Many polysaccharides are allowed for direct food use, where they serve a number of useful functions including dietary fiber, bulking agent, thickener, encapsulant, gelling agent, foam and emulsion stabilizer, protective colloid, emulsifier and suspending agent, adhesive and binder, flocculant, swelling agent, film/coat former, or syneresis inhibitor. Many of these polysaccharides have complex structures or are mixtures with different components. Over the years, NMR has been a premiere technique for characterizing these materials. NMR characterization can help identify the materials in question, quantify the different functional groups present, and detect minor components and impurities. Above all, the high resolution achieved in solution NMR can provide detailed structural information on composition, sequence distribution, substitution pattern, and molecular weights (in some cases) for individual polysaccharides. Concurrent application of other analytical techniques, such as methylation, esterification, fractionation, mass spectrometry, and chromatographic methods, has enabled structural information on even complex polysaccharides or mixtures to be obtained. In this article a review is given of the solution NMR of food polysaccharides, with emphasis on papers published in the past 20 years. Included in the review is a survey of 21 common food polysaccharides, the current understanding of their structures, and the techniques used for their determination.

Keywords  Carbohydrates, food gums, food polymers, hydrocolloid, nuclear magnetic resonance (NMR), polysaccharides

1. Introduction

A large number of polymers are allowed for direct food use; these are mostly of natural origin, some modified, and some made through microbial means.1–10 Many of them are polysaccharides or their derivatives, to which the terms “food gums” and “food hydrocolloids” are sometimes applied. Food polysaccharides are usually grouped by their origin; the following classification includes most of the common ones in commercial use.
Broadly speaking, these food polysaccharides come from three sources: (1) plant materials, for example, seeds, tubers/roots, plant exudates, and extracts of seaweeds and fruits; (2) micro-organisms, usually requiring fermentation processes for production, and (3) derivatives of cellulose and starch. A separate category of polymers approved for food use includes proteins from animal sources such as gelatin, casein, whey, zein, and soy protein; these are not covered in this review.

NMR spectroscopy is a powerful analytical technique that is often employed to study polymers. The detailed structural information obtained from NMR is frequently not available through other analytical techniques. Over the years, numerous publications have appeared on the NMR of food polysaccharides, and NMR has become a routine method for many analyses. This review aims at providing a selected survey of the papers published in this area especially in the past 20 years, with emphasis on studies of polysaccharide structures using solution NMR methods. For convenience, the following conventional nomenclature will be used for the monosaccharide residues:

\[
\begin{align*}
\text{Glc} &= \text{glucose}, \quad \text{Gal} = \text{galactose}, \quad \text{Fuc} = \text{fucose}, \quad \text{Rha} = \text{rhamnose}, \quad \text{Xyl} = \text{xylose}, \quad \text{Ara} = \text{arabinose}, \quad \text{Gul} = \text{gulose}, \quad \text{Man} = \text{mannose}; \\
\text{A} &= \text{uronic acid derivative}, \quad \text{p} = \text{pyranoside}, \\
\text{f} &= \text{furanoside}
\end{align*}
\]

The following abbreviations will be used: GC, gas chromatography; MS, mass spectrometry, ESI-MS, electrospray ionization mass spectrometry; CP/MAS, cross polarization/magic angle spinning, COLOC, correlated spectroscopy for long-range coupling; COSY, correlated spectroscopy; DOSY, diffusion ordered spectroscopy; HETCOR, heteronuclear correlated spectroscopy; HMBC, heteronuclear multiple-bond correlation spectroscopy; HMQC, heteronuclear multiple quantum correlated spectroscopy; HSQC, heteronuclear single quantum correlated spectroscopy; INADEQUATE, incredible natural-abundance double-quantum transfer experiment; NOESY, nuclear Overhauser enhancement spectroscopy; ROESY, rotating frame nuclear Overhauser enhancement spectroscopy, and TOCSY, total correlated spectroscopy.
2. Structure and Properties

Food polysaccharides serve many functions in food and food formulations. (Many of them are also used in non-food applications.) The following is only a partial list:

<table>
<thead>
<tr>
<th>(caloric) food</th>
<th>lubricant</th>
</tr>
</thead>
<tbody>
<tr>
<td>reduced calorie food</td>
<td>bulking agent</td>
</tr>
<tr>
<td>dietary fiber</td>
<td>crystallization inhibitor</td>
</tr>
<tr>
<td>thickener</td>
<td>encapsulant/microencapsulant</td>
</tr>
<tr>
<td>gelling agent</td>
<td>foam stabilizer</td>
</tr>
<tr>
<td>emulsion stabilizer</td>
<td>protective colloid</td>
</tr>
<tr>
<td>emulsifier</td>
<td>suspending agent</td>
</tr>
<tr>
<td>adhesive/binder</td>
<td>suspension stabilizer</td>
</tr>
<tr>
<td>flocculant</td>
<td>swelling agent</td>
</tr>
<tr>
<td>film/coat former</td>
<td>syneresis inhibitor</td>
</tr>
</tbody>
</table>

Most food polysaccharides are water-soluble or water-dispersible. The resulting aqueous solutions or dispersions may have increased viscosities and/or suspending and stabilizing properties. Many of them form gels, for example, agar, alginate, carrageenan, furcellaran, gelatin, pectin, and starch. The rheology and gelling properties of the solutions or dispersions depend on the polymers involved, pH, concentration, temperature, salt and other solutes present, and many other factors. For food use, food polysaccharides are preferably tasteless, odorless, colorless, and non-toxic.

In order to carry out rational product design and development, it would be desirable to characterize the structures of these polysaccharides and to improve the understanding of structure/property relationships. A fair amount of know-how has accumulated over the years. For example, many of the desirable properties have been correlated with structural features, such as molecular weight, type, and sequence distribution of sugar residues in the polysaccharide, and the presence (or the absence) of branching and charged functional groups.

Molecular weight has a significant effect on solution viscosity. As the molecular weight increases, the viscosity of the polymer solution also increases. When the molecular weight reaches a critical value, chain entanglement occurs, and the polymer solution tends to exhibit non-Newtonian behavior at low shear rates. Likewise, molecular weight distribution has a large effect on rheology. For example, a polymer with a high-molecular-weight component beyond the critical molecular weight may cause the polymer solution to exhibit non-Newtonian flow.

Types and sequence distributions of sugar residues in the polysaccharide are known to influence polysaccharide stiffness and solution behavior. Thus, cellulosic derivatives (with β-1,4 glycosidic bonds) tend to have higher solution viscosities than the corresponding starch derivatives (with α-1,4 glycosidic bonds). Starch in turn has a higher viscosity than dextrans (with mostly α-1,6 glycosidic bonds). Similarly, branching can have an effect on properties. Linear homoglycans, for example, cellulose and amylose, tend to associate and crystallize, and therefore dissolve either poorly or not at all in water. In contrast, branched polymers (e.g., amylopectin and gum arabic) tend to give more stable aqueous solutions which will not retrograde or precipitate.

Polysaccharides containing charged functional groups have properties expected of polyelectrolytes. Examples are pectin, alginates, and CMC which contain carboxylic
acids, and agar, carrageenan, and furcellaran which contain sulfates. With suitable cations, these anionic polymers can form gels. Other charged polysaccharides include xanthan, gellan, and gum arabic.

Solution NMR is very useful in structure/property studies of polymers. It can help identify the materials in question, quantify the different functional groups present, and detect minor components and impurities. In addition, the high resolution achieved in solution NMR can provide detailed structural information on composition, sequence distribution, substitution pattern, and molecular weight (in some cases). Many examples of the applications of solution NMR will be given in Section 5.

3. Experimental Techniques

The polysaccharide is often dissolved in D₂O in such a way that the –OH is fully deuterium exchanged. A variation is to use D₂O/d₆-acetone mixture, where the –OH or –NH can still be observable under the slow exchange condition. Another variation is to use a mixture of H₂O/D₂O (e.g., 9:1) for the same purpose. Another approach is to dissolve the polysaccharide in d₆-dimethylsulfoxide (DMSO). If the polysaccharide is peracetylated or permethylated, the spectrum may be obtained in deuterochloroform.

Different chemical shift reference compounds are often used. For ¹H TMS (tetramethylsilane), DSS (4,4-dimethyl-4-silapentane-1-sulfonate, sodium salt), and TSP (3-trimethylsilyl propionate, sodium salt) are the most popular references. The ¹H shifts relative to TMS (0 ppm) are DSS (0 ppm), TSP (−0.017 ppm), d₆-DMSO (2.50 ppm), and acetone (2.20 ppm). For ¹³C the reference compounds include TMS (0 ppm), DSS (−1.7 ppm), TSP (−1.8 ppm), dioxane (67.5 ppm), methanol (49.5 ppm), acetonitrile methyl (1.3 ppm), and d₆-DMSO (39.5 ppm). As different reference compounds are used by different workers, care is needed when shift comparisons are made.

Because the polysaccharide often has high molecular weight, NMR resonances may be broad. Higher temperatures and higher magnetic fields are helpful in producing better resolved spectra. Partial hydrolysis, using acids or enzymes, lowers the molecular weights and usually sharpens the resonances. An alternative is to use ultrasonication which also cuts down the molecular weight. At the data manipulation stage, overlapping signals can often be resolved through curve deconvolution. Computer techniques are also available that artificially narrow the resonances, for example, use of negative exponential multiplier, maximum entropy, and linear prediction methods.

Two-dimensional (2D) and three-dimensional (3D) NMR methods are now routinely used for NMR analysis. Commonly used experiments include:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COSY</td>
<td>Used for connectivity of adjacent ¹H resonances within a monosaccharide residue.</td>
</tr>
<tr>
<td>TOCSY</td>
<td>Used for correlation of all ¹H resonances within a monosaccharide residue.</td>
</tr>
<tr>
<td>HETCOR, HMQC, HSQC</td>
<td>Used to correlate the ¹³C and ¹H shifts of directly bonded C–H (i.e., ¹J_C–H).</td>
</tr>
<tr>
<td>HMBC, COLOC</td>
<td>Used to correlate the ¹³C and ¹H shifts of long-range ³JC–H across the glycosidic bond.</td>
</tr>
</tbody>
</table>
NOESY  Used for connectivity of $^1$H resonances through space (less than 4.5 Å). Can also be used to sequence oligosaccharides.

ROESY  Similar to NOESY, but carried out in the rotating frame. Particularly useful for mid-sized molecules (1000–3000 daltons) with weak conventional nuclear Overhauser effects.

INADEQUATE  Used for connectivity of adjacent $^{13}$C resonances within a monosaccharide residue.

DOSY  Used for connectivity of chemical shift and self-diffusion constant. This experiment often permits differentiation of molecules by size, mobility, binding or aggregation.

4. General Trends in Spectra

Solution NMR can be used very effectively to identify the type, the number, and the proportions of different sugar residues in a polysaccharide as well as different linkage configurations and positions in the polymer.\textsuperscript{17–27} Frequently there are good correlations between the chemical shifts of the polysaccharide and the chemical shifts of the component sugars. However, shift displacements do occur for the protons and the carbons near the glycosidic linkages.

Interpretation of the NMR spectra of monosaccharides and polysaccharides requires a good database of reference spectra and the appropriate use of NMR techniques. The large number of 2D and 3D NMR experiments available has been very helpful. Spectral assignments have been facilitated with the availability of web databases and shift prediction programs such as GLYCOSCIENCE.de\textsuperscript{39–40} and CASPER.\textsuperscript{41–43} Other computer-assisted approaches include the method developed by Kochetkov et al.,\textsuperscript{44} and artificial neural networks.\textsuperscript{45–47} Additional databases and computer approaches have been summarized in two reviews.\textsuperscript{48,49} For better elucidation of polysaccharide structure, data from other complementary analytical techniques are also helpful; some commonly used techniques are GC/MS, ESI-MS, high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD), matrix-assisted laser desorption ionization with time-of-flight detection (MALDI/TOF), affinity chromatography, and chemical methods (e.g., hydrolysis, methylation, acetylation, and reductive cleavage). Molecular modeling can also be useful in appropriate situations.

Although each polysaccharide has its own characteristic NMR features and needs to be studied individually, some general observations have been made concerning the trends in the NMR shifts.\textsuperscript{17–21} For pedagogical purposes, a summary table, initially made by Perlin and Casu,\textsuperscript{20} is given in Table 1. Thus, H-2 through H-6 resonate at 3.5–4.5 ppm, whereas the anomeric H-1 can be found at 4.5–5.8 ppm. Note that for pyranose rings, equatorial $^1$H is generally downfield from the axial $^1$H. Thus for a monosaccharide, an anomeric OH in the $\alpha$ position (which has an equatorial H) has the $^1$H resonances at 5.3–5.8 ppm, whereas the $\beta$ counterpart (having an axial H) resonates at 4.5–4.8 ppm. Any substituent on oxygen shifts the neighboring protons. The substituent shift values at the bottom of Table 1 indicate that the shifts usually move downfield, depending on the substituent in question.

In addition to the chemical shifts, spin-spin coupling constants can also be informative.\textsuperscript{17–21} For example, when –OH groups are fully deuterium exchanged, the splitting
Table 1
Representative $^1$H and $^{13}$C chemical shifts for nuclei of polysaccharides$^{a,b}$

<table>
<thead>
<tr>
<th></th>
<th>$^1$H shift (ppm)</th>
<th>$^{13}$C shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$C</td>
<td>$\sim$1.5</td>
<td>CH$_3$C $\sim$15</td>
</tr>
<tr>
<td>CH$_3$CON</td>
<td>1.8–2.1</td>
<td>CH$_3$COH) 20–23</td>
</tr>
<tr>
<td>CH$_3$CO$_2$</td>
<td>2.0–2.2</td>
<td>CH$_3$CO$_2$)</td>
</tr>
<tr>
<td>CH(NH)</td>
<td>3.0–3.2</td>
<td>CH$_3$C 38</td>
</tr>
<tr>
<td>CH$_3$O</td>
<td>3.3–3.5</td>
<td>CH$_3$O 55–61</td>
</tr>
<tr>
<td>H-2 to H-6’</td>
<td>3.5–4.5</td>
<td>CH(NH) 58–61</td>
</tr>
<tr>
<td>H-5</td>
<td>4.5–4.6</td>
<td>CH$_2$OH 60–65</td>
</tr>
<tr>
<td>H-1 (ax)</td>
<td>4.5–4.8</td>
<td>C-2 to C-5 65–78</td>
</tr>
<tr>
<td>H-C(OH)$_2$</td>
<td>5.2</td>
<td>C-X$^c$ 78–87</td>
</tr>
<tr>
<td>HO</td>
<td>5.0–5.4</td>
<td>C-1 (ax-O, red) 90–95</td>
</tr>
<tr>
<td>H-1 (eq)</td>
<td>5.3–5.7</td>
<td>C-1 (eq-O, red) 95–98</td>
</tr>
<tr>
<td>H-CO$_2$</td>
<td>5.9</td>
<td>C-1 (ax-O, glyc) 98–103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-1 (eq-O, glyc) 103–106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-1 (fur) 106–109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COOH 174–175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COOR 175–180</td>
</tr>
</tbody>
</table>

Substituent effects on $\alpha$-$^1$H and $\alpha$-$^{13}$C (ppm)$^d$,

<table>
<thead>
<tr>
<th></th>
<th>O-Alkyl</th>
<th>O-Acyl</th>
<th>O-Sulfate</th>
<th>O-Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>−0.2–0.3</td>
<td>+0.3–0.5</td>
<td>+0.3–0.6</td>
<td>+0.3–0.5</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>+7–10</td>
<td>+&lt;3</td>
<td>+6–10</td>
<td>+2–3</td>
</tr>
</tbody>
</table>

$^a$Taken from Perlin and Casu$^{26}$, with permission
$^b$Abbreviations: ax, axial; eq, equatorial; red, reducing; glyc, glycosidic; fur, furanosyl.
$^c$Non-anomeric $^{13}$C involved in glycosidic linkage.
$^d$Downfield, +; upfield, −.

Pattern of each proton on the ring can frequently give its position on the ring. Thus, the anemic H-1 usually shows up as a doublet (due to coupling with H-2), and H-2 as a pair of doublets (due to coupling with H-1 and H-3). The size of the coupling constant depends on the orientation of the protons. For axial/axial protons, $3J_{12}$ is 7–8 Hz. For axial/equatorial or equatorial/equatorial protons, $3J_{12}$ is 3–4 Hz. Thus, in theory the coupling pattern for any proton on the ring can be predicted. In practice, the $^1$H NMR spectrum tends to have overlapping lines in the 3.0–4.5 ppm region and may also exhibit line broadening (especially for high-molecular-weight polysaccharides), and the coupling information may not always be available, although multidimensional methods can be very helpful here.

$^{13}$C NMR has larger chemical shift dispersion and can often provide more information. A typical $^{13}$C spectrum of a polysaccharide (agar)$^{50}$ is given in Fig. 1. Representative $^{13}$C shifts$^{26}$ have also been summarized in Table 1. The resonances for C-2 to C-5 can be found at 65–78 ppm. The primary alcohol (C-6 for pyranoside) resonates at 60–70 ppm. The anomeric C-1 carbons are the most diagnostic; the shifts occur at 90–110 ppm and depend on their orientation (axial vs. equatorial) and chemistry (internal/non-reducing or chain end/reducing). Thus, from C-1 alone one can often determine the different types of sequences present and their relative proportions.
Substitution at oxygen usually moves the $^{13}$C shifts downfield, the extent of the shift depending on the nature of the substituent (Table 1, bottom). Thus, O-alkylation moves the $^{13}$C shifts downfield by 7–10 ppm and O-acylation moves less than 3 ppm. In addition, the next neighboring carbon on the ring tends to have an upfield shift ($\gamma$ effect) of about −1.5 ppm.

In addition to chemical shifts, the $^{13}$C coupling constants also provide information. For example, $^{1}$J_{C-H} for the anomeric carbon depends on the configuration at C-1: $\alpha$ anomer (170 Hz) has a higher $^{1}$J_{C-H} than the $\beta$ anomer (160 Hz). Another way for obtaining information on neighboring groups is deuterium isotope shifts; the information available is comparable to that obtained from coupling constants.

The literature on the NMR of polysaccharides has become rather extensive. Spectral data have been published for all common food polysaccharides. A survey of these polysaccharides is given below, with emphasis on the work published since 1990.

5. Survey of the NMR of Food Polysaccharides

5.1 Starch and Modified Starch

Starch is an abundant food material, available commercially from corn, potatoes, wheat, tapioca, rice, and other crops. It contains two major components with the following structures:

- amylose: $[(1,4)-\alpha$-D-Glcp]$_{n}$
- amylopectin: $[(1,4)-\alpha$-D-Glcp]$_{m}$-(1,4)-$\alpha$-D-Glcp-6 $\uparrow$
  1 $\rightarrow$
  $[(1,4)-\alpha$-D-Glcp]$_{m}$-(1,4)-$\alpha$-D-Glcp
Starch and related products have often been studied by NMR. Work done prior to 1990 has been reviewed by Gorin, Gridley, and McIntyre et al. Examples of published work since then include 2D NMR studies of starch and low-molecular-weight analogs and glycogen, and branching (α-1,4)/(α-1,6) ratio determination. As illustrations of the 2D data, COSY and HMBC plots of potato starch are shown in Figs. 2 and 3. As expected, the COSY plot permits the proton shifts within each anhydroglucose ring to be correlated. In the HMBC plot the 13C and the 1H spectra are depicted on the y and the x axis, respectively. Long-range couplings (2J_C-H and 3J_C-H) can be seen as cross-peaks. The 2D studies confirmed previous assignments and also permitted new assignments to be made. The assignments are summarized in Table 2.

Starch is often modified to improve its properties. It can be hydrolyzed by acids or enzymes to lower the molecular weight to produce acid-converted starches, maltodextrins, and corn syrup solids. It can be modified through oxidation, giving bleached starch and chlorinated starches. It can also be subjected to pyroconversion to form dextrins, which tend to have lower molecular weights and increased branching.
Maltodextrins and dextrins have often been studied by NMR as low-molecular-weight analogs of starch, for example, 2D NMR,\(^{62}\) \(^{13}\)C studies of corn syrup, maltodextrin and starch,\(^{58}\) and degraded amylopectin.\(^{63}\) Oxidized starches have been investigated occasionally, for example, \(^1H\) and \(^{13}\)C assignments,\(^{64}\) enzymatic hydrolysis and NMR analysis of the resulting oligosaccharides,\(^{65}\) and combined fractionation-NMR analysis.\(^{66}\)

### Table 2

\(^{13}\)C shifts and \(^1H\) shifts (in parentheses) for starch\(^a\) at \(80^\circ C\) and D\(_2\)O

<table>
<thead>
<tr>
<th>structure</th>
<th>C-1 (H-1)</th>
<th>C-2 (H-2)</th>
<th>C-3 (H-3)</th>
<th>C-4 (H-4)</th>
<th>C-5 (H-5)</th>
<th>C-6 (H-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1-4) Glc</td>
<td>102.1</td>
<td>74.2</td>
<td>75.8</td>
<td>80.1</td>
<td>74.0</td>
<td>63.3</td>
</tr>
<tr>
<td>(^{13})C shifts</td>
<td>(5.35)</td>
<td>(3.63)</td>
<td>(3.94)</td>
<td>(3.62)</td>
<td>(3.82)</td>
<td>(3.80)</td>
</tr>
<tr>
<td>(^1H) shifts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-reducing end</td>
<td>107.4</td>
<td>74.3</td>
<td>75.7</td>
<td>72.3</td>
<td>75.4</td>
<td>63.4</td>
</tr>
<tr>
<td>(^{13})C shifts</td>
<td>(5.33)</td>
<td>(3.58)</td>
<td>(3.69)</td>
<td>(3.41)</td>
<td>(3.71)</td>
<td>(3.75)</td>
</tr>
<tr>
<td>(^1H) shifts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1-6) Glc</td>
<td>(4.94)</td>
<td>(3.59)</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

\(^a\)Assignments from Nilsson et al.,\(^{56}\) TSP reference at 0 ppm.
Another approach for modifying starch is to crosslink it with adipate or phosphate. To be allowed for food use, the amount of crosslinking is controlled by government regulation, typically less than 0.14%. Other approaches include derivatization to form starch acetates, maleated starch, starch phosphates, starch sodium octenyl succinate, and hydroxypropyl starch. NMR is sometimes used to characterize these products. Examples include starch acetate and hydroxypropyl starch, and starch modified with octenyl succinyl anhydride and maleate.

Several papers have appeared on the use of $^{31}$P NMR to examine native starch, crosslinked starch, and starch phosphates. Native starch has been studied by Muhrbeck and Tellier, Lim and Seib, and Bay-Smidt et al., where the phosphate has been found to be bound to O-6 and O-3 only. Kasemsuwan and Jane and Lim et al. have identified and quantified the level of phosphate and phospholipids in native starches from different sources. For starch phosphates, Lim and Seib have found phosphorylation to occur at all three hydroxy groups on the anhydroglucose unit and the reactivity is O-6 > O-2 ~ 0.3. Crosslinked starch has been examined by Kasemsuwan and Jane. The POCl$_3$ reaction has been found to generate not only phospho-diester (crosslink) but also phospho-monoester. Amylose and amylopectin has been crosslinked together in the process, but amylose molecules have not crosslinked with each other. Starch crosslinking reactions with sodium trimetaphosphate and sodium polyphosphate have been studied by Sang et al., where $^{31}$P NMR has permitted different phosphate species to be identified.

5.1.1 Dextran. Dextrans are produced from sucrose by microbes of the Leuconostoc genus. They are used often in medical applications but much less so in foods. They have a $\alpha$-1,6-glucan structure with some $\alpha$(1-3) linkage and sometimes $\alpha$(1-2) and $\alpha$(1-4) linkages.

\[-[(1,6)-\alpha-D-Glcp]_n(1,6)-\alpha-D-Glcp-\]

\[X\]

\[\uparrow\]

\[1\]

$\alpha-D-Glcp$

(branching at position X)

The NMR spectra of dextrans have been studied extensively and previously reviewed. Using 2D NMR, have provided detailed $^1$H and $^{13}$C assignments (Table 3). The use of $^1$H NMR for routine analysis of dextran has also been demonstrated.

5.1.2 Pullulan. This is an extracellular polysaccharide produced by a fungus of the genus Aureobasidium (also called Pullularia). It can be used as a thickener, emulsion stabilizer, film-former, and adhesive in foods. It may also be used as a low-calorie food ingredient. It has a linear $\alpha$-glucan structure with the major $\alpha$-(1-6) maltotriose repeat structure:

\[[(1,6)-\alpha-D-Glcp-(1,4)-\alpha-D-Glcp-(1,4)-\alpha-D-Glcp]\]

There is also a variable amount of $\alpha$-(1-6) maltotetraose units.

Early work prior to 1981 has been reviewed by Gorin. Complete $^{13}$C NMR assignments have been made by McIntyre and Vogel, and by Arnosti and Repeta with the help of 2D NMR. In addition, the shifts for the reducing ends have been partly deciphered; the $\alpha$ anomer has H-1 shift at 5.27 ppm and C-1 shift at 92.62 ppm, and the $\beta$ anomer has H-1 shift at 4.69 ppm and C-1 shift at 96.53 ppm. More recently, pullulan-like polysaccharides
Table 3

$^{13}$C shifts and $^1$H shifts (in parentheses) for dextran$^a$ at 60°C and D$_2$O

<table>
<thead>
<tr>
<th>structure</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1-6) Glc</td>
<td>98.59</td>
<td>72.31</td>
<td>74.30</td>
<td>70.61</td>
<td>71.09</td>
<td>66.72</td>
</tr>
<tr>
<td>$^{13}$C shifts</td>
<td>(4.97)</td>
<td>(3.58)</td>
<td>(3.71)</td>
<td>(3.50)</td>
<td>(3.89)</td>
<td>(3.77)</td>
</tr>
<tr>
<td>$^1$H shifts</td>
<td>(3.77)</td>
<td>(3.98)</td>
<td>(3.97)</td>
<td>(3.86)</td>
<td>(3.85)</td>
<td>(3.83)</td>
</tr>
</tbody>
</table>

(1-3) Glc branch

α reducing end

$^1$H shifts | (5.32) | (3.59) | (3.75) | (3.44) | ? | ? |

β reducing end

$^1$H shifts | (5.23) | (3.53) | (3.71) | (3.49) | ? | ? |

$^{13}$C shifts and $^1$H shifts (in parentheses) for pullulan$^a$ in D$_2$O

Table 4

[(1,6)-$\alpha$-D-Glcp-(1,4)-$\alpha$-D-Glcp-(1,4)-$\alpha$-D-Glcp-(1,4)-$\alpha$-L-Rhap]

(partly acetylated)

5.1.3 Gellan. Gellan is an extracellular polysaccharide produced by Pseudomonas elodea and has good gelling properties.

A | B | C |
---|---|---|
$^{13}$C shifts | 100.83 | 74.10 | 76.31 | 80.20 | 73.26 | 63.65 |
$^1$H shifts | (4.96) | (3.61) | (4.01) | (3.66) | (3.86) | (3.92/3.86) |

Unit A

$^{13}$C shifts | 103.07 | 74.64 | 75.96 | 72.44 | 74.42 | 69.46 |
$^1$H shifts | (5.36) | (3.61) | (3.70) | (3.47) | (3.93) | (3.93/3.80) |

Unit B

$^{13}$C shifts | 102.66 | 74.50 | 76.23 | 80.20 | 74.26 | 63.38 |
$^1$H shifts | (5.39) | (3.64) | (3.96) | (3.66) | (3.85) | (3.88/3.83) |

$^a$Assignments from Delben et al.$^83$; $^1$H obtained at 25–70°C, TSP reference at 0 ppm; $^{13}$C obtained at 30 or 50°C, methanol reference at 51.75 ppm.
Table 5

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<tr>
<th></th>
<th>C-1 (H-1)</th>
<th>C-2 (H-2)</th>
<th>C-3 (H-3)</th>
<th>C-4 (H-4)</th>
<th>C-5 (H-5)</th>
<th>C-6 (H-6)</th>
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<td>(4.94)</td>
<td>(3.86)</td>
<td>(3.81)</td>
<td>(3.50)</td>
<td>(3.86)</td>
<td>(1.11)</td>
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</tr>
<tr>
<td>Unit A</td>
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<td>75.8</td>
<td>81.8</td>
<td>76.6</td>
<td>175.1</td>
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<td>13C shifts</td>
<td>(4.34)</td>
<td>(3.21)</td>
<td>(3.45)</td>
<td>(3.54)</td>
<td>(3.68)</td>
<td>—</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit I</td>
<td>103.3</td>
<td>75.1</td>
<td>83.6</td>
<td>69.5</td>
<td>77.3</td>
<td>62.2</td>
</tr>
<tr>
<td>13C shifts</td>
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<td>(3.22)</td>
<td>(3.41)</td>
<td>(3.28)</td>
<td>(3.29)</td>
<td>(3.72/3.53)</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Unit II</td>
<td>104.2</td>
<td>75.1</td>
<td>75.8</td>
<td>80.1</td>
<td>76.1</td>
<td>61.7</td>
</tr>
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<td>13C shifts</td>
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<td>(3.14)</td>
<td>(3.47)</td>
<td>(3.42)</td>
<td>(3.38)</td>
<td>(3.75/3.62)</td>
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<tr>
<td>1H shifts</td>
<td></td>
<td></td>
<td></td>
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</tr>
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</table>

*aAssignments from Bosco et al.86 and Jay et al.,85 TSP reference at 0 ppm for 1H, and dioxane reference at 67.4 ppm for 13C.*

A paper has been published on the calculated shifts.84 The full assignments of the 1H and 13C NMR spectra of intact gellan have been published by Jay et al.85 with the help of 2D techniques. The assignments have been revisited by Bosco et al.86 with the help of selective 1D experiments; their assignments are given in Table 5.

5.1.4 Xanthan. This well known extracellular polysaccharide is produced by Xanthomonas campestris and functions as a stabilizer for emulsions, suspensions, and dispersions. It is often used with locust bean gum and guar with which it has a synergistic viscosity effect.

\[
\begin{align*}
[(1,4)-\beta-D-Glcp-(1,4)-\beta-D-Glcp]_n \\
\beta-D-Manp-(1,4)-\beta-D-GlcpA-(1-2)-\alpha-D-Manp \\
(4,6-pyruvate 6-acetate ketal, 50%) 
\end{align*}
\]

NMR work prior to 1983 has been reviewed before.87 Because natural xanthan gum has a stiff and ordered conformation, the NMR resonances tend to be broad. Complete 13C assignments have been obtained by Rinaudo et al.87 and Horton et al.88 who used cellulase enzyme and ultrasound, respectively, to lower the molecular weights, and higher probe temperatures to achieve sharper NMR resonances. As for 1H NMR, the linewidth of 1H resonances can also be narrowed with similar treatments (Fig. 4), but except for the anomeric 1H's and the methyls from acetate and pyruvate, no assignments have been made.87,89 Even 2D NMR has not been helpful here.90
Figure 4. $^1$H NMR spectrum of partially depolymerized xanthan in D$_2$O at 90°C, from Rinaudo et al.$^{87}$ with permission.

Furthermore, the temperature dependence of chemical shifts has been studied in detail$^{91}$ and molecular weight degradation through an enzymatic method$^{92}$ and ultrasound$^{93}$ has been investigated. Low molecular weights have been obtained with enzymatic treatments (to pentasaccharides); the resulting line-narrowed $^1$H NMR resonances have been assigned.$^{92}$

5.1.5 Guar. Guar is a galactomannan with an approximate Gal:Man ratio of 1:2, produced by milling the seeds of the guar plant, *Cyamopsis tetragonolobus*. It is used as a thickener and stabilizer in food formulations.

\[
\beta-D-(1 \rightarrow 4)\text{Manp-D-(1} \rightarrow 4\text{-Manp-}
\]
\[
\alpha-D-\text{Galp}
\]

Because of its high molecular weight (about 2 $\times$ 10$^6$), unmodified guar gives a highly viscous solution even at 1% concentration. Grasdalen and Painter$^{94}$ have used partial acid hydrolysis to lower the molecular weight and obtained highly resolved $^1$H and $^{13}$C spectra. The anomeric protons are well separated, from which the Gal/Man ratio can be derived. Grasdalen and Painter$^{94}$ have also made assignments of the $^{13}$C spectra; particularly significant is the finding that C-4 of mannose is sensitive to diad sequences. Since then, several NMR studies have been made,$^{95-100}$ providing improvements in assignments or information content. Bociek et al.$^{95}$ have reversed the assignments for C-2 and C-3. Manzi et al.$^{98}$ have studied a similar galactomannan from another legume. At a higher magnetic field they have found C-6 of mannose to be sensitive to triad sequences and the other carbons of mannose also exhibiting splitting due to compositional and sequence sensitivity. The assignments are summarized in Table 6.

Several other NMR studies of galactomannan similar to guar have also been reported.$^{101-107}$ Although the compositions are different, the NMR assignments are similar.
Table 6

\(^{13}\)C shifts for guar and locust bean gum\(^a\) in D\(_2\)O at 90°C

<table>
<thead>
<tr>
<th>structure</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-D-Gal</td>
<td>101.70</td>
<td>71.33</td>
<td>72.18</td>
<td>72.36</td>
<td>74.02</td>
<td>63.99</td>
</tr>
<tr>
<td>β-D-Man</td>
<td>102.93</td>
<td>(\text{UU}72.89)</td>
<td>74.34</td>
<td>(\text{UU}79.26)</td>
<td>77.98</td>
<td>63.48</td>
</tr>
<tr>
<td>unbranched at O-6 (U)</td>
<td>(\text{BU}72.84)</td>
<td>74.25</td>
<td>(\text{BU}79.51)</td>
<td>77.92 (broad)</td>
<td>77.85</td>
<td></td>
</tr>
<tr>
<td>β-D-Man</td>
<td>102.80</td>
<td>(\text{UB}72.84)</td>
<td>74.25</td>
<td>(\text{UB}79.51)</td>
<td>76.34</td>
<td>(\text{UBU}69.80)</td>
</tr>
<tr>
<td>branched at O-6 (B)</td>
<td>(\text{BB}72.78)</td>
<td>split</td>
<td>(\text{BB}79.73)</td>
<td>76.27</td>
<td>(\text{BBB}69.68)</td>
<td></td>
</tr>
<tr>
<td>β-D-Man non-reducing chain end</td>
<td>102.35</td>
<td>72.89</td>
<td>75.80</td>
<td>69.51</td>
<td>78.40</td>
<td>63.48</td>
</tr>
<tr>
<td>β-D-Man reducing chain end</td>
<td>96.55</td>
<td>73.29</td>
<td>74.34</td>
<td>79.26</td>
<td>77.92</td>
<td>63.48</td>
</tr>
</tbody>
</table>

\(^a\)Assignments from Manzi et al.,\(^98\) DSS reference at 0 ppm.

5.1.6 Locust Bean Gum. This is another galactomannan, with Gal/Man ratio of approximately 1:4, obtained from the seeds of the carob tree, \(\text{Ceratonia siliqua}\). It is also used as a stabilizer and emulsifier and additionally forms a reversible gel with xanthan, carrageenan, and agar.

\[
\beta-D-(1\rightarrow4)\text{Manp}\]_3\{-\text{D-(1→4)-Manp}\}-_{6}
\text{ α-D-Galp}
\]

Because of the similarity in structure between locust bean gum and guar, these two polymers are often studied together, and the NMR assignments (Table 6) hold equally well for locust bean gum. McCleary et al.\(^96,97\) have hydrolyzed locust bean gum and carried out detailed studies of the oligosaccharides, including \(^1\)H and \(^{13}\)C analysis. The chemical shifts for disaccharides, trisaccharides,\(^96\) and higher oligosaccharides\(^97\) have been reported. The NMR analysis of a heptasaccharide has also been published.\(^99\) The locust bean gum samples from different tree populations have been analyzed and the Gal-Man diad frequencies compared.\(^108\)

5.1.7 Konjac Mannan. This polysaccharide is a glucomannan produced from a tuber (\(\text{Amorphophallus konjac}\)), with the following structure:

\[
\text{\{-[(1,4)-β-D-Manp]_{1.6}-(1,4)-β-D-Glcp\}_{1.0}\} }\text{ (partially branched)}
\]

This is a gel-forming material and can also be used as a protective coating or film, emulsifier and surfactant, drug delivery medium and encapsulant.\(^109\) Through enzymatic hydrolysis and analysis of oligosaccharides via NMR, capillary electrophoresis, and mass spectrometry, Cescutti et al.\(^110\) have deduced that the polymer backbone consists of random distribution of mannose and glucose. A detailed \(^{13}\)C NMR analysis by Katsuraya et al.\(^111\) has
confirmed the random distribution of mannose and glucose. In addition, they have found the ratio of terminal glucosyl versus mannosyl units to be ca. 2, and branching frequency ca. 8%, with β-C-1-linked C-6 carbon of glucosyl units as the branching units. Their \(^{13}\)C shift assignments are given in Table 7.

5.1.8 Gum Arabic. Gum arabic is an exudate from *Acacia* trees and is widely used as an emulsion stabilizer, thickener, and protective colloid in food.\(^{112}\) It has a complex structure, involving both polysaccharide and protein moieties conjugated together. The polysaccharide structure contains a core of β(1-3)-linked D-galactopyranose residues with β(1-6) D-galactopyranose branches. In addition, L-arabinose, L-rhamnose, and D-glucuronic acid are found in various branches. One of the many proposed structures\(^{35}\) is given below:

\[
\begin{align*}
\beta-\text{D-GlcpA} \\
1 \\
\downarrow \\
6 \\
X-3)\beta-\text{D-Galp} \\
1 \\
\downarrow \\
6 \\
-3)\beta-\text{D-Galp}(1\rightarrow3)\beta-\text{D-Galp}(1\rightarrow3)\beta-\text{D-Galp}(1\rightarrow3)\beta-\text{D-Galp}(1\rightarrow3) \\
6 \\
\uparrow \uparrow \uparrow \uparrow \\
1 \quad 1 \\
X\rightarrow3)\beta-\text{D-Galp} \\
6 \\
\uparrow \\
1 \\
X\rightarrow3)\beta-\text{D-Galp} \\
6 \\
\uparrow \\
1 \\
X\rightarrow3)\beta-\text{D-GlcpA} \\
X\rightarrow4)\beta-\text{D-GlcpA} \\
X &= \text{L-Araf(1-;} \text{L-Rhap(1-;} \text{α-D-Galp(1→3)-L-Araf(1-;} \\
\text{or} \quad \beta-\text{L-Arap(1→3)-L-Araf(1-};
\end{align*}
\]

\(^{a}\)Assignments from Katsuraya et al.,\(^{111}\) reference probably TMS at 0 ppm.
The first detailed $^{13}$C NMR structural study of gum arabic has been carried out by Defaye and Wong.

Although the spectrum is complex (Fig. 5), they have been able to achieve partial assignments. Anderson and Wang have reported the $^{13}$C shifts for seven samples of gum arabic from *Acacia senegal*; variations in the structures have been found in these samples. De Pinto has reported the $^{13}$C shifts for a related gum obtained from *Acacia xanthophloea*. A detailed study of gum Arabic structure has been done with 2D NMR by McIntyre et al. Through systematic use of TOCSY, NOESY, and HMQC experiments, many detailed assignments have been made. Tischer et al. have carried out an analysis with NMR, MS, reduction, and methylation of free reducing oligosaccharides in gum Arabic. Detailed structural elucidation and NMR structural assignments have been achieved.

5.1.9 Gums Ghatti, Karaya, and Tragacanth. These are exudates from plants with complex structures and used for their emulsification and thickening properties. Gum ghatti (from *Anogeissus latifolia*) has a glucomannan backbone with different neutral sugars branching from it. Recently several papers used NMR and methylation analysis to provide much better defined structures for gum ghatti. Gum karaya (from *Sterculia* trees) has a pectin-like core with galactose and gluconic acid branches. It is partially acetylated and has some proteins attached. The $^{13}$C NMR spectra of gum karaya and related gums have been published. Gum tragacanth (from *Astragalus spp.*) contains a branched pectin-like component and a branched arabinogalactan component. The structure of arabinogalactan obtained by ethanol precipitation of an aqueous solution of gum tragacanth has been studied by NMR, GC-MS, and ESI-MS.

5.1.10 Alginic Acid. Alginates are a family of polysaccharides extracted from brown algae with the following approximate structure:

\[-\{(1\rightarrow 4)-\beta-D-ManpA\}_m\cdot\{(1\rightarrow 4)-\alpha-L-GulpA\}_n\cdot\{(1\rightarrow 4)\cdot\beta-D-ManpA-(1\rightarrow 4)-\alpha-L-GulpA\}^p\]
Table 8

$^1$H and $^{13}$C assignments for alginate in D$_2$O

<table>
<thead>
<tr>
<th>structure</th>
<th>C-1 (H-1)</th>
<th>C-2 (H-2)</th>
<th>C-3 (H-3)</th>
<th>C-4 (H-4)</th>
<th>C-5 (H-5)</th>
<th>C-6 (H-6)</th>
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<tr>
<td>G</td>
<td>101.50</td>
<td>66.06</td>
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<td>(5.03)</td>
<td>(3.89)</td>
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<td>(4.10)</td>
<td>(4.44)</td>
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<td>M</td>
<td>101.20</td>
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<td>76.77</td>
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<td>(3.81)</td>
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<td>(3.81)</td>
<td>(3.65)</td>
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<td>67.8</td>
<td>71.8</td>
<td>82.6</td>
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<td>71.8</td>
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<td>69.9</td>
<td>177.3</td>
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<td>103.4</td>
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<td>71.8</td>
<td>82.6</td>
<td>69.9</td>
<td>177.3</td>
</tr>
</tbody>
</table>

*Reported by ref. 90 at 55°C, ref. 130 at 40°C, and refs. 125 and 126 at 90°C. All $^1$H data referenced to TSP at 0 ppm. For $^{13}$C, ref. 90 used dioxane reference at 67.4 ppm, and ref. 125 used TSP at 0 ppm.

*Assignment may be reversed.

The $\alpha$-L-mannuronic acid (M) and $\alpha$-L-guluronic acid (G) are arranged in both random and blocky sequences and the microstructure is essential for their properties. This material is used as a thickener and a coating material for various foods.

Alginates have been extensively studied by NMR, and assignments have been made, particularly by the Norwegian workers$^{90,125-130}$ (Table 8). $^1$H NMR can be used for the determination of composition, diad sequences and G-centered triads,$^{126}$ and $^{13}$C NMR for both the M-centered and the G-centered triads.$^{125}$ Through model fitting of M- and G-centered triad sequences, alginate composition has been shown$^{131}$ to be heterogeneous, with a range of microstructures and several components. Three commercial alginates have been subjected to coupled SEC-NMR analysis.$^{132}$ The diad and triad data can be treated with two-component first-order Markovian models, the first component reflecting a mostly G homopolymer, and the second component a MG copolymer with an almost random or alternating distribution; the relevance of the two components to the epimerization reactions has been noted.$^{132}$

More recently, Holtan et al.$^{133}$ have studied the hydrolysis mechanism of alginate using NMR and MS; the chemical shifts of MG oligomers (DP 2-5) have been elucidated by 2D NMR. Salomonsen et al.$^{134}$ have used chemometrics to study alginate monomer composition by solution and solid state NMR; $^{13}$C CP/MAS has been found to provide...
accurate M/G ratio of intact alginate powders. Vilen et al. have used diffusion-edited NMR to selectively suppress water signals in the determination of monomer composition in alginates.

5.1.11 Agar. This is a galactan isolated from red algae, primarily from species of *Gracilaria*, *Gelidium*, and *Pterocladia*, and possesses good gelling and colloidal properties. Agar can be separated into a low-sulfate, pyruvic acid-free agarose and high-sulfur, high-ash agaropectin. The structure consists of repeat units of two sugars (called unit A and unit B from left to right).

\[
\text{agarose} \\
- (1 \rightarrow 3) \beta - D - \text{Galp-(1} \rightarrow 4) - \alpha - 3, 6\text{-anhydro-L-Galp-} \\
(6\text{-methoxy-D-Galp also present in lesser amounts)}
\]

\[
\text{agaropectin} \\
\text{Similar to agarose with sulfate, pyruvic acid acetal, and 4, 6-O-(1-carboxyethylidene)-D-Galp.}
\]

Early on, $^1$H NMR and $^{13}$C NMR shift assignments have been made. Since then, a number of agar types have been studied. The $^{13}$C spectrum is shown in Fig. 1 and assignments given in Table 9. The precursor of agar:

\[
- (1 \rightarrow 3) \beta - D - \text{Galp-(1} \rightarrow 4) - \alpha - L - \text{Galp-} \\
| 6\text{-sulfate}
\]

and desulfated polymer have also been studied by $^{13}$C NMR. A combination of $^{13}$C NMR, HPAEC/PAD, and GC has been used to characterize three samples of agar. A good

<table>
<thead>
<tr>
<th>Table 9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$^{13}$C shifts of agar and carrageenan</strong></td>
</tr>
<tr>
<td>structure</td>
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<tr>
<td>agarose</td>
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<td>lambda diad</td>
</tr>
<tr>
<td>unit A (G2S)</td>
</tr>
<tr>
<td>unit B (D2S,6S)</td>
</tr>
</tbody>
</table>

$^a$Reported in d$_6$-DMSO (referenced to 39.6 ppm) at 80°C.

$^b$Reported in D$_2$O (referenced to DSS at 0 ppm) at 65°C.
review of the NMR assignments and physico-chemical properties of agar has been given by Lahaye and Rochas.\textsuperscript{141}

5.1.12 Carrageenan. Carrageenans are linear, partly sulfated galactans from red algae, used commonly in gelling and thickening applications.\textsuperscript{143–144} The three most important commercial types are kappa (e.g., from \textit{Eucheuma cottonii}), iota (e.g., from \textit{Eucheuma denticulatum}, also known as spinosum), and lambda (e.g., from \textit{Chondrus crispus}, \textit{Gigartina pistillata}, and \textit{Gigartina radula}). Like agar, the idealized structures consist of repeat sequences of two substituted sugars (called diads hereinafter):

\begin{align*}
\text{iota (\(\iota\))}, \text{ diad} &= G4S + DA2S \\
&(1\rightarrow3)\beta-D-Galp-(1\rightarrow4)\alpha-3,6\text{-anhydro-D-Galp-} \\
&| \\
&4\text{-sulfate} \quad 2\text{-sulfate} \\
\text{kappa (\(\kappa\))}, \text{ diad} &= G4S + DA \\
&(1\rightarrow3)\beta-D-Galp-(1\rightarrow4)\alpha-3,6\text{-anhydro-D-Galp-} \\
&| \\
&4\text{-sulfate} \\
\text{lambda (\(\lambda\))}, \text{ diad} &= G2S + D2S, 6S \\
&(1\rightarrow3)\beta-D-Galp-(1\rightarrow4)\alpha-Galp- \\
&| \\
&(2\text{-sulfate}) \quad (2, 6\text{-disulfate})
\end{align*}

Three other common forms, mu (\(\mu\)) and nu (\(\nu\)), are biological precursors of \(\kappa\) and \(\iota\) carrageenan, respectively, and theta (\(\theta\)), which is derived from \(\lambda\). The \(^{13}\text{C}\) NMR spectra of these carrageenans are shown in Fig. 6. A large number of papers have provided very detailed studies of carrageenan structure.\textsuperscript{144–149} \(^{13}\text{C}\) NMR assignments have been revised by van de Velde et al.,\textsuperscript{148} and \(^1\text{H}\) summarized by Campo et al.\textsuperscript{144} and Tojo and Prado.\textsuperscript{149} The latest assignments\textsuperscript{144,148,149} are given in Table 9. Using these shifts, one can readily determine the relative levels of the various diad structures in carrageenan samples. As for carrageenan oligomers, earlier work has been reviewed by van de Velde et al.\textsuperscript{147} Furthermore, Guibet et al.\textsuperscript{150} have reported the NMR assignments of oligosaccharides from \(\lambda\) carrageenan. In addition, methylation followed by \(^{13}\text{C}\) NMR has been shown to be useful to identify and to quantify major and minor components in carrageenan.\textsuperscript{151}

5.1.13 Furcellaran. This is a gelling polysaccharide extracted from red algae of \textit{Furcellaria} genus. The structure is similar to \(\kappa\) carrageenan except that the 4-sulfate is only partial. Thus, this polymer contains a mixture of \(\kappa\) and \(\beta\) diad structures:

\begin{align*}
\text{beta (\(\beta\))} \\
&(1\rightarrow3)\beta-D-Galp-(1\rightarrow4)\alpha-3,6\text{-anhydro-D-Galp-}
\end{align*}

Studies of furcellaran by \(^1\text{H}\) NMR\textsuperscript{145} and by \(^{13}\text{C}\) NMR\textsuperscript{142,152–154} have been reported.
Figure 6. Stacked plot of $^{13}$C NMR spectra of six carrageenan samples: C: iota, A: kappa, B: mu, D: nu, E: theta, F: lambda, in D$_2$O, pD 8, 80°C; shifts referenced to acetonitrile methyl at 0.7 ppm.

5.1.14 Cellulose. Cellulose is an abundant natural material and occurs in many food items. It is a $\beta$-glucan with a simple structure:

$$-[(1-4)-\beta-D-Glc\text{p}]_n-$$

It is not digestible and serves as roughage. Microcrystalline cellulose is used in many food formulations.

Cellulose is not soluble in common solvents, but it dissolves in some special solvents. The solution NMR spectra in several such solvents have been reported, for example, $N,N$-dimethylacetamide/LiCl,\textsuperscript{155,156} $N$-methylpyrrolidone/LiCl,\textsuperscript{155} NaOH-urea,\textsuperscript{157} NaOH-thiourea,\textsuperscript{158} and ionic liquid.\textsuperscript{159} The assignments are usually straightforward, for example,
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in NaOH/thiourea, C-1 (105.0 ppm), C-4 (89.3, 84.4 ppm), C-2, C-3, C-5 (75.2, 72.1 ppm, not individually assigned), and C-6 (65.6, 62.5 ppm).

5.1.15 Cellulosic Derivatives. A number of modified cellulosics are allowed for food use. These include carboxymethylcellulose (CMC), methylcellulose (MC), hydroxypropylcellulose (HPC), and hydroxypropylmethylcellulose (HPMC). These gums are made by reacting alkali cellulose with suitable reagents: sodium monochloroacetate (for CMC), methyl chloride (for MC), propylene oxide (for HPC), and methyl chloride/propylene oxide (for HPMC).

\[
\begin{align*}
\text{CMC: cellulose-CH}_2\text{COO}^- & & \text{MC: cellulose-CH}_3 \\
\text{HPC: cellulose-CH}_2\text{CH-OH} & & \text{HPMC: cellulose-CH}_3\text{ and -CH}_2\text{CH-OH} \\
\text{CH}_3 & & \text{CH}_3
\end{align*}
\]

These materials find many applications in food formulations. CMC is by far the most popular and can be used for formulations requiring thickening, water retention, adhesion, and suspension stabilization. MC and HPMC exhibit reversible thermal gelation (i.e., gelling at high temperatures, 45–90°C). In addition, they have film-forming, binding, water retention, and emulsion stabilization properties. HPC can be used as a coating material to impart an air and moisture barrier and also as a foaming aid and an emulsion stabilizer.

NMR has proved itself to be one the best techniques to characterize cellulosic derivatives. $^{13}$C is often preferred over $^1$H. In general, four approaches have been taken.

1. Analysis of the intact polymer

Because of high viscosity, the NMR spectrum tends to have broad resonances. Often the polymer is partly hydrolyzed to lower the molecular weight and to sharpen the NMR lines. CMC has been studied by a number of workers. $^{160-163}$ An example $^{164}$ is shown in Fig. 7. Rather detailed assignments have been attempted. $^{165}$ Similarly, MC has also been studied as intact polymer. $^{160,165-168}$ Both $^1$H and $^{13}$C NMR can be used to extract information on degree of substitution and (to a lesser extent) position of substitution; the $^{13}$C spectrum of a MC sample $^{165}$ is given in Fig. 8. HPC has also been studied as intact polymer. $^{169-171}$ From the methyl resonances, the terminal and the internal hydroxypropyl groups can be differentiated (Fig. 9).

2. Hydrolysis to monosaccharides

The second approach is to hydrolyze the cellulosics completely to monosaccharides using chemical or enzymatic methods. The analysis then reduces to the spectral assignments of glucose derivatives. This has the advantage that narrow NMR resonances are obtained and a wealth of information about polymer structure is available.

CMC has been completely hydrolyzed and studied by $^1$H NMR $^{172}$ and $^{13}$C NMR. $^{160,161,173,174}$ Reuben and Conner $^{174}$ have assigned the $^{13}$C resonances for all 16 isomers of carboxymethylglucose. The intensities have been matched with a theoretical kinetic model, assuming that the three hydroxy groups reacted independently. Excellent agreement has been found, indicating the validity of the kinetic model. The relative rates for O-2, O-3, and O-6 were 2.14:1.00:1.58.

In a follow-up work Reuben $^{175}$ has repeated the model calculations for hydrolyzed MC. The pattern of substitution conforms to a kinetic model whereby O-6 and O-2
reactivities are independent but the reactivity of O-3 depends on the state of substitution at O-2. For his samples, the relative reactivities for O-2, O-3 (unsubstituted at O-2), O-3 (substituted at O-2), and O-6 are 1.24:0.28:0.82:1.00.

HPC has been hydrolyzed to monomers and studied by $^{13}$C in three papers by Lee and Perlin.\textsuperscript{169,170,176} The spectra have been assigned and substitution information obtained.

3. Methanolysis

This approach is complementary to hydrolysis. Sometimes acid hydrolysis causes decomposition of the glucose derivatives. Sometimes the cellulosic derivative is not soluble in water and cannot be readily hydrolyzed. In such cases methanolic HCl may be used to hydrolyze the polymer and convert it to methyl glucosides.

Figure 7. $^{13}$C NMR spectrum of intact carboxymethylcellulose (CMC) in D$_2$O, 30°C, adapted from Cheng and Biswas\textsuperscript{164} with permission, TMS reference at 0 ppm (Color figure available online).

Figure 8. $^{13}$C NMR spectrum (100 MHz) of a sample of methylcellulose (MC), DS 2.0, in d$_6$-DMSO at 80°C, taken from Takahashi et al.\textsuperscript{165} with permission, TMS reference at 0 ppm.
derivatives. This approach has been used successfully for HPC\textsuperscript{176} and MC.\textsuperscript{175} A substantial advantage of this method is that it leads to a diminution of incompletely hydrolyzed fragments and bicyclic acetal, a byproduct of acid hydrolysis.\textsuperscript{177}

4. **Acetylation**

Tezuka et al. have developed this approach into a powerful technique to study cellulosic ethers. The idea is to acetylate the unsubstituted hydroxy groups of the cellulosic derivative and study the intact polymer by $^{13}$C NMR in DMSO at 100°C. They have successfully carried out detailed analysis of MC,\textsuperscript{177} HPMC,\textsuperscript{178,179} and HPC.\textsuperscript{180} The substitution patterns and relative reactivities of the three hydroxy groups can be estimated. In all these samples, the reactivities follow this trend: O-2 > O-6 > O-3. In the case of CMC, they have used two steps: the conversion of the carboxy group to methyl ester and the propanoation of unsubstituted –OH groups.\textsuperscript{181} This acetylation method has also been adopted by others.\textsuperscript{182}

5.1.16 **Pectin.** Pectin has the following putative structure:

$$[(1\rightarrow4)\alpha-D-GalpA(1\rightarrow4)\alpha-D-GalpA(1\rightarrow2)-L-Rhap(1\rightarrow4)-[\alpha-D-GalpA]_n\mid X_1\mid X_2\mid X_3]$$

- $X_1 = (D-Galp)_n(1\rightarrow)$
- $X_2 = \beta-D-Xylp(1\rightarrow; \alpha-L-Fucp(1\rightarrow2)-D-Xylp(1\rightarrow))$
- or $\beta-D-Galp(1\rightarrow2)-D-Xylp(1\rightarrow)$
- $X_3 = (L-Araf)_n(1\rightarrow)$

(with different degrees of methyl esterification)

Commercially pectin is obtained by extraction from citrus peel, apple pomace, and sugar beet. The properties depend on the degree of esterification (DE). High methoxyl
Table 10

$^{13}$C and $^1$H NMR chemical shifts (ppm) and assignments of galacturonate (G) and ester (E) units on pectins$^a$ in D$_2$O

<table>
<thead>
<tr>
<th>Position</th>
<th>unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>OCH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H assignments</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>E</td>
<td>5.00, 4.95$^b$</td>
<td>3.77</td>
<td>4.00</td>
<td>4.49</td>
<td>5.06–5.10$^c$</td>
<td>—</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>5.17, 5.12$^b$</td>
<td>3.77</td>
<td>4.00</td>
<td>4.44</td>
<td>4.67–4.75$^c$</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>$^{13}$C assignments</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>100.49,100.61$^d$</td>
<td>68.69</td>
<td>68–85</td>
<td>79.20</td>
<td>71.18</td>
<td>171.1–171.4$^e$</td>
<td>53.42</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>99.97,100.02$^d$</td>
<td>69.12</td>
<td>69.47</td>
<td>79.00</td>
<td>71.97</td>
<td>174.5–175.4</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Assignments after Neiss et al.$^{192}$, acetone reference at 2.22 ppm for $^1$H, acetonitrile methyl reference at 1.3 ppm for $^{13}$C.

$^b$Diads: EE 5.00, EG 4.95, GE 5.17, GG 5.12.

$^c$Triads: EEE 5.06, EEG 5.07, GEE 5.08, GEG 5.10, GGG 4.75, GGE/EGG 4.71, EGE 4.67.

$^d$Diads: EE 100.61, EG 100.49, GE 99.97, GG 100.02.

$^e$Triads: EEE 171.11, EEG/GEE 171.21/171.25, GEG 171.36.

pectin (DE $> 50\%$) forms gels with sucrose, and low methoxy pectin (DE $< 50\%$) forms gels with Ca$^{2+}$. In addition to gels, pectin can also be used as stabilizers and thickeners in a number of food items.

Pectin structure has been much studied by solution NMR. One line of approach is the sequence distribution of galacturonic acid (G) versus the corresponding ester (E). The assignments are summarized in Table 10. Work prior to 1988 has been reviewed by Perlin and Casu,$^{26}$ Sun,$^{183}$ and Grasdalen et al.$^{184}$ In their 1988 paper, Grasdalen et al.$^{184}$ have demonstrated that the anomeric protons are sensitive to diad sequences, and H-5 is sensitive to G-centered triad sequences. Westerlund et al.$^{185}$ from COSY experiments, have reversed the assignments for H-1 and H-5 in esterified residues. In a separate paper$^{186}$ they have also re-visited the $^{13}$C spectrum and showed C-6 of the esterified residues to be sensitive to triad sequences. In these papers,$^{184–186}$ alkaline de-esterification has been shown to produce random G/E sequence distributions. Andersen et al.$^{187}$ have examined the $^1$H spectrum at 600 MHz and found H-5 to be sensitive to triad and tetrad sequences. They have used these assignments to study pectins that have been de-esterified through alkaline$^{187}$ and enzymatic$^{187,188}$ methods. Tomato pectinesterase has been shown to attack in alternating sequences and preferentially de-esterifies linearly towards the reducing end.

As for methodology development, Cantoire et al.$^{189}$ have developed a depolymerization procedure (through simple heating) that permits NMR analysis at room temperature. A simple method to determine the degree of esterification by $^1$H NMR has been proposed by Rosenbohm et al.$^{190}$ Winning et al.$^{191}$ have used chemometrics to quantify the degree of blockiness in pectin using $^1$H NMR. Neiss and Cheng$^{192,193}$ have used NMR and statistical modeling to elucidate the microstructure of pectin and its fractions. Rather detailed information is available on G/E distribution from analyses of triad sequence intensities.

Another approach in the NMR studies of pectin structure is the elucidation of rhamnogalacturonan structure. After all, pectin is a rhamnogalacturonan, with $\alpha$-(1-4)-linked galacturonic acid residues interrupted at intervals by $\alpha$-(1-2)-linked rhamnose residues, to which neutral sugar side chains are attached. It has been found that these neutral sugars are
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found in specific regions of pectin, dubbed “hairy regions.” A fair amount of work (including NMR) has been done to characterize these hairy regions and their distribution along the pectin chain. Five papers provide good representation of the work done in this area.194–198

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