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Calculation and Comparison Proton Nuclear Magnetic Resonance (NMR) Spectrum for Serum of Normal and Testicular Cancer

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Abstract

An equally effective way is to vary the frequency of the radio frequency radiation while keeping the external field constant in every state by employing a magnetic field and measuring the radio frequency RF signal emitted by the sample. The goal of this study is to use NMR to study the physical and chemical aspects of differences in the intensity, concentration of hydrogen atoms, chemical shift, coupling constant (J), measuring magnitude of the splitting effect distance between the peaks in a given multiplet for Proton nuclear magnetic resonance for two cases (normal and testicular cancer) of H-NMR spectrum and to explain the number of peaks formed, by RF signal emitted by the specimen under the influence of the magnetic field, and then to compare the normal and pathological states (Testicular cancer) in order to determine the cause of this type of disease, and it can be applied to the rest of the diseases such as leukemia and others, for which we have found a treatment or a more accurate explanation of the pathologi In the cases mentioned above, the obtained results reveal a distinction between the pathological and normal states.

Keywords: Proton nuclear magnetic resonance (H-NMR), Serum of Testicular cancer, lyophilization (Freeze drying), Serum powder, coupling constant (J).

1. INTRODUCTION

Proton nuclear magnetic resonance is the application of nuclear magnetic resonance in NMR spectroscopy with respect to hydrogen-1 nuclei within a substance's molecules to determine the structure of its molecules (proton NMR, hydrogen-1 NMR, or 1H NMR).[1],[2],[3]. Understanding biological macromolecules' structure and dynamics is critical to understanding their biological function. Nuclear Magnetic Resonance (NMR) spectroscopy with high resolution is a popular method for studying protein structure and conformation in multidimensional space.,[4],[5]. Almost all of the hydrogen in samples containing natural hydrogen (H) is the isotope 1H (hydrogen-1; i.e., with a proton for a nucleus)[6],[7],[8]. Simple NMR spectra must be recorded in solution with no interference from solvent protons. Deuterated deuterium solvents, such as deuterated water (D2O), deuterated acetone (CD3)2CO), deuterated methanol (CD3OD), deuterated dimethyl sulfoxide (CD3)2SO), and deuterated chloroform (CDCl3), are preferred for use in NMR. A non-hydrogen solvent, such as carbon tetrachloride (CCl4) or carbon disulfide

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(CS2), can also be used [9],[10]. Historically, deuterated solvents were supplied with a small amount (typically 0.1 percent)of tetramethylsilane (TMS) as an internal standard for calibrating the chemical shifts of each analyte proton . [11],[12]. TMS is a tetrahedral molecule with chemically equivalent protons that produces a single signal that defines a chemical shift of 0 ppm. It's easy to recover a sample because it's volatile. Modern spectrometers can calibrate spectra using the amount of residual proton in the solvent (e.g., 0.01 percent in 99.99 percent CDCl3)[13],[11],[14]. For deuterated solvents, TMS is no longer necessary. 1H NMR spectroscopy is the most commonly used spectroscopic instrument in organic chemistry[15],[16]. Chemical shift values, denoted by, are not exact, but typical, and should only be used as reference. Typically, deviations are in the range of 0.2 ppm, but they can be higher.[17],[18],[19]. The chemical shift value is determined by the structure of the molecule, the solvent, the temperature, the magnetic field used to record the spectrum, and other functional groups in the vicinity. [20],[21]. Hydrogen nuclei are affected by hybridization and electronic effects of the atom to which the hydrogen atom is connected. Groups that draw electron density away from nuclei tend to deshield them. The resonance values of unshielded nuclei are higher than those of shielded nuclei. [22],[23].

2. Testicular Cancer

Testicular cancer develops in the testicles, which are located inside the scrotum, a loose sack of skin beneath the penis. The testicles are in charge of producing male sex hormones and sperm for reproduction.Testicular cancer is uncommon when compared to other types of cancer.[24],[25]. However, testicular cancer is the most frequent malignancy in guys aged 15 to 3. Even after the cancer has gone beyond the testicle, testicular cancer is highly curable. Depending on the type and stage of testicular cancer[26],[10],. Is often divided into two main types of testicular cancer. Germ cell cancers and stromal tumors. Germ cell tumors account for the vast majority of cases (90–95%) and have a favorable prognosis with modern therapies even in advanced stages [27],[28], [29],[30],[31],[32] . Although the World Health Organization (WHO) suggested significant modifications to the categorization system in 2016, germ cell cancers have generally been classified as seminoma or non-seminoma [33]. Stromal tumors (non-germ cell) are uncommon, accounting for just 5–10% of all testicular cancers. [34],[35]. Early-stage survival rates are relatively excellent, but survival rates for the 10–20 percent of patients who acquire malignant illness are worse than for their germ cell counterparts. [36],[37],[38].

3. Material and method

This research was carried out in the Quality Control Laboratories of the Ministry of Industry and Minerals - Industrial Research and Development Authority (Al-Razi Center) for Research and Production Medical Diagnostic Kits, and the available devices used in our measurements are: Electronic Analytical Scale, for the electric balance made by (KERN & Sohn), The sensitivity of this balance is up to (0.1 mg). This scale was used to measure the weight of normal and abnormal blood samples. Christ Alph 1-2 LD freeze drying (Freeze drying) is the process of removing water from frozen products under extremely low pressures. The method is commonly used to dry thermodegradable products that would otherwise be destroyed by heat drying. The samples were dried after being dried for three days and turned into a powder. The SIGMA 3-16L centrifuge is a device that separates the different components of a liquid using centrifugal force. This is accomplished by circulating liquid at a high rate within a container, separating liquids of different densities, such as liquids from solids. It causes the denser particles to sink to the tube's bottom, while the lighter particles rise to the top. To obtain blood serum, the blood samples were separated and then placed in a machine at 3000 r/m for 3 minutes. A water bath is a piece of lab equipment that consists of a container filled with hot water. It's used to incubate samples in water for an extended period of time at a constant temperature. To make the separation process easier, blood samples were placed in the water bath to form a clot. For 5 minutes, the samples were in the device. The use of NMR phenomena to study the physical, chemical, and biological properties of matter is known as NMR spectroscopy. The nuclei's resonant

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frequencies are then measured and converted into an NMR spectrum, which plots all of the correct frequencies as peaks. (H-NMR spectrum), as shown in fig.1. For more accurate results, use the Masternova NMR processing software, is spectral data analyzing software, which can be run on Windows, after compared according to the result, we show a highly significant between a normal person with a testicular cancer patient.

4. Sample preparation and conversion to powder Blood samples were taken from patients with testicular cancer in the Anbar Specialized Center for Oncology. Other blood samples were taken from healthy people without testicular cancer. Samples are placed in a gel tube and then in a water bath for 5 minutes to obtain coagulated blood. Coagulated blood was removed and centrifuged at 3000 rpm for 3 min to separate the blood and obtain serum. The serum is placed in the white tube and kept at a temperature of -20°C for the purpose of storage. The serum was dried by Lyophilyzer Christ Alph 1-2 LD, which freezes liquid materials (converting them to powder by cooling and pressure) for 3 days. were separated and weighed with an electronic analytical balance as shown in the following table(1). To obtain a solid's NMR spectra, it must first be dissolved in a suitable solvent, such as deuterium oxide (D2O). Because deuterium has a different magnetic moment and spin than protons, it is invisible in a spectrometer tuned to protons..

5. Results and Discussion

The signal obtained from NMR spectroscopy contains information about interactions between nuclei and electrons as well as interactions between nuclei, which can aid in determining the structure of a chemical complex. The resulting NMR spectrum is made up of one or more resonant peaks at a specific frequency. [39] [40] [41]. Using a magnetic field and observing the radio frequency RF (500MHz) signal emitted by the sample using NMR to study the physical and chemical aspects in terms of difference chemical shift, intensity, the concentration of hydrogen atoms, integration, multiplicity, coupling constant of Proton nuclear magnetic resonance for Testicular cancer of H-NMR spectrum between two cases (control, testicular) as shown in fig. (1) and its results shown in the tables (1). When we pick a wider segment of the two cases, for pathological state and control condition, as shown in figure (2), we can use Mestrenova NMR processing software, which is spectrum data analyzing program that can be used on Windows. When we take a larger section of the two cases, for pathological condition and control condition, as shown in figure (2), and compare the results, we see a highly significant valve in the intensity between a normal person and a testicular cancer patient, the valve increases for a testicular cancer patient than the normal person, the number of peaks for the normal person is about 36 peaks, while for testicular cancer patient 13 peaks this means the number of compounds appear less in the testicular cancer patient with a highly significant. Each signal in a proton NMR spectrum may or may not be split into one or more peaks, and the 1H-NMR spectrum provides information on signal multiplicity and spin – spin splitting As shown in fig. 2 and table (1), this is known as signal multiplicity, and it gives rise to terms like singlet, doublet, triplet, quartet, pentet, and multiplet . The location of absorption peaks in various NMR resonance signals is affected by the external magnetic field strength as well as the RF frequency. [41],[42],[43],[44] According to these references, the 1HNMR spectrum contains some crucial information, like as

1. as shown in fig.1The proton resonance is distributed along the frequency axis in two different cases. Each proton exists in a unique chemical environment, defined by its chemical shift. (δ).
2. As shown in fig. (1,2), different peaks in the spectrum appear with different intensities that are related to the number of protons producing the signal. In two cases, the signal intensities reflect the relative numbers of different types of protons present in the molecule. When recording a spectrum, an integrator determines the areas beneath the peaks.
3. When two protons couple, their peaks split.The coupling constant, also known as the J constant, is the distance between the peaks that is the same for both protons. This number is always expressed in hertz (Hz) and is calculated using the following formula:

J Hz = Δ ppm x instrument frequency

The difference in ppm between two peaks for a given proton is measured in Δ ppm. The frequency of the instrument is 500 MHz. Because of spin-spin coupling between neighboring protons, the signals of protons that are magnetically equivalent to other protons in geminal or vicinal positions are split. Calculating the

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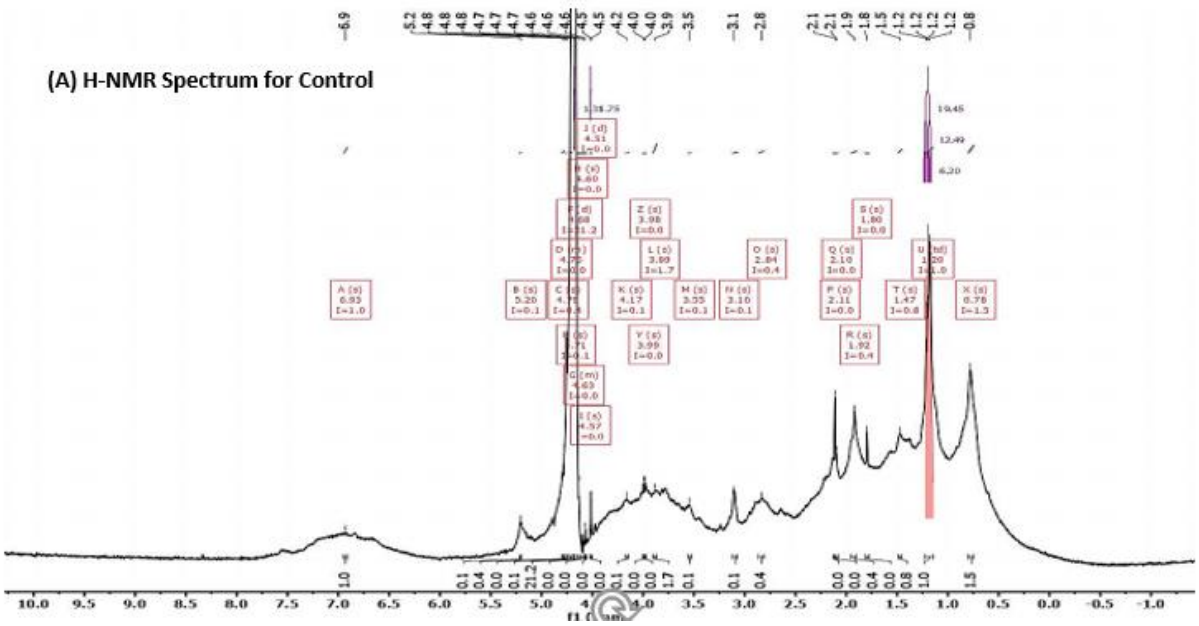
coupling constant of the relevant signal can be used to determine signal splitting (Quartet (q) as an example Worksheet for triplets (t) Example of a doublet of doublets (dd) Worksheet for doublets of doublets (dd). Example of a doublet of doublets (ddd) Worksheet for the doublet of doublets (ddd). Example of a triplet of doublets (td) Worksheet for triplets of doublets (td). Example of a triplet of triplets (dt) Worksheet for doublets of triplets (dt). The coupling constant is a constant value that is determined primarily by the extent of interaction between two proton systems and is independent of magnetic field strength. The coupling constant for a doublet is calculated simply by subtracting the two peaks. Triplet has three peaks. Because the proton couples equally with both neighboring protons, we can take the difference of either of the two peaks. In both cases, we get nearly identical results. The coupling constant for a quartet is calculated in the same way as it is for a triplet. The J value for a quartet can be calculated by taking the difference between any two consecutive peaks. Because it has two doublets, a double doublet has two coupling constants. Using the Mestre Nova NMR processing software, all of the values for the two cases (control and testicular cancer) are shown in fig.1,2 and presented in table(1). Any two sets of protons with the same coupling constant are likely to split each other's signals and are said to be coupled. This information can be extremely useful when assigning NMR signals to specific groups of protons in a molecule.

Calculating Coupling Constants in Mestre Nova: There are several ways to calculate coupling constants in MestreNova. Using the Multiplet Analysis tool is the simplest option. Go to Analysis Multiplet Analysis Manual (or just press the "J" key) to do this. Draw a box around each group of protons that are equivalent. Below each one, a purple version of the integral bar will appear, along with a purple box describing the splitting pattern and location in ppm. You can right-click the integral bar, select "Edit Multiplet," and set these integrals to whatever makes sense for that particular structure, just as you can with normal integrals. As we shown in fig.2.and data is presented in table (1) the difference in Multiple proton resonances can interact with neighboring atoms. The degree of interaction or coupling is indicated by the coupling constant (J). The absorption peak that appears in the ^{1}H-NMR spectrum is represented by the difference in the resonance frequency of a nucleus against the standard in units of ppm or chemical shift (δ) as shown in Figure 2.



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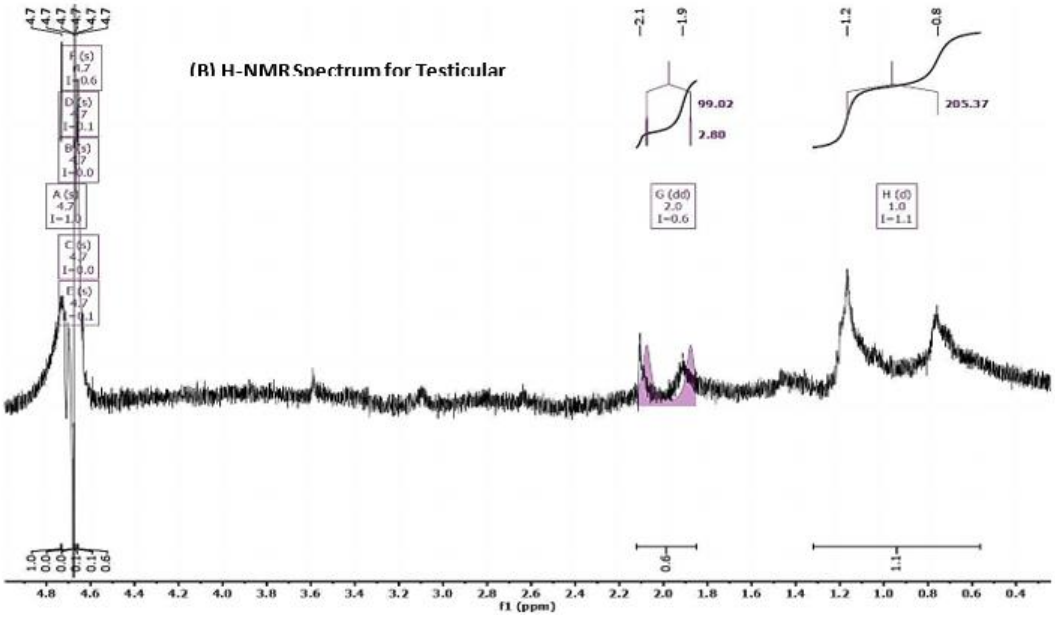
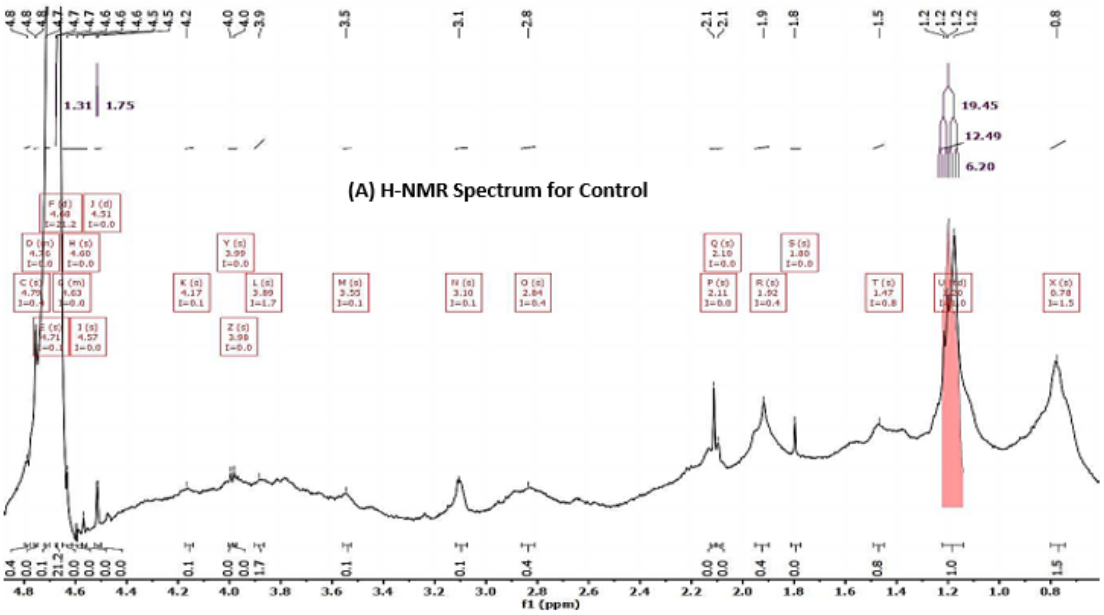


Figure 1. H-NMR Spectrum A: Control B: Testicular



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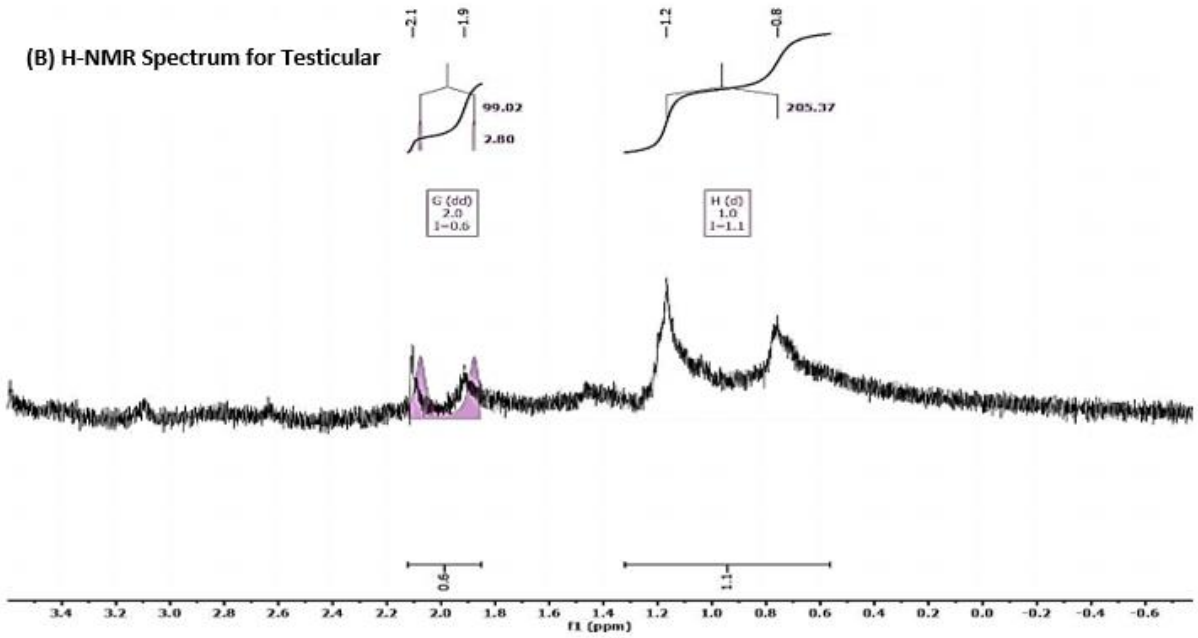

Figure 2. By using Mestre nova NMR processing software, which can be run on Windows, taking a larger section of the two cases, for pathological condition and the control condition of H-NMR spectrum.

Table (1) Multiplets of H-NMR spectrum for control and H-NMR Testicular

H-NMR (Control)								H-NMR (testicular)							
No of peaks	**signal**	**Shift (ppm)**	**Range**	**H's**	**Integral**	**Multip -liciy**	**J's**	**No of peaks**	**signal**	**Shift (ppm)**	**Range**	**H's**	**Integral**	**Multi-plicity**	**J's**
1	A	6.93	6.95 .. 6.91	1	1	s		**1**	A	4.73	4.73 .. 4.73	1	1	s	
2	B	5.2	5.22 .. 5.20	0	0.12	s		**2**	B	4.68	4.68 .. 4.68	0	0.01	s	
3	C	4.79	4.80 .. 4.78	0	0.42	s		**3**	C	4.67	4.68 .. 4.67	0	0.01	s	
4	D	4.76	4.76 .. 4.75	0	0.05	m		**4**	D	4.67	4.67 .. 4.67	0	0.08	s	
5	E	4.71	4.72 .. 4.70	0	0.13	s		**5**	E	4.67	4.67 .. 4.67	0	0.06	s	
6	F	4.68	4.68 .. 4.67	21	21.19	d	1.31	**6**	F	4.66	4.66 .. 4.66	1	0.65	s	
7	G	4.63	4.65 .. 4.61	0	0.02	m		**7**	H	0.96	1.32 .. 0.56	1	1.09	d	205.37
8	H	4.6	4.62 .. 4.57	0	0	s		**8**	G	1.98	2.12 .. 1.85	1	0.63	dd	2.80, 99.02
9	I	4.57	4.58 .. 4.55	0	0.01	s									
10	J	4.51	4.52 .. 4.50	0	0.02	d	1.75								
11	K	4.17	4.17 .. 4.14	0	0.09	s									
12	L	3.89	3.90 .. 3.87	2	1.66	s									
13	M	3.55	3.56 .. 3.53	0	0.05	s									
14	N	3.1	3.12 .. 3.07	0	0.11	s									
15	O	2.84	2.86 .. 2.81	0	0.36	s									
16	P	2.11	2.13 .. 2.11	0	0.02	s									
17	Q	2.1	2.10 .. 2.08	0	0.03	s									
18	R	1.92	1.95 .. 1.90	0	0.36	s									

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19	S	1.8	1.81 .. 1.77	0	0.01	s									
20	T	1.47	1.49 .. 1.45	1	0.76	s									
21	X	0.78	0.80 .. 0.74	1	1.45	s									
22	Y	3.99	4.00 .. 3.99	0	0.01	s									
23	Z	3.98	3.99 .. 3.97	0	0.02	s									
24	U	1.2	1.22 .. 1.14	1	1.03	td	12.49, 6.20, 19.45								

Conclusion

The study of the difference in spectral analysis such as H-NMR between two cases, the normal (control) state and the disease state (testicular) is very important by studying the difference in the increase or decrease of some structure and compounds for the two cases, any two sets of protons with the same coupling constant are said to be coupled since they are splitting each other's signals. When it comes to identifying NMR signals to specific groups of protons in a molecule, this knowledge can be extremely useful, which can be generalized by studying other spectra such as C, F, Si, P nuclear magnetic resonance, and others in order to reach a diagnosis and that help us treat some diseases. With this paper, we believe that this paper can be used as a basis for understanding and interpreting H-NMR and others data.

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