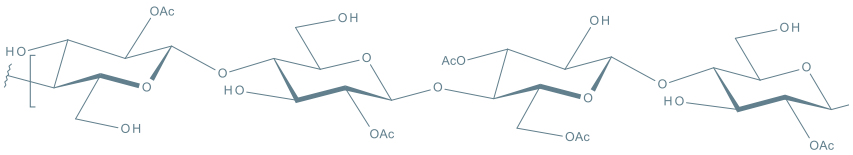


Fig 133R Cellulose acetate

8.7.9 Carboxymethyl Chitosan

Carboxymethyl chitosan (Fig 134R) has good solubility in water and also unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and good ability to form films, fibres and hydrogels (Muzzarelli, 1988; Chen, 2005). For this reason, it has been extensively used in many biomedical fields such as a moisture-retention agent, a bactericide, wound dressing agent, artificial bone and skin, blood anticoagulant and as a component in different drug delivery matrices (Farag, 2013). Thus, the reactive ligands COOH and NH_2 groups are available for metal chelation and dye binding.

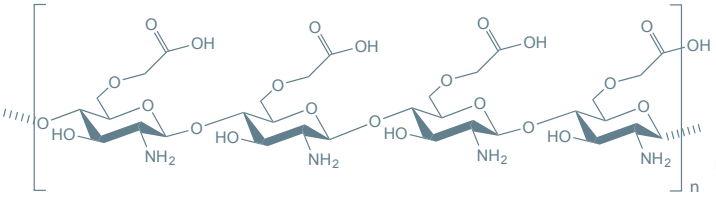


Fig 134R Carboxymethyl chitosan

8.7.10 Glycol Chitosan

Chitosan, a copolymer of β-1,4-linked D-glucosamine derived by deacetylation of natural chitin, is chemically modified to produce glycol chitosan by introducing ethylene glycol groups mostly at position 6, which yields a hydrophilic polymer that is soluble in water and culture media at neutral pH (Fig 135R). Glycol chitosan is non-cytotoxic and biocompatible and stimulates the growth of chondrocytes at low concentration. Glycol chitosan and a number of derivatives such as by adding cholesterol or folic acid to glycol chitosan, yields self-assemblies that contain a hydrophobic core and a hydrophilic shell and these can be used for targeted drug delivery (Yu, 2013).

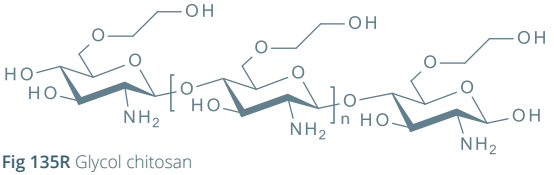


Fig 135R Glycol chitosan

8.7.11 Hyaluronate Fluorescein

Hyaluronic acid, a polysaccharide with alternating β-1,3 glucuronide and β-1,4 glucosamine residues-derived from *Streptococcus equi*, was labelled with 5-amino-fluorescein giving a yellow fibrous product, soluble in both water and electrolytes (de Belder, 1975) (Fig 136R). The degree of substitution was between 0.001 and 0.008 and the molecular weight determined by gel chromatography system gave a MW of 6.0×10^6.

Fluorescein-labelled hyaluronic acid may be used as a probe to follow the fate of hyaluronan *in vitro*. A FITC-labelled hyaluronic preparation greatly enhanced the visualization of the permeation of the substrate through skin (Yang, 2012). Other applications of fluorescein-labelled hyaluronic acid have been reported in cancer research (Cheng, 2014).

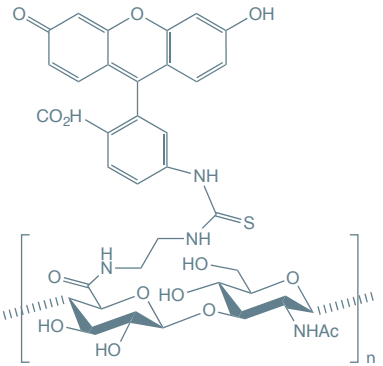


Fig 136R Hyaluronate fluorescein

Dextran Biotin and Rhodamine

Dextrans are widely used as both anterograde and retrograde tracers in neurons (Ferguson, 2001) and for numerous other applications. They are biologically rather inert as they have α-1,6 -linked glucose residues, which are resistant to cleavage by most endogenous cellular glycosidases. They usually have low immunogenicity.

These properties make dextrans effective water-soluble carriers for dyes (Fig 137R), indicators, and reactive groups in a wide variety of applications. Therefore, dextran conjugates make ideal long-term tracers for live cells. Fluorescent dextrans (Fig 138R) also serve as valuable markers for cell loading of macromolecules by micro- injection, vesicular fusion, and electroporation, as well as for the uptake and internal processing of exogenous materials by phagocytotic and endocytic pathways (Ansorge, 1988).

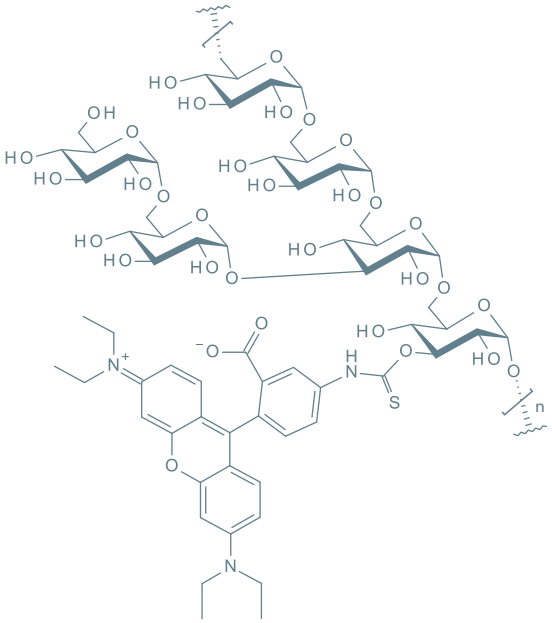


Fig 138R Dextran rhodamine

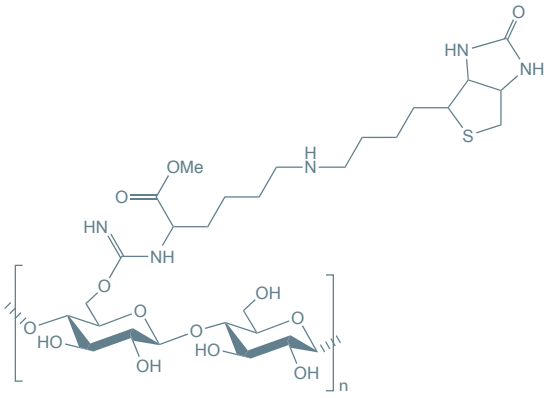


Fig 137R Dextran biotin

8.7.13 Dextran Sulphate

Dextran sulphate (Fig 139R) is a dextran derivative whose ulcer causing properties were first reported in hamsters (Ohkusa, 1985) and extrapolated a few years later to mice (Okayasu, 1990) and rats. The exact mechanisms through which dextran sulphate induces intestinal inflammation are unclear but may be the result of direct damage of the monolayer of epithelial cells in the colon, leading to the crossing of intestinal contents (e.g., commensal bacteria and their products) into underlying tissue and therefore induction of inflammation.

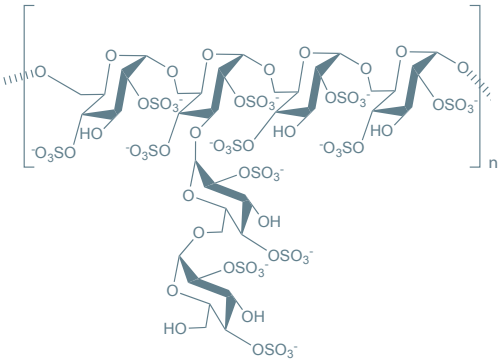


Fig 139R Dextran sulphate

8.7.14 Carboxymethyl Dextran

Carboxymethyl dextran (CM-dextran) is a white, odorless and tasteless powder which is freely soluble in water or electrolyte solutions. The product has a pronounced polyanionic character due to the high degree of carboxyl substitution (Fig 140R). The solution properties of CM-dextran are described in several publications (Gekko, 1979; 1981). Applications that have been described for CM-dextran include carriers of paramagnetic contrast agents (Rongved, 1991), preparation of conjugates of pharmacologically active compounds (Baudys, 1998; Ma, 2011) and CM-dextrans in biosensors (Situ, 2008). A number of other uses in cosmetics, agriculture, foods, paints and textiles have been the subject of patent applications.

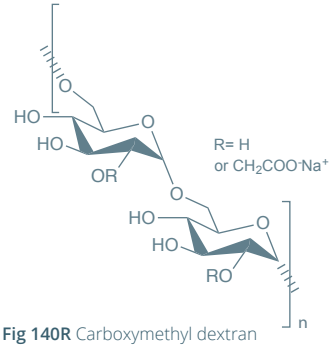


Fig 140R Carboxymethyl dextran

8.7.15 Diethylaminoethyl Dextran

DEAE-Dextran (Fig 141R) (DEAE-D) is a positively charged dextran derivative that can be used for vaccine production, gene therapy, protein stabilisation, dyslipidemia prevention, flocculating agents, and many other applications. DEAE-D is also used for transfecting animal cells with foreign DNA. DEAE-Sepharose, DEAE-650 and DEAE-Sephadex are commonly used in chromatography for the separation of biological molecules such as proteins and carbohydrates (de Belder, 1996).

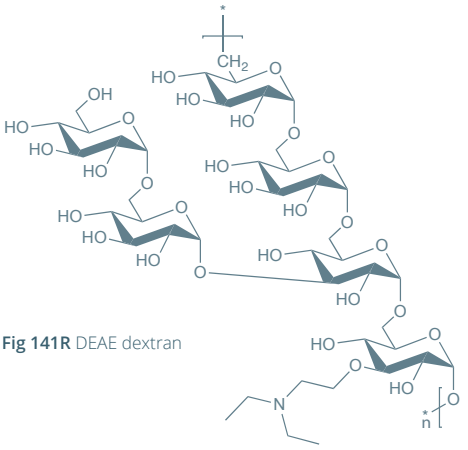


Fig 141R DEAE dextran

8.7.16 Fluorescein Isothiocyanate Dextran

Fluorescein isothiocyanate dextran (Fig 142R) is primarily used for studying permeability and transport in cells and tissues. An added benefit is that measurements of the fluorescence provide quantitative data on the permeability of healthy and diseased tissues. Such studies can be performed in real time by intravital fluorescence microscopy. The technique offers high sensitivity and concentrations down to 1µg/ml can be detected in tissue fluids. FITC-dextrans have also been used as a pH probe in cells (Geisow, 1981; Ohkuma, 1978). It may also be noted from polarisation experiments that the rotational freedom of fluorescein conjugated to dextran remains high and fluorescent lifetime of the excited state is similar to that before conjugation (Geisow, 1981).

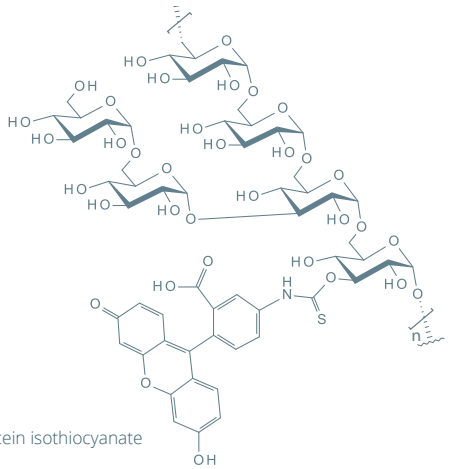


Fig 142R Fluorescein isothiocyanate dextran

8.7.17 Carboxymethyl Curdlan

Carboxymethyl curdlan (CMC) (Fig 143R) is widely used in the preparation of nanoparticles for biomedical applications. In a recent study, following the synthesis of superparamagnetic iron oxide nanoparticles capped with CMC for use in cellular and *in vivo* imaging applications, the stability and dispersibility of SPIN in water were greatly improved with the introduction of the CMC moiety (Lee, 2009). More recently, a green and simple route was proposed to synthesize Ag nanoparticles using carboxymethylcurdlan under UV irradiation (Wu, 2012).

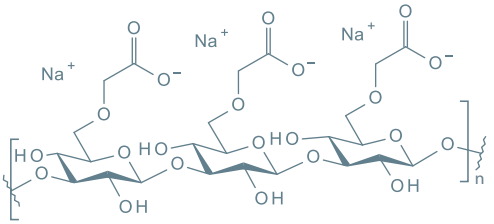


Fig 143R Carboxymethyl curdlan

8.7.18 Propylene Glycol Alginate

Propylene glycol alginate (Fig 144R) is a reaction product of propylene oxide and alginic acid (Steiner, 1951). At the 49th JECFA meeting (1997) it was resolved that the total dietary propylene glycol intake from all sources should be allocated an ADI of 0-25 mg/kg. Applications include as a stabiliser in beer foam due to electrostatic interaction between carboxyl groups on the glycol alginate molecules and amino groups on the peptides in the bubble wall (Jackson, 1980) and in ice cream by emulsifying the fat (Finney, 1972).

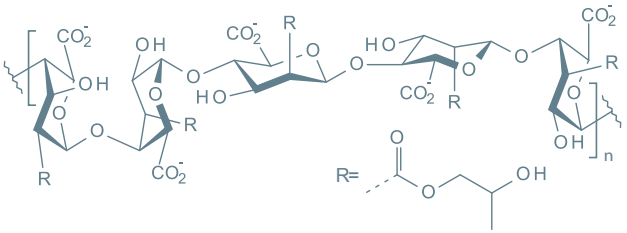


Fig 144R Propylene glycol alginate

8.8 Formulated Polysaccharides

8.8.1 Danaparoid

Danaparoid sodium (the active pharmaceutical ingredient in Orgaran; Merck Sharp and Dohme) is a biopolymeric non-heparin drug used as an anticoagulant and antithrombotic agent approved for the prophylaxis of postoperative deep-vein thrombosis. It consists of a mixture of three glycosaminoglycans: heparan sulphate, dermatan sulphate, and chondroitin sulphate (Üstün, 2011). Danaparoid has well established antithrombotic activity. The drug has a high antifactor Xa to antifactor IIa (thrombin) activity ratio, a low tendency to cause bleeding and minimal effects on the fibrinolytic system (Ibbotson, 2002).

8.8.2 Polyglycoplex

PolyGlycopleX, (α-L-glucurono-α-D-manno-β-D-manno-β-D-gluco), (α-L-gulurono-β-D- mannurono), (β-D-gluco-β-D-mannan (PGX)) is produced from a mixture containing proprietary proportions of three polysaccharides, konjac glucomannan, xanthan gum and sodium alginate. It has been subjected to a proprietary process (EnviroSimplex) including heat input after mixing the solid components. Recent hydrodynamic, rheological and analytical studies (Abdelhameed, 2010; Harding, 2011) have shown that the unexpectedly high viscosity of solutions of PGX is consistent with an interaction between a konjac glucomannan, xanthan gum complex and sodium alginate to form a new, ternary complex in solution. Human and animal feeding studies have shown that PGX can be used to control weight, lower the glycaemic index of foods and postprandial glycaemia (Brand-Miller, 2010; 2012). A monograph on the identification of PolyGlycoplex has recently been published (FCC, 2013).

8.9 Artificial Polysaccharides

8.9.1 Polydextrose

Polydextrose is a synthetic polymer of glucose. It is a food ingredient classified as soluble fibre by the U.S. Food and Drug Administration (FDA) as well as Health Canada, as of April 2013. It is frequently used to increase the non-dietary fibre content of food, to replace sugar, and to reduce calories and fat content. It is a multi-purpose food ingredient synthesized from dextrose (glucose), plus about 10 percent sorbitol and 1 percent citric acid. Its E number is E1200. It was approved by FDA in 1981.

Polydextrose is described in its Foods Chemicals Codex (FCC) Monograph as a randomly bonded (the 1,6-glycosidic linkage predominates) condensation polymer of D-glucose, sorbitol, and citric acid (Fig 145R). Commercial polydextrose also contains small amounts of free glucose, sorbitol, citric acid, and 1,6 anhydro-D-glucose (levoglucosan). Polydextrose has a broad molecular weight range (162 to 20,000) with 90 % of the molecules being between 504 and 5,000 MW. The average degree of polymerisation is 12: average molecular weight of approximately 2000. It is 0.1 times as sweet as sugar.

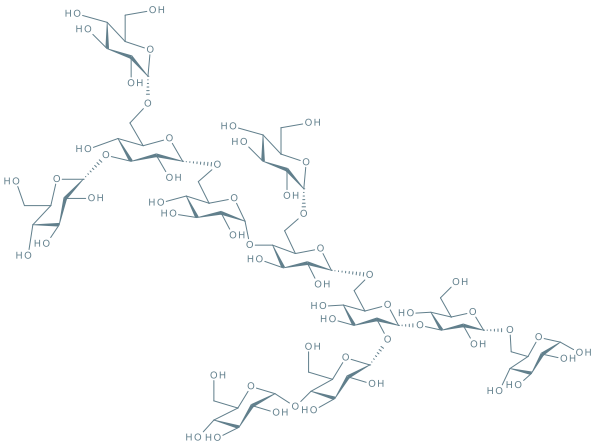


Fig 145R Polydextrose

Polydextrose is commonly used as a replacement for sugar, starch, and fat in commercial beverages, cakes, candies, dessert mixes, breakfast cereals, gelatins, frozen desserts, puddings, and salad dressings. Polydextrose is frequently used as an ingredient in low-carb, sugar-free, and diabetic cooking recipes. It is also used as a humectant, stabiliser, and thickening agent.

Polydextrose is a form of soluble fibre and has shown healthful prebiotic benefits when tested in animals. It contains only 1 kcal per gram and, therefore, is able to help reduce calories (Rennhard, 1973).

B

Section 9
References

References

Abdelhameed, A. S., Ang, S., Morris, G. A., Smith, I., Lawson, C., Gahler, R., et al., An analytical ultracentrifuge study on ternary mixtures of konjac glucomannan supplemented with sodium alginate and xanthan gum. Carbohydrate Polymers, **81**, 145–148, (2010)

Al-Kaisey, M.T., Wilkie, C.B., The polysaccharides of agricultural lupin seeds. Carbohyd. Res., **227**, 147-161, (1992)

Anderson D. M. W. and Bridgeman M. M. E., The composition of the proteinaceous polysaccharides exuded by astragalus microcephalus, A. gummifer and A. kurdicus-the sources of turkish gum tragacanth, Phytochemistry, **24**, 2301,2304, (1985)

Anderson, N.S., Dolan, T.C.S., Lawson C.J. and Rees D.A., The Masked Repeating Structures of ι and κ-Carrageenans. Carbohydrate Research, **7**, 368-473, (1968)

Ansorge W., Pepperkok R., Performance of an automated system for capillary microinjection into living cells, Journal of Biochemical and Biophysical Methods, **16(4)**, 283-292, (1988)

Arnott S., Fulmer A., Scott W. E., Dea I. C. M., Moorhouse R. and Rees D. A., The agarose double helix and its function in agarose gel structure, J Mol Biol, **90**, 269, (1974)

Aspinall G. O. and Sanderson G. R., Plant gums of the genus Sterculia. Part V. Degradation of carboxyl reduced Sterculia urens gum, J Chem. Soc., 2259, (1970)

Ballou, C. E. (1974). Some aspects of the structure, biosynthesis and genetic control of yeast mannans. In Advances in Enzymology, **40**, 239-270, Ed. A. Meister. New York, Wiley (1974).

Barclay T., Ginic-Markovic M., Cooper P. and Petrovsky N., Inulin-a versatile polysaccharide with multiple pharmaceutical and food uses, J Excipients and Food Chem., **1(3)**, 28-50, (2010)

Barker S. A., Bourne E. J., O'Mant D. M. and Stacey M., Studies of Aspergillus niger. Part VI. The separation and structures of oligosaccharides from Nigeran, J. Chem. Soc., 2448-2454 (1957)

Baudys M., Letourneur D., et al., Extending insulin action in vivo by conjugation to carboxymethyl dextran, Bioconj. Chem., **9**,176-183 (1998)

BeMiller J., Whistler R. L., (eds), Starch Chemistry and Technology, Food Science and Technology International Series, Academic Press, 3rd Edition, (ISBN 978-0-12-746275-2), (2009)

Bjorndal H., Hellerquist C. G., Lindberg B. and Svensson S., Gas-Liquid Chromatography and Mass Spectroscopy in Methylation Analysis of Polysaccharides, Angew. Chem. Internat. Ed., **9**, 610-619, (1970)

Blake J. D., Clarke M. L., Jansson P. E., and McNeil K. E., Fructan from Erwinia herbicola, Journal of Bacteriology, **151(3)**, 1595-1597, (1982)

Blanshard J. M. V. and Mitchell J. R., eds., Polysaccharides in Food, Butterworths, (1979)

Bouveng, H.O., Meier, H., Galactan from Norwegian spruce compression wood. Acta Chem. Scand., **22**, 1884-1889, (1959)

Bouveng H. and Lindberg B., Methods in Structural Polysaccharide Chemistry, Adv. Carbohydr. Chem., **15**, 58-68, (1960)

Brand-Miller J. C., Atkinson F. S., Gahler R. J., Kacinik V., Lyon M. R. and Wood S., Effects of PGX, a novel functional fibre, on acute and delayed post-prandial glycaemia, European Journal of Clinical Nutrition, **64**, 1488-1493, (2010)

Brand-Miller J. C., Atkinson F. S., Gahler R. J., Kacinik V., Lyon M. R. and Wood S., Effects of added PGX, a novel functional fibre, on the glycaemic index of starchy foods, British Journal of Nutrition, **108**, 245-248, (2012)

Brett C. T., Cellulose microfibrils in plants: biosynthesis, deposition, and integration into the cell wall, Int Rev Cytol., **199**, 161-99, (2000)

Carlsson, A., Lindman, B., Nilsson, P.-G., Karlsson, G. Polymer, **27**, 431, (1986)

Casu B., Structure and biological activity of heparin, Advances in Carbohydrate Chemistry and biochemistry, **43**, 51-134, (1985)

Catley B. J. and Whelan W. J., Obsevations on the structure of pullulan, Arch. Biochem. Biophys., **143**, 138-142, (1971)

Chen F., Tao Y., Jin C., Xu Y., Lin B–X., Enhanced production of polysialic acid by metabolic engineering of Escherichia coli, Applied Microbiology and Biotechnology, **99(6)**, 2603–2611, (2015)

Chen L., Du Y., Tian Z. and Sun L., Effect of the degree of deacetylation and the substitution ofcarboxymethyl chitosan on its aggregation behavior, J. Polym. Sci. Polym. Phys., **43**, 296–305, (2005)

Cheng D., Han W., Song K., et al., One-step facile synthesis of hylauronic acid functionalized fluorescent gold nanoprobes sensitive to hyaluronidase in urine specimens from bladder cancer patients, Talanta, **130**, 408-414, (2014)

Churms S. C., ed., CRC Handbook of Chromatography, Carbohydrates Volume I, CRC Press, (1982)

Dea I. C. M. and Madden J. K., Acetylated Pectic Polysaccharides of Sugar Beet, Food Hydrocolloids, **1 (1)**, 71-88, (1986)

Dea I. C. M., Morris E. R., Rees D. A., Welsh E. J., Barnes H. A. and Price J., Association of like and unlike polysaccharides: mechanism and specificity ion galactomannans, interacting bacterial polysaccharides, and related systems, Carbohydr. Res., **57**, 249-272, (1977)

Deavin L., Jarman T.J., Lawson C.J., Righelato R.C., Slocombe S., The Production of Alginic Acid by Azotobacter vinelandii in Batch and Continuous Culture. ACS Symp. Ser., **45**, 14-26, (1977)

de Belder A. N., Wik K. O., Preparation and properties of fluorescein-labelled hyaluronate, Carbohyd. Res., **44(2)**, 251-257, (1975)

De Belder A. N., Medical Applications of Dextran and its Derivatives, Polysaccharides, Medical Applications, Marcel Dekker, ed Dumitriu S., 505-523, (1996)

Delgado J. N., William A. in Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry. Lippincott-Raven Publishers, Wickford (1998)

Dimitrijevich S.D., Tatarko M., Gracy R.W., Linsky C.B. and Olsen C., Biodegradation of oxidized regenerated cellulose. Carbohydr. Res., **195**, 247–256, (1990)

Drummond G.S., Smith E.E. and Whelan W. J., On The Specificity of Starch Debranching Enzymes, Febs Letters, **9(3)**, 136-140, (1970)

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith., F., Colorimetric method for determination of sugars and related substances, Anal Chem., **28**, 350, (1956)

Dumitriu S., ed., Polysaccharides in Medicinal Applications, Marcel Dekker, Second Edition, (1996)

Dumitriu S., ed., Polysaccharides, Structural Diversity and Functional Versatility, Marcel Dekker, Second Edition, (2005)

Ebringerova A., Heinze T., Xylan and Xylan derivatives-biopolymers with valuable properties 1, Naturaly occurring Xylans structures, isolation procedures and properties, Macromol. Rapid Commun., **21**, 542-556, (2000)

Farag R. K., and Mohamed R. R., Synthesis and Characterization of Carboxymethyl Chitosan Nanogels for Swelling Studies and Antimicrobial Activity, Molecules, **18**, 190-203, (2013)

FCC, Alginate-Konjac-Xanthan Polysaccharide Complex, Second Supplement to FCC 8th Edition, 1743, (2013)

Fee M., Errington N., Illum K. J. L., Smith A. and Harding S. E., Correlation of SEC/MALLS with ultracentrifuge and viscometric data for chitosan, Eur. Biophys. J., **32**, 457-464, (2003)

Ferguson I. A., Xian C., Barati E., Rush R. A., Comparison of wheat germ agglutinin–horseradish peroxidase and biotinylated dextran for anterograde tracing of corticospinal tract following spinal cord injury, Journal of Neuroscience Methods, **109(2)**, 81–89, (2001)

Finney D. J., Ravenhill J. R. and Tait M. J., Ice Cream, US Patent 3914441 to Lever Brothers (1972)

Fischer M. H., Yu N., Gray G. R., Ralph J., Anderson L. and Marlett J. A., The gel-forming polysaccharide of psyllium husk (Plantago ovata forsk), Carbohydr. Res., **339(11)**, 2009-2017, (2004)

Friedrichs G. S., Kilgore K. S., Manley P. J., Gralinski M. R., Lucchesi B. R., Effects of heparin and N-acetyl heparin on ischemia/reperfusion-induced alterations in myocardial function in the rabbit isolated heart, Circulation Research, **75**, 701-710 (1994)

Geddes R., in The Polysaccharides, **3**, 316 (1985)

Geisow M. J., D'Arcy Hart P. and Young M. R., Temporal changes of lysosome and phagosome pH during phagolysosome formation in macrophages; studies by fluorescence spectroscopy, J. Cell. Biol., **89**, 645-652, (1981)

Gekko K., Solution properties of dextran and its ionic derivatives, ACS Symposium Series, **150**, 415-438, (1981)

Gekko K. and Noguchi H., Selective interaction of calcium and magnesium ions with ionic dextran derivatives, Carbohyd. Res., **69**, 323-326, (1979)

References (continued)

Gibb R. D., Johnson W. McR. Jr., Russell D A., Hasselblad V. and Alessio D. A D., Psyllium fibre improves glycemic control proportional to loss of glycemic control, Am. J. Clin. Nutr., **102 (6)**, 1604-1614, (2015)

Gloaguen V., Brudieux V., Closs B., Barbat A., Krausz P., Sainte-Catherine O., Kraemer M., Maes E. and Guerardel Y., Structural Characterization and Cytotoxic Properties of an Apiose-Rich Pectic Polysaccharide Obtained from the Cell Wall of the Marine Phanerogam Zostera marina, J. Nat. Prod., **73(6)**, 1087–1092, (2010)

Glushka J.N., Terrell M., York W. S., O'Neill M.A., Gucwa A., Darvill A.G., Albersheim P., Prestegard J.H., Primary structure of the 2-O-methyl α-L-fucose-containing side chain of the pectic polysaccharide, rhamnogalacturonan II. Carbohydr. Res., 338, 341,352, (2003)

Gorin P. A. J. and Spencer J.F.T., Exocellular alginate from Azotobacter vinelandii, Canad. J. Chem., **44**, 993-998, (1966)

Hacking A. J., Taylor I. W. F., Jarman T. R. and Govan J. R. W., Alginate biosynthesis by Pseudomonas mendocina, Journal of General Microbiology, **129**, 3473-3480, (1983)

Hakomori S., A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide, J. Biochem., **55**, 205-208, (1964)

Han B., Baruah K., Cox E., Vanrompay D., Bossier P., Structure-Functional Activity Relationship of β-Glucans From the Perspective of Immunomodulation: A Mini-Review, Front. Immunol., **11**, (2020)

Harding S. E., Challenges for the modern analytical ultracentrifuge analysis of polysaccharides, Carbohydr. Res., **340(5)**, 811-826 (2005)

Harding S. E., The Intrinsic viscosity of biological macromolecules, progress in measurement, interpretation and application to structure in dilute solution, Progress in Biophysics and Molecular Biology, **68**, 207-262, (1997)

Harding S.E., Smith I.H., Lawson C.J., Gahler R J. and Wood S., Studies on macromolecular interaction in ternary mixtures of konjac glucomannan, xanthan gum and sodium alginate, Carbohydrate Polymers, **83(2)** 329-338, (2011)

Harding S.E., Adams G.G., Almutairi F., Alzahrani Q., Erten T., Kok M.S. and Gillis R.B., Ultracentrifuge methods for the analysis of polysaccharides, glycoconjugates, and lignins. Methods in Enzymology. **562**, 391-439 (2015)

Harris P., ed., Food Gels, Elsevier Applied Science, (1990)

Henry R.J., Pentosan and 1,3,1,4)-β-Glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye, Journal of Cereal Science, **6(3)**, 253–258, (1987)

Hicks K.B., Scott M. and Doner L.W., Preparative chromatography of carbohydrates, uronic acids and related derivatives, Carbohydr. Res., **168 (1)**, 33-45, (1987)

Hirst, E.L., Jons, J.K.N., Walder, W.O., Pectic substances. Part 7: the constitution of the galactan from Lupinus albus. J. Chem. Soc., 1225-1229, (1947)

Hokputsa., Alexander K. J. C. and Harding S. E., A comparison of molecular mass determination of hyaluronic acid using SEC/MALLS and sedimentation equilibrium, Eur. Biophys. J., **32**, 450-456, (2003)

Ibbotson T., Perry C. M., Danaparoid, A Review of its Use in Thromboembolic and Coagulation Disorders, Drugs, **62 (15)**, 2283–2314, (2002)

Izydorczyk M. S and Dexter J. E., Barley β-glucans and arabinoxylans: molecular structure, physical properties, and uses in food products-a review, Food Research International, **41**, 850-868, (2008)

Jackson G., Roberts R. T. and Wainwright T., Mechanism of Beer Foam Stabilisation by Propylene Glycol Alginate, J. Inst. Brew., **86**, 34-37 (1980)

Johnson S.D., Lacher K.P. and Anderson J.S., Carbon-13 nuclear magnetic resonance spectroscopic study of teichuronic acid from Micrococcus luteus cell walls. Comparison of the polysaccharide isolated from cells with that synthesized in vitro, Biochemistry, **20(16)**, 4781,4785, (1981)

Kacurakova M. and Wilson R. H., Developments in mid-infrared FT-IR spectroscopy of selected carbohydrates, Carbohydrate Polymers, **44(4)**, 291,303, (2001)

Kaeffer B., Bénard C., Lahaye M., Blottière H. M., Cherbut C., Biological Properties of Ulvan, a New Source of Green Seaweed Sulphated Polysaccharides, on Cultured Normal and Cancerous Colonic Epithelial Cells, Planta Med., **65(6)**, 527-531, (1999)

Karunaratne D. N., Jayalal R. G. U. and Karunaratne V., Lichen Polysaccharides in The Complex World of Polysaccharides, Chapter 8, Intech (Open Science), 215-226, (2012)

Kato Y., Habu N., Yamaguchi J., Kobayashi Y., Shibata I., Isogai A. and Samejima M., Biodegradation of β-1,4-linked polyglucuronic acid (cellouronic acid), Cellulose, **9(1)**, 75–81, (2002)

Kato Y., Kaminaga J., Matsuo R. and Isogai A., Oxygen permeability and biodegradability of polyuronic acids prepared from polysaccharides by TEMPO-mediated oxidation, J. Polym. Environ., **13(3)**, 261–266, (2005)

Keogh, G.F., Cooper G.J., Mulvey T.B., McArdle B.H., Coles G.D., Monro J.A., Poppitt S.D., Randomized controlled crossover study of the effect of a highly beta-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men, The American Journal of Clinical Nutrition, **78(4)**, 711–718, (2003)

Kishimoto S., Kobayashi H., Shimizu S et al., Changes of colonic vasoactive intestinal peptide and cholinergic activity in rats with chemical colitis, Dig. Dis. Sci., **37**, 1729–1737, (1992)

Knutson C. A., Pittsley J. E., Jeanes A., Composition and properties of the extracellular polysaccharide produced by Arthrobacter stabilis NRRL B-3225, Carbohydr. Res., **73(1)**, 159-168 (1979)

Kobayashi K., Huang Ci. and Lodge T. P., Thermoreversible gelation of aqueous methylcellulose solutions, Macromolecules, **32**, 7070-7077, (1999)

Labavitch J.H., Freeman L.E., Albersheim P., Purification and characterisation of β-1,4-galactanase which degrades a structural component of the primary cell wall of dicots., J. Biol. Chem., **251**, 5904-5910, (1976)

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

Lawson C.J. and Rees D.A., Reinvestigation of the Acetolysis Products of Carrageenan, Revision of the structure of α-1,3-Galactotriose and a Further Example of the Reverse Specificities of Glycoside Hydrolysis and Acetolysis. J. Chem. Soc, 1301, (1968)

Lawson C.J. and Rees D.A., An Enzyme for the Metabolic Control of Polysaccharide Conformation and Function, Nature (London), 227, (1970)

Lawson C.J., Rees D.A., Stancioff D.J. and Stanley N.F., Repeating Structures of Galactan Sulphates from Furcellaria, Gigartina, Ahnfeltia, Gymnogongrus, Euchuma. J. Chem. Soc. Perkin Trans. 1, **19**, 2177-2182, (1973)

Lawson, C.J., Symes, K.C., Oligosaccharides produced by partial acetolysis of xanthan gum, Carbohydr. Res., **58**, 433, (1977)

Lawson C. J. and Symes K. C, Suspensions and gels of indican and their uses, US Patent, US 4372785 A, (1979)

Lindberg B., Longren J., Svensson S., Specific Degradation of Polysaccharides, Advances in carbohydrate Chemistry & Biochemistry, **31**, 185-240, (1975)

Lee C.M., Jeong H.J., Kim E.M., Cheong S.J., Park E.H., Kim D.W., Lim S.T. and Sohn M.H., Synthesis and characterization of iron oxide nanoparticles decorated with carboxymethyl curdlan, Macromolecular Research, **17**, 133-136, (2009)

Lienart Y., Heyraud A. and Sevenou O., β-1,4-D-glucuronane polymers and related polysaccharide and oligosaccharide pesticides and fertilizers. WO 2001000025, (2001)

Li X., He Y., Zeng P., Liu Y., Zhang M., Hao C., Wang H., Lv Z., Zhang L., Molecular basis for Poria cocos mushroom polysaccharide used as an antitumour drug in China, *Journal of Cellular and Molecular Medicine*, **23(1)**, 4-20, (2019)

Ma H., Li X. et al., High antimetastatic efficacy of MEN4901/T-0128, a novel campothecin carboxymethyldextran conjugate, J. Surg. Res., **171(2)**, 684-690, (2011)

Mackie W., Semi-quantitative estimation of the composition of alginates by infra-red spectroscopy, Carbohydr. Res., **20(2)**, 413-415, (1971)

Maeda M., Shimahara H. and Sugiyama N., Detailed examination of the branched structure of konjac glucomannan, Agric. Biol. Chem., **44(2)**, 245-252, (1980)

Manners D. J., Massan A. J. and Patterson J. C., The structure of a β-1,3 glucan from yeast cell walls, Biochem. J., **135**, 19-30, (1973)

Manners D J., Recent developments in our understanding of glycogen structure, Carbohydrate Polymers, **16(1)**, 37-82, (1991)

Marchessault R. H. and Sundararajan P. R., in The Polysaccharides, (Aspinall G. O., ed.), Academic Press, New York, **2**, 25-65, (1983)

Mazeau K., Hervé du Penhoat C., Pérez S., Pectic Arabinogalactans & Rhamnogalacturonans, Plant Physiology and Biochemistry, **38(1–2)**, 37–55, (2000)

McCleary B.V. and Matheson N.K., Enzymic Analysis of Polysaccharide Structure, Advances in Carbohydrate Chemistry & Biochemistry, **44**, 147-276, (1987)

Meena D. K., Das P., Kumar S., Mandal S. C., Prusty A. K., Singh S. K., Akhtar M. S., Behera B. K., Kumar K., Pal A. K. and Mukhergee S. C., Beta-glucan an ideal immunostimulant in aquaculture, Fish Physiology and Biochemistry, **39 (3)**, 431,457, (2013)

References (continued)

Morales-Lange B., Bethke J., Schmitt P. and Mercado L., Phenotypical parameters as a tool to evaluate the immunostimulatory effects of laminarin in Oncorhnchus mykiss, Aquaculture Research, **46(11)**, 2707-2715, (2015)

Morris G.A., Ralet M-C., Bonnina E., Thibault J-F and Harding S. E., Physical characterisation of the rhamnogalacturonan and homogalacturonan fractions of sugar beet (Beta vulgaris) pectin, Carbohydrate Polymers, **82**, 1161–1167, (2010)

Mourão P. A. S., Pereira M. S., Pavão M. S. G., Mulloy B., Tollefsen D. M., Mowinckel M-C. and Abildgaard U., Structure and Anticoagulant Activity of a Fucosylated Chondroitin Sulphate from Echinoderm, Sulphated Fucose Branches on The Polysaccharide Account For Its High Anticoagulant Action, J. Biol. Chem., **271**, 23973-23984, (1996)

Muzzarelli, R.A.A., Carboxymethylated chitins and chitosans, Carbohyd. Polym., **8**, 1–21, (1988).

Nelson T. E. and Lewis B. A., Separation and characterisation of the soluble and insoluble components of insoluble laminaran, Carbohydr. Res., **33**, 63-74, (1974)

North S. J., Hitchen P. G., Haslam S. M., and Dell A., Mass spectrometry in the analysis of N- and O-linked glycans, Curr Opin Struct Biol., **19(5)**, 498–506, (2009)

Ohkuma S. and Poole B., Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH of by various agents, Proc. Natl. Acad. Sci. USA, **75**, 3327-3331, (1978)

Ohkusa T., Production of experimental ulcerative colitis in hamsters by Dextran Sulphate Sodium and changes in intestinal microflora, Nihon Shokakibyo Gakkai Zasshi, **82(5)**, 1327-36, (1985).

Okayasu I., Hatakeyama S., Yamada M. et al., A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice, Gastroenterology, **98**, 694–702, (1990)

Olafsdottir E. S., Ingolfsdottir K., Barsett H., Paulsen S. B., Jurcic K., Wagner H., Immunologically active 1,3-1,4)-α-D-Glucan from Cetraria islandica, Phytomedicine, **6(1)**, 33-39, (1999)

Palma A. S., Liu Y., Zhang H., Zhang Y., McCleary B. V., Yu G., Huang Q., Guidolin L. S., Ciocchini A. E., Torosantucci A., Wang D., Carvalho A. L., Fontes C. M. G. A., Mulloy B., Childs R A., Feizi T and Chai W., Unravelling Glucan Recognition Systems by Glycome Microarrays Using the Designer Approach and Mass Spectrometry, Molecular & Cellular Proteomics, **14**, 974-988, (2015)

Peat S., Turvey J. R. and Rees D. A., Carbohydrates of the red alga, Porphyra umbilicalis, J. Chem. Soc., 1590-1595, (1961)

Peat S., Whelan W. J., Turvey J. R., Morgan K., The structure of isolichenin, J. Chem. Soc., 623-9, (1961b)

Percival E. and McDowell R. H., The Chemistry and Enzymology of Marine Algal Polysaccharides, Academic Press, (1967)

Pérez S., Mazeau K., Hervé du Penhoat C., The three-dimensional structures of the pectic polysaccharides, Plant Physiol. Biochem., **38(1/2)**, 37−55, (2000)

Perlin A. S., Suzuki S., The Structure of Lichenin: Selective Enzymological Studies, Canadian Journal of Chemistry, **40(1)**, 50-56, (1962)

Radley J. A., Starch and its Derivatives, 4th Edition, Chapman and Hall, (1968)

Ravno A. B. and Purchase B. S., Dealing with Dextran in the South African Sugar Industry, Proc. S. Afr. Sug. Technol. Ass., **79**, 28, (2005)

Ray B. and Lahaye M., Cell-wall polysaccharides from the marine green algae Ulva 'rigida' (Ulvales, Chlorophyta) — 1 Extraction and chemical composition, Carbohydr. Res., **274**, 251–261, (1995a)

Ray B. and Lahaye M., Cell-wall polysaccharides from the marine green algae Ulva 'rigida' (Ulvales, Chlorophyta) — 2 Chemical structure of ulvan. Carbohydr. Res., **274**, 313–318, (1995b)

Rees D. A., The Shapes of Molecules, Carbohydrate Polymers, Oliver & Boyd, (1967)

Rees D. A. and Conway E., The structure and biosynthesis of Porphyran, a comparison of some samples, Biochem J., **84**, 411,416, (1962)

Rees D. A., A double helix structure in food, Science Journal, **6(12)**, 47-51, (1970)

Rees D. A., Structure conformation and mechanism in the formation of polysaccharide gels and networks, In Advances in Carbohydrate Chemistry and Biochemistry, eds. Wolfrom M.L. and Tipson R. S., **24**, 267, (1969)

Rees D.A. et al, Covalent structure of the extracellular polysaccharide from Xanthomonas campestris: evidence from partial hydrolysis studies, Carbohydr. Res., **46**, (1976)

Rees D. A., Polysaccharide Shapes, Chapman and Hall, (1977)

Rennhard H., Polysaccharides and their preparation, US Patent, US 3766165 A (1973)

Roberfroid M., Dietary fibre, inulin and oligofructose: a review comparing their physiological effects, Critical Reviews in Food Science and Nutrition, **33(2)**, 103-148, (1993)

Robyt J.F., Essentials of Carbohydrate Chemistry, Springer Advanced Texts in Chemistry, (1998)

Rongved P. and Klaveness J., Water soluble polysaccharides as carriers of paramagnetic contrast reagents for magnetic resonance imaging; Synthesis and relaxation properties, Carbohyd.Res., **214**, 315-323, (1991)

Ross-Murphy S. B., Structure-property relationships in food biopolymer gels and solutions, J. Rheol., **39**, 1451-1463, (1995)

Ross P., Mayer R. and Benziman M., Cellulose Biosynthesis and Function in Bacteria, Microbiological Reviews, **55(1)**, 35-58, (1991)

Saito H., Ohki T. and Sasaki T., A ^{13}C-nuclear magnetic resonance study of polysacchaccharide gels. Molecular architecture in the gels consisting of fungal, branched 1,3)-β-D-glucans (lentinan) and (schizophyllan), Carbohydr. Res., **74(1)**, 227-240, (1979)

Sandford P.A. and Laskin A., Extracellular Microbial polysaccharides, eds., ACS Symposium Series **45**, (1977)

Schauer R., Sialic acids as regulators of molecular and cellular interactions, Current Opinion in Structural Biology, **19 (5)**, 507–514, (2009)

Sidebotham R. L., Dextrans, Advances in Carbohydrate Chemistry and Biochemistry, **30**, 371,444, (1974)

Situ C., Wylie A.R.G., Douglas A., et al., Reduction of severe bovine serum associated matrix effects on carboxymethylated dextran coated biosensor surfaces, Talanta, **76**, 832-836, (2008)

Skriptsova A. V., Shevchenko N. M., Zvagintseva T. N. and Imbs T. I., Monthly changes in the content and monosaccharide composition of fucoidan from Undaria Pinnatifida, J. Appl. Phycol., **22**, 79-86, (2010)

Smith I. H., Lawson C. J., Harding S. E., Gahler R. J. and Wood S., Viscosity development during aqueous dispersion and dissolution: A comparison of PGX with other dietary supplements and individual polysaccharides, Food Hydrocolloids, **38**, 152-162, (2014)

Smith F. and Montgomery R., The Chemistry of Plant Gums and Mucilages, Reinhold, New York, 1959

Steiner A. B., McNeely W. H., Organic Derivatives of Alginic Acid, Ind. Eng. Chem., **43(9)**, 2073–2077, (1951)

Stephen A. M., Churms S. C. and Vogt D. C., Exudate Gums, in Methods in Plant Biochemistry, **2**, 484-522, (1990)

Stephens E., Maslen S. L., Green L. G., and Williams D. H., Fragmentation Characteristics of Neutral N-Linked Glycans Using a MALDI-TOF/TOF Tandem Mass Spectrometer, Anal. Chem., **76 (8)**, 2343–2354 (2004)

Symes K. C., Lipophilic Polysaccharides, Carbohydr. Polymers., **2**, 276-281, (1982)

Szymanska-Chargot M., Chylinska M., Pieczywek P. M., Rosch P., Schmitt M., Popp J., Zdunek A., Raman imaging of changes in the polysaccharides distribution in the cell wall during apple fruit development and senescence, Planta, **243**, 935-945, (2016)

Timell T. E., Vegetable ivory as a source of a mannan polysaccharide, Canad. J. Chem., **35**, 333-338, (1957)

Tipper D. J., Structure and Function of Peptidoglycans, International Journal of Systematic Bacteriology, **20(4)**, 361,377, (1970)

Tomulescu C., Stoica R., Sevcenco C., Casarica A., Moscoviki M., Vamanu A., Levan - A Mini Review, Scientific Bulletin. Series F. Biotechnologies, **20**, 309-317, (2016)

Tung K. K., Nordin J. H., Evidence for a buried location of Nigeran in the cell wall of Aspergillus niger, Biochem. Biophys. Res. Commun., **28(4)**, 518-524, (1967)

Usov A. I., Yarotskii S. V., Esteves M. L., Algal polysaccharides. XXXII. Polysaccharides of the red algae Galaxaura squalida kjellm., Bioorganicheskaya Khimiya, **7(8)**, 1261-70, (1981)

References (continued)

Usov A. I., Dobkina I. M., Polysaccharides of algae. 43. Neutral xylan and sulphated xylomannan from the red seaweed Liagora valida., Bioorganicheskaya Khimiya, **17(8)**, 1051-8, (1991)

Üstün B., Sanders K. B., Dani P. and Kellenbach E. R., Quantification of chondroitin sulphate and dermatan sulphate in danaparoid sodium by ^{1}H NMR spectroscopy and PLS regression, Analytical and Bioanalytical Chemistry, **399(2)**, 629–634, (2011)

Vidal S., Doco T., Williams P., Patrice Pellerin P., York W. S., O'Neill M.A., Glushka J., Darvill A.G., Albersheim P., Structural characterization of the pectic polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain. Carbohydr. Res., **326**, 277-294, (2000)

Vieira R. P., Mulloy B. and Mourão P. A. S., Structure of a fucose-branched chondroitin sulphate from sea cucumber. Evidence for the presence of 3-O-sulpho-beta-D-glucuronosyl residues, J. Biol. Chem., **266**, 13530-13536, (1991)

Vincken J-P., Schols H. A., Ronald J.F.J., Oomen R. J. F. J., McCann M. C., Ulvskov P., Voragen A. G. J., and Visser R. G. F., If Homogalacturonan Were a Side Chain of Rhamnogalacturonan I. Implications for Cell Wall Architecture1, Plant Physiology, **132(4**), 1781-1789, (2003)

Vipul D. and McCarthy S.P., Review of Konjac Glucomannan, Journal of Environmental Polymer Degradation, **5**, 237-241, (1997)

Vuksan V. et al., Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes. A randomized controlled metabolic trial, Diabetes Care, **22(6**), 913-919, (1999)

Wang J., Zhang Q., Zhang Z., Zhang H. and Niu X., Structural studies on a novel fucogalactan sulphate extracted from the brown seaweed Lamonaria japonica, International Journal of Biological Macromolecules, **47(2)**, 126-131, (2010)

Ward K. and Seib P. A., in The Carbohydrates, 2nd edn., (Pigman W., Horton D. and Herp A., eds.) Academic Press, New York, **2A**, 413-415, (1983)

Wefers D. and Bunzel M., Arabinan and Galactan Oligosaccharide Profiling by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), J. Agric. Food Chem., **64(22)**, 4656–4664, (2016)

Weiβ G., Knochc A., Laichera A., Stanislausa F. and Daniels R., Simple coacervation of hydroxypropyl methylcellulose phthalate (HPMCP) II. Microencapsulation of ibuprofen, International Journal of Pharmaceutics, **124(1)**, 97–105, (1995)

Whistler R. L., ed., Methods in Carbohydrate Chemistry, General Polysaccharides, Academic Press, Volume V, (1965)

Whistler R. L. and BeMiller J. N., eds., Industrial Gums, Polysaccharides and Their Derivatives, Academic Press, Third Edition, (1993)

White C. A., ed., Advances in Carbohydrate Analysis, JAI Press, Volume I, (1991)

Willför S., Sundberg K., Tenkanen M., Holmbom B., Spruce-derived mannans, a potential raw material for hydrocolloids and novel advanced natural materials, Carbohydrate Polymers. **72(2)**, 197–210, (2008)

Wood P.J., Siddiqui I.R., Isolation and structural studies of a water-soluble galactan from potato (Solanum tuberosum) tubers, Carbohyd. Res., **22**, 212-220, (1972)

Wu J., Zhang F. and Zhang H., Facile synthesis of carboxymethyl curdlan-capped silver nanoparticles and their application in surface enhanced Raman spectroscopy, Carbohydrate Polymers, **90(1)**, 261–269, (2012)

Yang J-E., E-S. KimE-S., Kwon J. H., et al., Transdermal delivery of hyaluronic acid–human growth hormone, Biomaterials, **33**, 5947-5954, (2012)

Yapo B. M., Rhamnogalacturonan-I: A Structurally Puzzling and Functionally Versatile Polysaccharide from Plant Cell Walls and Mucilages, Polymer Reviews, **51** (4), 391-413, (2011)

Ying-qing Z., Xie Bi-jun., Gan X., Advance in the application of konjac glucomannan and it's derivatives, Carbohydrate Polymers, **60**, 27-31, (2005)

Yu J., Xie X., Wu J., Liu Y., Liu P. and Xu X., Folic acid conjugated glycol chitosan micelles for targeted delivery of doxorubicin: preparation and preliminary evaluation in vitro, Journal of Biomaterials Science, Polymer Edition, **24**, 606-620, (2013),

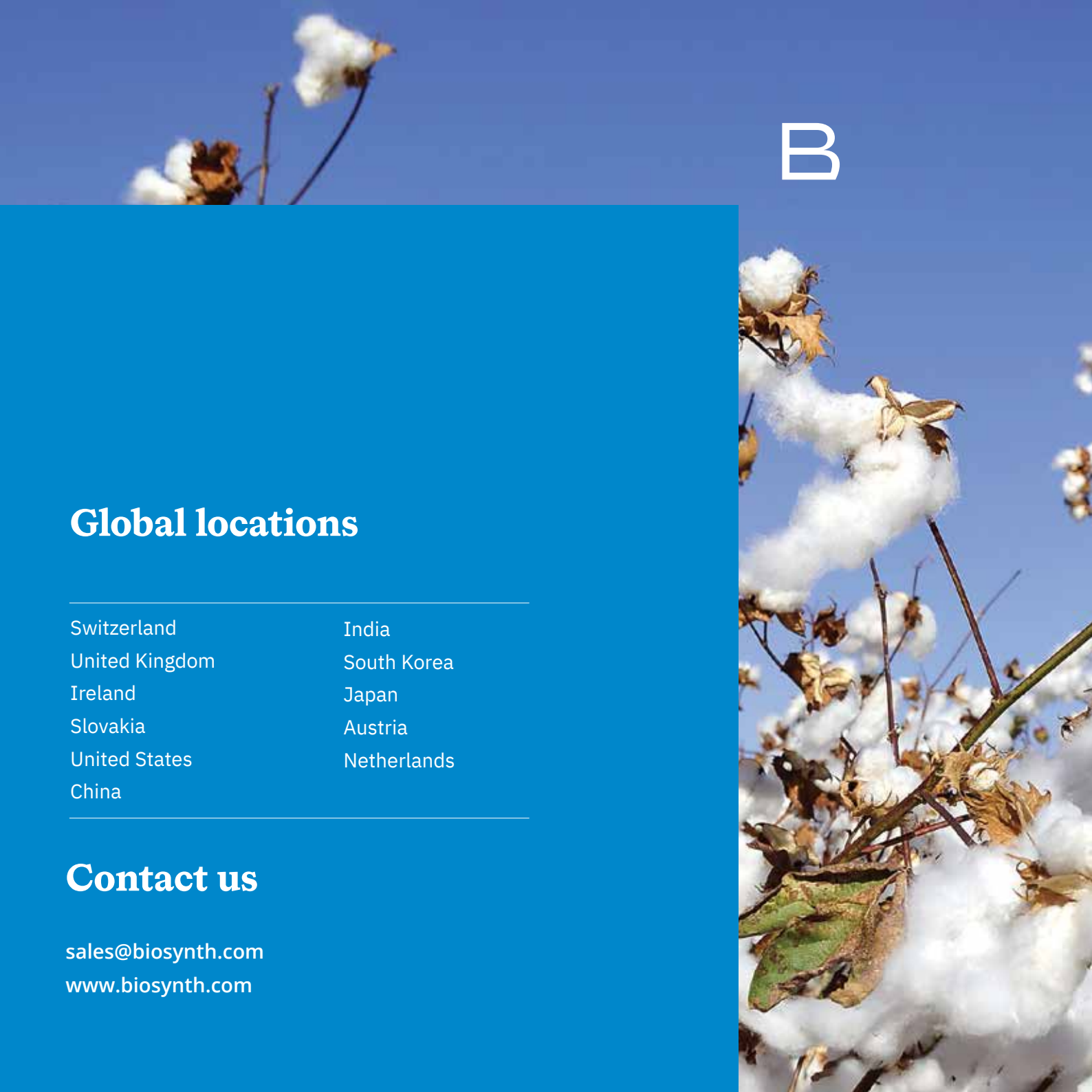
Zhang F., Zhang Z. and Linhardt R., Glycosaminoglycans, in Handbook of Glycomics, eds. Cummings R. D. and Pierce J. M., 59-80, (2009)

Zhang Q., Zhao T. et al., Chemical characteristics of a polysaccharide from Porphyra capensis, Carbohydr. Res., **340**, 2447-2450, (2005)

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