CHAPTER-1

INTRODUCTION

(A) Importance of the knowledge of structure of polysaccharide and its application:

In the seeds of many plants their exist giant molecules called 'Polysaccharide', 'Gums' or 'Mucilages' formed by the polymerisation of several monosaccharide units (usually ten to thousand). These giant molecules are produced in the seeds of various plants by the tremendously complicated process of photosynthesis and act as reserve or storage matter for cellular reactions.

The past decade has seen a tremendous growth and expansion of the knowledge of carbohydrate chemistry and biology including the knowledge about polysaccharides, gums and mucilages. This has resulted in the discovery of a wide ranging biological functions and activities of these compounds. They can form gels, can interact with proteins forming glycoproteins, form lipopolysaccharides with lipids, functions as enzymes and antibodies, possess non-cytotoxic antitumour activity, can inhibit viruses, induce interferon formation in cell cultures and can exist as plant lectins. Knowledge of the structural
features of these giant molecules as revealed by organic chemists can help a great deal in understanding these biological processes and present a model for the synthesis of biologically active carbohydrates. Several of these polysaccharides like starch, cellulose, gums (guar, tamarind, ghatti, arabic etc), mucilages, dextrans etc. have wide ranging industrial application and there is also a demand to develop modified and derivatised polysaccharides for specific application. Structural knowledge is, therefore, essential for developing such products in this area.

These naturally occurring polysaccharides are essential constituents of almost all living organisms widely distributed in plant and animal kingdom\(^1\). They are also found in seaweeds, fungi, capsules of micro-organisms, mucosa, joint fluids, cartilage, skin and exoskeleton of insects.

The potential sources of polysaccharides are abundant in the form of gum exudates and seeds. Generally starch is stored in seeds for use by embryonic plant in initial growth but many other polysaccharides as mentioned earlier also occur naturally in the form of reserve food materials in the seeds of plants which have been exploited extensively for industrial utilization.
The polysaccharides have been classified in various ways but the most logical and satisfactory classification is the one based on their chemical compositions and structures. According to it they can be classified as follows:

**PLANT POLYSACCHARIDE**

- **HOMOGLYCAN**
  - (Built up of a single monosaccharide repeating unit)
  - **NITROGENOUS POLYMERS**
    - Glucosamine Polymers
  - **NEUTRAL POLYMERS**
    - i) Glucans (starch, cellulose)
    - ii) Mannans
    - iii) Galactans
    - iv) Fructans (inulin)
    - v) Xylans
  - **ACIDIC POLYMERS**
    - i) Ploygalacturonic acid (pectin)
    - ii) Polymannuronic acid

- **HETEROGLYCAN**
  - (Built up of two or more type of monosaccharide repeating unit)
  - **NITROGENOUS**
    - i) Mucopolysaccharides
    - ii) Glycoproteins
  - **NEUTRAL**
    - i) Hemicellulose
    - ii) Arabinogalactans
    - iii) Glucomannans
    - iv) Galactomannans
  - **ACIDIC**
    - i) Gums
    - ii) Mucilages
    - iii) Alginates (seaweeds)
The use of polysaccharides goes back to earliest times. From time immemorial primitive people have used them as food. Even now, one of the important uses by man is as a component of food material, being used in the confectionary trade, bakery and in the preparation of peppermints and beverages. In addition they are frequently employed in textile, paper, printing, dyeing, Cosmetics and pharmaceutical industries. The polysaccharide present in the husk of the seeds of *Plantago ovata Forsk* (Ispaghula husk) is widely used as a prophylactic in the treatment of large bowel disorders.\(^2\) The husk has a very high content of mucilage polysaccharide that gels over a wide range of concentration. Beside these uses, polysaccharides are also used as flocculants or settling aids to concentrate ores in the mining industries, for quick settling of clarified juice in sugar industry, for e.g. the seed of polysaccharide of *Cassia tora*\(^3\) and as clarificants in gur manufacture such as the extracts of *Hibiscus ficulneus* (deola), *Hibiscus esculortus* (bhindi), *Bambax malabaricum* (Semal), *Grewia asciatica* (Phalsa) etc.

Due to this immense industrial importance, the structures of polysaccharides are always a subject matter of keen interest. It has been observed that the physical properties of the polysaccharides like gel formation, solubilities, viscosities\(^5\) etc. depend not so much on the
actual building unit (although this is an important consideration) as upon the overall fine molecular architecture of the polysaccharide. Polysaccharides containing uronic acid units specially provide a fascinating and challenging problem to organic chemist due to their complex structures as compared to those of neutral polysaccharides. While neutral polysaccharides in general give rise to one or two types of monosaccharide units on hydrolysis, the seed polysaccharides containing uronic acids liberate various types of sugars and it becomes exciting to discover the order, point and the nature of these linkages. It is interesting to note here that the gelling mechanisms of pectins, Isphaghula husk (*Plantago ovata*), Caragenans, etc. have been revealed by the knowledge of their fine structures. Even the fine structure of neutral polysaccharide like starch and cellulose explain the wide difference in their properties although both contain the same sugar unit --- glucose. Even the two fractions of starch, ie, amylose and amylopectin differ significantly in their properties.

(B) Uronic acid containing polysaccharide – Occurrence

Uronic acids are widely distributed in different plant and animal polysaccharides and gums. Nearly fifty percent of the polysaccharides known to mankind bear uronic acid units in the polymeric chain.⁶
Among all the uronic acids D-glucuronic acid is the most common natural uronic acid but D-galcturonic acid is also present in many plant gums and animal polysaccharides. It constitutes the main unit of pectic acid. D-mannuronic acid is the major unit of alginic acid. D-glucuronic acid units occur in polysaccharides both as interchain units and as non-reducing chain units. In animal and microbial polysaccharides, D-glucuronic acid usually occurs as a chain unit while in plant gums and hemicellulose, D-glucuronic acid is found most commonly in the side chain or as non-reducing end units.

Gum exudates are also good sources of uronic acids. Many gums contain D-glucuronic acid acid units which either occur as side chain units or as non-reducing terminal units. In most gums, the glucopyranosyluronic acid units are unmethylated. The partial acid hydrolysis of exudates gums yields individual sugar units and the acid resistant aldobiouronic acids. The most common aldobiouronic acid is 6-O- (β-D-glucopyranosyl-uronic acid)-D-galactose, which was first isolated from gum Arabic (Acacia verek) \(^{7,8}\) as well as from Prunus domestica\(^9,10\) gum, Acacia cyanophylla\(^11\) gum, etc. Similarly 2-O-(D-glucopyranosyluronic acid)-D-mannose has been obtained from Prunus insitia\(^12,13\) gum, Prunus virginia\(^14\) gum, Ghatti\(^15\) gum etc. 4-O-(D-glucopyranosyluronic acid)-D-galactose has been isolated from
Citrata maxima\textsuperscript{16} (grape fruit) and Citrus limonia\textsuperscript{16} (lemon) gum. 4-O-(methyl-D-glucopyranosyluronic acid)-D-galactose has been obtained from Prosopis juliflora\textsuperscript{17} and Khaya grandifolia\textsuperscript{18} gum.

The 4-O-methyl-D-glucuronic acid is very widespread in wood hemicellulose. The hemicellulose of sunflower heads\textsuperscript{19} yields 3-O-(\alpha-D-glucopyranosyluronic acid)-D-xylose; the hemicelluloses of Pinus radiata, Pinus pinaster\textsuperscript{20} and wheat straw\textsuperscript{21} yield 3-O-(4-O-methyl-\alpha-D-glucopyranosyluronic acid)-D-xylose. Jute has been reported to yield a similar aldobiuronic acid except that the methyl group is at position-3. The softwoods and annual plants are reported to contain L-arabino-(4-O-methyl-D-glucurono)xylans. Beechwood hemicellulose\textsuperscript{22} has a high content of uronic acid. A polysaccharide from European Beechwood (Fagus sylvatica) contains 9.5\% of 4-O-methyl-D-glucuronic acid.

(C) Uronic acid containing polysaccharide—Methods for structural elucidation

The determination of the detailed structure of polysaccharides is a complicated task in which answers must be given to questions of homogeneity, molecular weight, composition, sequence of
monosaccharide units, their point of linkages and the configurational aspect of these linkages. The structural elucidation of uronic acid containing polysaccharides becomes even more cumbersome due to following reasons- i) They are resistant towards the usual hydrolytic methods ii) The uronic acid acetates are not sufficiently volatile for analysis by gas chromatography and iii) Repetitive methylation by Hakomori method generally induces a β-elimination depolymerisation reaction. Due to these difficulties some modifications in the formal approach is necessary. The commonly encountered methods for these acidic polysaccharides are discussed as follows:

1. Complete acid hydrolysis:
Complete hydrolysis of acidic polysaccharides (those containing uronic acid) moiety is often complicated due to the resistance of glycosidic bonds formed by these moieties and hence a complete liberation of all the sugars is rarely possible without accompanying decomposition\(^{23,24}\). A useful approach in such cases is to activate the uronic acid groups with a water- soluble carbodiimide, which may then be reduced with sodium borohydride to acid labile neutral polymer. However, if the corresponding aldose is also a constituents of the polymer this approach will not be able to distinguish between
the two type of aldoses, i.e., the neutral constituents and the one formed by the reduction of acidic group.

2. Methanolysis:

The cleavage of all the glycosidic bonds between the uronic acid and neutral sugar units is rarely achieved by the acid hydrolysis of such polysaccharides and hence this leads to an incorrect stoichiometric determination of its constituents. In contrast to this, methanolysis is much more reliable method. When polysaccharides containing uronic acid moieties are cleaved with dry hydrogen chloride in anhydrous methanol, the sugar units occur as 1-methyl glycosides and the uronic acid occur as 1-methyl glycoside-6-methyl esters which are very stable under acidic conditions during the period required for completing the reaction (3-4 hours). Even highly acid sensitive sugar such as 3,6-anhydrogalactose is recovered and analysed. The glycosidic linkages between the uronic acids and other sugars are sufficiently cleaved during methanolysis and hence quantitative reproducibility increases greatly. Following methanolysis, only a simple silylation step is required for derivatising the remaining hydroxyl groups (trimethyl silyl ether derivatives). After that, neutral sugars and uronic acids can be analysed by GLC in the same chromatogram which leads to considerable saving of time and labour as compared to formal
analytical approach. However, the main drawback in methanolysis GC analysis is the complexity of the chromatogram, i.e., methanolysis yields a mixture of α and β-isomers and pyranose & furanose forms for each sugar unit. This problem has been overcome by the use of capillary columns in which nearly all the peaks of methyl glycosides have been resolved in the same chromatogram. The presence of multiple peak patterns having constant relative retentions and peak area proportions allows the identification of sugars unambiguously.

3. Methylation analysis:
Methylation of polysaccharides followed by acid hydrolysis of the methylated product, reduction, acetylation and identification of the resulting partially methylated alditol acetates by GLC and or GC-MS helps to reveal the point of linkages between the various monosaccharide units. Different methylation methods have been applied by different workers, e.g., Haworth’s, Purdie’s Kuhn’s and Hakomori’s methods but Hakomori method\(^{25}\) seems to be more effective for the methylation of acidic polysaccharides. In the Hakomori method use of a strong base, sodium or potassium dimly ensures complete alkoxide formation and hence efficient etherification and esterification is achieved in a single step\(^{26}\). The methylation of
Uronic acid polymers must be achieved in a single step because repetitive operations lead to extensive degradation of the polymer. It is due to the fact that the uronic acid esters formed during methylation are very susceptible to base catalysed β-elimination reaction. However, in practice complete methylation of uronic acid containing polysaccharides is not always achieved in a single step, hence a second methylation step by Purdie or Kuhn method may be necessary in such cases.

Another major difficulty encountered in the methylation analysis of acidic polysaccharides is their low solubility in specific solvents, which leads to undermethylation. But this problem can be overcome by lyophilisation and sieving of the polysaccharide before dissolution.

One of the most important experimental difficulties encountered in the methylation of polysaccharides is the destruction and demethylation of the different methylated sugars during hydrolysis of the methylated polysaccharide. With acidic polysaccharides this problem is further complicated by an unusual difficulty in obtaining complete methylation and also by resistance to hydrolysis of uronic acid glycosidic bonds. Thus, hydrolysis of methylated acidic polysaccharides requires more drastic conditions with chances of
increased degradation and demethylation of sugar moieties. Complete depolymerisation of the methylated uronic acid containing polysaccharide is usually possible only after the reduction of the uronic acid ester groups formed by methylation, to the corresponding hexose residues. This reduction may be performed with lithium aluminium hydride (or deuteride)\textsuperscript{28}, in tetrahydrofuran, lithium borohydride (or deuteride), lithium triethylborohydride (or deuteride called Superdeuteride) or even with diborane after the deesterification of the ester groups with dilute sodium hydroxide. With Lithium triethyl borohydride (or borodeuteride) reduction can be brought about at room temperature and no prolonged refluxing is needed as with lithium aluminium hydride. This ensures less destruction and side reactions of the material.

Uronic acid moieties in acidic polysaccharides may not be identified by simple methylation studies\textsuperscript{29}. However if reduction of the permethylated polysaccharide is performed with labeled reagents containing deuterium atoms (eg.lithium aluminium deuteride or lithium triethyl borodeuteride) the resulting hexoses (derived from uronic acid moieties) will contain two deuterium atoms (COOH––CD\textsubscript{2}OH) which could be easily identified by mass spectrometric
fragmentation pattern in the GC-MS analysis. The neutral hexoses will give fragments corresponding to CH$_2$OH group only. Further, another way to distinguish uronic acid moieties is to methylate, reduce and remethylate the polysaccharide and identify the methylated sugars. The remethylated polysaccharide (after reduction) will give a methylated aldose (from the uronic acid) in which the C-6 position will be methylated along with other positions whereas in the reduced but not remethylated polysaccharide the C-6 position in the corresponding methylated aldose would be free (without methylation). This will indicate that the C-6 methylated position in the aldose is formed by the methylation of the C-6 OH group in the aldose formed by the reduction of the uronic acid.

4. Partial Acid Hydrolysis:

Partial acid hydrolysis of the polysaccharides followed by isolation and characterisation of degraded polysaccharide or oligosaccharide gives detailed information about the sequence and anomeric configuration in addition to providing conformation of linkage types. In case of linear polysaccharides containing uniform linkages and assuming that all glycosidic bonds are equally susceptible to hydrolysis, partial hydrolysis leads to the information of polymer
homologous series of oligosaccharides (di, tri, tetra, penta, hexasaccharides)\textsuperscript{32}. Similarly for multilinkage type of polysaccharides, given the susceptibilities to hydrolysis of different linkages are approximately equal) a representative selection of all the possible oligosaccharides from different regions is liberated\textsuperscript{33}. However, rates of hydrolysis of different glycosidic linkages are in fact sufficiently different so that all the possible oligosaccharides are not liberated in a single step. Particularly the polysaccharides containing uronic acid, because of their resistance to acid hydrolysis\textsuperscript{34-39} require more drastic conditions. When uronic acid moiety is present in the main core graded hydrolysis is often required to isolate the acidic disaccharide (aldobiouronic acid). The polysaccharides containing uronic acid on partial hydrolysis produce mixture of neutral and acidic disaccharides or oligosaccharides. The acidic oligosaccharides in these mixtures are separated from neutral oligosaccharides by passing the neutralised hydrolysate through a column of weakly basic ion exchange resins in their acetate or formate form. The acidic oligosaccharides are absorbed on the resin whereas the neutral oligosaccharides are deabsorbed by elution with water. The acidic oligosaccharides are then eluted with dilute acetic acid or formic acid.
Mixtures of neutral oligosaccharides may be separated by chromatography on cation-exchange resin or gel filtration on sephadex or Biogel. Whereas acidic oligosaccharides are resolved on a weak base anion exchange resin column (in acetate or formate form) by elution with increasing concentration of acetic or formic acid or even by gel filtration chromatography on Biogels$^{40-42}$.

The complete characterization of oligosaccharides or even degraded polysaccharides is then achieved by methylation, reduction (in case of acidic oligosaccharides), hydrolysis, sodium borohydride reduction, acetylation followed by GLC and GC-MS analysis$^{43-46}$. NMR analysis of the oligosaccharides is also useful for linkage determination and anomeric configuration$^{47-49}$.

5. Periodate Oxidation:

Oxidation with periodate ion, resulting in 1,2-glycol cleavage is one of the most widely used reaction in the structural elucidation of polysaccharides since it furnishes information regarding the point of linkage. Particularly when the uronic acids are present in the polysaccharides molecule as terminal non-reducing residues this reactions become more valuable as these are oxidized completely to
formic acid and oxalic acid, provided the temperature and pH are effectively controlled. The reaction mixture can be analysed by titrimetric methods to determine periodate consumed, by acid-base titration to measure formic acid liberated, and by various colorimetric methods for formaldehyde produced. In addition, the presence of sugar residues that are substituted in a manner that leaves no diol groups susceptible to oxidation may be ascertained by the liberation of the unchanged sugar after hydrolysis.

Non-ideal behavior of polysaccharides during periodate oxidation arises from both over oxidation and underoxidation. Overoxidation is frequently encountered when oxidation gives rise to tartonic acid half aldehyde derivativess, from hexuronic acid end groups or to tartron-dialdehyde derivatives from hexafuranosides or heptapyranosides.

There are many reasons behind the incomplete oxidation of sugars moieties. Most commonly, hexacetal formation between aldehyde fragments in oxidatively cleaved residues and hydroxyl group is adjacent but not yet oxidised residues protects the latter unit from oxidation. The highest degree of incomplete oxidation occurs primarily in 4-linked polysaccharides whose sugars residue
donot carry primary hydroxyl groups at C-6\textsuperscript{28}. Another reason is the hydrogen bonding between one of a pair of hydroxyl groups, normally susceptible to oxidation. At low pH and temperature the oxidation of 4-linked β-D-glucuronic acid residues is negligible when flanked by 3-linked residues of a 2-acetamido-2-deoxy-β-D-hexapyranose as in chondroitin-4-sulphate\textsuperscript{54}. Periodate resistant α-D-glucuronic acid residues are also encountered in heparin. Although no detailed hydrogen bonding scheme has been proposed to account for this observation, the importance of cooperative interresidues hydrogen bonding is indicated since periodate resistant glucuronic acid become susceptible to oxidation after scission of the periodate-oxidised polysaccharide in alkaline medium\textsuperscript{55}.

Steric hindrance and electrostatic repulsions in many polysaccharide structures are also responsible for the inhibition of periodates oxidation. Painter et al\textsuperscript{56,57} has demonstrated that there is inhibition of oxidation in β-D-glucose or β-D-glucuronic acid residues linked through position 1 and 4. The low reactivities of these residues are due to steric hindrance. Steric hindrance is also responsible for the faster oxidation of cis-1,2-diols than the corresponding trans isomers. Further it has been demonstrated that
there is an electrostatic repulsion between periodate ion and the uronate ion. This is borne out by the fact that the rate of periodate oxidation of uronic acid containing polysaccharides can be increased by varying the ionic strength of the reaction mixture. This is because inorganic salts diminish the mutual electrostatic repulsion between the uronate anion and the attacking periodate ion. This phenomenon is known as Donnan effect or primary salt effect which is always significant for reactions between two ionic species. For uronic acid containing polysaccharides such as gums and mucilages, a decrease in pH has the same effect (increase in ionic strength).

The above described effects could be used as a tool for bringing a highly selective oxidation in uronic acid containing and many other kinds of polysaccharides. This is particularly useful for Smith degradation wherein after partial oxidation with periodate, the hydrolysis product would lead to useful alternative information to that obtained by partial hydrolysis experiments.

6. Smith degradation

The Smith degradation involves a controlled acid hydrolysis of the reduced (by sodium borohydride) oxidised polysaccharide in which hydrolysis of acyclic acetals from cleaved sugar units occurs with
dilute acid at room temperature without significant hydrolysis of all the glycosidic linkages. The reaction sequence results in the isolation from polysaccharides of those sugar residues that resisted oxidative cleavage by periodate. Depending on the relative placing of such periodate-resistant sugar residues, the degradation may result in the formation of an isolated unit of low molecular weight in which the sugar residues are present as simple glycosides of fragments such as glycerol or a tetritol which can be analysed by GLC.

The success of Smith degradation depends not only on ensuring that all the potential vulnerable diol and triol groups have been oxidised but also upon the selectivity in the acid hydrolysis step. It has been observed that the fragments resulting from the oxidative scission of hexuronic acids are more resistant to hydrolysis than the corresponding fragments from neutral sugar residues and hence analysis becomes complicated. However, as discussed earlier, it may provide a useful alternative to partial acid hydrolysis study.

7. Application of Mass Spectrometry:
Mass spectrometry has become an important and versatile technique in the structural elucidation of complex polysaccharides particularly when it is used in conjunction with Gas liquid chromatography. The
combined GC-MS is a major tool in the characterization of mono and disaccharides derivatives. Most underivatized mono and disaccharides are nonvolatile and thermally unstable and are, therefore, unsuitable for GC-MS analysis. For GC-MS, the sugars must be converted into thermally stable volatile derivatives. In terms of thermal stability and ease of interpretation of spectra, permethylated alditol acetates (formed on reduction of permethylated and hydrolysed polysaccharides with sodium borohydride followed by acetylation) are the most widely used derivatives for the characterisation of methylated sugars by mass spectrometry.

With the adoption of GC-MS for the analysis of methylated sugar derivatives it is now possible to determine the glycosidic linkages between sugars in a polysaccharide without the preparative scale isolation of methylated sugars from their mixtures. The resolving power of GC coupled with the fragmentation patterns of MS helps in the identification of the various glycosidic linkages in a polysaccharide. This is because the GLC retention time and the mass spectrometric fragmentation pattern are characteristics of the substitution patterns (acetoxy and methoxy groups) in partially methylated alditol acetates derivative.
GC-MS is also useful for the structural elucidation of uronic acid containing polysaccharides. Due to resistance to hydrolysis of glycosiduronic acids complete hydrolysis is usually possible only after reduction to the corresponding hexose residues. This reduction may be performed after permethylation of the polysaccharide with labelled reagents (for example superdeuteride or Lithium borodeuteride). Reduction with labelled reagents converts the uronic acid group (COOH) to primary alcoholic group with the incorporation of the two-deuterium atoms (CD₂OH). This helps in distinguishing those hexose residues formed from uronic acids when mass spectrometry is used for the identification of the partially methylated alditol acetates.

The main limitation of the use of partially methylated alditol acetates for the characterisation of methylated sugars lies in the structural symmetry, which may exist when the primary hydroxyl group (O-5 in pentoses and O-6 in hexoses) is not etherified. This difficulty can be overcome by reduction of the sugars with sodium borodeuteride which introduces deuterium at C-1. This will create a isotopic shift in the primary fragmentation of MS.
8. Nuclear Magnetic Resonance Spectroscopy:

Nuclear magnetic resonance spectroscopy has become an important tool in the structural elucidation of complex polysaccharides as it has a number of characteristics that makes it more advantageous over other physical methods. NMR spectroscopy is non-destructive and the material can be recovered back. The method requires only a few milligrams of the compound thus avoiding large scale isolations. Generally $^1$H NMR and $^{13}$C NMR techniques complement each other in the structural studies of the polysaccharides.

Although NMR spectroscopy provides valuable information about the structure of polysaccharides, it is not always easy to interpret the spectra due to poor resolution of signals. However, high frequency instruments (360 MHz) now available are capable of resolving all the protons into singlets or multiplets. FT mode is also advantageous for $^1$H spectra. As polysaccharides are generally soluble in water solutions are prepared in deuterium oxide ($D_2O$). The preparation of the solution of a polysaccharides requires prior exchange treatment with $D_2O$ of high isotopic content (preferably 99.96%). Nevertheless, a strong peak due to residual water (HOD signal) is often obtained whose chemical shift at room temperature ($\delta=4.8$) obscures the vitally important anomeric region. However, at a
higher temperature (70-80°C) the HOD signal shifts upfield (Δ=4.5), thereby exposing the anomeric region of the spectrum. The anomeric protons are then easily distinguished.

Other factors that complicate the acquisition of ¹H NMR spectra of polysaccharides are interference by exchangeable protons (O-H, N-H) and line broadening of signals in aqueous solution. However this can be overcome by recording the spectra in deuterated dimethyl sulphoxide (DMSO-d₆). Because of low exchange rates in this medium, hydroxyl and amino proton resonance are clearly observed in the spectra and provide valuable structural information. The most useful information from ¹H spectra is obtained from the anomeric region of protons. These signals are well separated (in both ¹H and ¹³C NMR) from those produced by other nuclei. This greatly helps in determining the number of different monosaccharide residues in a polysaccharide and also in estimating their relative proportion. It is much easier to assess the degree of molecular complexity (which is a measure of different kinds of sugar residues and their ratios) of a polysaccharide from ¹³C NMR spectra rather than the ¹H NMR spectra because the signals in the former are much better dispersed than the signals of the latter. Owing to the low natural abundance
(1.1%) and low sensitivity of the $^{13}$C nucleus the $^{13}$C spectra are always recorded in the FT mode.

NMR may also be employed for the identification and estimation of constituent sugars in a polysaccharide hydrolysate. Especially $^{13}$C chemical shifts for each of the various sugars known to be its constituents are well distinctive.