Spinsolve

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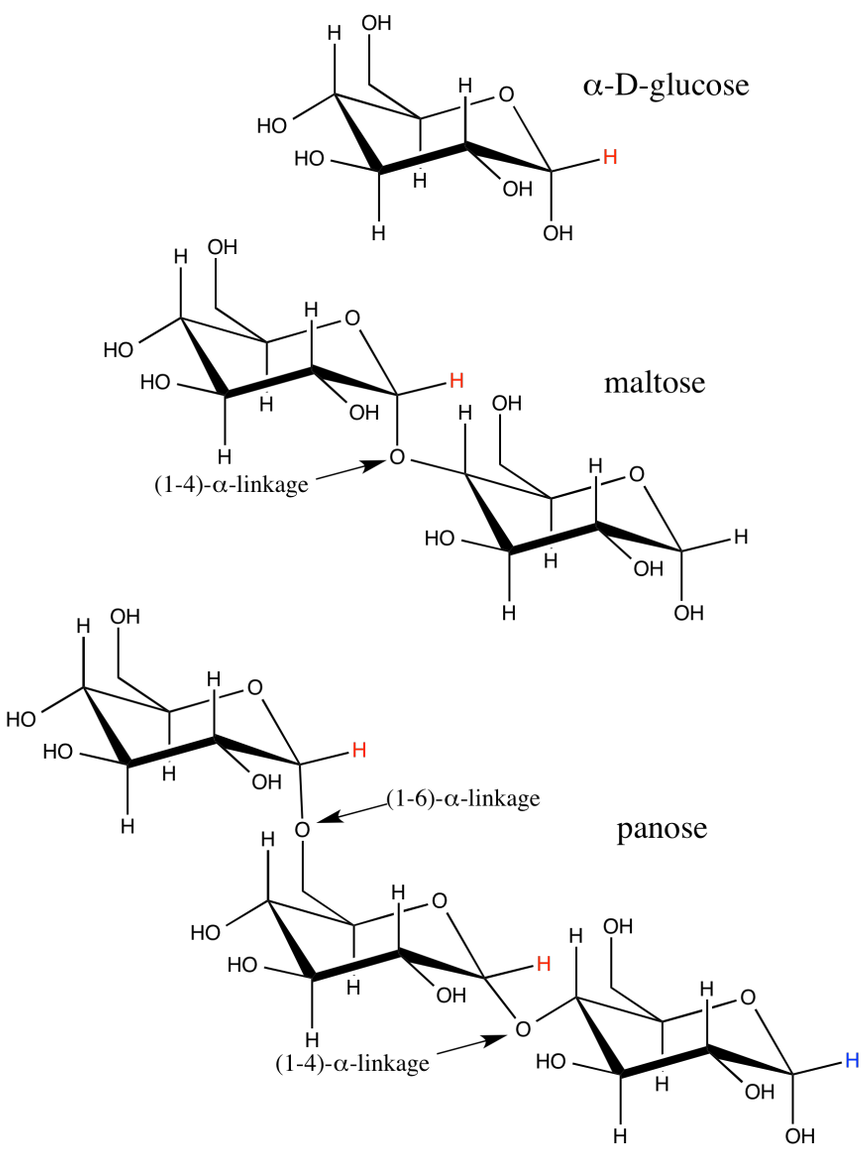
Application Note 8: Polysaccharide chain analysis



Background

Sugar is a common energy source for many living organisms. It can be found in monomer form such as glucose, linked in long chains such as starch, or anywhere in-between. Maltose is a disaccharide made of two glucose molecules bonded by (1 → 4)-α- linkage and the main energy source for yeast when fermenting beer. Maltodextrins are slightly longer and are considered to have approximately 3-20 glucose molecules joined by glycosidic bonds, while starch is made of very long chains and are a common energy storage molecule for plants. The (1 → 6)-α- linkage is found in branched starches. A starch molecule with primarily linear chains made up of (1 → 4)-α- linkages is called amylose and starch that is highly branched with (1 → 6)-α- linkages is called amylopectin. If three glucose units are combined with one (1 → 4)-α- linkage and one (1 → 6)-α- linkage you have panose. These linkages and the reducing protons can be observed by ^{1}H NMR on the Spinsolve spectrometer and allows one to infer information about chain lengths and degree of branching of smaller polysaccharides.

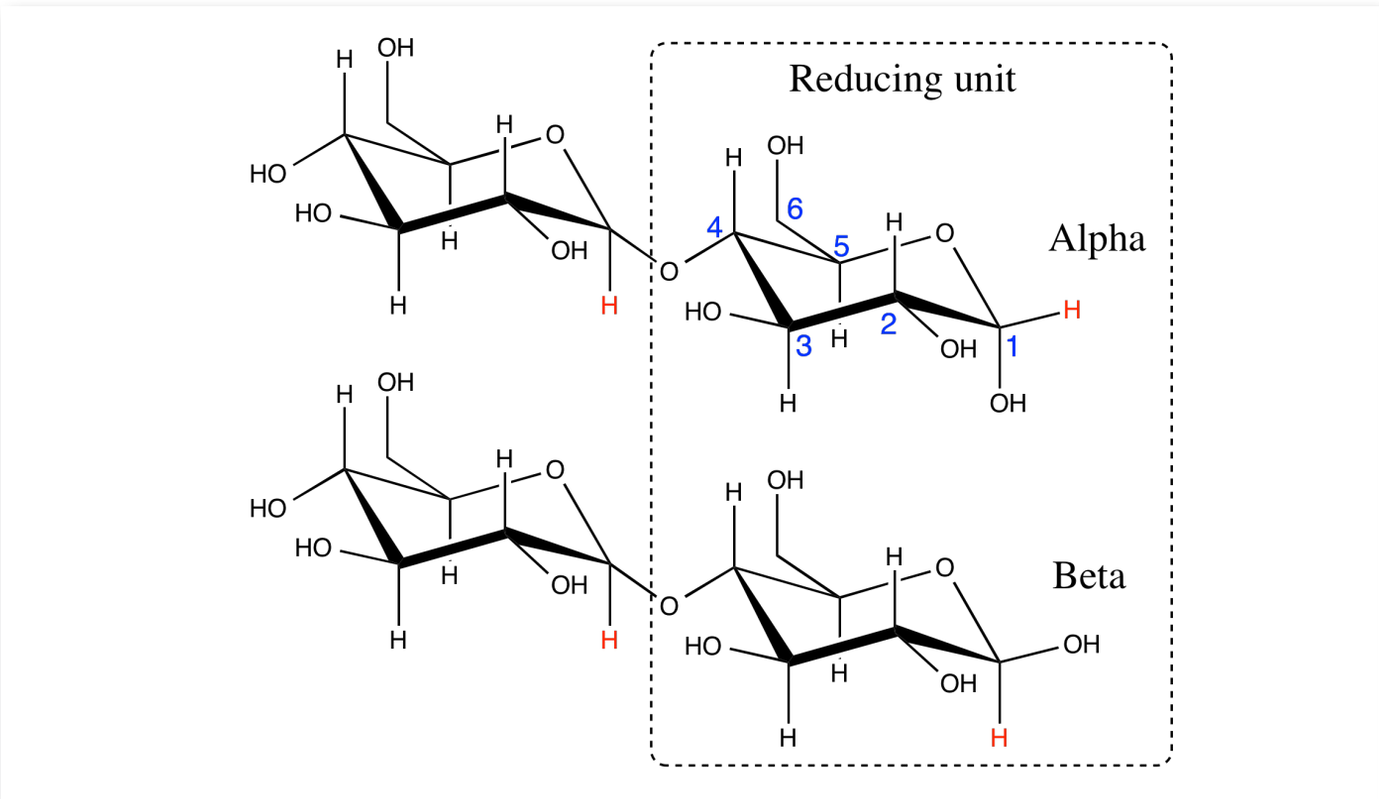


Figure 1: The *alpha* and *beta* anomeric conformations of maltose are shown in the reducing unit.

Observing the anomeric protons in maltose

Maltose is a dimer composed of two glucose molecules. Like glucose, the reducing end glucose remains mostly in the cyclic pyranose form in two conformational anomers shown in Figure 1. These two forms interconvert via an aldehyde chain and can be observed by monitoring the ^{1}H NMR resonances of the anomeric protons shown in red in Figure 1. The equatorial (*alpha*) anomeric proton resonates further down field (5.3 ppm) from the axial (*beta*) proton (4.7 ppm) making these two anomer populations distinguishable by ^{1}H NMR even at low field (Figure 2).

Note: More information is available about the glucose anomeric proton in Application note #5, “Glucose anomers”.

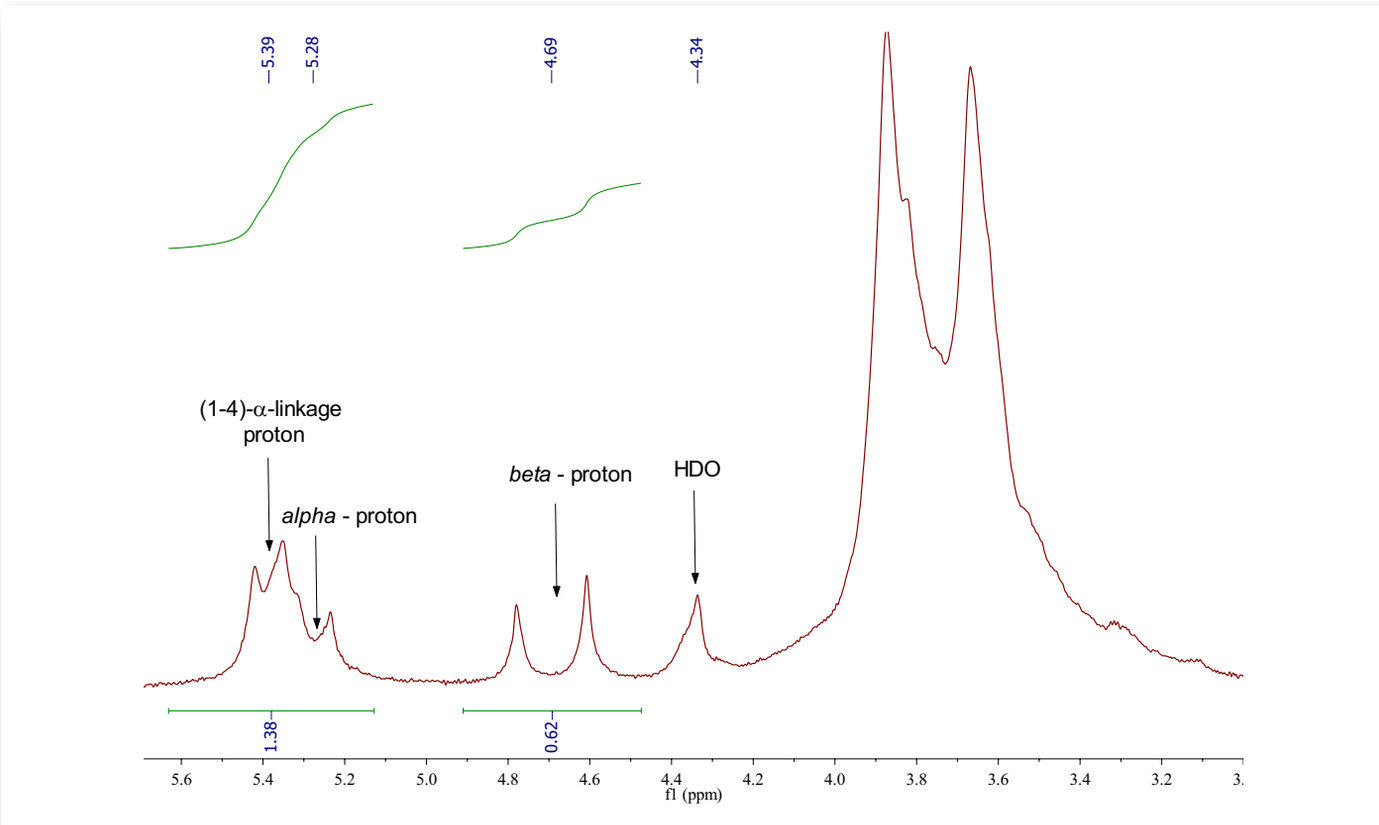


Figure 2: ^{1}H-NMR spectrum of maltose.

Observing (1 ⟶ 4)-α- linkage in maltose

The (1 ⟶ 4)-α- linkage is observed by the presence of the H-1 linkage proton NMR resonance at 5.36ppm as demonstrated in the maltose spectrum (Figure 2). You will also see the anomeric proton peak of the reducing unit at two positions relating to the equatorial (*alpha*) and axial (*beta*) anomers at 5.28 and 4.69 ppm, respectively.

The *alpha* anomeric proton and the (1 ⟶ 4)-α- linkage proton resonances are overlapping, so they cannot be integrated individually, but the total integral of the three peaks should be equivalent to 2.00 representing both H-1 protons from each glucose subunit in maltose. The *alpha* and *beta* equilibrium of the reducing anomeric proton should be 36 and 64%, respectively. Therefore, if the total integral value is fixed to 2.00 the individual integrals should be about 1.36 for the (1 ⟶ 4)-α- linkage and *alpha* H-1 protons and 0.64 for the *beta* anomeric H-1 proton as shown in Figure 2.

Observing (1 → 6)-α- linkage in panose

There are two types of starch molecules present in cereal grains, amylose and amylopectin. Amylose has mostly (1 → 4)-α- linkages and forms straight chains, while amylopectin is branched and has both (1 → 4)-α- linkages to form the chains and (1 → 6)-α- linkages at the branching points. Panose is a special trisaccharide with one (1 → 4)-α- linkage and one (1 → 6)-α- branching linkage, as seen below, and is commonly used to demonstrate the NMR signature of each linkage.

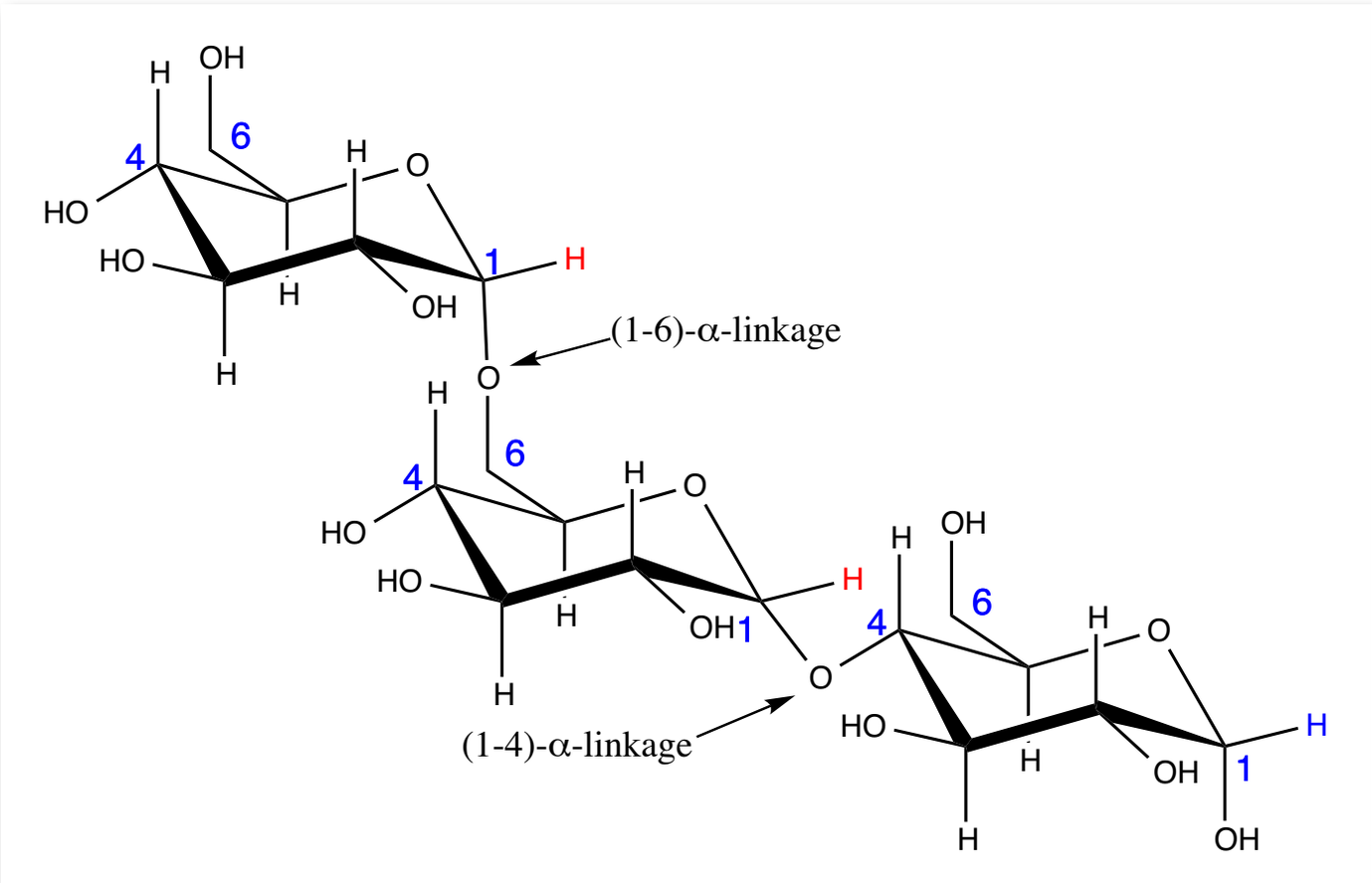


Figure 3: Structure of panose showing the two different glucosidic bonds.

By looking at the ^{1}H NMR resonance of the H-1 proton, it is possible to determine the linkages present (Gidley, 1985). Figure 4a shows the ^{1}H NMR spectrum of the H-1 protons for both maltose (maroon) and panose (blue). For panose, this region of the spectrum is composed of four resonances at 5.37, 4.98, 5.25, and 4.67 ppm corresponding to the (1 → 4)-α- linkage, (1 → 6)-α- branching point, *alpha* reducing proton, and *beta* reducing proton. Maltose does not have a (1 → 6)-α- bond and therefore lacks the resonance at 4.98 ppm.

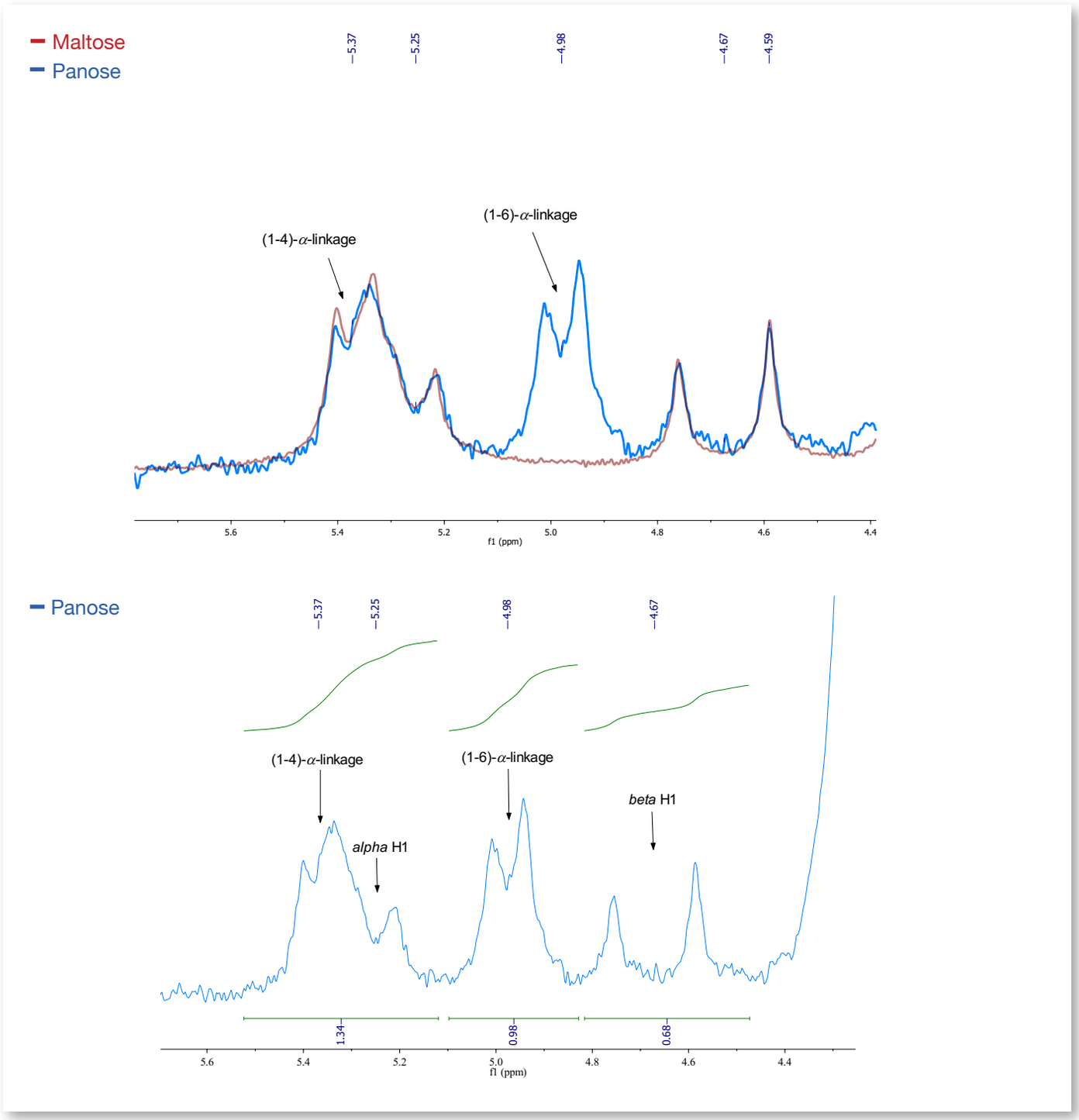


Figure 4 (a) An over lay of maltose (maroon) and panose (blue) shows the presence and absence of the (1-6) bond and (b) the integrals of panose show the expected fraction for the two bonds and two anomers.

For panose the sum of the integrals should be equivalent to the number of glucose molucules in the chain (*i.e.* three). The linkages should be equal to 1, the *alpha* anomeric proton equal to 0.36, and the *beta* anomeric proton equal to 0.64. Again, the *alpha* proton and the (1 → 4)-α- linkage cannot be resolved, so their combined integral should be 1.36 (Figure 4b).

Measuring chain length

It is easy to measure the chain length of maltose and panose, but can this be applied to longer polysaccharides called maltodextrins? A general structure for polysaccharides is shown in Figure 5, where the chain length or degree of polymerization will be equal to n+2. Three maltodextrins of varying dextrose equivalents were obtained, the hydroxyl protons were exchanged to simplify the spectra, with deuterium and their ^{1}H-NMR spectra were acquired. The *beta*-anomer proton peak was used to calibrate the integrals. By setting this value to 0.64, the sum of the linkage and anomer integrals will be equal to the number of units in the chain. A comparison of all the spectra is shown in Figure 6. As the chains become longer, the anomeric protons becomes a smaller fraction of the signal, therefore there is a limit to the chain length one can accurately measure.

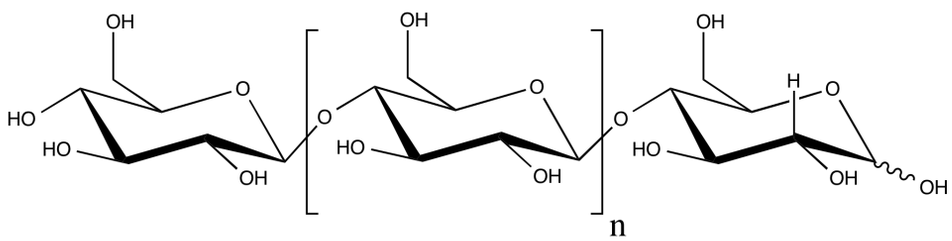


Figure 5: Molecular structure of dextrin.

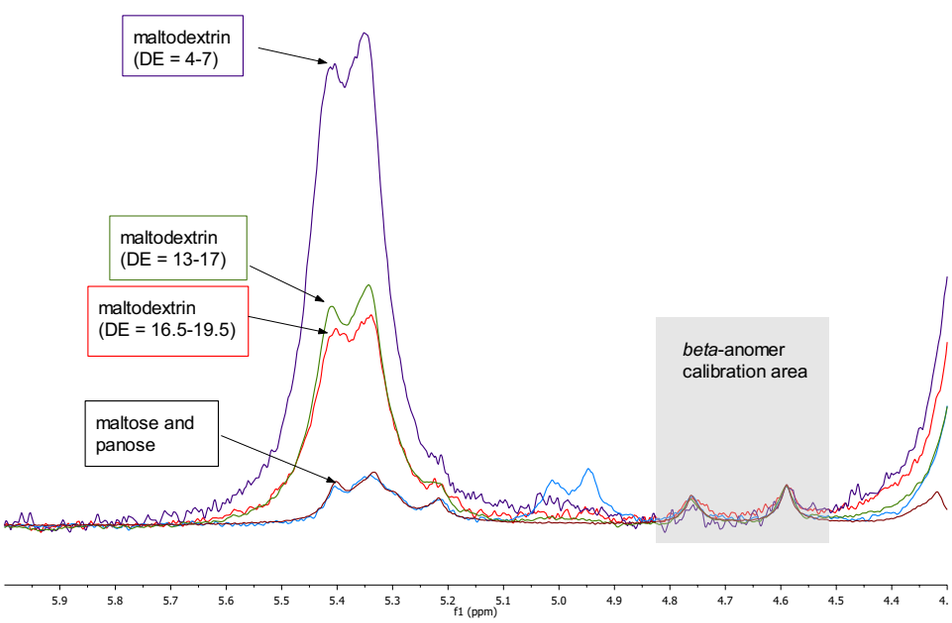


Figure 6: An overlay of the three maltodextrins, maltose, and panose shows how the signal for the (1 → 4)-α- linkage scales as the average chain length increases. The spectra were scaled using the *beta* anomer peak height.

The samples were also run at 300 MHz to confirm the integral values from well resolved NMR peaks. For the most part, the resonances were well resolved from each other and the HDO peak, as shown in Figure 7. Even though the spectra are better resolved at 300 MHz than 43 MHz, the integrals are very similar (Table 1).

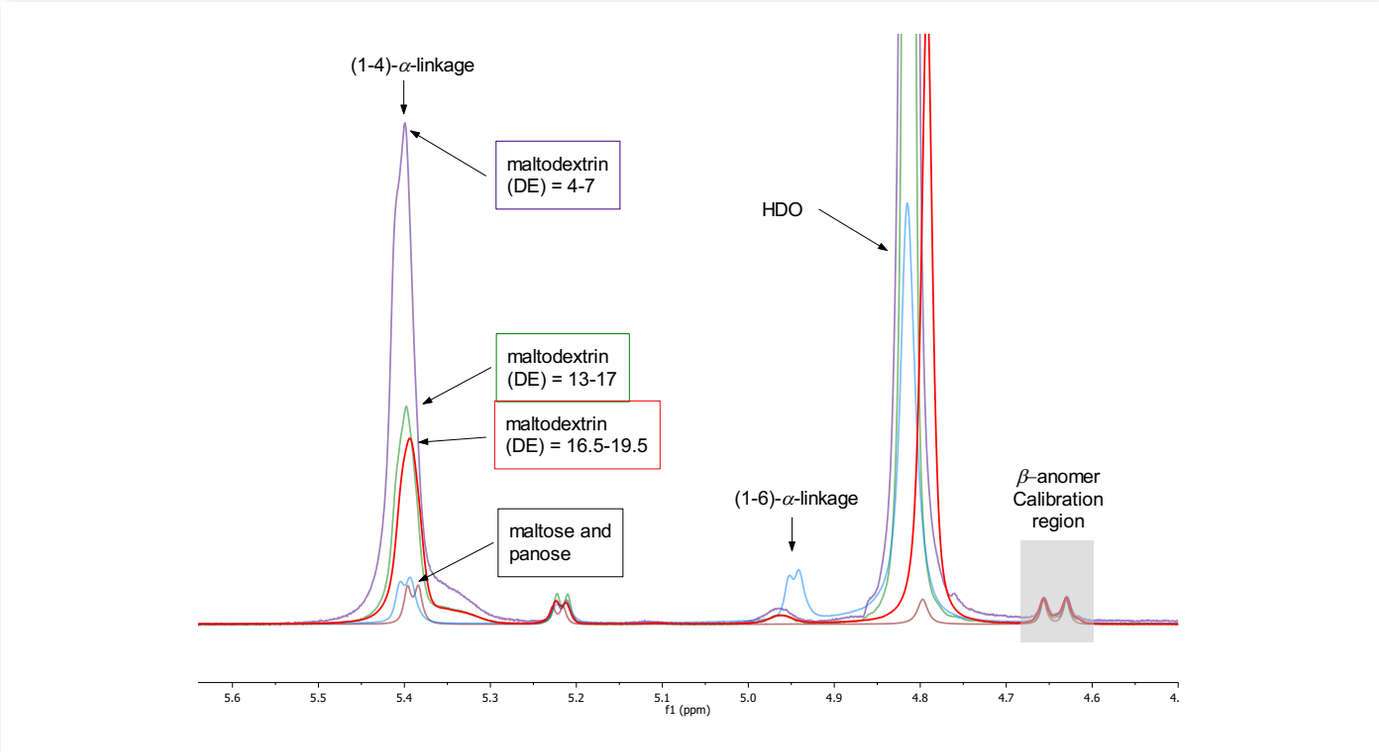


Figure 7: An overlay of 300 MHz spectra of the three dextrins, maltose, and panose shows good spectral resolution of each of the peak. The spectra were scaled using the beta anomer peak height.

Maltodextrins are usually created by hydrolysis of starch, either by enzyme or acid catalysis, resulting in a polydispersed mixture of chain lengths opposed to a single value. The degree of hydrolysis tends to be described in *dextrose equivalent*, which is determined by measuring the reducing power of the digest as a percentage of that expected for glucose (dextrose). Glucose therefore has a dextrose equivalent of 100, maltose about 50, panose about 33 and starch about zero. From this a model can be created:

DE = 100/DP;

where DE is the dextrose equivalent and DP is the degree of polymerization or mean chain length. By using maltose, panose, and three maltodextrins a plot of the degree of polymerization measured by NMR (mean chain length) versus the dextrose equivalent is created (Figure 8). The equation DE = 100/DP was plotted over top of the data as a guide.

Polysaccharide	Dextrose Equivalent (DE)	Mean chain length 42 MHz (Spinsolve)	Mean chain length 300 MHz
Maltose	52	2.0	2.0
Panose	33.3	2.9	3.1
Maltodextrin 1	16.5-19.5	6.0	6.6
Maltodextrin 2	13-17	7.2	7.1
Maltodextrin 3	4-7	15	15

Table 1. The dextrose equivalent and measured mean chain length by NMR at 43 MHz and 300 MHz is listed for each maltodextrin, maltose, and panose.

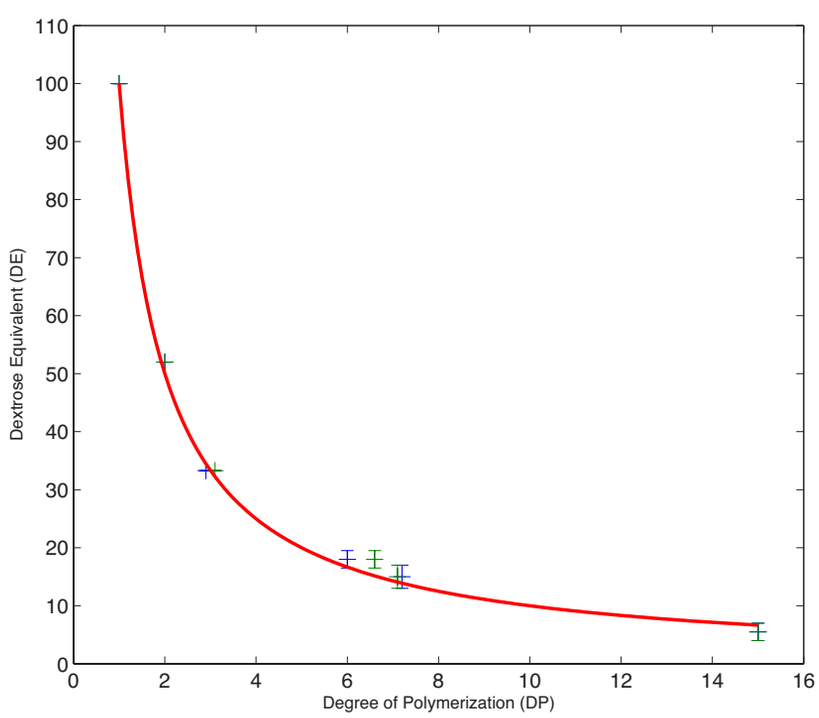


Figure 8. The dextrose equivalent was plotted against the mean chain length measured by NMR. The first point for glucose (1, 100) was inferred as the definition of dextrose equivalent. The green markers represent measurements made 300 MHz and the blue 43 MHz. The measurements of degree of polymerization were the same at both 43 and 300 MHz for DE = 1, 2, and 15.

Experimental

The samples were prepared by exchanging the hydroxyl protons with D_2O and dissolving them back into D_2O for NMR analysis. Exchanging the protons reduces the HDO peak in the spectrum. The samples were heated to >90 °C to shift the water peak up-field from the region of interest. Once the samples reach 90 °C, they are inserted into the Spinsolve and the spectrum was collected after waiting five times the longest T_1. The spectrum was acquired in a single scan usinga 90° excitation pulse. At 90 °C the HDO peak shifts to about 4.1 ppm, which is mostly out of the range of interest. Spectra collected at 300 MHz were run at room temperature.

Conclusion

It is possible to analyse the linkages and chain lengths of polysaccharides using a Spinsolve benchtop NMR spectrometer. The (1 → 4) and (1 → 6)-α- linkages are observed by the presence of resonances at 5.4 and 5.0 ppm respectively. The number of glucose units is observed in small chain polysaccharides by comparing the *beta* anomeric proton resonance integral with the linkage proton resonances. The dextrose equivalent can be calculated from this chain length.

Reference:

Gidley, M. J. Quantification of the structural features of starch polysaccharides by NMR Spectroscopy. *Carbohydrate Research*. **1985**, 139, 85-93.

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