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Nuclear Magnetic Resonance (NMR) Analysis of D - (+) - Glucose: A Guide to Spectrometric Structural Elucidation of Sugars

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Abstract: *NMR spectroscopy has a wide range of applications including exchange phenomena, the identification and structural studies of complex biomolecules. 1D ^{1}H-NMR without water suppression, 1D Carbon, 1D ^{13}C-DEPT135, 2D Cosy, 2D HSQC, 2D TOCSY, 2D HMQC, and 2D HMBC techniques were used to completely elucidate the structure of glucose with spectral induced at 400MHz.. The spectral were analysed using spinworks 3. The results obtained from the spectral data were systematically combined to elucidate the structure of the D-glucose. Full characterisation of D-glucose was achieved by assigning ^{1}H and ^{13}C signals, starting from the known to unknown signals.*

I. Introduction

NMR spectrometry is a technique widely used by scientists for structural elucidation and identification of chemical species [1-3], and to gain information about types, numbers and connectivity of particular atoms to deduce structures of organic, inorganic and biological molecules [4-5].

The principle of NMR spectroscopy is based on the magnetic properties of some nuclei [6]. Depending upon the atomic number and mass number of a nucleus, there is an associated angular momentum spin number. For a particular spin number, an isotopic nucleus may give rise to a magnetic field that can absorb energy from a pulsed radio frequency in a strong magnetic field and subsequently the energy can be released when the radio frequency is removed [7]. The release of energy will simultaneously give a weak signal, which represents the structural information about the individual nucleus and its surroundings. The released signal is detected, analyzed, and expressed as chemical shift (measured in ppm) and spin coupling. The most useful nuclei in carbohydrate research are ^{1}H and ^{13}C [8]. ^{1}H NMR signals are much more sensitive than ^{13}C signals due to their natural abundance. Therefore, using one dimensional proton NMR alone to solve a structural problem of complex organic molecules is very difficult [9 -11].

The most recent development in two and multi-dimensional NMR techniques has significantly improved the resolution and sensitivity of NMR spectroscopy. For example, homonuclear correlated spectra are extremely useful for assigning 1H resonances while the complete assignment of ^{13}C-resonances is achieved by ^{1}H-^{13}C heteronuclear correlation [6, 12-14].

Product of reactions unless properly characterized, amounts to uncertainty and lack of control over the structures. To deduce the structure of a compound, there is the need to measure, analyse and interpret spectral data. However, spectral is not always simple and straight forward, thus the chemist would need a range of available techniques for analysis and interpretation such spectra [9].

(β-glucose) (α-glucose)

Step by step analysis of D-(+)-glucose was provided by processing and interpreting the measured data of the sample using spinworks. Fourier transformed 1-Dimensional NMR was combined with Fourier transformed 2-Dimensional NMR spectrometry for the interpretation of the spectral, because ^{2}D has the advantage of having more accurate assignments.

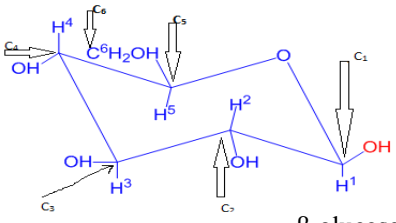
II. Materials And Methods

2.1 Sampling and sample preparation

D-glucose (98% $^{13}C_6$) with molecular formula $C_6H_{12}O_6$ and molecular weight of 180.16 Daltons used was obtained from sigma and Aldrich. D-glucose (89mg) was dissolved in deuterated dimethyl sulfoxide (D_6-DMSO, 0.7ml), and 5ml pipetted into a clean and dried NMR tube, which was then sealed with paraffin, ready for spectral analysis. The sample spectral of 1D ^{1}H-NMR without water suppression, 1D carbon 1D ^{13}C-DEPT135, 2D COSY, 2D HSQC, 2D TOCSY, 2D HMQC, and 2D HMBC techniques were determined at 400MHZ, in Manchester Interdisciplinary Biocentre using Bunker NMR, and spectral analysed using spinworks

III. Results And Discussion

The results obtained from the spectral data were systematically combined to elucidate the structure of the D- glucose. The analysis of the individual spectrum shows that the structure of D-glucose cannot be deduced completely from just one spectrum. Where a portion of an interpretation proved difficult, the next appropriate spectral that will make the interpretation easier was considered.



β-glucose

The six carbons in β-glucose are labelled from C_1 to C_6 in anticlockwise direction and each of the hydrogen attached to them were also labelled as H_1 to H_6 respectively.

3.1 ^{1}H-NMR technique

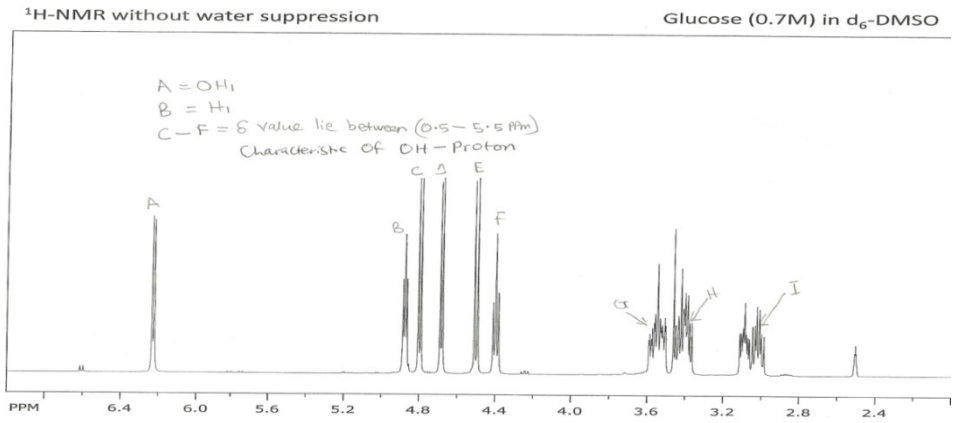


Figure 1 Spectrum of 1D ^{1}H NMR (peaks labelled from A to I)

Table1 Analysis of the 1D ^{1}H NMR spectral

Proton	δ	Multiplicity	J in Hz	Integral
OH_1	6.2344	d	4.6870	1.0000
H_1	4.8947	t	4.2550	1.0892
OH_4	4.8156	d	5.5500	1.0046
OH_3	4.7014	d	4.9950	1.0052
OH_2	4.5197	d	6.4750	1.0455
OH_6	4.4138	t	5.9200	1.0192

From figure 1, integration of A = 1.0000, B-F = 5.2169, G-H = 4.4190, I = 2.0900, Ratio of 1:5:4:2 from left to right, G-I shows multiplicity that was not clear in this spectrum. Total number of proton environment to be accounted is 12. From figure 1, 12 protons were detected. Using spinworks, peaks appeared as either a doublet (A, C, D and E) or as a triplet (A and F). Peaks G to I showed multiplicity, thus not clear; a problem which could only be solved using a 2D shift correlated NMR spectroscopy.

A doublet appears at 6.2338δ (peak A) which was in consistence with the presence of a proton that is bonded to a carbon (C_1) that is bonded to two oxygen atoms. Peak A was identified as OH_1 and peak B as H_1, since OH_1 will be most deshielded followed by H_1 attached to the same carbon (C_1). Peaks C to F were characteristic of OH protons (0.5-5.5ppm). The ^{3}JH,H coupling constant values in Table 1 falls within the theoretical value (0-18Hz) (Akit, 1992) . Integration shows that there were 12 proton environments.

3.2 1D ^{13}C NMR technique

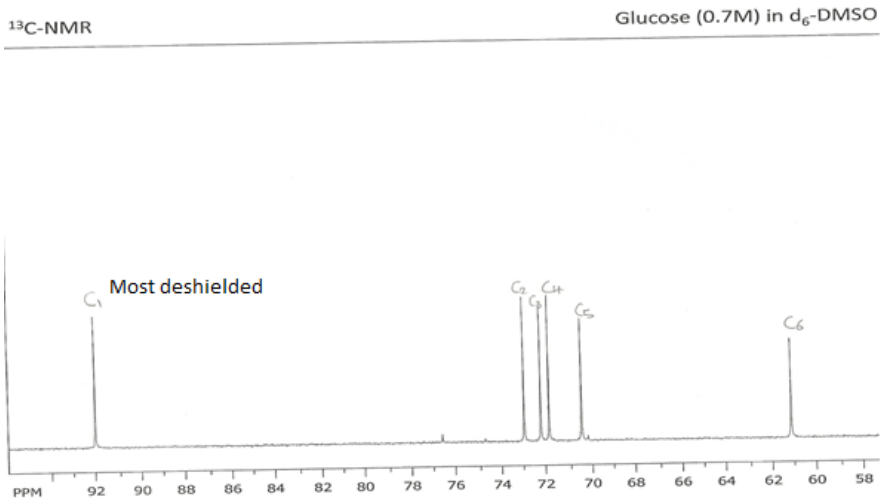


Figure 2 Spectrum of 1D ^{13}C NMR

Table 2 Analysis of the 1D ^{13}C NMR spectral

Carbon	Chemical shift(δ)
C_1	92.1375
C_2	72.9877
C_3	72.2516
C_4	71.8865
C_5	70.4292
C_6	61.0899

There are 6 carbon environments and all were visible, indicating that there was no symmetry. The Carbon that appears at 92.1375 ppm (table2) is C_1 because the most deshielded carbon will be the carbon that is attached to two oxygen atoms.

3.3 ^{13}C DEPT-135 technique

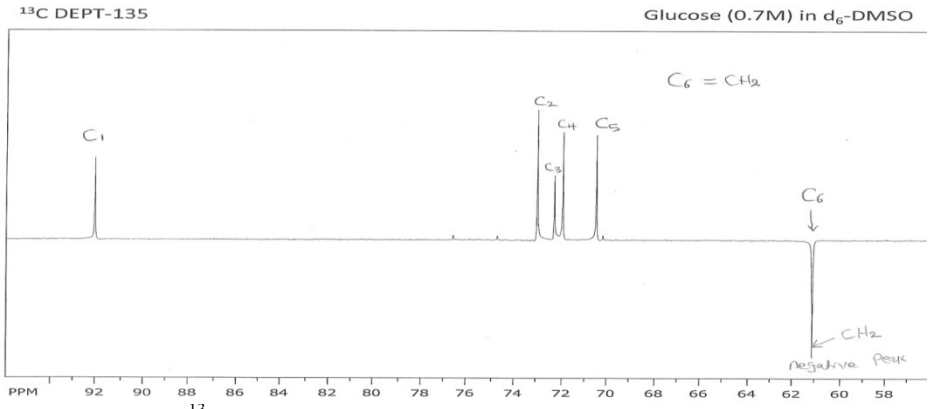


Figure 3 ^{13}C DEPT-135 Showing CH_2 inverted and CH and CH_3 not inverted

The only inverted peak at 61.0899 ppm was CH_2 (even number of H) and so identified as C^6. The remaining peaks were either for CH_3 or CH (odd number of H), but there was no CH_3 in the compound; hence the peaks might be for CH.

3.4 2D HSQC (Heteronuclear Single Quantum Correlation)

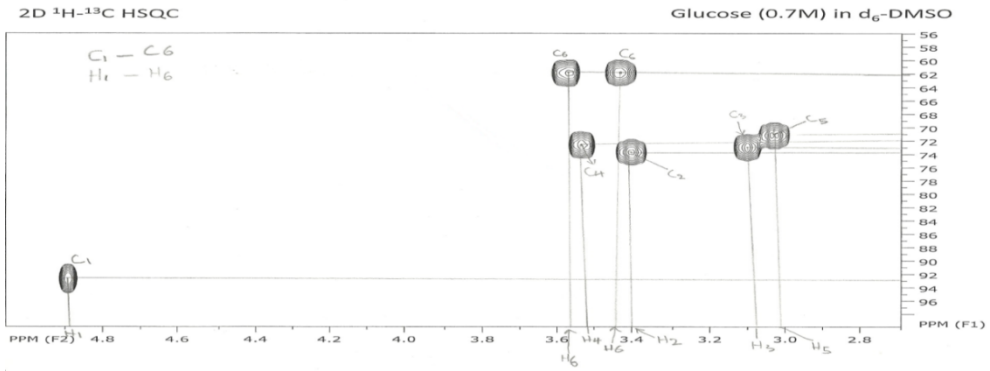


Figure 4 2D HSQC (^{1}H-^{13}C) Direct Proton – Carbon Correlation

Table 4 Analysis of 2D HSQC (^{1}H-^{13}C) spectral

C_1 92.9158 Correlate with	H_1 4.9007
C_2 72.9748 " "	H_2 3.4040
C_3 72.3613 " "	H_3 3.0956
C_4 71.9151 " "	H_4 3.5371
C_5 70.5208 " "	H_5 3.0276
C_6 61.0397 " "	H_6 3.5696, 3.4335

From Table 4, C_1 to C_6 are directly bonded to H_1 to H_6 respectively using their chemical shift values.

3.5 2D Cosy

COSY is a ^{1}H homonuclear shift correlation spectrum which contains information on spin coupling networks within a constituent residue through the observation of cross peaks off the diagonal [14]. The strategy of assigning a COSY spectrum is to find one unmistakably characteristic signal from which to begin the tracing of a spin system or network. An anomeric proton is often chosen as the starting point because it is connected to a carbon bearing two oxygen atoms, which is probably the most down field [2]. ^{1}H signal correlation Spectroscopy (^{1}H-^{1}H Correlation) correlates proton that are three bonds away .

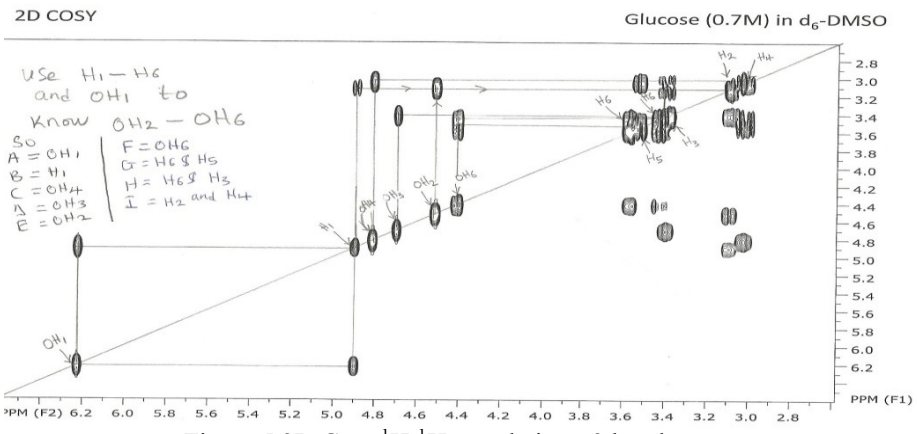


Figure 5 2D Cosy ^{1}H-^{1}H correlation - 3 bonds away

Table 5 Analysis of the 2D Cosy - 3 bonds away

Proton	Correlate with	Proton	Correlate with
OH_1	H_1	H_1	H_2
OH_2	H_2	H_2	H_3
OH_3	H_3	H_3	H_4
OH_4	H_4	H_4	H_5
OH_6	H_6	H_5	H_6*2

Correlation results between protons that are coupled to each other (form squares from diagonal and cross peaks) are presented in Table 5. This is particularly useful because of the complicated signals in peaks from G to I. From figure 5, the diagonal and cross peaks formed by joining solid lines shows which protons are coupled to each other (mutual scalar coupling). OH_1 to OH_4 correlates with H_1 to H_4 respectively and OH_6 correlates with H_6. Also, H_1 to H_5 correlates H_2 to H_6 respectively but H_5 correlates with two H_6 at 3.5604ppm and 3.4171ppm.

3.6 2D ^{1}H-^{13}C HMBC

The HMBC experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity. This technique is very valuable for detecting indirectly quaternary carbons coupled to protons and is especially useful if direct carbon-13 is impossible to obtain due to a low amount of material available [12].This very useful sequence provides information about the skeleton of a molecule. In HMBC, carbon correlates with hydrogen 2-4 bonds away [13].

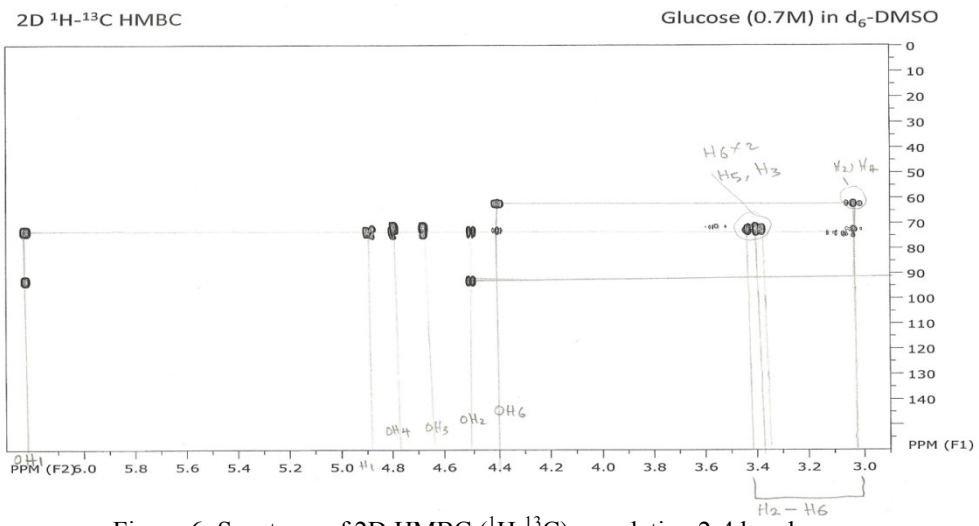


Figure 6: Spectrum of 2D HMBC (^{1}H-^{13}C) correlation 2-4 bonds away

Table 6: 2D HMBC (^{1}H-^{13}C) Correlation 2-4 Bonds away

Carbon	H_1	H_2	H_3	H_4	H_5	H_6	OH_1	OH_2	OH_3	OH_4	OH_6
C_1							*	*			
C_2	*						*	*	*	*	
C_3	*							*	*		
C_4	*	*				*		*	*	*	*
C_5				*		*			*	*	
C_6			*		*						*

From Table 5, the cells marked with * signifies correlation. The appearance of at least a * in a column shows that there was a correlation between all nuclides of interest (carbon and hydrogen), C_1 to C_6 with OH_1, OH_2, OH_3, OH_4 and OH_6, and H_1 to H_6 (two H_6).The results in Table 5 also indicated that a carbon sees at least a proton and vice versa.

3.7 2D TOCSY

Total correlated spectroscopy (TOCSY), also known as homonuclear Hartmann-Hahn spectroscopy (HOHAHA), correlates protons that are in the same spin system and yields both long range and short range correlations. It is useful for establishing the scalar connectivity if the proton signals are from within a spin system, especially when the multiplets overlap or there is extensive second order coupling [14-15]. All protons of a coupled system are known from here. All the protons are coupled to each other as shown in figure 6

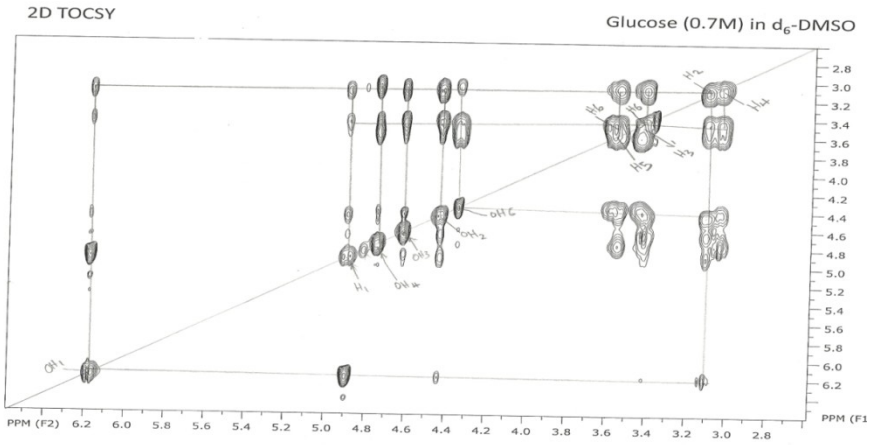


Figure 7 ^{1}H-^{1}H Correlation – 5 bonds away

3.8 ^{2}D^{1}H-^{13}C HSQC-TOCSY

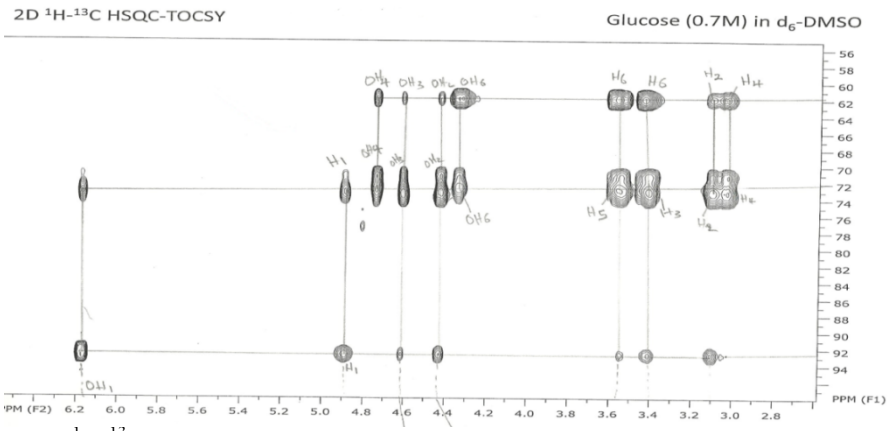


Figure 8 2D ^{1}H-^{13}C HSQC-TOCSY Showing coupling between all hydrogen and Carbon atoms

All coupled nuclides become strongly coupled due to complete correlation between all the carbons with the hydrogen atoms. This was used to confirm the coupling of all the coupled spin system to elucidate the full structure

IV. Conclusion

NMR spectroscopy has been demonstrated to be the most powerful tool in structural analysis.Structural elucidation is systematic and coherent. A COSY experiment will overcome most of the overlap problems in the 1D spectrum with the assistance of TOCSY NMR. Full characterization of D- glucose was done by assigning ^{1}H and ^{13}C signals, starting from known to unknown signals.

Acknowledgement

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