

**Analysis of Carbohydrates by Mass Spectrometry**

**Literature Seminar**

**By**

**Mirela L. Maxim**

**Literature Advisor: Dr. Carolyn J. Cassady**

**October 2<sup>nd</sup>, 2007**

**Shelby Hall, Room 151, 12:45 p.m.**

## Introduction

There are four major classes of biomolecules: proteins, lipids, carbohydrates, and nucleic acids. Carbohydrates are the most abundant group of the natural molecules, produced in large quantities by photosynthesis by plants and microorganisms. There are also a major part of cellular structure of plants and animals.<sup>1</sup> For a long time proteins and nucleic acids were considered the crucial compounds in organisms that conserved and transferred biological information, while carbohydrates were studied as a source of glucose that provided energy for living organisms and were the main supportive structure of the plant tissues. In recent years, they have become the third bioinformative macromolecule with the given name of Glycom next to the well-known Genom and Proteom.<sup>2</sup> They play an important role in the working process of the immune system, fertilization, pathogenesis, blood clotting, and development.<sup>3</sup>

The initial step in the structural characterization of the carbohydrates was the work of Emil H. Fisher who established in 1888 the relation between three of the monosaccharides well known today: glucose, mannose, and fructose. This was followed by the establishment of the stereochemical configuration of all known sugars with possible isomers in 1890 and the successful synthesis of glucose, mannose, and fructose from glycerol.<sup>4</sup>

The next steps in the carbohydrate analysis included various analytical techniques like specific chemical reactions (acidic hydrolysis, enzymatic hydrolysis, methylation analysis)<sup>5</sup>, nuclear magnetic resonance (NMR)<sup>6</sup>, and gas chromatography with mass spectrometry electron ionization (GC-MS-EI). Due to the complexity of large carbohydrate molecules like oligosaccharides and polysaccharides, their structural

analysis could not be determined only by the NMR method which requires pure samples in relatively large amounts.<sup>2</sup>

Until the 1990s carbohydrate structural analysis by mass spectrometry was limited to electron ionization (EI) or fast atom bombardment (FAB). These methods presented disadvantages because they could not give satisfactory results for large molecules.

The development of desorption/ionization techniques enabled the full characterization of large carbohydrates by matrix assisted laser desorption/ionization (MALDI) and/or electrospray ionization (ESI).<sup>7</sup> The use of MALDI source gives the possibility to analyze biomolecules with masses higher than 200 kDa in their native state with an improved sensitivity. This paper will focus on the comparison of different mass spectrometry techniques and determining factors in the structural determination of large carbohydrate molecules.

### **Structures of the most common carbohydrates**

The general term carbohydrate includes monosaccharides (e.g., glucose, fructose), oligosaccharides (2 to 10 units) and polysaccharides (> 10 units). They have the chemical formula  $C_nH_{2n}O_n$ , where  $n = 3$  (trioses) to 9 (nonoses) and  $n = 6$  is the most abundant.<sup>8</sup> The number of possible isomers for each monosaccharide increases with the number of carbons in the molecule due to the asymmetrical constituents. Thus, for complex molecules like oligo or polysaccharides, simple analytical techniques can't differentiate between different isomers and give an accurate structural conformation. Structures of the most common isomers of glucose, mannose and fructose are shown in figure 1:

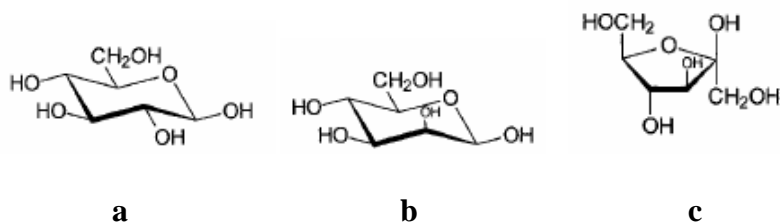


Fig. 1 Chemical structure of monosaccharides: (a) glucose ( $\beta$ -D-glucopyranose); (b) mannose ( $\beta$ -D-mannopyranose); (c) D-fructose

Monosaccharide units can be linked by glycosidic bonds leading to formation of oligosaccharides. Structures of the sucrose (table sugar), and lactose<sup>9</sup> which are also called disaccharides, streptomycin and kanamycin A<sup>10</sup>, (oligosaccharides pharmaceutically relevant) are shown in figure 2:

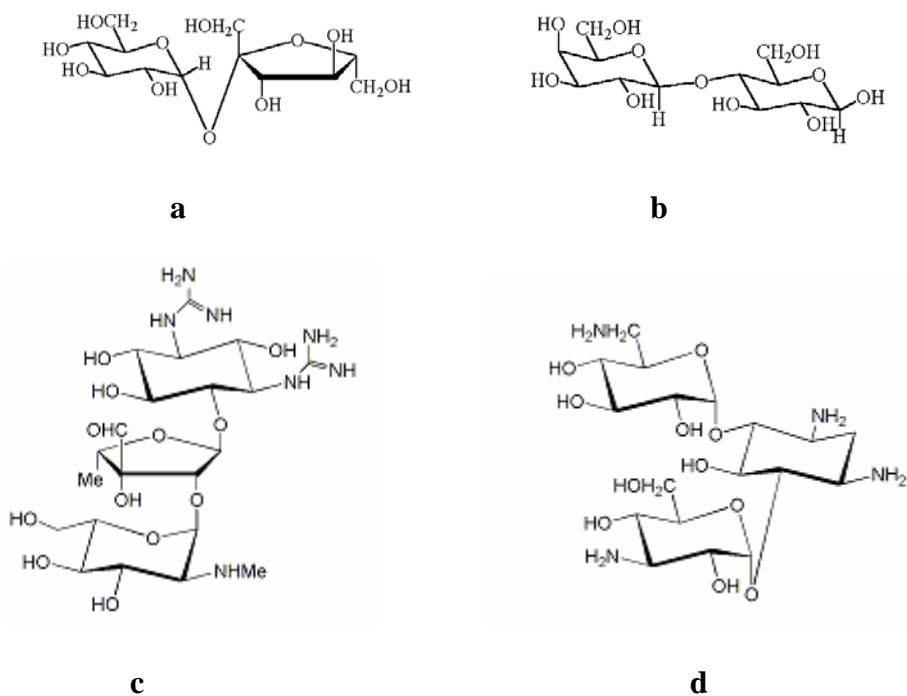


Fig. 2: Structures of oligosaccharides: (a) sucrose, (b) lactose, (c) streptomycin, (d) kanamycin A

Examples of natural polysaccharides are starch – used by plants to store glucose, cellulose – a structural molecule which supports the plants parts, chitin – a major part of the insects and sea animal’s shells. Example structures<sup>11</sup> are shown in the figure 3:

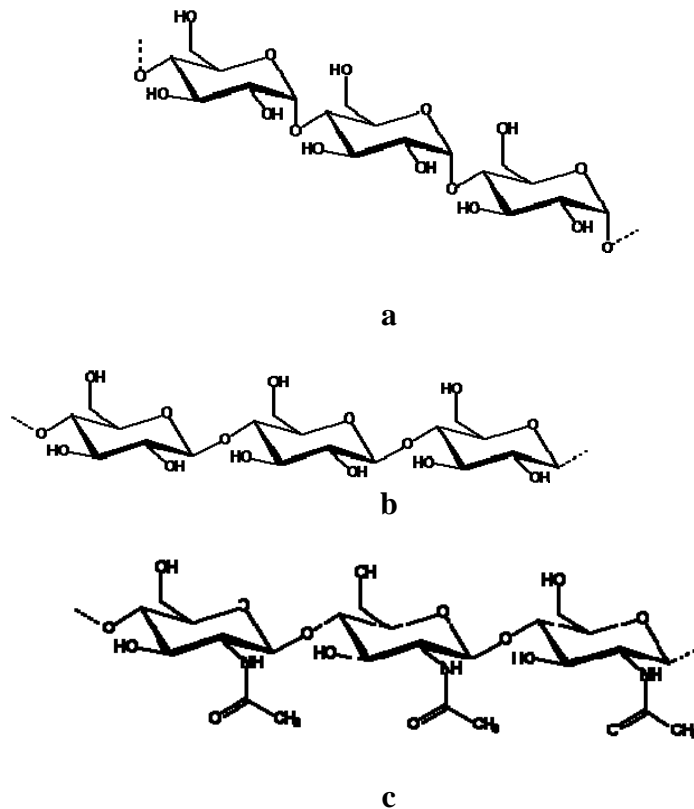


Fig. 3: Structures of polysaccharides: (a) amylose (starch), (b) cellulose, (c) chitin

Polysaccharides exist in a large structural diversity, linear or branched. There are also glycoconjugated compounds which are glycoproteins or glycolipids. These compounds are proteins or lipids that have covalently attached to their structure carbohydrates (glycans).

Carbohydrates are of great importance to science and especially the medical field (nutrition, biomedicine and pharmacy). By fully characterizing these compounds, valuable information can be obtained for diagnosis, treatment and cure for several diseases<sup>2</sup>. The methods used for analysis of carbohydrates need to have high degree of

sensitivity, high throughput and capacity of analyzing mixtures.<sup>7</sup> This is where mass spectrometry techniques come in very handy for all types of carbohydrates.

### Mass Spectrometry

Mass spectrometry is an analytical technique that generates ions from compounds (organic or inorganic) and separates them by mass-to-charge ( $m/z$ ) ratio. The ions are then detected qualitatively and quantitatively by their respective  $m/z$  and abundance.<sup>12,13</sup> A mass spectrometer consists of a sample inlet and an ion source which converts analyte molecules into gas-phase ionic species. During ionization the molecules may break into characteristic fragments. In the mass analyzer the molecular ion and the charged fragments are separated based on their mass-to-charge ratio. The ion detector is collecting the data and sends it to a data system where it is processed and translated into a mass spectrum. The instrument is operated under high vacuum except for the sample inlet which can be either under vacuum or atmospheric pressure. A schematic of the described components is presented in figure 4.

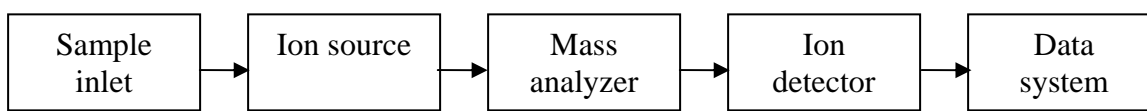


Fig. 4 Schematic diagram with components of a mass spectrometer

Mass spectrometry techniques in general provide molecular weight and structural information, have a high specificity, low limits of detection, require minute amounts of sample, and can analyze mixtures with rapid results and for a wide variety of possible samples.

Although mass spectrometry techniques provide high quality information, there is not an ideal one that will give all the necessary information for all the compounds. For

such a wide range of structural types like carbohydrates there is a need for different analytical techniques that would be complementary and would make a full analysis for a certain compound.

### **Mass Spectrometry Techniques for Structural Determination of Carbohydrates:**

A full characterization of a carbohydrate contains knowledge of the monosaccharide composition, sequence and branching of each monomer, stereochemistry, interglycosidic linkage positions, and anomeric configuration of each glycosidic bond.

The first mass spectrometry technique used for carbohydrate analysis was the electron ionization (EI) and although it is a simple method to use it has certain limitations: it requires gaseous samples prior to ionization (i.e., volatile samples), creates uncertainty in molecular mass determination of the original sample when derivatization is necessary, and it gives extensive fragmentation for molecules larger than 1000 Da.

Another method used for carbohydrate analysis was fast atom bombarding (FAB) which increased the range of molecules size to 5000 Da. The sample is dissolved in a polar, relatively less volatile and inert liquid matrix (e.g., glycerol) and is bombarded with a beam of high-energy atoms or ions (Ar, Xe, Ar<sup>+</sup>, Xe<sup>+</sup>, Cs<sup>+</sup>). The presence of matrix increases the sample lifetime and the reproducibility of the spectra but it may influence the detection sensitivity due to the excessive matrix background. This problem can be solved by using a different sample introduction device called continuous-flow CF-FAB probe that will reduce matrix-to-sample ratio and consequently the matrix background.<sup>14,15</sup>

MALDI MS was introduced in the late 1880s and it proved to be very effective for the analysis of low volatility organic compounds. The analyte is mixed with a matrix (solid or liquid) and is ionized by laser irradiation. The matrix's function is to absorb the energy from the pulsed laser and to transfer it to the analyte which will be ionized. The irradiation of the laser is adjustable by a rotating UV filter and also the duration of emitted pulses can vary in the ns range. These factors (matrix, laser irradiation, and duration of pulse) are important to prevent the degradation of the sample. A schematic<sup>16</sup> of the ionization process by laser absorption is shown in the figure 5.

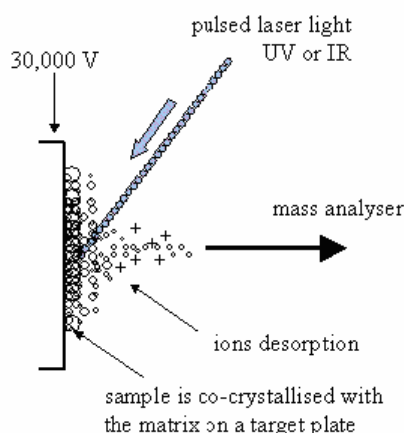


Fig. 5 Ionization process by laser absorption

For oligosaccharides the matrix used is usually 2,5-dihydrobenzoic acid (DHB) or combined matrices that have DHB as the main component.<sup>13,17</sup> Because oligosaccharides have a high affinity to alkali metal ions, they can be added to the matrix-analyte solution to result in the cationization of the neutral analyte.<sup>13,18</sup>

### Applications of MALDI on Carbohydrate Analysis

There are several factors that need to be considered for determination of carbohydrate structure. Different types of carbohydrates require different matrices although the main matrix used is still 2,5-dihydroxybenzoic acid (DHB). The

carbohydrates are not easily ionizable to form  $[M+H]^+$ , so they need the addition in the matrix of a metal ion that they can bind to and form detectable ions like  $[M+Na]^+$ . But, the affinity of carbohydrates for alkali metals varies: cesium has the highest affinity but is unable to ionize low mass carbohydrates,<sup>19</sup> sodium has a medium affinity, and hydrogen has the smallest affinity of all.<sup>20</sup>

Sample preparation is important considering that natural carbohydrates occur in mixtures. Purification of the sample needs to be made without the loss of any quantitative information. Separation of the mixtures can be done by enzymatic or chemical release. Derivatization can also be used to enhance the mass spectrometry signal and to have a well-defined fragmentation pattern.

Studies have shown that under different ionization conditions the fragmentation patterns are similar and depending on determining factors can be classified into two groups:

- glycosidic cleavages of bond linking two sugar rings
- cross-ring cleavages – breaking of two bonds

The first group provides information about sequence and branching and the second group about linkage.

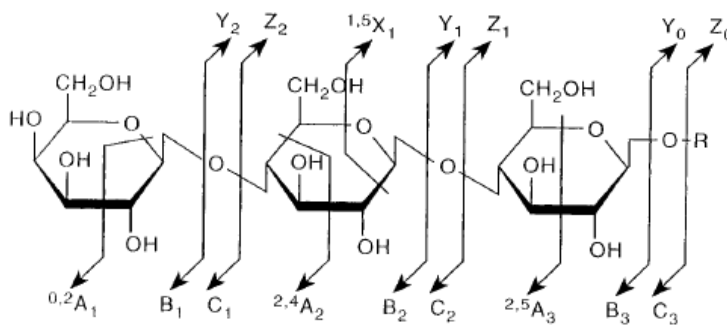


Fig. 6 Schematic nomenclature describing the major fragment ions from carbohydrates

A schematic describing the fragmentation pattern for carbohydrates was introduced in 1988 by Domon & Costello and it is shown in figure 6.<sup>21</sup> Ions that retain the charge at the reducing terminus are X (cross-ring), Y, and Z (glycosidic) and the complementary ions are A, B, and C respectively. For the A, B, and C the rings are numbered from the non-reducing end and for the others from the reducing end. Fragments from branched chain glycans are distinguished by Greek letters, with  $\alpha$  representing the largest chain. Cross-ring fragments are given superscript numbers to show the cleaved bonds like in figure 7.<sup>22</sup>

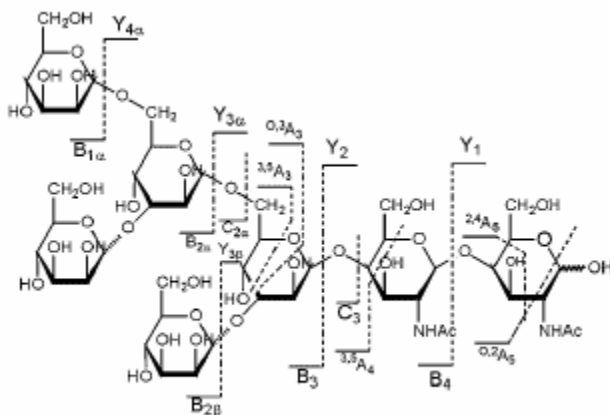


Fig. 7 Fragmentation of (Man)<sub>5</sub>(GlcNAc) from a Q-TOF mass spectrometer fitted with a MALDI ion source

## Conclusion

The structural analysis of carbohydrates depends on the complexity of the analyte studied, the technique used, and the conditions that the analysis is made under. Current studies show that the improved techniques like MALDI sources using ion trap mass analyzers (ITM) make easier the approach of complex mixtures of compounds with the full structural characterization.

## References:

- 
- <sup>1</sup> Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry*; Wiley-VCH: Weinheim, FRG, 2003; pp 1
- <sup>2</sup> Campa, C.; Coslovi, A.; Flamigni, A.; Rossi, M. *Electrophoresis* **2006**, *27*, 2027-2050
- <sup>3</sup> <http://en.wikipedia.org/wiki/Carbohydrate>
- <sup>4</sup> [http://nobelprize.org/nobel\\_prizes/chemistry/laureates/1902/fischer-bio.html](http://nobelprize.org/nobel_prizes/chemistry/laureates/1902/fischer-bio.html)
- <sup>5</sup> Pazur, J. H. *Carbohydrate analysis a practical approach*; J. F. Kennedy ed., Oxford: IRL Press, 1986, pp 55-96
- <sup>6</sup> Duus, J.; Gotfredsen, C. H.; Bock, K. *Chemical Reviews* **2000**, *100*, 4589-4614
- <sup>7</sup> Fernandez, L. E. M. *Carbohydrate Polymers* **2007**, *68*, 797-807
- <sup>8</sup> Harvey, D. J. *Mass Spectrometry Reviews*, **1999**, *18*, 349-451
- <sup>9</sup> <http://www.uwplatt.edu/~sundin/354-7/1547-44.htm>
- <sup>10</sup> [http://www.biosynth.com/index.asp?topic\\_id=225&g=19&m=276](http://www.biosynth.com/index.asp?topic_id=225&g=19&m=276)
- <sup>11</sup> <http://tonga.usip.edu/gmoyna/biochem341/lecture34.html>
- <sup>12</sup> Kientz, H. *Einführung Massenspektrometrie*; Verlag Chemie: Weinheim, 1968
- <sup>13</sup> Gross, H. J. *Mass Spectrometry – A Textbook*; Springer-Verlag: Berlin, 2004, pp 3
- <sup>14</sup> Daas, C. *Fundamentals of Contemporary Mass Spectrometry*; John Wiley & Sons, Inc.: Hoboken, NJ, 2007, pp 34
- <sup>15</sup> Caprioli, R. M. *Anal. Chem.* **1990**, *62*, 477A-485A
- <sup>16</sup> <http://qbab.aber.ac.uk/roy/mss/tofspec.htm>
- <sup>17</sup> Karas, M.; Ehring, H.; Nordhoff, E.; Stahl, B.; Strupat, K.; Hillencamp, F.; Grehl, M.; Krebs, B. *Org. Mass Spectrom.* **1993**, *28*, 1476-1481
- <sup>18</sup> Coates, M. L.; Wilkins, C. L. *Biomed. Mass Spectrom.* **1985**, *12*, 424-428
- <sup>19</sup> Cancilla, M. T.; Penn, S. G.; Carroll, G. A.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1996**, *118*, 6736-6745
- <sup>20</sup> Mohr, M. D.; Bornsen, K. O.; Widmer, H. M. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 809-814
- <sup>21</sup> Domon, B.; Costello, C. E. *Glycoconjugate J.* 1988, *5*, 397-409
- <sup>22</sup> Harvey, D. J. *Mass Spectrom. Reviews* **2006**, *25*, 595-662