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STRUCTURE AND MECHANICAL PROPERTIES OF POLYSACCHARIDES

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Abstract This article serves both as a review of polysaccharides as gelling and thickening agents and as an introduction to polysaccharides, other than starch and cellulose, their source and extraction, and how molecular detail is reflected in their macromolecular and supramolecular properties. The whole is summarised for workers in the synthetic polymer area interested in new "green" polymers. We also introduce results for a new polysaccharide extracted from a Nigerian tree pod, and describe details of its structural and physicochemical characterisation including light scattering and rheological measurements.

INTRODUCTION

A technologically useful group of naturally occurring ("green") polymers are the polysaccharides, constructed formally by polymerisation of sugar (saccharide) units. On a volume basis, the most widely utilised are starch and cellulose, both derived from plants. However, there are many other less well known polysaccharides, and several are of technological importance in their own right. For example, many are polyelectrolytes, some of which form stiff chains in solution, and are therefore very effective viscosifiers. Others can be induced to form gels under appropriate conditions, and these also have a wide range of applications. This paper will serve both as an overview of these less common polysaccharides as archetypal green polymers, and also introduce more recent work on a new polysaccharide extracted from a Nigerian plant. This material, detarium gum, which may become a useful commercial viscosifying agent has been characterised using biochemical and physico-chemical techniques, including dynamic mechanical and light scattering measurements.

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THE POLYSACCHARIDES

Polysaccharides are branched or linear polymers of saccharides (sugars). In nature, they serve as an energy reserve, since they can be broken down by enzymes to produce oligomeric saccharides, or as hydrated networks in the exocellular and endocellular material of plant and marine organisms. They may also be exuded by extracellular microbes, or exist as intra-cellular hydrated material in plants. *In vivo* they can occur as essentially pure macromolecules or as part of highly specific structures, whose properties are supramolecular.

Since both starch and cellulose are discussed by others in this Volume and are by far the most important polysaccharides commercially, we concentrate in this article on the wide range of other polysaccharides, many of which are of technological interest. In Tanford's classic monograph, penned in 1961 he states "most of these substances have so far received relatively little attention from physical chemists" (Ref.1). Since this time there has been a significant growth of interest in these, inspired originally by the work of D.A. Rees and co-workers in the 1960s and 1970s. They showed how the hydrodynamic properties and the function of different polysaccharides could be related back to the detailed inter-saccharide residue linkage geometry (Ref.2). Subsequent work has extended this, although this article can do no more than act as an introduction to the area, and a guide to other work (Ref.3)

POLYSACCHARIDE SHAPES

As with synthetic polymers, few of the functional properties of polysaccharides are directly governed by the primary sequence structure, more crucial are the space-filling conformations adopted by the biopolymers. The inter-residue bond rotation between sugar units in polysaccharides is, in general, restricted so that only rather specific conformations are allowed. This means, for example, that the β-(1,4) linkage of cellulose produces a local "zigzag" shape that is propagated as an extended ribbon structure and which facilitates a high degree of ordered chain packing. The cellulose macromolecule commonly occurs in fibrous and highly crystalline sheets. By contrast the α-(1,4) linked amylose chain is geometrically constrained to give a more sinuous chain profile, with a further partiality to form helical structures. For all polysaccharides, the secondary structure is responsible for the overall macromolecular or tertiary structure by affecting the chain persistence (or Kuhn) length. Long chain branching, for example via (1,3) or (1,6) linkages may also occur. Rees' paperback (Ref.2) describes how such structures are explicitly related to the sugars and their specific linkage pattern. At a larger distance scale, quaternary structure such as the arrangement of crystalline chains in starch granules (Ref.4) may also be

important. Many plant polysaccharides are laid down either as a part of the complex cell-wall network structure (Ref.5) or in vacuoles of almost pure polysaccharide (Ref.6)

PLANT POLYSACCHARIDES

Starch, cellulose, pectin and guar are amongst the most important members of this group. In view of our earlier remarks, we restrict discussion to the latter two, and some other less common polymers. Pectins, found for example in fruits (apples, lemons) and other fibrous sources (sugar beet), as the intercellular cement within the plant cell wall, consist predominantly of sequences of galacturonic acid, with occasional interruptions by rhamnose residues. Pectins of low degree of esterification gel with divalent ions. The more esterified materials gel under conditions of low pH and decreased water active, and the junction zones are thermoreversible at about 40°C.

Effective polysaccharide thickeners include polymers such as the galactomannans (guar gum and locust bean gum), glucomannans (konjac) and xyloglucans (tamarind). The galactomannans are leguminous polysaccharides consisting of a poly(mannose) backbone, with galactose substituents distributed as sidearms along the chain (Ref.7). The number and the pattern of substitution of galactose units is rather specific to the original plant source. Since they are of quite high molecular weight (typically $> 10^6$), and not so flexible (Kuhn length ~ 10 nm), they have high $[\eta]$ for a given M_w (Ref.8) and are therefore efficient thickeners. Consequently, c^* ($\sim 1/[\eta]$) occurs at or below 0.1%; at concentrations above ~ $10c^*$ (~1%) their viscosity ~ c^4.

MARINE POLYSACCHARIDES

The most important of these are ι- and κ-carrageenan, agar(ose), and alginate and all can form gels under appropriate conditions. Evidence suggests that the carrageenans and agar form gels via a coil-helix (order/disorder transition). On heating above the helix-coil transition temperature (for the charged carrageenans this depends crucially on ionic strength and cation species, but typically in the range 20-50°C), they become disordered. On recooling they form gels, in which the cross-links (junction zones) are formed by ion mediated aggregation of double helical regions (Ref.9). Alginate gels by comparison are not thermoreversible but are heat stable up to >100°C. Their formation can only be induced by certain, specifically divalent, cations, and their structure involves so-called "egg-box" packing (Ref.10).

MICROBIAL POLYSACCHARIDES

A number of polysaccharides of interest occur outside the cells of certain cultured microbes, either covalently attached or secreted into the growth media. Some of these are plant pathogens, and blockage of water flow by the polysaccharide slime appears to be an important factor in pathogenicity. Some of these are cultured commercially by fermentation. This is a perfect example of a "green" production route, since feeding low molecular sugars and salts to the bacterium allows this to perform the polysaccharide synthesis. and production. On a volume basis, the two major members of this group are gellan, an anionic polysaccharide produced by *Auromonas elodea* (Ref.11) and xanthan from *Xanthomonas* strains (Ref.12). Gellan has a complex tetrasaccharide repeat unit, and gels in the presence of multivalent cations, via a double helical intermediate, similar to the mechanism for the carrageenans described above. The bulk mechanical properties are sensitive to the degree of acylation of the chain.

Although many strong polysaccharide gels close to the critical gel concentration may have weak gel-like properties, in this article we will restrict our attention to xanthan gum. This is a microbial exopolysaccharide produced by culturing *Xanthomonas campestris*. Xanthan, which has a pentasaccharide sequence, can form strong gels, but only under extreme conditions. More usually it displays the rheological properties of a "yield stress fluid", with a very pronounced shear rate dependent viscosity, with no clear indication of a low shear rate Newtonian viscosity plateau. Such behaviour is of considerable interest, because this suggests that such xanthan solutions will suspend small particles (where they exert a shear stress smaller than the yield stress). This so-called "weak gel" or "stabiliser" behaviour (Ref.13) has applications in the oil extraction, cosmetics, health care and food industry sectors. At low temperatures and/or in the presence of salt, xanthan is a stiff (Kuhn length around 240 nm, Ref.14) conformationally ordered polysaccharide. Although such properties are exhibited to a limited extent by some other stiff polysaccharides, for example schizophyllan, only xanthan is produced commercially on a large scale.

CHARACTERISATION OF A NEW PLANT POLYSACCHARIDE

The seed flour of the African leguminous plant, *Detarium senegalense* Gmelin, is used traditionally in Nigeria as a food condiment for the thickening of soups and stews. Preliminary chemical and physical analysis has indicated that the detarium seeds have a high level of water-soluble non-starch polysaccharide (~60g/100g dry weight) (Ref.15). Full characterisation involves a number of different techniques including: (1) *Extraction and basic sugar analysis*. To determine the type and proportion of saccharide units. (2) *Enzymatic analysis*. To determine the pattern of

glycosidic linkage at the oligosaccharide residue level. This helps to establish the class of polysaccharide. (3) *Dilute solution viscometry*. This allows an estimate of M_w from [η] and also an estimate of its potential viscosifying power, through c*. (4) *Semi-dilute rheometry*. This involves both steady and oscillatory shear rheometry, to confirm the potential usage of the material as a thickener; and (5) *Integrated light scattering*. This measures M_w and the radius of gyration, and (ideally) the Kuhn length or the presence of long chain branching, from the angular dependence. The work described below has been published in detail elsewhere (Ref.15, 16), so only essential methods are given.

Extraction And Basic Sugar Analysis

Detarium senegalense Gmelin is a leguminous seed crop, and its tree is small to medium-size, normally 5-7 meter high, mainly found in West Africa, Chad and Sudan. The pods, each of which contain one seed, are usually rounded, oval or flattened and are about 4 cm in diameter. The seed samples here were purchased at a market in Nsukka, Enugu State, Nigeria and stored at -20°C.

The seed coats (testae), which are a deep brown purple colour, were removed after boiling in water. The cotyledons were left to soak in cold water overnight, and then air-dried and ground into a fine powder. The powder were further dried at room temperature and stored at -20°C. The polymer was extracted and purified by a modification of the method of Girhammer and Nair (Ref.17), involving ethanol extraction, and enzyme treatment to remove contaminant protein.

Total acid hydrolysis (Ref.18) of the purified s-NSP fraction was carried out to produce the constituent sugars, which were then converted to the alditol acetate derivatives. The derivatives were analysed by GLC and the uronic acid content was determined by a sulphuric acid-dimethylphenol colorimetric assay.

TABLE 1. Monosaccharide composition of the detarium gum polysaccharide

	Polysaccharide extract (g/100g dry matter)	
Monosaccharides	**Mean**	**Standard deviation (±)**
Arabinose	1.75	0.14
Xylose	30.49	0.53
Mannose`	0.72	0.14
Galactose	15.91	0.52
Glucose	42.19	1.21
Uronic acid	0.75	0.14
Total	91.81	1.71

Enzymatic Analysis

An extract of detarium gum, and a control sample of tamarind gum, were digested with a pure (1- 4)-β-D-glucanase of fungal origin in Prof. J.S.G. Reid's laboratory in Stirling, Scotland, and analysed with HPLC. The results (Ref.15) confirmed that the new polymer was indeed a member of the xyloglucan family, although the precise pattern of short chain branching was significantly different to that of tamarind gum.

Dilute Solution Viscometry

The intrinsic viscosity of the polysaccharide from detarium was found to be ~ 8.9 ± 0.2 dl/g, which is significantly higher than the value reported for tamarind gum ([η]=6.0 ± 0.5 dl/g)(Ref.19). This difference could be due either to the difference in substitution pattern increasing the Kuhn length or, more likely that the detarium xyloglucan is of higher M_w. If we assume that the exponent a in the Mark-Houwink-Sakurada equation is 0.5-0.8 and use published data for tamarind xyloglucan (Ref.19), we can estimate M_w for detarium gum to be ~ 1.3 to 1.9 x 10^6.

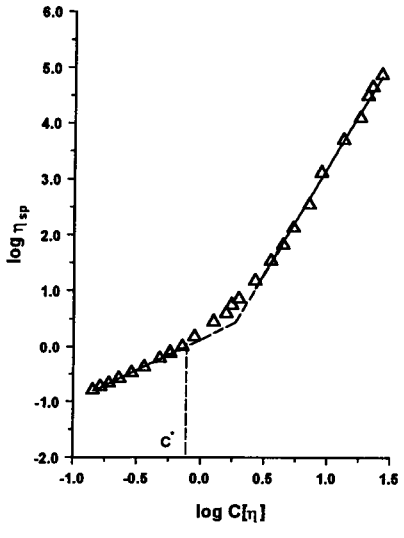

Fig.1 Zero shear specific viscosity of detarium xyloglucan solutions plotted against C[η]

Semi-Dilute Steady Shear Viscosity

The viscosity at concentrations above ~ 0.2% w/w is very shear rate dependent. However, using the empirical Cross equation, it is possible to obtain good estimates of the zero shear specific viscosity), even where this cannot be measured directly. The concentration dependence of the zero shear rate specific viscosity η_{sp} (= $\{\eta_0 / \eta_s\}$-1, where η_s is

the viscosity of water) can be presented as a double logarithmic plot of η_{sp} against the coil overlap parameter $C[\eta] = C/C^*$ (Fig.1). Two distinct linear regimes of slope can be identified which is consistent with data seen for almost all synthetic and biopolymer solutions. The first regime with a slope of 1.3 corresponds to the dilute solution, and the second one with a slope of 4.0 represents semi-dilute solutions. This means that when $C<C^*$, $\eta_{sp} \propto C^{1.3}$, while at $C>C^*$, $\eta_{sp} \propto C^{4.0}$, which is similar to data published for guar gum (Ref.8). and other polysaccharide solutions discussed above. The highest slope value (i.e. 4.0) is typical for linear polymers interacting purely by topological entanglements.

Integrated Light Scattering

Results from the light scattering measurements carried out in the laboratory of Prof. W. Burchard in Freiburg-i-Br, Germany are summarised in Table 2. Samples were autoclaved to produce molecular dispersions (Ref.20). The weight average molecular weights, MW_w, from independent zero angle and zero concentration extrapolations matched well in almost all cases, and good data were obtained for the (root of z-average mean square) radius of gyration, R_g (Table 2). The observation that MW_ws obtained are effectively independent of heating temperature and time strongly suggests that we are measuring a true molecular property, rather than a time- and temperature-dependent molecular weight-degradation effect. This was confirmed by re-measuring $[\eta]$ for heated samples.

TABLE 2. Summary of static light scattering results

Sample	Treatment	$Mw_w (x10^6)$	R_g /nm
XG1	130°C for 20 mins	2.75	119
XG3	Repeat of XG1	2.55	112
XG4	130°C for 120 mins	2.75	123
XG5	160°C for 20 mins	2.72	114

Fig.2 shows a Kratky plot ($u^2P(u)$ vs. u) of this data. Here, $u = qRg$, $P(u) \equiv R_\theta/R_0 = 0$ is the so-called particle scattering factor, which reflects the angular dependence of the scattered light, and q is the scattering vector ($= 4\pi\lambda/\sin(\theta/2)$). The parameter u, which is dimensionless, measures the intramolecular probe distance relative to the incident light wavelength, and P(u) can be calculated for different chain architectures. The data from different experiments are in very good agreement (this is a particularly testing strategy) and show that at wide angles (high u) they reach an apparent plateau, where $u^2P(u)$ is ca. 1.5.

The data lie much below the expected profile for a Gaussian (flexible) chain, which itself is below that for semi-flexible chains or rods (not illustrated). The data lie above the curve for a very

high degree of random homogeneous branching, and qualitatively, at least, resemble the traces calculated for low degree (three or four arm) star-branched macromolecules. Such a model cannot be taken too literally without including the effects of polydispersity, but what can be stated unequivocally is that the scattering profile for detarium gum is not consistent with that of a linear

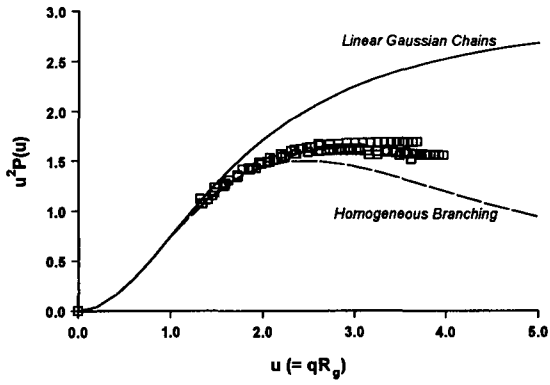

Fig.2 Kratky plot for detarium gum samples. Theoretical curves calculated according to Ref.21

macromolecule, but instead strongly suggests a small element of long chain branching. The level of such branching need not be very great, say perhaps 2-10 branch points per chain. This is much below the level detectable biochemically (J.S.G. Reid, personal communication), but is quite sufficient to affect the overall chain profile. The difference between the MW_w measured here, and that estimated from $[\eta]$ is, of course, due to assuming in the latter, that the original sample was linear.

CONCLUSIONS

As well as reviewing work on the less widely known polysaccharides, this article illustrates, by application to a new polysaccharide, the range of techniques required to fully characterise such a new material. If, in the future, green polymers are required where synthetic polymers are now the rule, this introduction should prove of value to a wider audience in synthetic polymer science.

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