Identification and Characterization of Sugar-Borate Ester Complexes Formed by Reaction of Calcium and Magnesium Salts with Borate and Different Sugars Using Multinuclear Liquid and Solid-State NMR

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Overview of Study

Different sugar borate esters (SBE) are found in fruits, vegetables, nuts and legunes and they are naturally absorbed by animal cells. Fructose and glucose borate esters are most common SBEs. In food they serve as soluble borate with potential health beneficial effects. The samples used foor this study are magnesium and calcium sugarborate complexes formed by the reaction of boric acid with different sugars (fructose, glucose, galactose, mannose and sucrose) and magnesium or calcium carbonate. Liquid and solid-state ^{13}C and ^{11}B NMR was utilized to establish a baseline for product quality and to establish a robust testing method for both identification and quantification of the mono-complex and the di-complex present in the product, as well as free borate and free sugar that is present in the finished product. The effect of varying the salt form and the sugar component of the borate complex component was studied by ^{11}B and ^{13}C NMR to establish the relative amounts of free borate, free sugar, and mono-/di-complex present in the products formed by the manufacturing recipe changes.

Background

Boron is naturally occurring and essential element for plant and animal life. There are many different biological compounds that can form complexes with boron. Compounds capable of complexing with boric acid include sugar alcohols, pyranose and furanose sugars or their derivatives, organic acids. Boric acid forms esters and complexes with a wide variety of mono-, di-, and polyhydroxy compounds (Woods, 1996). One of the most stable esters of boric acid are complexes where boric acid is a bridge between two carbohydrate molecules, e.g. fructose-boron-fructose. The examination of boron complexation in plants and plant extracts by ^{11}B NMR demonstrated the majority of the boron was associated with a diester complexes of diols and hydroxycarboxylic acids in radish and apple respectively (Matsunaga & Nagata, 1995). The authors made the conclusion that fructose is the most significant boron complexing molecule. Later these hypotheses were verified (Brown & Shelp, 1997 and Hu, et al., 1997) after successful isolation and full characterization of soluble boron complexes from higher plants. Calcium fructoborate (CF) is most commonly found in fresh fruits and vegetables. As a dietary supplement it is manufacture by VDF FutureCeuticals, Inc under the commercial name Fruitex-B®(FrxB) based on the US patent 5.962.049 (Miljkovic, 1999). The characterization of this complex has been reported previously (Rotaru et al., 2010) using thermal analysis, X-rays diffraction, ICP-MS, Raman spectrometry techniques. In this study we investigate molecular composition, stability and identification of FrxB used as a dietary supplement for human nutrition (Dinca & Scorei, 2013, and Reyes-Izquierdo et al., 2012) using liquid- and solid-state ^{11}B and ^{13}C NMR.

Materials and Method

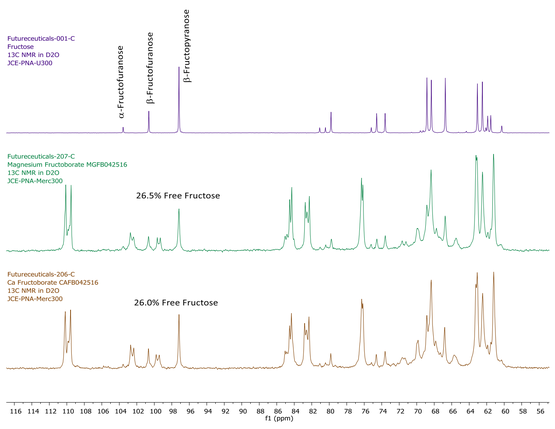
Materials

Fruitex-B ® formulations containing magnesium and calcium salts of fructoborate (MgFruB and CaFruB) were manufactured along with similar magnesium and calcium salt complexes formed with glucose (MgGluB and CaGluB), mannose (MgManB and CaManB), galactose (MgGalB and CaGalB) and sucrose (MgSucB and CaSucB) and provided for analysis by Futureceuticals, Momence IL, USA according to the Miljkovic patent (US 5, 962,049).

NMR Spectroscopy

Liquid-state ^{11}B, ^{13}C, and ^{1}H NMR was performed on a Varian Mercury 300MVX NMR spectrometer equipped with a 5mm Varian ATB Probe at a resonance frequencies of 96.14 MHz (^{11}B), 75.36 MHz (^{13}C) and 299.67 MHz (^{1}H), respectively. ^{11}B spectra were acquired with a 45 degree tip angle pulse width, a relaxation delays of 0.2 seconds, an acquisition time of 80 ms with 8K points acquired with a spectral width of 100 kHz, and 1024 pulses were averaged. The data was zero filled to 65K points. The ^{13}C NMR was acquired with a 30 degree tip angle pulse width, a 5 seconds relaxation delay, 0.96 second acquisition time, with 24K points acquired with a spectral width of 25 kHz, and 10-12,000 pulses were averaged. The data was zero filled to 131K points. The data was acquired in a quantitative manner with inverse gated decoupling of protons during the acquisition of the ^{11}B and ^{13}C experiments. All samples were dissolved in D_2O (Cambridge Isotope Laboratories). No pH adjustments were performed on the samples after dissolution.

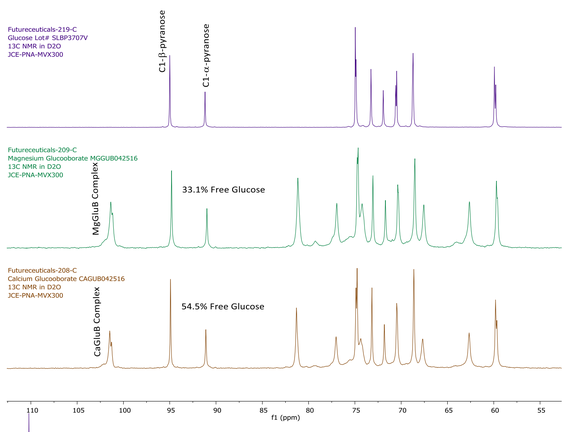
Solid-State ^{13}C (50.30 MHz) and ^{11}B (64.17 MHz) NMR spectra were obtained on a Varian UnityPlus-200 NMR spectrometer equipped with a Doty Scientific 7mm Supersonic CP-MAS probe. Magic angle spinning (MAS) speeds of around 6 kHz were employed. The ^{13}C NMR data was acquired using cross polarization which prepares the magnetization on the protons initially and then transfers the spin locked magnetization to the ^{13}C nuclei. The advantage of this experiment is the fact that the experiment is performed at the spin-lattice relaxation rate (T_1) of protons in the sample which is considerably shorter than the T_1 of ^{13}C nuclei in the same sample. Thus, one obtains a significant enhancement of the ^{13}C signal from the polarization transfer and can pulse at a shorter pulse-repetition rate. The ^{13}C experiment on calcium and magnesium "sugar borate" complex were acquired with an 8 second relaxation rate, and acquisition time of 25.6 ms, with 1K points being acquired over a spectral with of 40 kHz, and 4096 pulses were averaged. The exception to these acquisition parameters were those used for pure crystalline fructose. The ^{11}B NMR spectra were acquired with MAS and with the sample remaining static in the NMR probe. The experiments were acquired with a central transition selective pulse width, a 0.2 second relaxation time, with 1K points being acquired in an acquisition time of 10.2 ms, and with a spectral width of 100 kHz. Solid-State 13C NMR on the pure sugar samples required .

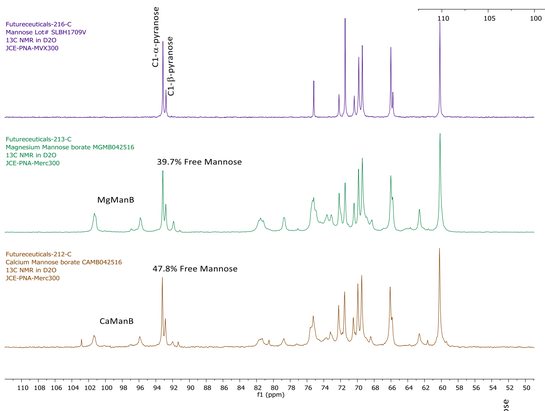


Liquid-State ^{13}C NMR

In the series of figures below we show the stacked plot comparison of each sugar borate with the calcium and magnesium sugar borate complex. The anomeric region of the 13C spectrum can be utilized to identify the signals from free sugar molecules mixed intimately with the sugar borate complex. The sum of the integration of the free sugar signals compared to the total integral of the anomeric region allows the free sugar percentage to be calculated. This value is utilized to determine which sugar and metal combination provides the most effective complex formation.

The tabulation of the free sugar concentrations is provided in Table I below. From the results it is determined that under the current manufacturing conditions fructose yields the highest concentration of sugar borate complex and there is little difference observed in the fructoborate complex between magnesium and Calcium forms of the complex. The mannose sugar complex is formed effectively, however there appears to be a difference in the effectiveness of the complex formation with the reaction of magnesium providing a higher concentration of complex. This behavior is mirrored in the higher concentration of complex formed in the magnesium glucoborate complex.

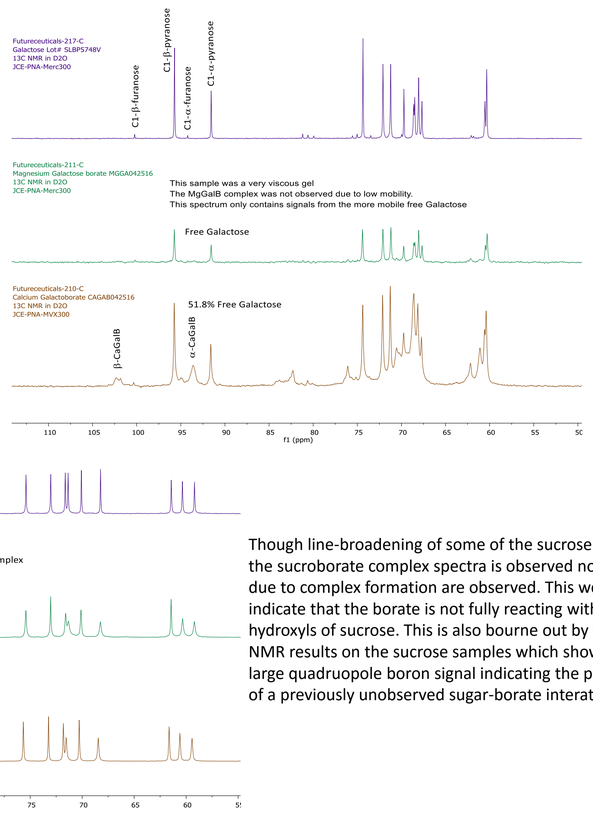


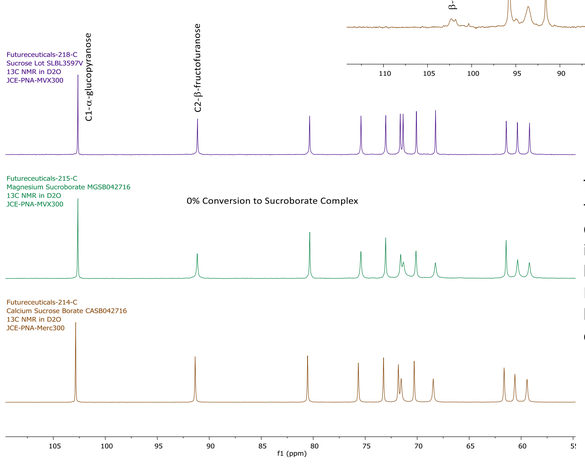


It was noted that though complex formation is seen to occur in the calcium galactoborate mixture the magnesium galactoborate shows a strong complex formation that forms a very effective gel. This gel formation renders the complex carbon signals unobservable due to low molecular mobility. Only the mobile free galactose entrained in the gel is observed and the lack of complex signals prevents a calculation of the absolute value of the free galactose concentration.

Table I: Free Sugar % in Calcium And Magnesium Sugar-Borate Complexes

Sample	Free Sugar %C
Calcium Fructoborate	26.0
Magnesium Fructoborate	26.5
Calcium Glucoborate	54.5
Magnesium Glucoborate	33.1
Calcium Galactoborate	51.8
Magnesium Galactoborate	?
Calcium Mannoborate	47.8
Magnesium Mannoborate	39.7
Calcium Sucroborate	100.0
Magnesium Sucroborate	100.0





Though line-broadening of some of the sucrose peaks in the sucroborate complex spectra is observed no peaks due to complex formation are observed. This would indicate that the borate is not fully reacting with the hydroxyls of sucrose. This is also bourne out by the ^{11}B NMR results on the sucrose samples which shows a very large quadruopole boron signal indicating the presence of a previously unobserved sugar-borate interation.

Liquid-State ^{11}B NMR

The Figure and Table II, shown below, provide a stacked plot of the ^{11}B NMR spectra obtained on the sugar-borate complexes formed in the presence of either calcium or magnesium salts.

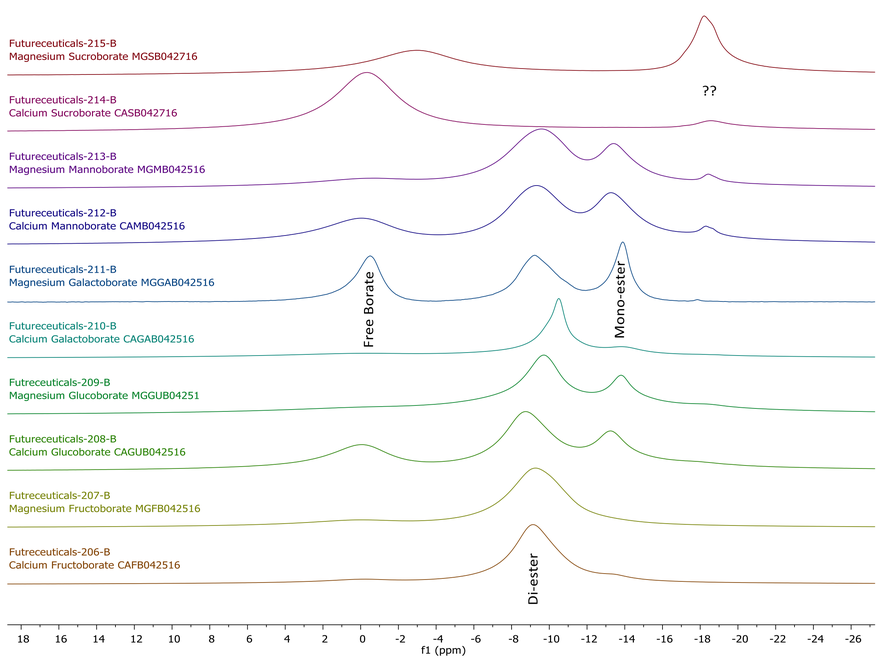


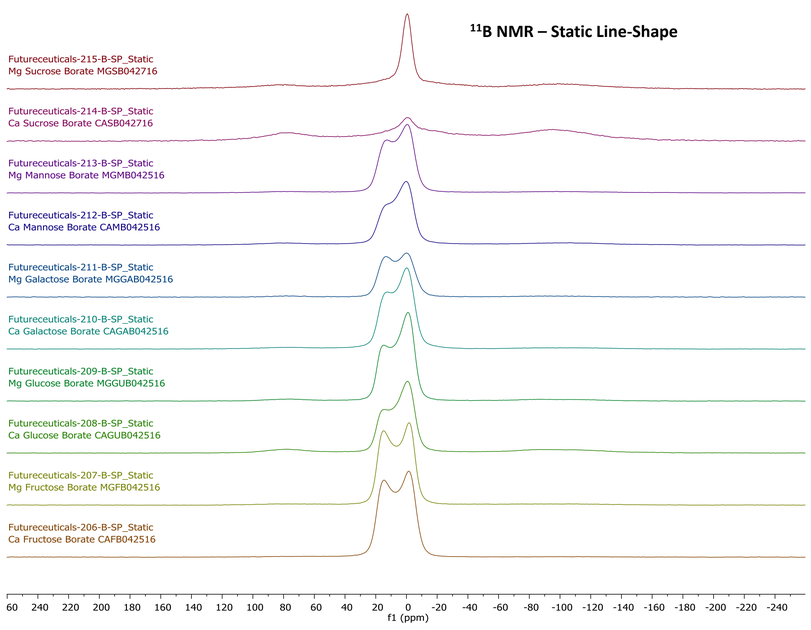
Table II: % Free Borate, Di-Ester Complex, Mono-Ester Complex and Unknown Boron Coordination Species found in Calcium & Magnesium Sugar-Borate Complexes

Sample	Free Borate %B	Diester %B	Mono-Ester %B	Unknown %B
Calcium Fructoborate	1.5	95.2	3.3	0
Magnesium Fructoborate	9.9	90.1	0.0	0
Calcium Glucoborate	23.3	49.9	25.4	1.4
Magnesium Glucoborate	9.9	61.5	26.5	2.1
Calcium Galactoborate	13.4	74.4	11.1	1.1
Magnesium Galactoborate	29.7	44.9	25.0	0.4
Calcium Mannoborate	21.7	43.6	30.5	4.2
Magnesium Mannoborate	6.2	67.7	24.5	1.6
Calcium Sucroborate	91.8	0	0	8.2
Magnesium Sucroborate	51.7	0	0	48.3

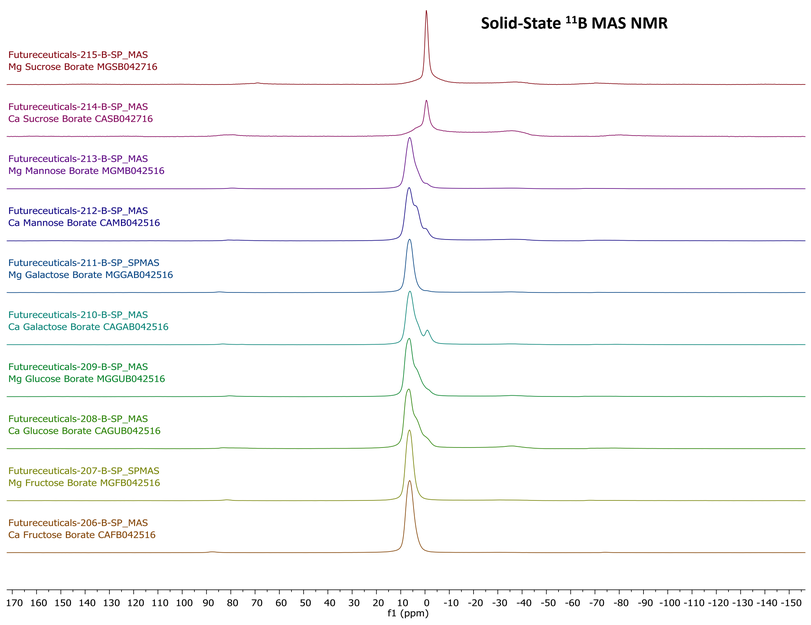
The liquid-state ^{11}B NMR shows the presence of different amounts of free borate as well as boron coordinated in di-ester and mono-ester sugar-borate complexes. Again the results underline the fact that fructose forms the most stable complex. The other sugar-borate complexes show high concentrations of mono-ester (borate coordinated to a single sugar). Interestingly, though no ^{13}C signal was observed for the gel forming magnesium galactoborate, an ^{11}B signal was obtained for the complex in this sample. We are not sure why the ^{11}B signal was not observed for the same reason as the ^{13}C signal being non-observable. Also, in all the sugar-borate complex samples other than fructoborate a fourth component is observed at -18 ppm. This seems to correspond to a boron center with a non-symmetric coordination that is found particularly in the solid-state ^{11}B NMR of the sucroborate samples. The solid-state NMR data will be discussed below.

Solid-State ^{11}B NMR – Magic Angle Spinning and Static Line-shape Experiments

In the figures below we present the solid-state ^{11}B NMR spectra obtained with magic angle spinning and also as static line-shapes. ^{11}B is a quarupolar nucleus which means that it yields "non-symmetric" line-shapes whose shape and width are effected by the symmetry of the boron center coordinated in the sugar borate complex as well as any other small molecule boron species present in the mixture. It is plain that there is a lower quadrupole coupling constant line-shape present in all of the sugar-borate samples (line-shapes centered around 0 ppm) as well as a high quadrupole coupling constant boron center that yields a much broader low symmetry signal that extends across the 80 to -110 ppm region of the static line-shape spectrum.

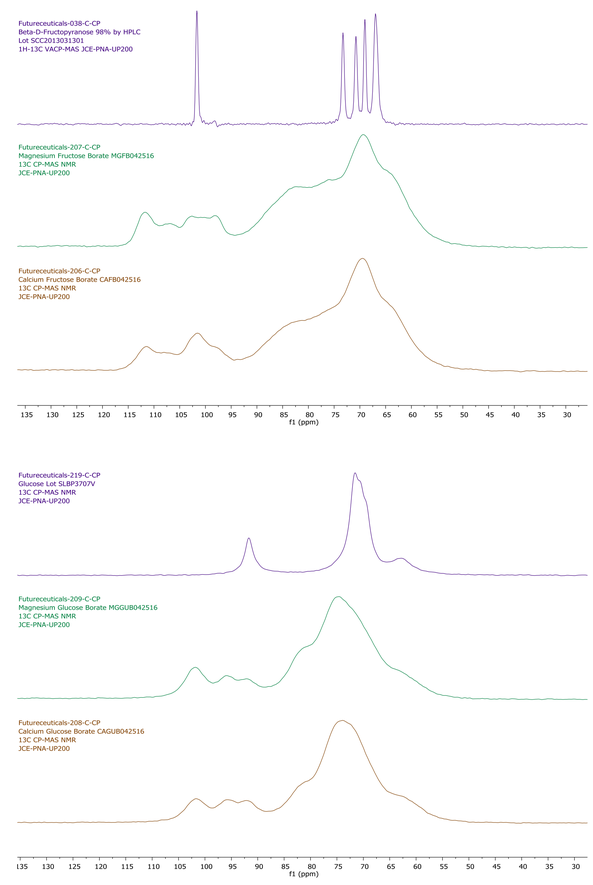


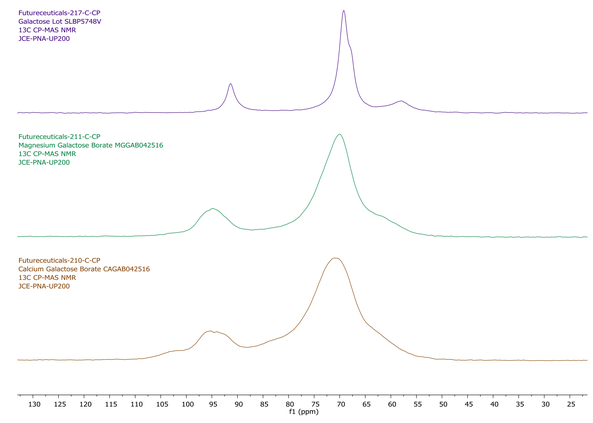
In the ^{11}B magic angle spinning data it is easier to see that there are 3 or 4 signals present in the NMR spectrum representing multiple boron centers in different coordinations in the mixture. In the case of fructoborate there appears to be essentially one type of boron center and from other NMR experiments it seems that the signal for the Di-ester complex is represented by the fairly symmetrical narrow line at 6.6 ppm. The broad ^{11}B signal narrows slightly with the spinning and those broad unsymmetrical peaks at 80 to -40 ppm seem to line up with the higher free borate concentration indicating that signal is form free borate. The mono-ester boron center yields a signal at 3.5 ppm that appears as a "shoulder" on the peak at 6.5 ppm. The unknown boron signal that gives rise to a liquid-state peak at -18 ppm shows up as a peak at -0.6 ppm in the solid-state MAS data.

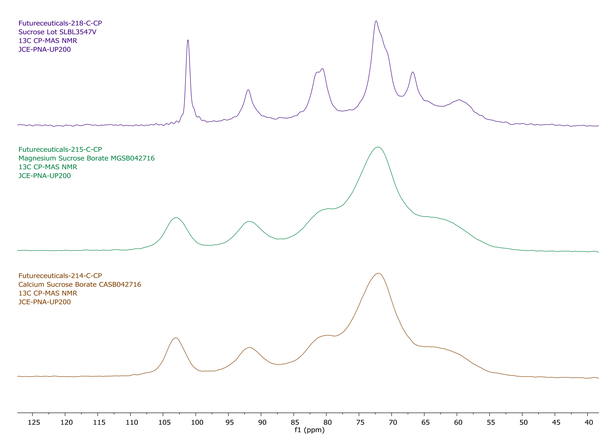


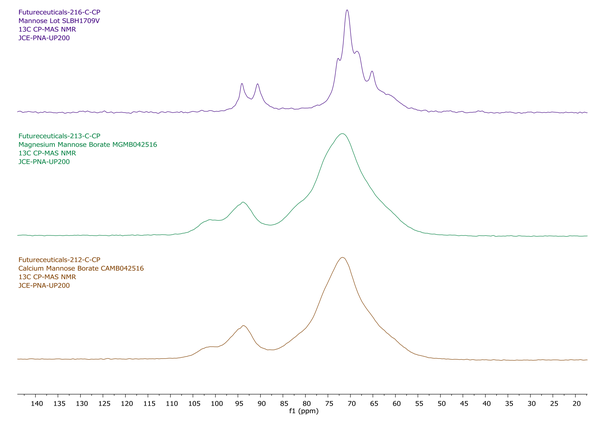
Solid-State ^{13}C NMR – cross-polarization with magic angle spinning

In the series of figures below we show the solid-state ^{13}C NMR spectral comparison of each sugar borate combination. It was observed that the pure sugar spectra did not appear in the complex samples which indicates that the "free sugar" found in these samples is either formed upon dissolution or it is intimately mixed with the complex in the solid-state that it is effectively completely amorphous. Experiments were performed with very long relaxation delays in order to rule out the non-observation of pure sugars being attributable to saturation due to relatively short relaxation delays required for the complex carbon signals









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