A COMPARISON OF THE 80MHz, 200MHz AND 500MHz NUCLEAR MAGNETIC RESONANCE SPECTRA OF HOMOEOPATHIC SULPHUR 30CH.

by

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I, Angela Cason, do hereby declare that this dissertation is representative of my own work.

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Dedicated with deepest love and gratitude
to my parents

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ABSTRACT

The purpose of this study was to investigate whether frequency strength is a parameter requiring consideration when conducting NMR spectroscopy studies on homoeopathic potencies. To this end, samples of Sulphur 30CH and a Lactose control were analysed using NMR spectrometers operating at three different frequency strengths of 80MHz, 200MHz and 500MHz. It was hypothesized that differences existed in the spectra of respective Sulphur samples, control samples, and between parallel samples of Sulphur and control. It was further hypothesized that differences between parallel samples of Sulphur and control would be more noticeable at the lower frequencies. This hypothesis was based on the assumption that a higher frequency strength would have more intense resonance effects on the structure of the homoeopathic potency, thereby disturbing the micro-structural changes induced during potentisation.

The design of the investigation was that of a scientific experiment. Potencies of Sulphur and a lactose-based control were prepared to a 30CH potency each, in 87% ethanol. The final prepared volumes (10ml) of Sulphur and control were blinded by means of colour codes by a third party prior to analysis. The blinded samples were transported to the University of Natal, Pietermaritzburg, where they were subjected to analysis using the following instruments:

1) A Varian FT80A 80MHz instrument
2) A Varian Gemini 200MHz instrument
3) A Varian Inova 500MHz instrument

At each instrument NMR spectroscopy was conducted on ten (10) samples from each group (Sulphur and control). The samples were prepared in coaxial tubes using acetone as both an external lock and reference, and NMR spectra were recorded for each sample. All the samples were run at a thermostatically controlled temperature of 24^0C ($\pm$ $0,2^0$C), and the laboratory was maintained at a constant temperature of 22^0C. The spectra and data of all the samples were recorded in terms of the chemical shift and integration values of their respective CH_2, H_2O and OH signals.

The data thus obtained was subjected to statistical analysis. The Oneway Analysis of Variance (ANOVA) was used to test differences between the frequency strengths within each group. If the null hypothesis was rejected then the Bonferroni Multiple Comparisons test was conducted in order to determine where exactly these differences were to be found. The Independent Samples t-Test was used to determine differences between the Sulphur and control at parallel frequency strengths. The level of significance used for all tests was $\alpha = 0.05$.

The intra-group comparison of the Sulphur group showed significant differences in the chemical shift values of the CH_2, H_2O and OH signals between the three frequency groups. The same result was obtained when comparing the control chemical shift values, except in two instances, where no difference was found at the CH_2 signal between the 500MHz and 200MHz readings ($p = 0.108$), and at the OH signal, where no difference was found between the 500MHz and 80MHz readings ($p = 0.073$). Intra-group comparisons of the integration values also revealed statistically significant differences between the three spectrometer frequencies. It was discovered, however, that the integration values are calculated within a 5% error, and therefore the statistically significant differences obtained lacked credibility. The inter-group comparisons comparing Sulphur and control at parallel frequency strengths showed no differences at the CH_2, H_2O an OH signals of both the chemical shift and integration values. Standard deviations and variances were shown to be very small across all groups.

The initial assessment of the data supported the hypothesis that the different frequency strengths produce different results, however problems were noted that diminished the reliability of such an interpretation, the most notable being the indeterminable influence of temperature during the NMR experiment.

The investigation was not able to provide conclusive evidence that frequency strength is an influential parameter when conducting NMR experiments on homoeopathic potencies, and further studies are required. The hypothesis that differences between the Sulphur and control would be more noticeable at the lower frequency range was not confirmed in this

study, however the investigation has highlighted the possibility that the applied radiofrequency may not affect the structure of the sample whilst measuring it. It was hypothesized that this may be due to differing resonance frequencies of the applied radiofrequency and the potency particles (other than protons).

The study has served to highlight problems previously unconsidered within the field of NMR studies on homoeopathic potencies. Recommendations were made to address these problems, the most important of which is the need for standardization in this field of research. There is a need for further assessment of NMR as a tool for analysis of homoeopathic potencies in order that greater understanding regarding the nature of homoeopathic potencies can be achieved. Inter-disciplinary collaboration is a necessary step towards this goal.

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TABLE OF ABBREVIATIONS

ANOVA - Analysis of Variance

B_0 - static magnetic field

CH - Centesimal Hahnemannienne

δ - delta; represents the chemical shift

Hz - Hertz; represents the frequency

I - nuclear spin

RF - radiofrequency

m_I - magnetic quantum number

DEFINITIONS OF TERMS

Ångström (Å): A unit of measurement. $1Å = 10^{-10}$ m.

Analysis of Variance: a parametric statistical method used to analyse data relating to three main effects.

Chemical shift: a molecular quantity which is a function of the nucleus and its environment. It is always measured from a suitable reference compound and is therefore a parameter independent of spectrometer frequency. It's measurement is in parts per million (ppm).

Electromagnetic waves: the effects of oscillating electric and magnetic fields that are capable of travelling across space, ie. They do not require a medium through which to be transmitted.

Frequency: describes the number of complete wavelengths or cycles produced in one second, and is measured in Hertz (Hz).

Integration: the measurement of the area of individual NMR peaks, the intensity of which is proportional to the number of protons that give rise to that signal. The integration values reflect the relative number of protons within individual groups of molecules.

Magnetic field: the region of space in which a magnetic body exerts its force. Magnetic fields are produced by moving charged particles and represent a force with definite direction.

Magnetic moment (μ): the intrinsic magnitude of a magnetic dipole, which itself is generated by the overall spin of a charged nucleus along its spin axis.

Mean (x): the quotient of the sum of the values in a sample.

NMR spectroscopy: an experimental technique which analyses the interaction of protons in a static magnetic field with an applied radiofrequency, thereby providing detailed information regarding the three dimensional structure of the substance.

Potency: the stage of altered remedial activity to which a drug has been taken by means of the potentisation process.

Potentisation: also referred to as 'dynamisation'. It is a mechanical and mathematico-physical process peculiar to homoeopathy whereby a substance is modified by means of serial dilution and succussion, or trituration, according to a specific scale.

Radiofrequency (RF): an oscillating electromagnetic field (see electromagnetic waves).

Standard deviation (S_x): a frequently used measure of dispersion, which uses all of the data points in a sample to indicate the variation existing within a data set.

Structure: the three-dimensional arrangement of atoms and molecules, and the interactions between them, or within a mixture.

Succussion: the action of shaking up, or the condition of being shaken up, vigorously of a liquid dilution of a homoeopathic medicine in its bottle, where each stroke ends with a jolt, usually by pounding the hand engaged in the shaking action against the other palm.

Trituration: the act of prolonged grinding of a crude substance, in combination with lactose powder, with a pestle and mortar to reduce a homoeopathic drug to a fine powder.

T-Test: a statistical analytical method used to determine differences between the means of two groups of normal distribution. There are two types: paired and unpaired.

CHAPTER ONE: INTRODUCTION

According to Bellavite and Signorini (1995: 1), Homoeopathy is a distinctly singular phenomenon in the history of medicine. Two hundred years have passed since the observations and experiments of Samuel Hahnemann lead to its development as a healing art, yet the mechanisms of its action remain largely unknown, despite the advancements in biomedical technology and research.

A particular problem which has hindered acceptance of Homoeopathy by conventional medical practitioners is the apparent lack of scientific explanation for the mechanism of action of its remedies. More specifically, there has been little concensus regarding the physico-chemical changes occurring during the potentisation process. Inability to account for the therapeutic activity of substances which have been diluted beyond theoretical levels of bioactivity has resulted in the action of homoeopathic medicines being labeled as being mainly placebo effects (Bellavite and Signorini, 1995: 1-3).

Several analytical methods have been explored in attempts to find a scientific model for the mechanisms of homoeopathic potentisation. Nuclear magnetic resonance spectroscopy has proved useful in this regard (Lessell, 1994: 37). Despite the apparent success of NMR as a method of analysis, it has been hampered by difficulties in interpretation of results, as well as problems with reproducibility, and as a result there has been an increased awareness of the need for standardization of NMR studies conducted on homoeopathic potencies [Bol (1997), Demangeat and Poitevin (2001)].

This study will attempt to highlight the problems encountered in NMR research to date, specifically those relating to the operating frequencies of the NMR spectrometers used.

1.1 THE AIM OF THE STUDY

The aim of the study is to compare the 80MHz, 200MHz and 500MHz Nuclear Magnetic Resonance (NMR) spectra of Homoeopathic Sulphur 30CH and a lactose control in order to determine whether the frequency value is relevant when conducting NMR research on homoeopathic potencies.

1.2 THE STATEMENT OF THE OBJECTIVES

1.2.1 The first objective

The first objective is to analyse the 80MHz, 200MHz and 500MHz NMR spectra of homoepathic Sulphur 30CH and a lactose control, in terms of the chemical shift and integration values of the CH_3, CH_2, H_2O and OH signals produced at each frequency.

1.2.1 The second objective

The second objective is to statistically evaluate any similarities and/or differences existing between the analyses of the NMR spectra of the homoeopathic Sulphur 30CH at the three different frequencies mentioned above.

1.2.3 The third objective

The third objective is to statistically evaluate any similarities and/or differences existing between the analyses of the NMR spectra of the lactose control at the three different frequencies mentioned above.

1.2.4 The fourth objective

The fourth objective is to statistically evaluate any similarities and/or differences existing between the Sulphur 30CH and lactose control at the three different frequencies mentioned above.

1.2 THE HYPOTHESES

1.3.1 Hypothesis one

It is hypothesised that the Sulphur 30CH will show statistically significant differences in the chemical shift and integration values of the CH_3, CH_2, H_2O and OH signals between the 80MHz, 200MHz and 500MHz frequencies.

1.3.2. Hypothesis two

It is hypothesised that the lactose control will show statistically significant differences in the chemical shift and integration values of the CH_3, CH_2, H_2O and OH signals between the 80MHz, 200MHz and 500MHz frequencies.

1.3.3 Hypothesis 3

It is hypothesized that there will be statistically significant differences between the Sulphur 30CH and the lactose control at the three frequency values, and that these differences will be more notable at the lower frequency range.

CHAPTER TWO: THE REVIEW OF RELATED LITERATURE

2.1 INTRODUCTION

In order for Homoeopathy to gain the credit it deserves there needs to be accurate research on which to base its theories, something which has been lacking within the field of homoeopathic research so far (Schulte, 1994: 171). It is therefore necessary to ensure that current analytical methods being used to investigate the molecular nature of homoeopathic potencies are accurate and reliable.

Nuclear magnetic resonance spectroscopy is an experimental technique which has proven useful in the field of research on homoeopathic potencies (Schulte, 1999), however problems have been reported by various investigators assessing this tool [Bol (1997), Demangeat and Poitevin (2001)]. In order that reliable hypotheses be made regarding the mechanisms of homoeopathic potentisation it is necessary that any problems relating to NMR research be highlighted, in order that they may be appropriately addressed. This study aims to contribute to the assessment of NMR spectroscopy as a reliable and suitable tool for the analysis of homoeopathic potencies.

2.2 THE PHENOMENON OF HOMOEOPATHIC POTENCY

In the field of Homoeopathy, potency describes the state of a medicine after its modification by the process of potentisation. Potentisation, often referred to as dynamisation, is a process peculiar to Homoeopathy which involves the mechanical grinding (trituration) of the crude medicinal substance in lactose, followed by its serial dilution and succussion in water or an ethanol-water solution, according to a specific scale (Hahnemann 1995: 288 - 295). It has been observed that many important properties which are not evident in a drug's crude state become manifest when potentised. Similarly, inert substances and toxic substances become remedial in action only once processed into a potency. (Gaier 1991:433)

In homoeopathic pharmacy there are three scales employed for the manufacturing of homoeopathic potencies, namely decimal (denoted as D, DH or x), centesimal (denoted as C, CH or c) and quinquagenimillesimal, or fifty millesimal (denoted as LM). These are produced according to dilution ratios of 1:10, 1:100 and 1:50000 respectively. The 'H' indicates preparation of a potency in accordance with the methods instructed by Hahnemann in his 'Organon' (Gaier, 1991:448).

There are three forms of diluent used in the preparation of potencies namely; water, ethanol and lactose. In his 'Organon' (1995:288-290), Hahnemann describes these substances as 'indifferent vehicles', the primary function of which was to provide a medium within which the natural substance could be subjected to some degree of friction. The addition of kinetic energy - by means of trituration in lactose, or by vigorous shaking (succussion) in an ethanol-water solution - was seen to be the vital step to the development and strengthening of the latent therapeutic properties of the natural substance, rendering it therapeutically active.

During this dynamisation process a critical point may be reached, whereby the deconcentration exceeds Avogadro's number - a constant value of 6.022×10^{23} which indicates the number of elementary particles available per mol of substance (Fremantle, 1987:91). The potencies of 24x and 12C (equivalent deconcentrations of 1×10^{-24}) are the first to exceed this limit, after which all subsequent potencies have no chemically active substance left within it. Various investigators have proposed that therapeutic activity is achieved due to the ability of the diluent substance to retain and transfer the chemical imprint of the original substance by means of structural changes within the diluent, a phenomenon often referred to as 'the memory of water' (Del Guidice,1994: 117; Schiff, 1994: 9).

2.3 MEMORY AND INFORMATION

It requires no evidence to make the statement that information is a powerful and vital requirement in life. Communication and action is prompted and made possible only through the presence of information. According to Resch and Gutmann (1991:194) the word 'information' is derived from the Latin word 'informare', which could be translated as 'to shape or form anew by adding and working in something from the outside'. They add that, despite the absence of a clear definition, it is agreed that the nature of information is such that it must serve a purpose and must be understandable. Other sources say that information may be defined as the ability to establish order, or as 'the power to direct what is done' (Bellavite and Signorini, 1995:136).

Information has qualitative as well as quantitative components. The quantity of information does not in itself comprise the meaning (quality) of the information, and thus it is the meaning which differentiates two quantitatively similar 'bits' of information. The meaning of information lies in the interaction between the information itself and the receiving system, and in the result produced by this interaction. It is important to note here then, that interactions convey meaning without being information itself. It can therefore be said that information is not only in molecules, but also in the 'way' molecules relate to the receiver system (Resch and Guttman, 1987:199; Bellavite and Signorini, 1995: 137). Nucleic acids, the building blocks of DNA, are a good example of this. They interact in such a way so as to produce and dictate the vital codes of living organisms. They have a substantial degree of *order*, a major degree of *complexity*, and great physico-chemical *stability* (emphasis mine), all of which are requirements in order to be a good vehicle of information (Bellavite and Signorini, 1995:138).

In science so far it has seemed that the only way of storing information was to require a solid-state array of atoms or molecules, or some form of space ordered pattern (Del Guidice, 1994:117). Resch and Gutmann (1991), however, state that it is not justified to

associate information with the presence of a certain species of molecules, nor even with a certain structure. One has to look at the *whole interacting system* and the *dynamically ordered relationships within it*. There is a complimentarity between the available molecules and the actual motion pattern, so that the physical or chemical characterization of the highly differentiated motion pattern is related to the information content, but is independent of particular molecules. This might explain how the solvent water contains information without there being any molecules of the original substance present, that is, by virtue of its structural arrangement and modification by the solute. This idea is supported by Bellavite and Signorini's (1995:137) definition of information as *'an intrinsic function of every spatiotemporal structure, capable of being transmitted to another spatiotemporal structure and, thus, of modifying it in a specific manner'*.

Thus it can be seen that in order for the diluent substance to 'remember' the original crude substance, that is in order for information to be transmitted, there need not be any physical presence of chemically active molecules. There does, however, need to be some form of dynamic, ordered system in operation, one which will be able to not only receive the information but to send it as well (that is, a certain basic conformity of sender and receiver is required).

Aside from molecules, it is important to note that information is also transmitted by frequencies such as sound and electromagnetic waves (Bellavite and Signorini, 1995:138). Extensive research on the function and effects of electromagnetic frequencies within living systems by Smith and Best (1989:26) support this view, as their studies have prompted them to state that it is highly likely that Nature is using highly coherent electromagnetic signals within and between living systems. That is, coherent electromagnetic signals may function as carriers of information.

2.4 LIQUID WATER AS A VEHICLE FOR INFORMATION

Water is the most common compound on Earth, and is the only compound found naturally as a liquid, solid and gas. Water is a covalent molecular compound with a relatively high dipole moment. It has excellent solvent properties due to the polar nature of the water molecules. Liquid water is remarkably well structured and highly differentiated. A three dimensional network is dynamically maintained by means of flexible hydrogen bonds. Each oxygen atom is connected to four other oxygen atoms by hydrogen bonds. The length and strength of the hydrogen bonds, as well as the bond angles, vary in various regions (Resch and Guttman, 1987:333).

Hydrogen bonding accounts for some of the anomalous physical properties of water compared to other liquids, such as its abnormally high freezing and boiling points and surface tension, as well as its enthalpies of vaporisation and fusion per gram, which are higher than almost any substance. Water is unique in that its maximum density is at +4°C, which means that ice floats in water (Fremantle, 1987: 80). A further interesting observation about water is that, at varying temperatures, the molecular dipole moment remains constant, whilst the permittivity of water undergoes considerable changes (Schulte, 1994:110). Also worth noting is the observation that, at 100°C and at 1 atm, water molecules in the vapour phase, mutually distant at 36 Angstroms, suddenly keep rushing one towards another and increase the density by a factor of 1600. This phenomena occurs at a sharp temperature without any gradual evolution (Del Guidice, 1994: 118). These facts indicate the increased complexity of liquid water compared to other liquids. The flexibility of water, as seen by its structure and physical properties, allows for enormous differentiation, and an extensive variety of only slightly different structural features within a given liquid structure (Resch and Guttman, 1987:302).

This variety of only slightly different structural features is further extended by the presence of isotopic diversity in water. Isotopes are atoms of the same chemical element

having a fixed number of protons but a different number of neutrons in their nuclei, and is a feature of most atoms. According to Berezin (1994:139), isotopes are highly flexible and have a very high density of information storage, and he proposes that the distribution of stable isotopes in water or other substances can play the role of an information-carrying pattern during potentisation. It is worth noting then, that water has 18 isotopes (Schulte, 1994:113), which contributes to an increased complexity of micro-structural permutations available within the macrostructure of liquid water.

The tendency of water molecules to form hydrogen bonds is said to allow the formation of polymeric aggregates within the liquid phase, termed *clusters* (Lessell, 1994:14; Schulte, 1994:105) or *clathrate*s (Agnostatos, 1991:121-127), and it has been proposed that the 'memory of water' may be attributed to the formation of aggregates which are shape-specific to the dissolved solute. These so-called 'geometric theories' (Lessell 1994:18) have been expanded and improved upon by several investigators, whose hypotheses will be discussed further on.

In studies of biological systems, the well known molecular interactions between water and surfaces has been extended by the introduction of the hypothesis of 'vicinal water' (Bellavite and Signorini, 1995:248), a term which describes water which is near to solid surfaces or macromolecules and which is influenced by these structures. According to this phenomenon vicinal water undergoes structural modifications which extend much further than the specific surface interactions. It has a density greater than normal water, freezes only at temperatures many degrees below zero, and its solvent properties are also altered (Bellavite and Signorini, 1995:248; Smith, 1994:190). Although the presence of vicinal water has greatest implications in the biological system, it is interesting to note the ability of water to behave in what appears to be an organized, co-operative manner over extensive areas.

Worth noting is the statement by Schulte (1994:105) that in physics, physical chemistry and quantum chemistry, water is known as a complex substance in all its states of

aggregation. According to Del Guidice (1994:118), the generally accepted theory of the liquid state is still unable to explain many basic features of real liquids, and the physics community cannot yet claim to understand liquids satisfactorily. Liquid water is thus a complex substance to understand completely. It is precisely this complexity, however, which makes it not only an excellent choice of solvent in the preparation of homoeopathic potencies, but also a likely vehicle for the receiving and transmitting of information from solute particles of the chemical substance during potentisation.

2.5 ETHANOL

Ethanol is the second major diluent used in preparing homoeopathic potencies. Considering the fact that potencies are often prepared in high percentages of alcohol, it is necessary to consider its physical properties as well as its role within the potentisation process.

Ethanol is an excellent solvent of organic materials and inorganic salts and is highly miscible with water in all proportions. Ethanol, rather like water, has a relatively high boiling point of 78°C. This partly relates to its propensity to form hydrogen bonds and thus polymers. However this propensity differs from that of water. Compared to water the structural network of liquid ethanol is less developed, as ethanol has only one hydrogen atom available for hydrogen bonding and hence for contributing to network formation. In this way ethanol is not so highly structured as water, nor as flexible, a quality which may be beneficial by way of contributing to stability of the ethanol-water structure during potentisation (Lessell, 1994:29-30; Resch and Gutmann, 1987:324).

It has been suggested that one of the favourable properties of ethanol is its action in lowering surface tension. This creates a situation where less energy is required to fracture surface films during succussion, which may explain why potentisation proceeds more readily in the presence of ethanol (Lessell, 1994:30).

2.6 LACTOSE

Lactose (milk sugar) is lactose monohydrate $C_{12}H_{22}O_{11}.H_2O$, and is a natural product contained in the milk of all mammals (Resch and Gutmann, 1987: 273). As was mentioned earlier, lactose is a diluent substance used in the potentisation process.

The structure of lactose is such that the lactose molecules are connected with each other to a highly flexible, three-dimensional network. The end groups of lactose molecules are interconnected by water molecules and by ten flexible hydrogen bonds of various bond lengths (Resch and Gutmann, 1987: 273).

According to Resch and Gutmann (1987: 274-284), a characteristic feature of milk sugar is its highly developed structural differentiation and the high flexibility of the whole structural framework. Like water, it is proposed that the various components of the lactose structure form a system organization, with various regions being assigned particular roles and heirarchies during the potentising process. Thus lactose, like water, seems to possess the properties of complexity and order which are necessary for adequate transfer of information (see 2.3).

The effect of grinding is said to increase the system differentiation and strengthen the system organization of lactose in a manner particular to the solute. The whole system becomes energetically more differentiated, and this new information is maintained through the oscillating pattern of the whole solution. It is this oscillating pattern upon which the total information content depends, and which proceeds to imprint itself on the solvent in subsequent dilution in water.(Resch and Gutmann, 1987: 274-284).

2.7 ELECTROMAGNETIC FIELDS AND WATER

Extensive research by Smith and Best (1989:98) on the interaction between electromagnetic fields and living systems has contributed to the interesting observations already noted regarding the interaction and behaviour of water molecules. The extensive

clinical trials conducted by these authors have led them to hypothesise that allergies may be attributed to the existence of electrical sensitivity in patients. It was observed that the patients' symptoms appeared and disappeared when exposed to specific electromagnetic frequencies (individual to each patient) – termed the 'triggering' and 'neutralising' frequencies respectively. A notable observation was that the allergic reaction was found to be neutralised when the patient held a vial of water which had been exposed to the neutralising frequency, and that water treated in this way was found to be clinically effective for 1-2 months. Thus it appears possible that externally applied electromagnetic fields may affect the structure of water in some manner.

This view is supported by Del Guidice (1994: 119), who has observed that externally applied electric fields are able to produce sizeable static or quasi-static polarization fields over large regions in water, which last a long time. The author attributes this effect to the collective motion of water molecules, which makes them much more polarizable than in an incoherent configuration.

Further evidence is provided by the comments of Towsey and Hasan (1995) on a radionic technique of preparing homoeopathic potencies termed the 'magneto-geometric preparation' of medicines. This process, developed by Malcom Rae, involves the placing of a vial of water on a white card which is imprinted (in metallic ink) with a circular geometric pattern specific to the medicine required. An electric current leads to the center of the pattern, which 'modulates' the magnetic field. This modulated field in turn 'structures' the water just as if solute molecules were present. The authors went on to conclude that magnetic and electric fields are able to 'imprint' crystalline and molecular structures.

2.8 EXPERIMENTAL MODELS FOR POTENTISATION AND THE ROLE OF ELECTROMAGNETIC RADIATION

Certain hypotheses regarding the nature of homoeopathic dilutions look to the diluent as being representative of the electromagnetic characteristics of the solute molecules. These models have been classified by Lessell (1994:18) as 'dynamic field' hypotheses. The various investigators in this field propose that oscillations within the structure of the diluent are vital to maintaining the chemical imprint of the solute molecules, and that the structure is perpetuated by means of dynamic interactions between oscillations (frequencies) of various components throughout the system (Resch and Gutmann, 1991; Del Guidice, 1994; Antonchenko and Ilyin, 1992; Smith, 1994).

A collective interaction of liquid water is supported by the work of Resch and Guttman (1991), who propose a 'supermolecular system organization' of liquid water. They propose that the solute molecules (termed 'structure breakers') modify surrounding diluent molecules, with an increased local density. The structural differentiation induced by the solute extends throughout the whole solution and causes the hydrophobic gas molecules (termed 'structure makers'), located in the spaces between the water molecules, to begin to oscillate. The oscillation of the 'structure makers' is modified according to the boundary conditions at the inner surfaces of the holes. At the same time they in turn exert an influence on the inner surface, and hence on the properties of the whole solution, until 'synchronisation' is established (Resch and Gutmann, 1994: 301 – 312). In this way any changes in the structure of the solution are dynamically maintained by the oscillation of these 'synchronisation nodes'.

Del Guidice (1994: 118) also places importance on the presence of long-range interactions between water molecules during potentisation. Studies have shown that atoms or molecules, when closely packed and under the organizing influence of an electromagnetic radiation field, have a collective behaviour and act coherently, rather

than as isolated objects – a phenomenon termed superradiance (Bellavite and Signorini, 1994:250; Schiff, 1994:15).

Del Guidice and Preparata's (Del Guidice, 1994: 118) theory states that, beyond a certain threshold of density, the particle and electromagnetic fluctuations within liquid water couple and oscillate with a constant phase relationship within certain regions of the solution, termed 'coherent domains'. The energy of these coherent domains is lower than the rest of the liquid, which creates a stable state resistant to outside molecular influence (Del Guidice, 1994: 118). The existence of a protective 'shell' of strong hydrogen bonds surrounding these domains is also reported as having a stabilizing effect. It is proposed that the force of succussion during potentisation relaxes these domains, thereby allowing an external field (such as that generated by the solute) a chance to interact with the polarisation field of water and assign it its new frequencies. At the end of succussion the shell re-forms, thereby protecting the new frequency from outside influences (Bellavite and Signorini, 1995:251-252). As in radio communications, the low frequency fields of the coherent domain should not be disturbed by the higher frequencies fields produced by single molecules in the liquid, and in this way the selective aspects of the message are conserved (Del Guidice, 1994:119). The size of the domain is related to the wavelength of the electromagnetic radiative field (Del Guidice, 1994:119).

Studies conducted by Smith and Best (1987: 115) led them to hypothesise that the action of homoeopathic remedies could be explained if water were able to take up a structure having the properties of an electrical resonator, and that in order to account for magnetic field effects and a memory for frequency, it was desirable to think in terms of a helical structure in water. Antonchenko and Ilyin (1992) incorporate this view into their experimental model for potentisation. They view solvent water as a system of flickering 'embryos' of all possible clusters, or hydration shell structures, which are shape-specific to the dissolved solute. Surrounding the hydration shells are microclusters of water, which are stabilized as static structures at the expense of hydrogen bonds. Stability is proposed to be a result of a soliton mechanism along these structures, whereby coherent

proton conductivity occurs along the spiral path suggested by Smith and Best above. It is proposed that conditions for the stability of these 'dissipative structures' are connected with charge transfer processes, hence the dissipative structures must have radiation spectral characteristics (Antonchenko and Ilyin , 1992) During potentisation the water acquires new radiative characteristics conditioned by the specific dissolved substance.

In addition, Antonchenko and Ilyin (1992) state that all homoeopathic medicines have their own frequency spectrum of radiation. This idea finds support in observations made by Ludwig (quoted by Smith and Best, 1987), who found the principal frequency for Phosphorus 6x to be 300Hz, and that of Arnica 1000x to be 9.725kHz. Lessell (1994:24), however, concludes that the recorded frequencies are more likely a function of serial succussion, and correspond more to the level of potency, rather than to any specific molecular electromagnetic frequency of the solute that might characterize it individually. Thus, if frequency is indeed an expression of potency, it can be surmised that the frequency may be considered as the vibration that carries the healing information from the drug substance. Any disturbance to this structural vibration implies a disturbance in the medicines ability to act.

Lessell (1994:44) reports on what he terms an 'orbital energy theory' put forward by Sharma, which suggests that the supplied energy during potentisation causes the lone pair of electrons of the oxygen atom of the OH group to be resonantly promoted to a new orbital status possessing the energies characteristic of the highest orbitals of the solute. Lessell (1994:51) has extended this suggestion, and hypothesizes the production and interaction of virtual particles (a concept used by quantum theorists) during the collision of solute and diluent molecules during potentisation. The virtual particles, termed orbitons, are only observable or measurable in terms of their effect, and not directly. These orbitons communicate energetic information from the electrons of the higher orbitals of the solute molecules to the lone pair electrons of the hydroxyl group or groups of the diluent (Lessell, 1994:51). During succussion, the orbitons become attached to the lone pair electrons until a sufficient number of identical frequency have been

accumulated on a particular electron. Once this has occurred the electron will jump to the appropriate target orbital (corresponding to the energy of the appropriate higher orbital of the solute molecule), thus contributing to the change of the diluent molecule from an 'ordinary' solvent to water which contains characteristic vibrations of the solute. At the end of each succussion stage, not all the diluent molecules will have promoted electrons characteristic of the solute. It is hypothesized that the gradual loss of orbitonic quanta from unpromoted lone pair electrons is capable of contributing to the production and maintenance of an electromagnetic dynamic field. The energy of this field, through conversion to kinetic energy, causes the molecules of the diluent to oscillate in harmony and with the same frequency (Lessell, 1994:51). Thus Lessell supports Del Guidice's application of the phenomenon of superradiance when describing the mechanisms of potentisation.

In light of the concepts and hypotheses introduced in this chapter, it is worth mentioning that it has been stated that the activity of high dilutions can be destroyed by electromagnetic radiation (Bellavite and Signorini, 1994:244; Lessell, 1994:47), although it has not been stated in what frequency range this may occur.

2.9 METHODS OF MEASUREMENT OF THE PHYSICOCHEMICAL STRUCTURE OF HOMOEOPATHIC POTENCIES

Many methods have been used to determine physicochemical differences between ethanol-water mixtures and homoeopathic potencies. Investigations which have been carried out include measurements of electrical conductivity, relative permittivity (dielectric constant), and surface tension. The use of NMR (nuclear magnetic resonance) spectroscopy, Raman laser spectroscopy, UV (ultraviolet) spectroscopy and light polarizers have also been utilized (Lessell, 1994: 37).

From this available range of techniques, NMR spectroscopy is a method of analysis which has been deemed favourable for the detection of structural differences between succussed and unsuccussed homoeopathic dilutions as well as ethanol-water solutions (Bol, 1997).

2.10 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

2.10.1 The principles of Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique which makes use of the nuclear magnetic resonance phenomenon to study the chemical, physical and biological properties of matter.

The physical foundation of NMR spectroscopy lies in the magnetic properties of atomic nuclei, the fundamental property of which involved is the nuclear spin. Nuclear spin (*I*) is quantised in multiples of ½ and can be positive or negative. Several nuclei, including the proton, possess angular momentum (L), which in turn is responsible for the fact that these nuclei also exhibit a magnetic moment (μ). With this in mind, if a proton in isolation is considered, it may be pictured as behaving as a tiny magnet with a north and south pole (figure 1)(Hornak, 2001).

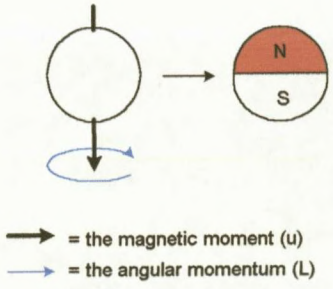
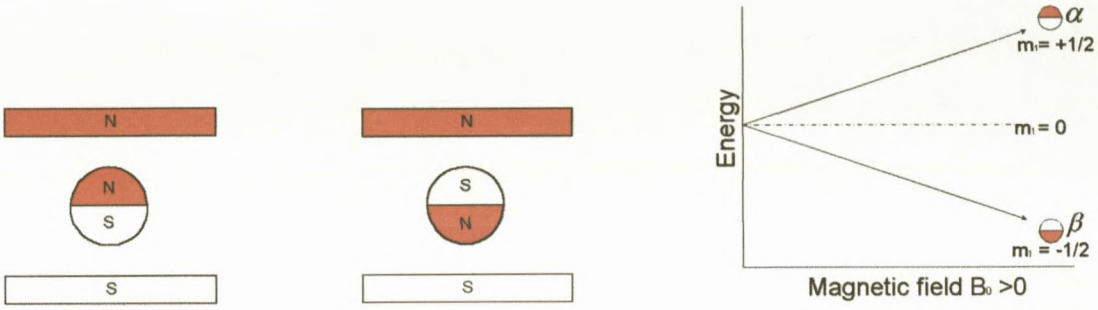


Figure 1. The magnetic moment of a proton pictured as a tiny magnet.

When placed in an external magnetic field, the spin vector of the particle aligns itself with the external field, just as a magnet would. Depending on the orientation of the proton, a high energy (α) or low energy (β) state configuration results. The nuclear moments orient themselves with only certain allowed orientations as we are considering a quantum mechanical system. A nucleus of spin I has $2I + 1$ possible orientations, thus a proton of spin value +½ has two allowable orientations (see figure 2).



A. NN-SS orientation = proton in high energy (α) state

B. NS-NS orientation = proton in low energy (β) state

C. Allowable orientations of protons in a static magnetic field B_0

Figure 2. Energy states of a proton when placed in a static magnetic field. (m_I = magnetic quantum number) [adapted from Hornak, 2000: and Gunther, 1987:3]

Through addition of a high frequency transmitter, transitions between the allowable spin states is stimulated. Transition between the two spin states is possible by the absorption of a photon. A particle in the lower energy state absorbs a photon and ends up in the upper energy state. The energy of this photon must exactly match the energy difference between the two states. The energy of the photon is related to its frequency, and the quantity of energy which exactly matches the difference between the two energy states is called the resonance frequency, or Larmor frequency. For ^{1}H NMR spectrometers this Lamor frequency is usually between 60 and 800MHz and is directly dependant on the strength of the external magnetic field B_0 (Hornak, 2001) (see figure 3).

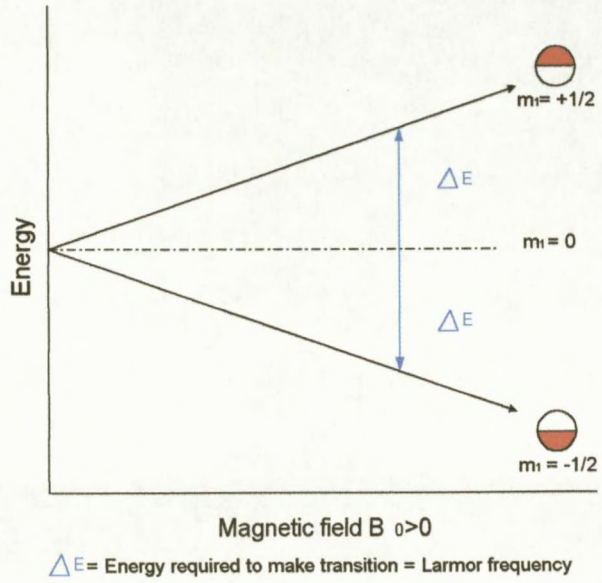


Figure 3. Energy diagram depicting Lamor frequency (adapted from Hornak, 2000:)

Once the radiofrequency pulse has been removed, the perturbed system will begin to relax back towards its equilibrium condition by means of two separate processes defined as the relaxation times T_1 (spin-lattice relaxation) and T_2 (spin-spin relaxation) (Abraham and Loftus, 1980:85). The absorption and release of energy during stimulation is detected, amplified and recorded as a spectral line, or resonance signal (see figure 4). (Gunther, 1980: ix).

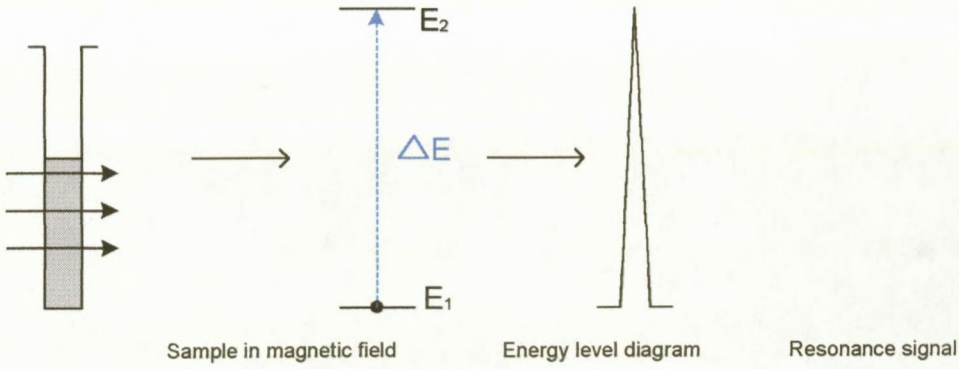


Figure 4. Production of the resonance signal (Günther, 1980: ix)

NMR spectroscopy is thus a measurement of the resonance or exchange of energy at a specific frequency between the spins of the sample and the spectrometer. The NMR spectrum provides information concerning the spatial arrangements of the molecules within the sample, and has thereby proved to be a useful tool in determining molecular structure of substances, both known and unknown, in the biological, chemical and physical sciences.

2.10.2 Features of the proton (^{1}H) NMR spectrum

An important feature of the NMR spectrum is the chemical shift, which may be defined as the nuclear shielding divided by the applied field (Gunther, 1980:13). The electron density around each nucleus in a molecule varies according to the types of nuclei and bonds in the molecule. These factors influence the resonance frequency of an individual nucleus, as the opposing field and therefore the effective field at each nucleus will vary. This phenomenon is called the chemical shift, and is measured in frequency units (Hz) or in parts per million (ppm). The chemical shift is always measured from a suitable reference compound, and is a parameter independant of the spectrometer frequency when measured in ppm (Hornak, 2001: 12, Watson 2001). Factors which may affect the chemical shift in ppm are the sample conditions, such as sample concentration, solvent used, and temperature (Abraham and Loftus, 1980:13). A positive chemical shift signifies a decrease in shielding (Gunther, 1980:16).

The integration value of each peak, representing the peaks area intensity, is another important characteristic of the spectrum line. The area under the resonance signal is proportional to the number of protons that give rise to that signal. Only the relative number of protons can be determined by integration, and these ratios are automatically calculated by an electronic integrator built into the spectrometer (Gunther, 1980:19, Watson, 2001).

Relaxation times are a further feature of NMR spectra already mentioned in 2.7.1. Relaxation time is a complex parameter resulting from the dipolar magnetic interaction between inter- and intra-molecular vicinal protons and the presence, if any, of para-magnetic substances (certain metals, molecular oxygen, free radicals) (Gaier, 1991:260). Relaxation times have not been considered in this study.

2.10.3 The NMR Spectrometer and Experiment

Basic requirements for all high resolution NMR spectrometers include a radiofrequency (RF) source and a magnetic field, both of which have to be stable and homogenous to a very high degree (Abraham and Loftus, 1980: 6) The magnet producing the static magnetic field B_0 consists of a superconducting solenoid, immersed in a dewar of liquid helium (at a temperature of about 0 K), which in turn is surrounded by a high vacuum region acting as a thermal buffer between the room temperature air and the liquid helium (Hornak, 2001: 21), with a liquid nitrogen dewar specifically to reduce heat input through the necessary access ports. A bore hole extends through the center of the assembly, and

immediately within the bore of the magnet are the shim coils which are used to correct minor inhomogeneities in the B_0 field.(Hornak, 2001: 21) In a nuclear magnetic resonance experiment the sample is contained in a glass tube about 5mm in diameter and situated in the probehead of the spectrometer between the pole pieces of the magnet. The probe contains a RF coil which firstly produces the radiation necessary to rotate the spins within the sample, and secondly detects the resultant NMR signal. It also contains the coil for locking to the deuterium frequency of the solvent In high resolution pulsed NMR spectroscopy the current flowing through the RF coil is turned on and off very rapidly over a small value of time, with a signal of sufficient band width to match all the resonant frequencies of the sample. When the resonance condition is satisfied for the nuclei under observation, the sample absorbs energy emitted from the RF coil. The subsequent relaxation and release of energy, and the resulting signal is detected on the RF coils, amplified and recorded (Abraham and Loftus, 1980: 7). The recorded information is further analysed by the hardware of the spectrometer, where the data is transformed from representation in the time domain into the frequency domain via Fourier transformation (Gunther, 1980: 231) (see figure 5).

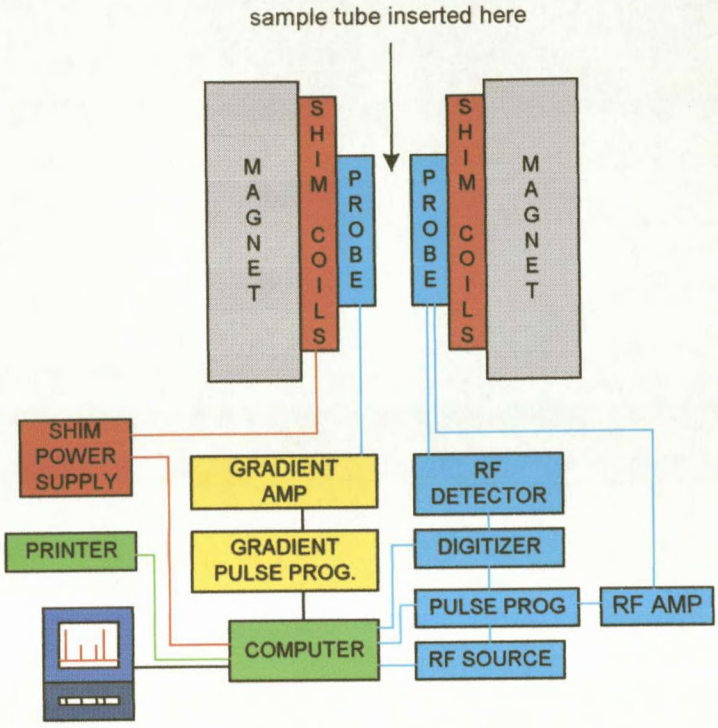


Figure 5. Schematic diagram of the components of an NMR spectrometer (adapted from Hornak, 2000)

Stability of the frequencies is achieved by the fact that the spectrometer has all its frequencies 'locked' to a single master oscillator, whilst compensation for any slight variations in physical conditions at the sample is automatically made by the software through the monitoring of the deuterium lock signal (Abraham and Loftus, 1980:93).

2.10.4 Summary

From the foregoing it can be seen that NMR spectroscopy is capable of detecting very small differences between the spin states in substances, and of recording these differences at very specific frequencies. Such sensitivity has made NMR a popular choice in recent

years within the field of scientific homoeopathic research, in fact NMR Spectroscopy has been used more frequently than any other measurement technique for this purpose (Schulte, 1999). NMR research has made fundamental contributions to the search for answers regarding the nature of ultra high dilutions of homoeopathic preparations, allowing useful and necessary hypotheses to arise and be seriously considered.

2.11 NMR RESEARCH IN HOMOEOPATHY

Smith and Boericke (1966) conducted some of the first significant NMR studies in homoeopathy. Results showed a difference in the hydroxyl (OH) group of the spectrum of succussed and unsuccussed dilutions when compared to the alcohol controls. Similar results were obtained by Sacks (1983) whose NMR spectra also showed clear differences between succussed high dilutions and the ethanol-water control.

Weingärtner (1990) found statistically significant relative intensities of the OH *and* H_2O signals of potentised Sulphur 23x compared to the solvent alone. He demonstrated that the peaks of the homoeopathic dilutions of Sulphur are significantly lower and broader compared to the peaks for the solvent alone.

In a study of quite a different nature, Demangeat *et al.* (1992), in their assessment of relaxation times, found changes in T1 and T1/T2 ratios of potentised Silica samples as opposed to those of distilled water (Bellavite and Signorini, 1995: 261).

NMR research into LM potencies (homeopathic potencies produced at a serial dilution of 1:50 000) conducted by Ross (1997) noted statistically significant differences between ascending potencies within each group, and it was concluded that distinct physico-chemical entities are produced by the process of homoeopathic potentisation (Ross, 1997: 64). This was confirmed in a more elaborate follow-on study by Power (1999), as well as a recent NMR study conducted by Davies (2001), in which the Hahnemannian and Korsakovian methods of preparing potencies were compared. Significant differences were found between the various potency levels used (9C, 30C and 200C) for both methods, with the Hahnemannian method showing a greater amount of differences overall). These differences were present for almost all the peaks (ie the CH_3, CH_2, H_2O and OH signals), and not only the OH signal, as reported by investigators already mentioned. Davies commented on the possibility that the potentising process induces certain changes within a sample, irrespective of whether the sample is from the treatment or control group, and that small electric fields are may be responsible for differences observed.

The available literature suggests that NMR spectroscopy is a powerful technique that should continue to be employed in homoeopathic research (Bol, 1997). Difficulties in interpretation of the results have been noted, however this factor is outweighed by the useful demonstration of differences in the mobility and organization of molecules of water in homoeopathic dilutions [Schulte (1999) Demangeat *et al.* (1992), Bol, (1997)]. This position is further maintained by Schulte (1999), who states that NMR-spectroscopy is a technically well-developed, easily accessible, sensitive and accurate means of

analysis, especially at a microscopic level. These investigations at a molecular level has provided useful information for the construction of theories relating to the phenomenon of potentisation [(Smith and Boericke (1966), Smith and Boericke (1968), Demangeat *et al.* (1992), Ross (1997)].

2.12 PROBLEMS WITH NMR RESEARCH IN HOMOEOPATHY

Despite the success of NMR as a method of analysis of homoeopathic remedies to date, examples have arisen that demand closer evaluation. Milgrom (2001) has highlighted a set of low resonance NMR studies conducted by Conte *et al.* (quoted by Milgrom, 2001) which reported large, irreproducible differences in T2 values between parallel succussed and unsuccussed dilutions of Nitric acid. Initial attempts by Milgrom to reproduce these results were successful. However, the variations disappeared on re-measurement of the samples when they were placed in boro-silicate glass tubes instead of soda glass tubes, which were reportedly used in Conte *et al.* 's studies. It was concluded by Milgrom that the significant results obtained by Conte *et al.* were, in fact due to experimental artefact originating in the glassware of the NMR tubes used, i.e. the significant results were a result of trace amounts of silica leached from the soda-glass tubes.

Conte's construction of theories regarding the nature of homoeopathic dilutions, subsequent to obtaining what are seemingly inaccurate results, was highly criticised by Milgrom (2000). The controversy highlights the need for awareness and accuracy when

conducting NMR research on homoeopathic dilutions, in order that results may be meaningful and reliable.

It is interesting to note that the various NMR studies cited above (see 2.10) were conducted using different NMR spectrometers, operating at different frequencies (see Appendix A). Thus it is difficult to correlate the various studies done, as they were performed under differing conditions. Schulte (1999) supports this argument by adding that it is difficult to assess the significance of the reported results in NMR research on homoeopathic dilutions due to failure of researchers to report any modifications made to the experimental procedure, as well as important parameters usually mentioned in NMR measurements. Demangeat *et al.* (1992, 2001) have been influential in making steps toward appropriate and standardized NMR studies on homoeopathic dilutions.

There is further evidence to support the emerging difficulties of NMR research on homoeopathic dilutions. Attempts by Aabel *et al.* (2001) to reproduce experiments conducted by Weingärtner (1990) and Demangeat *et al.* (1992) were a failure, leading them to conclude that there is no experimental evidence that homoeopathic remedies make any kind of imprint on their solvent, which can be detected with NMR spectroscopy. Demangeat and Poitevin (2001) responded by pointing out several flaws in Aabel *et al.*'s (2001) experimental procedure , which may have contributed to the unfavourable results obtained. Criticism included the use of plastic tubes instead of glass tubes, using frequency and temperature values which differed from Demangeat *et al* 's experimental model, and failure of Aabel *et al.* to follow the specific recommendations

made by Demangeat *et al.* which were deemed essential if conducting NMR measurements on homoeopathic dilutions.

In attempting to understand the discrepancy between theirs and Weingartners' (1992) work, Aabel *et al.* provided several explanations which ranged from varying geometric properties in the glass capillary tubes, and varying levels of dissolved gases in the samples being measured (due to varying waiting periods between succussion), to varying levels of dissolved carbon dioxide – which could alter the pH and thereby the hydroxyl (OH) resonances of the sample.

These problems highlight the myriad of factors, ranging from the preparation of samples to the experimental design, which might possibly influence results obtained. There is a need for refinement of protocol in NMR research conducted on homoeopathic potencies. Demangeat and Poitevin (2001) support this claim by remarking that published NMR research to date should be considered carefully, and that the first step in NMR research in homoeopathy at present is to define the methodological bases which will have to be implemented. A solid base is required in order that theoretical constructions may be firstly reliable, and secondly, that various works can be correlated and expanded upon, thereby providing much needed answers in this field of research.

The critical reviews of NMR, provided by researchers to date, have failed to mention a further possible influential parameter in NMR research on homoeopathic dilutions – the NMR instrument itself. The possible influence of electromagnetic fields, such as those

applied by the NMR spectrometer, on the activity of homoeopathic dilutions requires further evaluation.

2.13 SUMMARY

Given the fact that there is still no certain theory regarding the mechanisms of homoeopathic potentisation, it is vital to ensure that the information obtained by various analytical methods is reproducible and reliable. Further evaluation of NMR spectroscopy as a method of analysis in homoeopathic research is a necessary step toward these goals.

From the wide range of examples given it can be seen that, when dealing with homoeopathic dilutions, it is reasonable to consider that externally applied electromagnetic fields may influence the stability of the molecular structure of the homoeopathic potency. Given that NMR spectroscopy operates by way of applying an external electromagnetic field, and given the existing uncertainty regarding the exact manner in which homoeopathic potencies remain stable, it is necessary to consider whether the applied frequency may influence the potency as it measures it, and at what frequency strength this might occur. The fact that NMR studies to date have been conducted using instruments operating at different frequency strengths leads to questioning of the qualitative value of these results. Which method has been, or is most suitable for the measurement of homoeopathic potencies? For these reasons it is proposed that a single homoeopathic potency of Sulphur 30CH and a lactose control be analysed using a range of three different frequency strengths, in order to determine whether

frequency strength is a parameter requiring further consideration in NMR studies of homoeopathic potencies.

CHAPTER THREE: MATERIALS AND METHODS

3.1 THE PREPARATION OF POTENCIES

Potencies of Sulphur and the Lactose control were prepared by hand at the Department of Homoeopathy, Technikon Natal, Durban. Preparation of the samples was completed in accordance with the methods provided in the German Homoeopathic Pharmacopaeia (GHP), specifically Method 6 for trituration of the solid basic drug materials (GHP 1985:22), and Method 8a for the preparation of liquid potencies from the triturations (GHP 1985:23) [See Appendix B(i) and B(ii)]. It must be noted that the manufacturing method was altered in the following manner:

Method 8a indicates the employment of 43% ethanol as the diluent from the 9CH potency upward (GHP 1985:23). For the purposes of this research it was necessary to prepare the potencies in a final ethanol concentration of 87%. This percentage ethanol was deemed necessary for two reasons, the first being to ensure separation of the OH and H_2O signals when analyzing the samples using NMR spectroscopy (Watson, 2000)], and secondly, that the final prepared samples should be analogous to potencies used in previous NMR research [Weingärtner (1990) [*87%*], Ross (1997) [*95%*], Power (1999) [*95%*], Davies (2001)[87%]. The preparation method was changed in such a manner that the indicated 43% ethanol was replaced with 87% ethanol from the 15CH upwards. This was the only change made during the preparation of the Sulphur and Control.

At the first stage of manufacture of the Sulphur potencies, one part chemically pure Sulphur was added to 33 parts pure lactose powder and triturated in the manner described in Appendix B (i). The preparation of the Lactose control was identical in manner, however at the first stage of manufacture one part Sulphur was replaced with one part pure lactose powder and triturated with 33 parts pure lactose powder, as described in Appendix B (i). The manufacturing process of both samples was identical thereafter. Thus the only difference between the Sulphur and Lactose control samples was the presence or absence of sulphur respectively at the initial manufacturing stage.

Rigorous steps were taken to ensure that no variables or contaminants were introduced during the manufacturing process. All potencies were prepared under Laminar flow (employing a Labaire unit at a maintained air velocity of 150 Pascals) without the implementation of ultraviolet lighting. All glassware employed was washed, rinsed in tap water, then rinsed in distilled water three times before being sterilized. Sterilisation was by method of dry heat in a baking oven (180^0C for 40 minutes) for all the 25ml amber glass bottles, whilst the remaining glassware (reagent bottles, beakers, measuring cylinders etc) were sterilized by autoclave at 121^0C for 15 minutes (British Pharmacopoeia, 1887:A208).

All potencies were prepared from a single container each of pure lactose powder, distilled water and ethanol (30%, 43% and 96%). The 87% ethanol was prepared using calculated quantities of 96% ethanol and distilled water, employing the use of an alcoholmeter as the measure of the correct final percentage. The percentage alcohol of both the 30% and 43% ethanol were also checked using the alcoholmeter before their employment. The prepared 87% alcohol was stored in 2 screw top reagent bottles in volumes of 250ml each. This was done to diminish absorption of water into the ethanol from the environment during the manufacturing process. The small volumes (0.15ml) transferred to the diluent at each potentisation level [see Appendix B(ii)] were measured by means of a micropipette, and a new micropipette tip was used each time in order to avoid cross contamination of samples. A parallel process of production was employed to minimize differences in environmental conditions surrounding the manufacture of the parallel potencies.

For the purposes of this research the highest potency level, for both Sulphur and Lactose, was the 30^{th} Hahnemannian centesimal (30CH). These were prepared to 36ml volumes.

3.2 THE PREPARATION OF SAMPLE VOLUMES

Upon completion of the entire manufacturing process as outlined in Appendix B(i) and B(ii), it was necessary to prepare sample volumes sufficient for the purpose of the study. Three volumes of 10ml each were transferred from the final 36ml volumes of Sulphur 30CH and Lactose 30CH into six 20ml amber glass bottles respectively. Each group, consisting of 3 volumes of 10ml each of Sulphur 30CH and Lactose 30CH respectively, was subsequently blinded by a third party, plus one witness, with one group being labeled as 'red' and the other group as 'blue'. For the purposes of this research blinding was not essential, but was nevertheless utilised as an added precaution against experimental bias.

3.3 THE MEASUREMENT OF SAMPLES

Measurement of the samples and recording of their NMR spectra was carried out at the Department of Chemistry (UNP) by the resident NMR technician, Mr Martin Watson. The sample bottles containing the Sulphur 30CH and Lactose 30CH (described above in 3.2) were never taken into the NMR laboratory, but were stored in an adjacent chemistry laboratory, where the test samples for the experiment were subsequently prepared. This precaution was taken in order to exclude any possible electromagnetic influence on the samples, prior to their measurement, from the spectrometer's magnet.

Each sample (0.6ml) was drawn into an NMR tube (Wilmod 505-p5, 5mm outside diameter, Wilmod Glass Co., Buena, N.J.) made of boro-silicate (quartz) glass. Previous studies have shown boro-silicate to be the most suitable glass for NMR studies on homoeopathic potencies (Demangeat and Poitevin, 2001). An external lock of acetone was used, which was contained in a sealed capillary reference tube (KIMAX-51, 1.6mm outside diameter, Kimble Products, USA), and inserted into the NMR tube containing the remedy. No solvent was added to the sample so as not to compromise its physico-chemical structure. Ten samples of each substance were drawn from the provided volumes using an overlapping process (i.e. initial samples of each group were drawn and measured, before proceeding to the drawing of second samples, third samples etc.), and

NMR spectra recorded for each sample. Each sample was drawn by means of a micropipette, with a clean capillary tube being used for each sample. Each sample was inside the NMR laboratory and instrument for the same length of time, which included centralizing in the receiver coil, shimming and acquisition time.

Three different spectrometers were employed for the measurement of samples, the specifications of which are as follows:

1) A Varian FT80A 80MHz instrument (Varian Inc., Palo Alto, California) equipped with a 5mm Omega broadband probe and running at 79.542MHz for protons.
2) A Varian Gemini 200MHz instrument (Varian Inc., Palo Alto, California) equipped with a 5mm $^{1}H/^{13}C$ probe and running at 199.975MHz for protons.
3) A Varian Inova 500MHz instrument (Varian Inc., Palo Alto, California) equipped with a 5mm inverse detection switchable probe and running at 499.982 MHz for protons.

Samples were run non-spinning on the 500MHz instrument, but spinning at 20Hz (±2Hz) on both the 200MHz and 80MHz instruments (a requirement for the necessary resolution of resonances) (Watson, 2001). All the samples were run at a thermostatically controlled temperature of $24^{0}C$ (± $0,2^{0}C$), measured using a standard methanol temperature probe supplied by the manufacturer. Each laboratory was maintained at a constant temperature of $22^{0}C$. In order to obtain as homogenous a field as possible for each sample, shimming was performed before each acquisition (details of pulse widths and acquisition times employed at each instrument are detailed in Appendix C).

Logistically it was impossible to carry out an overlapping process of measurement with regards to the spectrometers (i.e. measurement of initial samples at each spectrometer before proceeding to measurement of the second samples, third samples etc.). The samples were measured over three days, with all the samples from each group being measured on each spectrometer on each of the three days.

3.4 THE RECORDING OF DATA

The NMR spectra (listing chemical shift and integration values) were recorded at the NMR spectroscopy laboratories of the Department of Chemistry, University of Natal, Pietermaritzburg using the following software:

1) 80MHz:. Varians standard (unnamed) sequence, acquired and processed with Varians NJ FT80A programme on a SPERRY V-77-200.
2) 200MHz: Varians 5S pul, acquired and processed with Varians GEMINI 6.3E programme on a Motorola 68000 processor.
3) 500MHz: Varians 5S pul, acquired and processed with Varians 6.2C VNMR programme on a SUN Ultra-1 computer running Solaris 7 (Watson, 2001).

3.5 SORTING OF DATA

The data was recorded using the blinding codes 'Red' and 'Blue'. Once all the tests were completed and the data recorded, the blinding envelope was opened and the data was sorted into the treatment and control groups. The Sulphur 30CH had been coded as 'Red' and Lactose 30CH as 'Blue'. The crude data was subsequently tabulated (see Appendix D), and statistically analysed.

3.6 STATISTICAL ANALYSIS

The chemical shift (δ, ppm) values (to eight decimal places) of the CH_3, CH_2, H_2O and OH signals of each sample were recorded as well as their respective integration values (to eight decimal places). Data was not rounded off to fewer decimal places in order to retain as accurate a representation of the crude data as possible before its statistical manipulation. Only the CH_2, H_2O and OH values were use in subsequent statistical analysis. In the case of the CH_2 δ-values, it was necessary to derive the mean value of the four resonance signals for the purposes of statistical manipulation. This was done by calculating the mean value of the two centre peaks of the CH_2 resonance signal, denoted

by their higher intensity values. Data thus manipulated in each case underwent statistical evaluation according to two methods. The first method used was the Oneway Analysis of Variance (ANOVA), which compared the respective CH_2, H_2O and OH values within respective Sulphur and Lactose groups, obtained from the 500MHz, 200MHz and 80MHz instruments. Where necessary the Bonferroni Multiple Comparisons test was applied. The second statistical method employed was the Independent Samples *t*-Test, which was used to compare the respective CH_2, H_2O and OH signals of the Sulphur versus the Lactose control at each parallel frequency strength.

In each case the null hypothesis (H_0) states that there is no difference between the groups being compared. The alternative hypothesis (H_1) states that there is a difference between the groups being compared. The null hypothesis was accepted if the resultant p-value was greater than or equal to the level of significance (α) set for each test. The null hypothesis was rejected if the p-value was less than α. For all comparisons α was set at 0.05 (Thomas, 2001).

The statistical package SPSS (version 9.0 for Windows 1998) was used to facilitate statistical analysis.

3.6.1 THE ONEWAY ANALYSIS OF VARIANCE (ANOVA) METHOD

The Oneway ANOVA method is used for the analysis of data relating to three main effects. The statistical model for this method is given as follows:

$Y_{ijk} = \mu + T_j + \varepsilon_{ijk}$

where

Y_{ijk} is the observation in cell (i,j,k)

μ is the common effect

T_j is the effect of treatment j, j = 1,...,k

ε_{ijk} is the random error in cell (i,j,k)

k = 3 = number of treatments in the experiment

i.e. The total variation = variation due to the true differences between the three groups + variation due to random error.

The method compares the effects of the three chosen frequency strengths on the test samples of each group. To this end the hypotheses are stated as follows:

- H_0: There is no difference between the frequency strengths ($\mu_1 = \mu_2 = \mu_3$)
- H_1: At least one frequency strength is different from the rest ($\mu_i \neq \mu_j$; $i \neq j$)

The hypotheses were accepted or rejected according to the following decision rule:

- Accept H_0 if $p \geq \alpha$
- Reject H_0 if $p < \alpha$

If the null hypothesis was rejected (alternative hypothesis accepted), then a multiple-comparison of the means was carried out using the Bonferroni test.

3.6.2 BONFERRONI MULTIPLE COMPARISONS TEST

The function of the Bonferroni Multiple Comparisons Test is to determine which two groups (of the three analysed) are different. The decision is based on the p-value, as before (see 3.5.1).

3.6.3 THE INDEPENDENT SAMPLES *t*-TEST

The Independent Samples *t*-Test is used to determine the difference between the means of two samples that are independent, small in size (less than 30), and are taken from population groups of normal or approximately normal distribution.

In each case the test was used to determine whether any differences existed between the Sulphur and Control potencies at parallel frequency strengths. The hypotheses are stated as follows:

- H_0: There is no difference between Sulphur and Control ($\mu_1 = \mu_2$)
- H_1: There is a difference between Sulphur and Control ($\mu_1 \neq \mu_2$)

The hypotheses were accepted or rejected according to the following decision rule:

- Accept H_0 if $p \geq \alpha$
- Reject H_0 if $p < \alpha$

α is the significance level of the test ($\alpha = 0,05$).

CHAPTER FOUR: THE RESULTS

4.1 THE CRITERIA GOVERNING ADMISSIBILITY OF DATA

Precautions were taken to ensure appropriate storage, handling and analyses of the samples once they had been accurately prepared and labeled (see Appendix Ai, Aii and Aiii). All samples were subjected to the same treatment during the analytical procedure. The bottles containing the homoeopathic potencies were all kept under the same conditions at all times, and all the samples were prepared outside of the NMR laboratory under the same conditions. All samples used were drawn from the same bottle for each group (Sulphur and Lactose control) and identified appropriately. All samples were drawn in an overlapping process and pipetting equipment was not used across samples.

The crude data obtained was subjected to statistical analysis described in 3.5. The initial analysis consisted of a comparison of the chemical shift (δ) values of the CH_2, H_2O and OH signals of each respective sample. The analysis was subsequently repeated using the integration values of the samples. In each case comparisons were made both within respective Sulphur and conrol groups (e.g. Sulphur 30CH measured at 500MHz, 200MHz and 80MHz) and between parallel Sulphur and control potencies (e.g. Sulphur 30CH versus Lactose 30CH at 80MHz). No data was excluded from analysis.

4.2 COMPARISON OF CHEMICAL SHIFT (δ) VALUES

4.2.1 CHEMICAL SHIFT VALUES OF THE SULPHUR GROUPS

A comparison of the means of the CH_2 signal of Sulphur 30CH measured at 500MHz, 200MHz and 80MHz was made. The means of the three groups differ within the second decimal value. The very low standard deviation (S_x) and variance (S_x^2) values are notable and were found to be consistently low throughout the samples.

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	3.036006	3.03885	3.0205
S.D. (S_x)	0.00133968	0.00033747	0.00368932
Variance (S_x^2)	$1.79473X10^{-6}$	$1.13889X10^{-7}$	$1.36111X10^{-5}$

Table 4.0 Summary statistics: CH_2 δ-values – SULPHUR 30CH

A statistically significant difference between the three groups was indicated after analysis using the Oneway Analysis of Variance (ANOVA) method (Table 4.2). Subsequent analysis using the Multiple Comparisons Bonferroni Method (Table 4.3) revealed statistically significant differences between 500MHz and 200MHz ($p = 0.028$), 200MHz and 80MHz ($p = 0.000 < .001$), and 500MHz and 80MHz ($p = 0.000 < 0.001$).

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	1.951×10^{-3}	2	9.754×10^{-4}	188.549	**.000** (<.001)
Within Groups	1.397×10^{-4}	27	5.173×10^{-6}		
Total	2.091×10^{-3}	29			

Table 4.1 **Oneway ANOVA**: CH_2 δ-values – SULPHUR 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$-2.844x10^{-3}$*	**0.028**
500MHz	80MHz	$1.55.x10^{-2}$*	**0.000** (<.001)
200MHz	80MHz	$1.853x10^{-2}$*	**0.000**. (<.001)

*The mean difference is significant at the 0.05 level

Table 4.2 **Bonferroni Multiple Comparisons**: CH_2 δ-values-SULPHUR 30CH

Similar statistical evaluation of the Sulphur H_2O (Tables 4.3 - 4.5) and Sulphur OH (Tables 4.6 - 4.8) revealed similar results. In each case the mean values differed within the first and second decimal values, and ANOVA and Bonferroni revealed statistically significant differences between all three groups.

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	4.026415	4.0512	4.041
S.D. (S_x)	0.000818647	0.001549193	0.003162278
Variance (Sx^2)	6.70183X10-7	2.4X10-6	1X10-5

Table 4.3 Summary statistics: H2O δ-values – SULPHUR 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	3.104×10^{-3}	2	1.552×10^{-3}	356.177	**.000** (<.001)
Within Groups	1.176×10^{-4}	27	4.357×10^{-6}		
Total	3.221×10^{-3}	29			

Table 4.4 **Oneway ANOVA:** H_2O δ-values – SULPHUR 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	-2.478×10^{-2}*	**0.000** (<.001)
500MHz	80MHz	-1.458×10^{-2}*	**0.000** (<.001)
200MHz	80MHz	1.020×10^{-2}*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.5 **Bonferroni Multiple Comparisons:** H_2O δ-values- SULPHUR 30CH

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	4.770634	4.791	4.765
S.D. (S_x)	0.000589241	0.001154701	0.005270463
Variance (S_x^2)	3.47204X10-7	1.33333X10-5	2.77778X10-5

Table 4.6 Summary statistics: OH δ-values – SULPHUR 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	3.742×10^{-3}	2	1.871×10^{-3}	190.526	**.000** (<.001)
Within Groups	2.651×10^{-4}	27	9.819×10^{-6}		
Total	4.007×10^{-3}	29			

Table 4.7 **Oneway ANOVA**: OH δ-values – Sulphur 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	-2.037×10^{-2}*	**0.000** (<.001)
500MHz	80MHz	5.634×10^{-3}*	**0.001**
200MHz	80MHz	2.600×10^{-2}*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.8 **Bonferroni Multiple Comparisons**: OH δ-values- SULPHUR 30CH

4.2.2 CHEMICAL SHIFT VALUES OF THE CONTROL GROUPS

A comparison of the CH_2 means of Lactose 30CH measured at 500MHz, 200MHz and 80MHz was made. The means of the three groups differ within the second decimal value (Table 4.9). Analysis of the data using Oneway ANOVA revealed statistically significant differences between the three groups (Table 4.10). Further evaluation using Multiple Comparison Bonferroni revealed that these differences were to be found between two of the three groups, namely the 500MHz and 80MHz groups ($p = 0.000 < 0.001$) and 200MHz and 80MHz groups ($p = 0.000 < 0.001$). There were no differences between 500MHz and 200MHz groups ($p = 0.108$) [Table 4.11].

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	3.03677	3.0389	3.0205
S.D. (Sx)	0.000434795	0.000394405	0.003689324
Variance (Sx2)	1.89047×10^{-7}	1.55556×10^{-7}	1.36111×10^{-5}

Table 4.9 Summary statistics: CH2 δ-values - CONTROL GROUPS

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	2.026×10^{-3}	2	1.013×10^{-3}	217.759	**.000** (<.001)
Within Groups	1.256×10^{-4}	27	4.652×10^{-6}		
Total	2.152×10^{-3}	29			

Table 4.10 **Oneway ANOVA**: CH_2 δ-values – CONTROL 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$2.131x10^{-3}$	**0.108**
500MHz	80MHz	$1.627x10^{-2}$*	**0.000** (<.001)
200MHz	80MHz	$1.840x10^{-2}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.11 **Bonferroni Multiple Comparisons**: CH_2 δ-values- CONTROL 30CH

Statistical analysis of the chemical shift values of the H_2O values of the control revealed statistically significant differences between all three groups (Tables 4.12 – 4.14). The means differed within the first and second decimal places.

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	4.026975	4.0516	4.042
S.D. (S_x)	0.000737145	0.000966092	0.006324555
Variance (S_x^2)	$5.43383X10^{-7}$	$9.33333X10^{-7}$	$4X10^{-5}$

Table 4.12 Summary statistics: H2O δ-values – CONTROL GROUPS

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	3.081×10^{-3}	2	1.541×10^{-3}	111.424	**.000** (<.001)
Within Groups	3.733×10^{-4}	27	1.383×10^{-5}		
Total	3.454×10^{-3}	29			

Table 4.13 **Oneway ANOVA**: H_2O δ-values – CONTROL 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$-2.463x10^{-2}$*	**0.000** (<.001)
500MHz	80MHz	$1.502x10^{-2}$*	**0.000** (<.001)
200MHz	80MHz	$9.600x10^{-3}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.14 **Bonferroni Multiple Comparisons**: H_2O δ-values- CONTROL 30CH

Analysis of the OH δ- values using ANOVA showed inequality of variances (Table 4.16). Further analysis confirmed statistically significant differences between 500MHz and 200MHz ($p = 0.000 < 0.001$) [Table 4.15), and between 200MHz and 80MHz (P=0.000 <

0.001) [Table 4.16]. The null hypothesis was accepted for 500MHz versus 200MHz, where $\alpha \geq 0.05$ ($p = 0.073$) [Table 4.17].

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	4.772029	4.7928	4.764
S.D. (Sx)	0.002893016	0.001229273	0.012649111
Variance (Sx2)	$8.36954X10^{-6}$	$1.5111X10^{-6}$	0.00016

Table 4.15 Summary statistics: OH δ-values – Control groups

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	4.418×10^{-3}	2	2.209×10^{-3}	39.008	**.000** (<.001)
Within Groups	1.529×10^{-3}	27	5.663×10^{-5}		
Total	5.947×10^{-3}	29			

Table 4.16 **Oneway ANOVA**: OH δ-values – Lactose 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$-2.077x10^{-2}$*	**0.000** (<.001)
.500MHz	80MHz	$8.029x10^{-3}$	**0.073**
200MHz	80MHz	$2.880x10^{-2}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.17 **Bonferroni Multiple Comparisons**: OH δ-values- CONTROL 30CH

4.2.3 COMPARISON OF CHEMICAL SHIFTS IN PARALLEL POTENCIES OF SULPHUR AND CONTROL

A comparison of the CH_2, H_2O and OH values of Sulphur and Control was made at parallel frequency strengths. A statistically significant difference was found between the OH chemical shift values of Sulphur and control measured at 200MHz. The remainder of the tests revealed no differences between parallel frequency strengths.

4.2.3.1 THE CH_2 CHEMICAL SHIFTS

No difference was detected between the means of the CH_2 chemical shift values of Sulphur and Control when measured at 500MHz (Table 4.18), 200MHz (Table 4.19) and 80MHz (Table 4.20).

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	6.045	0.024	-1.714	18	**0.104**
Equal variances not assumed			-1.714	10.875	**0.115**
					p-value ≥ 0.05

Table 4.18 Independent Samples t-test: CH_2 δ-values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.233	0.635	-3.05	18	**0.764**
Equal variances not assumed			-3.05	17.580	**0.764**
					p-value≥ 0.05

Table 4.19 Independent Samples t-test: CH_2 δ-values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.000	1.000	0.000	18	**1.000**
Equal variances not assumed			0.000	18.000	**1.000**
					p-value≥ 0.05

Table 4.20 Independent Samples t-test: CH_2 δ-values – Sulphur vs Control at 80MHz

4.2.3.2 THE H_2O CHEMICAL SHIFTS

As was the case in the comparison of the means of the CH_2 chemical shift values, no differences between the Sulphur and Control were revealed when analysed at parallel frequency strengths (Tables 4.21 – 4.23).

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.221	0.644	1.608	18	**0.125**
Equal variances not assumed			1.608	17.806	**0.126**
					p-value ≥ 0.05

Table 4.21 Independent Samples t-test: H_2O δ-values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.887	0.359	-6.93	18	**0.497**
Equal variances not assumed			-6.93	15.08	**0.499**
					p-value ≥ 0.05

Table 4.22 Independent Samples t-test: H_2O δ-values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	4.327	0.052	-0.447	18	**0.660**
Equal variances not assumed			-0.447	13.235	**0.662**
					p-value ≥ 0.05

Table 4.23 Independent Samples t-test: H_2O δ-values – Sulphur vs Control at 80MHz

4.2.3.2 THE OH CHEMICAL SHIFTS

Statistical analysis of the OH chemical shift values revealed no differences between Sulphur and control at all three frequency strengths (Tables 4.24 - 4.26). The null hypothesis ($\alpha \geq 0.05$) was accepted in each case.

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	Df	Sig. (2-tailed)
Equal variances assumed	4.637	0.045	-1.494	18	**0.152**
Equal variances not assumed			-1.494	9.745	**0.167**
					p-value ≥ 0.05

Table 4.24 Independent Samples t-test: OH δ-values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	Df	Sig. (2-tailed)
Equal variances assumed	5.607	0.029	-2.027	18	**0.058**
Equal variances not assumed			-2.027	15.638	**0.060**
					p-value ≥ 0.05

Table 4.25 Independent Samples t-test: OH δ-values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	5.114	0.036	0.231	18	**0.82**
Equal variances not assumed			0.231	12.034	**0.821**
					p-value ≥ 0.05

Table 4.26 Independent Samples t-test: OH δ-values – Sulphur vs Control at 80MHz

Bar graphs constructed using the means of the chemical shift values of the CH_2, H_2O and OH signals (see figures 6-8, Appendix D) clearly illustrate the results obtained from the statistical analysis (see Tables 4.27 – 4.29).

	Results of statistical analyses		
	500MHz vs 200MHz	200MHz vs 80MHz	500MHz vs 80MHz
CH_2	*	*	*
H_2O	*	*	*
OH	*	*	*

* null hypothesis: rejected

Table 4.27 Intra-group comparison of the δ-values of SULPHUR 30CH

	Results of statistical analyses		
	500MHz vs 200MHz	200MHz vs 80MHz	500MHz vs 80MHz
CH_2	---	*	*
H_2O	*	*	*
OH	*	*	---

* null hypothesis: rejected
--- null hypothesis: accepted

Table 4.28 Intra-group comparison of the δ-values of the CONTROL

	Results of statistical analyses		
	500MHz	200MHz	80MHz
CH_2	---	---	---
H_2O	---	---	---
OH	---	*	---

* null hypothesis: rejected
--- null hypothesis: accepted

Table 4.29 Inter-group comparison of SULPHUR 30CH and CONTROL

The bar graphs reveal no observable trends when making intra- and inter-group comparisons using the mean values. For the intra-group comparisons the chemical shift values of the CH_2 and OH signals of both groups show the 200MHz readings to consistently be the highest, followed by 500MHz and 80 MHz. This trend is altered in the H_2O signal readings, where 200MHz remains highest, but the 80MHz is higher than the 500MHz reading.

When making inter-group comparisons using the mean values, it can be seen that the control readings from both the 500MHz and 200MHz instruments are slightly higher than the Sulphur for all three signals (ie CH_2, H_2O and OH), with the 200MHz reading of the CH_2 signal having near equal values for both the Sulphur and control. Similarly, when looking at the readings from the 80MHz instrument, it can be seen that the control reading is again slightly higher than Sulphur, but this is only evident at the H_2O signal. Both the CH_2 and OH signals for 80MHz show the control to have a slightly lower reading than the Sulphur.

4.3 COMPARISON OF THE INTEGRATION VALUES

4.3.1 INTEGRATION VALUES OF THE SULPHUR GROUPS

A comparison of the CH_2 means of lactose 30CH measured at 500MHz, 200MHz and 80MHz was made. The means of the three groups differ within the first and second decimal values. As was noted in the corresponding chemical shift value comparison, the standard deviation (S_x) and variance (S_x^2)values are consistently low throughout the samples.

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	2.003403	2.04507	1.981393
S.D. (S_x)	0.003746278	0.005197018	0.011409883
Variance (S_x^2)	$1.4346X10^{-5}$	$2.7009X10^{-5}$	0.000130185

Table 4.30 Summary statistics: CH_2 Integration values – SULPHUR 30CH

A statistically significant difference was found between all three groups when analysed using the Oneway Analysis of Variance (ANOVA) method (Table 4.31). Further analysis using the Bonferroni Multiple Comparisons Method (Table 4.32) revealed that these differences exist between the 200MHz and 80 MHz ($p = 0.000 < 0.001$) and between the 80MHz and 500MHz ($p = 0.000 < 0.001$). There were no differences between the 500MHz and 200MHz groups ($p = 1.000$).

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	3.23×10^{-3}	2	1.61×10^{-3}	30.612	.000 (<.001)
Within Groups	1.42×10^{-3}	27	5.27×10^{-5}		
Total	4.654×10^{-3}	29			

Table 4.31 **Oneway ANOVA**: CH_2 integration values – SULPHUR 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	**200MHz**	**0.000**	**1.000**
500MHz	80MHz	$2.201x10^{-2}$*	**0.000** (<.001)
200MHz	80MHz	$2.201x10^{-2}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.32 **Bonferroni Multiple Comparisons**: CH_2 integration values - SULPHUR 30CH

Analysis of the Sulphur H_2O groups revealed statistically significant differences between all three groups (Tables 4.33 -4.35).

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	1.391466	1.41655	1.336721
S.D. (S_x)	0.00502339	0.012655017	0.025901992
Variance (S_x^2)	$2.52344X10^{-5}$	0.000160149	0.000670913

Table 4.33 Summary statistics: H2O integration values – SULPHUR 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	3.333×10^{-2}	2	1.666×10^{-2}	58.384	**.000** (<.001)
Within Groups	7.707×10^{-3}	27	2.854×10^{-4}		
Total	4.104×10^{-2}	29			

Table 4.34 **Oneway ANOVA**: H_2O integration values – SULPHUR 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$2.508x10^{-2}$*	**0.008**
500MHz	80MHz	$5.475x10^{-2}$*	**0.000** (<.001)
200MHz	80MHz	$7.983x10^{-2}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.35 **Bonferroni Multiple Comparisons**: H_2O integration values - SULPHUR 30CH

Analysis of the Sulphur OH groups by means of ANOVA revealed statistically significant differences between all three groups (Table 4.37). Further analysis using Bonferroni showed that these differences were to be found between 500MHz and 200MHz (p=0.016) and 200MHz and 80MHz (p = 0.012). There was no difference between 500MHz and 80MHz (Table 4.38). The means differed within the second decimal place, and the standard deviation and variances were low (Table 4.36).

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	1.012016	1.02767	1.011486
S.D. (Sx)	0.002204244	0.010954659	0.016574254
Variance (Sx2)	$4.85869X10^{-6}$	0.000120005	0.000274706

Table 4.36 Summary statistics: OH integration values – SULPHUR 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	1.691×10^{-3}	2	8.454×10^{-4}	6.348	**.006** (<.001)
Within Groups	3.596×10^{-3}	27	1.332×10^{-4}		
Total	5.287×10^{-3}	29			

Table 4.37 **Oneway ANOVA**: OH integration values – SULPHUR 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$1.565x10^{-2}$*	**0.016**
500MHz	80MHz	$5.302x10^{-4}$	**1.000**
200MHz	80MHz	$1.618x10^{-2}$	**0.012**

*The mean difference is significant at the 0.05 level

Table 4.38 **Bonferroni Multiple Comparisons**: OH integration values - SULPHUR 30CH

4.3.2 INTEGRATION VALUES OF THE CONTROL GROUPS

Statistical analysis of the Control groups produced results that were largely similar to the Sulphur groups. Again, the standard deviation (S_x) and variances (S_x^2) were low throughout the samples. Results from both the Control CH_2 (Tables 4.39- 4.41) and Control H_2O groups (Tables 4.42 – 4.44) revealed statistically significant differences between all three groups ($p < 0.05$).

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	2.004365	2.047108	1.98803477
S.D. (Sx)	0.002709581	0.005714288	0.012475735
Variance (Sx2)	$7.34183X10^{-6}$	$3.26531X10^{-5}$	0.000155644

Table 4.39 Summary statistics: CH_2 integration values – CONTROL 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	1.861×10^{-2}	2	9.305×10^{-3}	142.694	**.000** (<.001)
Within Groups	1.761×10^{-3}	27	6.521×10^{-5}		
Total	2.037×10^{-2}	29			

Table 4.40 **Oneway ANOVA**: CH_2 integration values – CONTROL 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	4.274×10^{-2}*	**0.000** (<.001)
500MHz	80MHz	1.633×10^{-2}*	**0.000** (<.001)
200MHz	80MHz	5.907×10^{-2}*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.41 **Bonferroni Multiple Comparisons**: CH_2 integration values – CONTROL 30CH

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	1.3899492	1.42016	1.3488524
S.D. (S_x)	0.003453574	0.009040796	0.018378881
Variance (S_x^2)	1.19272×10^{-5}	8.1736×10^{-5}	0.000337783

Table 4.42 Summary statistics: H_2O integration values – CONTROL 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	2.562×10^{-2}	2	1.282×10^{-2}	89.077	**.000** (<.001)
Within Groups	3.883×10^{-3}	27	1.438×10^{-4}		
Total	2.950×10^{-2}	29			

Table 4.43 **Oneway ANOVA**: H_2O integration values -CONTROL 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	3.021×10^{-2}	**0.000** (<.001)
500MHz	80MHz	4.110×10^{-2}	**0.000** (<.001)
200MHz	80MHz	7.131×10^{-2}	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.44 **Bonferroni Multiple Comparisons**: H_2O integration values – CONTROL 30CH

When the OH means of the Control groups were analysed using Oneway ANOVA, statistically significant differences were found between all three groups (Table 4.46). Subsequent analysis using Multiple Comparison Bonferroni revealed statistically significant differences between two of the three groups, namely the 500MHz and

200MHz (p =0.016), and the 200MHz and 80MHz (p=0.000 < 0.001). No difference was found between the 500MHz and 80MHz groups (p=0.139) [Table 4.47], a result which is similar to that previously recorded in the CH_2 integration values analysis above.

	500MHz	200MHz	80MHz
Sample size	10	10	10
Mean (x)	1.011326	1.02559	1.001457
S.D. (S_x)	0.002134662	0.007756639	0.016439807
Variance (S_x^2)	$4.55678X10^{-6}$	$6.01654X10^{-5}$	0.000270267

Table 4.45 Summary statistics: OH integration values – CONTROL 30CH

Source of Variation	Sum of squares	DF	Mean Square	F	Sig.
Between Groups	2.944×10^{-3}	2	1.472×10^{-3}	13.184	**.000** (<.001)
Within Groups	3.015×10^{-3}	27	1.117×10^{-4}		
Total	5.959×10^{-3}	29			

Table 4.46 **Oneway ANOVA**: OH integration values -CONTROL 30CH

(I) ID	(J) ID	Mean Difference (I-J)	Sig.
500MHz	200MHz	$-1.426x10^{-2}$*	**0.016**
500MHz	80MHz	$9.869x10^{-3}$	**0.139**
200MHz	80MHz	$2.413x10^{-2}$*	**0.000** (<.001)

*The mean difference is significant at the 0.05 level

Table 4.47 **Bonferroni Multiple Comparisons**: OH integration values – CONTROL 30CH

4.3.3 COMPARISON OF INTEGRATION VALUES IN PARALLEL POTENCIES OF SULPHUR AND CONTROL

A comparison of the CH_2, H_2O and OH values of Sulphur and Contriol was made at parallel frequency strengths. No differences were found in any of the comparisons.

4.3.3.1 THE CH_2 INTEGRATION VALUES

The means of the CH_2 integration values of Sulphur and Control were analysed using the Independent samples t-Test. No differences were found between Sulphur and Control when measured at 500MHz (Table 4.48), 200MHz (Table 4.49) and 80MHz (Table 4.50)

	Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.543	0.471	-0.658	18	**0.519**
Equal variances not assumed			-0.658	16.393	**0.52**
					p ≥0.05

Table 4.48 Independent Samples t-test: CH_2 integration values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.029	0.866	-0.834	18	**0.415**
Equal variances not assumed			-0.834	17.84	**0.415**
					p ≥0.05

Table 4.49 Independent Samples t-test: CH_2 integration values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	**Sig. (2-tailed)**
Equal variances assumed	0.04	0.844	-1.242	18	**0.23**
Equal variances not assumed			-1.242	17.858	**0.23**
					p ≥0.05

Table 4.50 Independent Samples t-test: CH_2 integration values – Sulphur vs Control at 80MHz

4.3.3.2 THE H_2O INTEGRATION VALUES

When the means of the H_2O integration values were analysed using the Independent Samples t-Test, the null hypothesis ($p \geq 0.05$) was accepted in each case, and no differences were found at any of the three frequencies (Table 4.51 – 4.53).

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	1.579	0.225	0.787	18	**0.442**
Equal variances not assumed			0.787	15.954	**0.443**
					p ≥0.05

Table 4.51 Independent Samples t-test: H_2O integration values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.509	0.485	-0.734	18	**0.472**
Equal variances not assumed			-0.734	16.288	**0.473**
					p ≥0.05

Table 4.52 Independent Samples t-test:H_2O integration values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	1.51	0.235	-1.208	18	**0.243**
Equal variances not assumed			-1.208	16.23	**0.244**
					p ≥0.05

Table 4.53 Independent Samples t-test: H_2O integration values – Sulphur vs Control at 80MHz

4.3.3.3 THE OH INTEGRATION VALUES

As was the case for both the CH_2 and H_2O integration values, no differences were found between Sulphur and Control groups at any of the three frequencies. (Tables 4.54 – 4.56).

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.007	0.934	0.711	18	**0.486**
Equal variances not assumed			0.711	17.982	**0.486**
					p ≥ 0.05

Table 4.54 Independent Samples t-test: OH integration values – Sulphur vs Control at 500MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	1.195	0.289	0.49	18	**0.63**
Equal variances not assumed			0.49	16.212	**0.631**
					p≥ 0.05

Table 4.55 Independent Samples t-test: OH integration values – Sulphur vs Control at 200MHz

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
Equal variances assumed	0.077	0.785	1.359	18	**0.191**
Equal variances not assumed			1.359	17.999	**0.191**
					$p \geq 0.05$

Table 4.56 Independent Samples t-test: OH integration values – Sulphur vs Control at 80MHz

Bar graphs constructed using the means of the integration values of the CH_2, H_2O and OH signals (see figures 9-11, Appendix D)) illustrate the results obtained from the statistical analysis (see Tables 4.57 – 4.59).

	Results of statistical analyses		
	500MHz vs 200MHz	200MHz vs 80MHz	500MHz vs 80MHz
CH_2	---	*	*
H_2O	*	*	*
OH	*	*	---

* null hypothesis: rejected

--- null hypothesis: accepted

Table 4.57 Intra-group comparison of the integration values of SULPHUR 30CH

	Results of statistical analyses		
	500MHz vs 200MHz	200MHz vs 80MHz	500MHz vs 80MHz
CH_2	*	*	*
H_2O	*	*	*
OH	*	*	---

* null hypothesis: rejected

--- null hypothesis: accepted

Table 4.58 Intra-group comparison of the integration values of the CONTROL

	Results of statistical analyses		
	500MHz	200MHz	80MHz
CH_2	---	---	---
H_2O	---	---	---
OH	---	---	---

--- null hypothesis: accepted

Table 4.59. Inter-group comparison of SULPHUR 30CH and CONTROL

The bar graphs reveal few observable trends when making intra- and inter-group comparisons using the mean integration values. When making intra-group comparisons it is observed that in both the Sulphur and Control groups the 200MHz readings are consistently highest, followed by the 500MHz and then the 200MHz readings. This trend was partly observed in the chemical shift values of both groups (see 4.2.3.3).

Inter-group comparisons reveal mixed trends. The Control values at 500MHz, 200MHz and 80MHz are all slightly higher than those of at the CH_2 signal. This trend is completely reversed within the OH group, where the Sulphur values are all slightly higher than the Control for all three frequency values. The H_2O group shows a mix of these two trends, with the Sulphur values being slightly higher than the Control at 500MHz, and slightly lower at both 200MHz and 80MHz.

CHAPTER FIVE: DISCUSSION

The results of this investigation clearly indicate differences between the spectra derived at the three chosen spectrometer frequencies for both the Sulphur and Control groups respectively. Comparison of the spectra at parallel frequency strengths, however, revealed no differences between the Sulphur and Control.

5.1 CHEMICAL SHIFT VALUES

The results within the Sulphur group indicated statistically significant differences between all three frequencies for respective CH_2, H_2O and OH values. The Lactose control group also revealed statistically significant differences between all three frequencies for respective CH_2, H_2O and OH signals, bar two results, which will be discussed shortly. According to NMR theory, the ppm scale of the chemical shift is a 'dimensionless' scale, calculated from a suitable reference compound and therefore independent of spectrometer frequency (Günther, 1980:17). The chemical shift values for both the Sulphur and Control groups should thus, theoretically, be the same regardless of the applied frequency utilized. The results obtained are therefore unexpected within the normal NMR experiment, although they do satisfy the first two hypotheses made within this study (see 1.2.1 and 1.2.2).

The chemical shift reflects the molecular environment of the nucleus, and denotes differences in electron density, types of nuclei and types of bonds present (See 2.10.2). A change in chemical shift therefore reflects a change in the structural configuration of the samples. It is assumed that the Sulphur samples used at each instrument were physico-chemically identical prior to their measurement, having each been originally drawn from a single container of the final prepared potency (see 3.2). This assumption holds true for the Control group samples as well. The differences in chemical shift values are therefore unlikely to be a reflection of physico-chemical differences present prior to measurement, but are rather a result of an aspect of the analytical procedure.

With regards to the analytical procedure, it is known that the chemical shift varies only with varied solvents, sample concentration and temperature (Abraham and Loftus, 1980:13), the former two of which do not apply in this study. In addition to this it has been stated that the ppm scale is only applicable in cases where the sample itself does not change with field strength (Jackson, 2002). Thus two likely explanations present themselves: the significant results were due to the NMR radiofrequency modulating the sample during its measurement, or that the significant results obtained were due to variations in temperature between experiments. The effect of these and other factors to the results obtained will be discussed in further detail.

The groups which showed no difference in chemical shift values also require mentioning before the discussion continues. No difference in chemical shift values was found in only two instances, both of which occurred within the Control group of the study. The results were as follows:

- CH_2 signal: No difference found between 500MHz and 200MHz
- OH signal: No difference found between 500MHz and 80MHz.

This could indicate stability of both the CH_2 and OH signals by way of different processes. Past NMR research hypothesises that, of the various components of the ethanol molecule, structural changes are most likely to be taking place at the OH pole (and more specifically the H_2O-OH interaction) during potentisation [(Smith and Boericke (1966), Weingärtner (1990)]. This explains how the OH pole of the molecule, which is usually relatively unstable (Jackson, 2001), could become stabilized in some manner during potentisation. The stability of the CH_2 pole could be a result of it having a very minor role in the physico-chemical changes induced during potentisation. Of course, all hypotheses are meaningless without a clear explanation for the cause of the results, thus attention will be turned to the discussion of such matters.

It has been mentioned that the reasons for the unusual results obtained have been narrowed down to electromagnetic or temperature effects during the NMR experiment. One of these factors must explain how the intra-group comparisons revealed mostly statistically significant differences, whilst the inter-group comparisons of Sulphur and

Control groups at parallel frequencies revealed no differences at all. Also, there needs to be some explanation as to how or why the 200MHz readings are consistently the highest of the three instruments used, as well as the lack of an obvious trend in the results obtained.

Temperature has a definite influence during the NMR experiment. Changes in temperature of the sample will alter the Boltzmann distribution of spins in the various energy levels (Becker, 1980). Thus temperature control is imperative during the NMR experiment. With regards to the NMR instruments the 500MHz is claimed consistent within 0,1 ^{0}C, whilst the older two instruments, the 200MHz and 80 MHz, have consistency within 0,2^{0}C – 0,5^{0}C (Watson, 2002). When examining the data it can be seen that there are very low standard deviations and variances within the samples of each group (see chapter 4). Thus it can be confirmed that the temperature control was well-maintained at each instrument. The problem arises when trying to determine the temperature equality of the instruments relative to each other. Temperature calibrations performed subsequent to the study provided chemical shift values for the ethanol molecule for every 1^{0}C shift in temperature. It is possible for very small chemical shift changes to occur with slight changes in temperature. Thus, hypothetically, there may have been 0,2^{0}C difference between one instrument and another, which would have created small differences in the chemical shift values (CH_2: +0,00002 , OH: -0.005) (Watson, 2002). The problem is that it is impossible to know at which instrument the temperature difference is present, nor by what value it is different. Investigations of temperature effects during the NMR experiment have been ultimately problematic. It is a difficulty in NMR, and this study in particular, that all results are relative, and that there are no absolute values with which to work (Watson, 2002). This created much difficulty in pin-pointing specific temperature influences during the NMR experiment.

The discussion now moves to the possible influence of the NMR instrument itself on the results obtained. The instrument has several areas where electromagnetic influences are exerted, firstly the standing magnetic field of the elctromagnet, secondly the shim coils

(discussed later) and thirdly the applied radiofrequency. The field strengths of the magnets are as follows:

- 500MHz – 11.9 Tesla
- 200MHz – 4.7 Tesla
- 80MHz – 1.9 Tesla

The initial assessment of the results was that the frequency strength modulated the sample structure during resonance, thereby shifting or 're-calibrating' the resonance frequencies of the samples in a subtle yet particular manner for each frequency. This is a feasible idea if one considers the 'coherent domains' theory of Del Guidice and Preparata (Bellavite and Signorini, 1995:249,. Del Guidice 1994). This theory hypothesizes the existence of stable domains in the solvent which vibrate at a coherent frequency reflective of the frequencies of the solute particles dissolved during potentisation. These domains communicate throughout the solution by way of their frequencies, and thereby maintain the structural changes taking place during potentisation. The applied radiofrequency of each instrument could possibly interact with these domains during resonance and somehow alter the domain frequency as a result. This could then explain the lack of differences between the Sulphur and Control groups, as both groups would have been modulated in the same way by each instrument. This is not such a strange idea when one considers the phenomenon of resonance.

The property possessed by a wave that distinguishes it from any other physical phenomenon is its ability to form interference and diffraction patterns. When different parts of a wave are recombined after traveling different distances they reinforce each other or cancel each other out depending on whether the two path lengths differ by an even or odd number of wavelengths. When two frequencies reinforce each other an amplification of that frequency wave results (Rae, 1990:7). Thus, in nature, huge concrete bridges begin to ripple in an earthquake of the same frequency, and a wine glass shatters at some distance from an opera singer singing the note that resonates with the natural frequency of that glass. It was therefore assumed by the investigator that the resonance effects induced during the NMR experiment would somehow destroy the delicate

structural balance present within the sample in a similar manner. There are several problems with this theory, however, all of which will be highlighted for the purposes of attempting to find some answers, if not in this study then in further studies of similar nature.

The first problem appears when examining the trends of the data. When analyzing the graphs (see chapter 4) it can be seen that the 200MHz readings are consistently highest, followed by 500MHz and 80 MHz. This trend is altered in the H_2O signal readings, where 200MHz remains highest, but the 80MHz is higher than the 500MHz reading. There is an apparent lack of trend in the results, and intra-group comparisons of the Sulphur and Lactose at parallel frequency strengths also reflects this (see 4.2.3.2). The higher 200MHz readings also seem unexpected, as one would expect frequency effects to be present in a linear fashion, with increased effects with increased frequency or vice versa. It may be hypothesized that the 200MHz is significant in terms of resonance effects, and that it causes increased de-shielding of the nucleus by some mechanism. However, the shadow of doubt cast by the problem with temperature takes the force out of any assumptions being made.

A further problem with the results is that they do not correlate with the findings of past NMR research. Past research studies, for the most part, obtained significant differences between the Treatment and Control groups, and this was using varying NMR instruments, potency levels and potency preparation methods (see 2.11). Although it is easy to assume some fault in the manner in which the study was conducted, it must be noted that the research guidelines provided by Schulte (1994:172) were strictly adhered to, and much attention was placed on accuracy of sample manufacture and analysis (see chapter 3). In addition to this, it is worth noting that it has been said that poor reproducibility of results in homoeopathic studies may be explainable in terms of intrinsically chaotic mechanisms occurring in the process of information transfer (Bellavite and Signorini, 1995:290).
Chaos theory may provide a mathematical model to demonstrate the principle of self-similarity in homoeopathy (Garner and Hock,1991), and if this theory proves significant it can be expected that minimal variations in the conditions of the experiment will express

themselves in the form of substantial variations in the results. Factors possibly contributing to the variations noted in this study are highlighted towards the end of this chapter.

If the possible influence of chaotic mechanisms is ignored, it appears that the results obtained by other investigators, mentioned above, immediately favour the argument that the frequency of the instrument does not modulate the sample as it measures it, or they too would have obtained no differences between treatment and control. Further contemplation of this matter has led to the consideration that other interactions exist which are unaffected during proton resonance. It may be possible that the proton resonance which occurs within the sample during the NMR experiment does not interfere with other aspects of electromagnetic stability of the remedy. Lessell's (1994:44-46) 'sub-molecular' theory looks to the *electron* configurations as providing the frequency information of the particles. The theory states that electrons which have not been fully promoted to a higher orbital during succussion, but which are partially promoted, contribute to the production and maintenance of an electromagnetic dynamic field. This field is of lower frequency and is stated to be in the kHz range (Lessell, 1994:68). Thus the coherent oscillation of the solution is a result of electron movement. Del Guidice's (1994) coherent domain theory describes the low frequency of these coherent oscillations, and that they are not disturbed by the higher frequencies of single molecules in the solution. It may be possible that the applied frequencies in NMR, despite causing proton resonance, do not in fact interfere with the coherent domains, as the domains are oscillating at a different frequency. The frequencies applied by the instrument are perhaps much higher than the coherent domain frequencies, and therefore resonance is not induced. It is worthwhile mentioning again that the principal frequency for Phosphorus 6x was found to be 300Hz, and that of Arnica 1000x to be 9.725kHz (Lessell 1994:24)(see 2.8). Thus, although NMR may be providing some information regarding the relationship of protons to each other, it provides no information as to the more subtle electromagnetic interactions, essential to the structure and function of the potency, occurring microscopically within the sample. This conforms with Resch and Gutmann's (1991) 'supermolecular'view of potentised remedies, where oscillating effects between

various components of the system, molecules and gas bubbles alike, contribute to its overall information content.

Although this hypothesis does not provide an explanation of the results, it is quite an exciting prospect and is worth being considered in further studies of Homoeopathic potencies using NMR.

5.2 INTEGRATION VALUES

Similar results to those of the chemical shift values were obtained when analyzing the integration values. The integration values of the Sulphur group showed statistically significant differences between frequencies for all the signals except two. The CH_2 signal showed no difference between the 500MHz and 200MHz readings, and the OH signal showed no difference between the 500MHz and 80MHz instruments. Lastly, the Lactose group showed no difference at the OH signal between 500MHz and 80MHz. The rest of the Lactose group showed statistically significant differences throughout.

The integration values indicate the number of protons giving rise to the signal. It is worth noting that only the relative number of protons can be determined by integration, and in this case the values were calculated relative to the CH_3 signal which was set at a value of 3 (Watson, 2001). The result of such a calculation is that the values with which one is working are not absolute values, and this makes interpretation of the data difficult. An additional factor is that, when determining the integration values, it is expected that the margin of error be within 5% [(Gunther 1980:21) (Watson, 2001)]. Since the statistical analysis is working with a 95% confidence interval, it can be hypothesised that the integration values will most likely be statistically significant, before any analysis has taken place.

Jackson (2002) states that it is the chemical shift and relaxation times which are most likely to change between instruments, not the integration values. The important factor with integration values is their ratio to each other, and not the numerical value obtained.

If the ratios are not consistent then a fault in the instrument is implied. The data shows that, despite slight numerical differences, the ratios of CH_3:CH_2:H_2O:OH are all 3:2:1:1 at each instrument and for each group. Thus it seems that no major influence on the integration values has occurred, and the statistically significant results can be largely attributed to the 5% error already mentioned.

Having said this it is interesting to note the almost identical integration values between the Sulphur and Control groups within each frequency strength, but not between instruments. This again suggests some uniformity of influence at each frequency strength, although this cannot be confirmed from the total results obtained.

This set of results was found to be difficult to interpret, due to the narrow range of comparisons used. The addition of higher potencies for comparison may render clearer distinctions between a homoeopathic potentised substance and a control. Ross's (1997) study showed greater differences at the highest potency (LM10) compared to the LM6 and LM2 potencies, and Davies' (2001) results were more significant at the 200C than the 30C and 9C. Thus the employment of higher potencies may provide more distinctive results, as well as aid in establishing clearer trends. It must be mentioned that the 30CH potency chosen for this study was deemed appropriate for two reasons. Firstly the sufficient number of published studies which all obtained some observable difference using this potency, or comparable and lower potencies [Smith and Boericke (1968)[*60x*], Sacks (1983)[*30C*], Weingärtner (1990)[*23x*], Davies (2001)[*30CH*], and secondly to compare, to some degree, the results obtained with those studies.

Further consideration should be given to the effects of the dilution scale on the development of the system organization of the potencies during potentisation. The dilution scale has been mentioned as having an effect on the imprinting dynamics (Lessell, 1994:75). The volumes used at each dilution level need also to be considered here. According to the model of Resch and Gutmann (1994: 327), the presence of fewer solute molecules at each dilution stage allows for greater development and imprinting of the solute information by the system. Thus the LM scale, which is a deconcentration of

1/50 000 could possibly produce more distinctive changes at each dilution level compared to the 1/100 deconcentration employed for CH potencies due to increased solvent to solute ratio. Resch and Gutmann (1994: 327) go on to describe how, as dilution increases, there is diminished mutual interaction by the structure breakers and better development of the individual properties of the solute molecules. There is also a greater development of static conditions, and thereby integrity of the solution. In this way it may be possible that a smaller drop allows greater development of the solute information at each dilution level. In this study larger volumes were used at each dilution level (0,15ml : 14,85ml), which may have affected the overall structural configuration at 30CH versus a 30CH that was made in other studies, or using smaller volumes [e.g 0,03ml : 2,97ml (Davies, 2001)].

The presence and effect of lactose during potentisation is an aspect of this type of study which requires further attention. Resch and Gutmann (1987: 274-284) describe how grinding of the solute in ordinary lactose facilitates modification of the lactose in such a manner as to incorporate the characteristics of the solute into its structure. The system organization is strengthened through the dilution and grinding process, and is increasingly able to impose its own aspects of order onto the pure, 'unstructured' lactose diluent at each trituration level. The authors state that the total information content does not depend on the presence of certain molecules, but rather on the oscillating pattern of the whole solution, as shaped by the solute molecules originally present (see 2.6). This suggests that the Lactose Control used in this study, which would usually be considered as inert, has a more developed system organization than ordinary untriturated lactose, and is thereby 'active' to a certain degree during the potentisation process. The effects of triturating the drug substance in lactose, versus dilution only, at the initial stages of potentisation remains unknown. Thus the effect of triturated lactose on the structural organization in liquid homoeopathic potencies, and thereby the NMR spectra of these potencies, is not certain. This fact contributes to difficulties in interpretation of the results. A better comparison between samples might be obtained if a third reference is used which does not contain lactose, such as ethanol. The ethanol control will have none

of the suggested structural developments described above, which may aid in obtaining clearer results.

Further aspects of the NMR experiment, previously unexamined, have come to light during this study. The finer aspects of the NMR experiment are taken into consideration here. Each experiment requires certain parameters to be set, such as acquisition time, delay time, pulse width etc. Different parameters were required for each of the three instruments employed in this study, all of which have been accurately detailed for the purposes of reproduction of this study (see Appendix C). In hindsight it was noted that the samples were subjected to the slightly differing conditions for each instrument (beyond the expected difference in the operating frequency), and differences were noted as follows:

- The 80MHz had a longer acquisition time, and was subjected to the applied frequency for a longer period (±1 second) than the other two groups.
- The number of data points collected also differed between samples (80MHz had the lowest number and 500MHz the highest number), thereby varying the accuracy of data between instrument readings (a higher number of data points provides more detailed spectra).

Thus the 80MHz instrument provided less refined spectra compared to the 200MHz and 500MHz (see Appendix D).

In conventional NMR experiments the acquisition time, digital resolution, sweep width and number of data points are all related. Different spectrometers will not have the same pulse length and acquisition time (Jackson, 2001). Unfortunately this fact has contributed to difficulty in interpreting of results, the specifics of which have are noted above.

In addition to these factors the shimming process requires further consideration. Several small shim coils are present in the probe of the NMR instrument, each providing its own current, and thereby a local electromagnetic field. The operator can adjust the current in individual shim coils in order to modulate the static field B_0 and maintain a homogenous

field around the sample. A homogenous static field B_0 provides more accurate results, thus the important role of the shimming process (Watson, 2001). The notably low standard deviation and variances within each group suggest that the structure remained unaffected by the varied shim processes per sample. The problem relating to this study, however, is that the shimming process varies with each sample, thus introducing varied electromagnetic effects into the experiment beyond that of the applied frequency.

It has also been stated that the absence of an absolute energy scale makes the comparison of nuclear magnetic resonance spectra difficult if agreement cannot be reached upon a universal reference (Gunther, 1980:18). This has implications for further NMR studies in homoeopathy, as investigators must ensure that the same reference is used as in other NMR experiments.

It has therefore been discovered that the model for the one dimensional NMR experiment is such that there are several problems which present themselves when attempting to compare results obtained from different instruments. Standardisation in this field is certainly not a simple matter, and many further tests need to be conducted to attempt to find out what influences the more subtle aspects of the NMR experiment, such as the static field strength of the instrument, and the shimming and acquisition times, have on the results obtained. These aspects of the NMR experiment relating to homoeopathic research need to be addressed, and hopefully the problem can be overcome in some way. If not, the question of the effect of frequency (specifically the NMR spectrometer's frequency) on homoeopathic dilutions needs to be addressed in a different manner, the suggestions for which follow shortly.

As a final point of discussion the problem of matter and its measurement is highlighted. Quantum theory describes an undivided whole, a continuum within which all observable matter arises as a result of discontinuities within this continuum (Resch and Guttmann, 1987:75). Quantum mechanics describes particles within this continuum as possessing the property of 'wave-particle duality' (Rae, 1990:8). Wave and particle properties are

complementary rather than contradictory properties, which means that they can never actually be observed simultaneously. An electron, for example, will be observed by the investigator as having only wave or only photon properties, depending on how the experiment is set up (Rae, 1990:11). Such limitations inherent in any experimental set up should be kept in mind. The instrument will measure only one aspect of a whole interaction. This is confirmed by Heisenberg's uncertainty principle, which states that it is impossible to determine, with infinite precision, the velocity (or any related property such as energy or momentum) and position of a particle simultaneously (Resch and Guttmann, 1987:75). By measuring one aspect the other is automatically altered or masked.

Thus it can be seen that the experimental process used in this study is not able to observe the system in its totality. Resch and Guttmann (1991) maintain that the inclusion of continuous relationships between molecules is required when attempting to understand the qualitative changes involved during the potentisation process. This concept is thoroughly extended and supported by Einstein's theory of relativity which proposes that, ultimately, the entire universe has to be understood as a single, undivided whole, in which analysis into separately and independently existent parts has no fundamental status (Resch and Guttmann, 1987:108). Such radical abstraction is not useful when attempting to obtain quantitative information about a system, but it serves to highlight the inherent problem of measurement, which is the alteration of a system being measured, through the very act of measuring it! Perhaps it was such understanding which led David Bohm to state that '... the original and creative insight within the whole field of measure is the action of the immeasurable' (Resch and Guttmann, 1987:271).

These insights aid in understanding the difficulties inherent in any attempt to scientifically measure homoeopathic potencies. Difficulties in interpretation of NMR studies on homoeopathic potencies have been mentioned by Bol (1997) and Schulte (1999), and this study certainly supports the views of these authors.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

It is not evident from the results whether frequency strength is a factor to be considered when conducting NMR spectroscopy on homoeopathic potencies. The Oneway ANOVA analyses of both Sulphur and the control showed statistically significant differences in both the chemical shift and integration values between the three frequency strengths employed. These differences satisfied the first two hypotheses of the study, and led to the initial hypothesis that a varied frequency strength does indeed influence the results obtained. The t-Tests showed that no differences existed between the Sulphur 30CH and the Lactose control at all three frequency strengths. This made confirmation of the original hypothesis very difficult, but did prompt the consideration of influential factors previously unconsidered in studies of this nature.

In chapter one it was hypothesised that differences between the Sulphur and Lactose control would be present, and that these differences would be more noticeable at the lower frequency range. This hypothesis, based on the assumption that a higher frequency strength would have more intense resonance effects on the structure of the homoeopathic potency, was not confirmed in this study. It is possible that the applied radiofrequency may not affect the structure due to differing resonance frequencies between the spectrometer and potency particles (other than protons). The resonance occurring during the NMR analysis might not affect the electromagnetic field interactions that maintain the microstructural arrangement of the remedy. This hypothesis is supported by the coherent domains theory of Del Guidice and Preperata. The absence of differences between Sulphur and Control also prohibited assessment of an appropriate frequency strength to be used when conducting NMR studies on homoeopathic potencies.

The effects of the applied radiofrequency remain undetermined at this point, and further studies regarding this factor are worthwhile and necessary. The role of temperature on the

varied chemical shift values was also difficult to determine exactly, and this problem requires further attention.

The results of this study have stimulated further evaluation of the usefulness of NMR spectroscopy as a tool of analysis in homoeopathic research. The difficulties in interpretation of NMR spectra of homoeopathic potencies mentioned by Bol (1997) and Schulte (1999) have been confirmed, and possible causes for these difficulties have been highlighted. This study has also confirmed the views of Demangeat and Poitevin (2001) that there is a need for standardization in NMR research on homoeopathic potencies, and this must extend from the initial stage of potency manufacture through to the final stage of analysis. Extensive investigations need to be completed in order to determine the reliability and usefulness of NMR spectroscopy as a method of analysis of homoeopathic potencies.

The interaction of the NMR radiofrequency with the physico-chemical structure of homoeopathic potencies remains undetermined, but cannot be ignored. Results are such that there are enough questions to warrant further investigation into this matter. Many questions have arisen during the study and it is hoped that future research will attempt to find the much needed answers in this field of research.

6.2 RECOMMENDATIONS

In their appraisal of Homoeopathy, Bellavite and Signorini (1995 :302) describe how the theoretical and experimental basis on which homoeopathy rests is indeed gaining ground. The vast body of experimental evidence available is highlighting, with increased consistency, the convergence between Homoeopathy and the fields of immunology, biology and physics. Investigations into the nature and mechanisms of homoeopathic potencies have created new insights regarding matter and life, and possible explanations are to be found particularly in the fields of quantum physics and mathematical theories (Bellavite and Signorini, 1995: 292). The fields of research open to investigators are therefore extremely broad-ranging, and all contributions are necessary and useful. The way forward is through co-operation and collaboration between investigators of the various disciplines involved. This is essential for continued appraisal of current theories, as well as the establishment of new experimental methods and theories and in this field of study.

The following recommendations will aid the search for accurate and meaningful results when using NMR spectroscopy in the analysis of homoeopathic potencies:

1. An increased range of potencies

This study should be repeated using the 30CH potency, and extended to include the 200CH and 1M potency levels. It is hypothesized that the NMR spectra of higher potencies will show differences between Sulphur and Control, and thereby provide clearer information regarding the possible effects of the applied radiofrequency on the samples.

2. The inclusion of a lactose-free control

The problem of lactose was described in Chapter 5, and it is recommended that further studies include a lactose-free control, such as ethanol. The ethanol would be the same percentage as the treatment potencies, and both succussed and unsuccussed samples could be utilised. These may prove useful comparisons once all the tests have been completed.

3. The use of modern instruments for analyses

Modern instruments, built with superior technology, will provide accurate and reliable data for further studies. The problem of temperature control encountered in this study will be minimized, and the chemical shift and integration values will be accurately recorded. Unfortunately, conventional chemistry and physics laboratories are using the higher frequency spectrometers due to their highly penetrative nature, and this has led to phasing out of older, lower frequency instruments (Watson 2002). Follow-on studies attempting to test a range of frequencies may be hindered somewhat by this factor.

4. Re-evaluation of the medium and frequency of NMR spectroscopy

Further studies must be done regarding the relevance of the applied radiofrequency strength when conducting NMR studies on homoeopathic potencies. The recommendations found in this chapter are to be noted regarding this matter. The influence of the following factors, relating to the NMR experiment, also require further investigation:

- Static magnetic field strength
- Schimming process
- Acquisition time

Further studies must be done in order to determine whether these factors influence the homoeopathic samples during the analytical procedure.

5. Standardisation of NMR procedures

One dimensional NMR studies, and studies investigating relaxation times are two very different processes requiring different sets of guidelines. As far as one dimensional studies are concerned, it is necessary that all further studies include very detailed reports of potency preparation, samples analysis, and spectrometer parameters used in order that the studies be reproducible. A multitude of further studies is demanded in order for a standard protocol for NMR spectroscopy of homoeopathic potencies to emerge.

6. The use of 'non-invasive' methods to investigate the effects of frequency

It has been observed in this study that direct comparison of the spectra with each other does not clearly show the presence of any changes that may have taken place, as all samples have been subjected to the same changes. The spectra are difficult to interpret if they are the only source of information. In order to obtain more meaningful results the samples would need to be assessed by means of a second, more objective, means. Samples which have not been analysed by an NMR instrument can thus be included as comparisons in the study.

7. Repetition of experiments

If chaos and complexity play a role in the dynamics of potentisation, it is necessary for all experiments to be repeated several times. Bellavite and Signorini (1995:290) explain how the same phenomena will not be observed in the repetition all experiments performed, and that there has to be a certain type of reproducibility present, whereby a particular result must be obtained in a significant number of the overall cases. Time and funding is necessary in order to do this.

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APPENDIX A

TABLE OF NMR SPECTROMETERS USED IN PUBLISHED RESEARCH TO DATE

This list includes only those authors referenced in the 'literature review'.

NAME OF RESEARCHER(S)	SPECTROMETER MODEL	OPERATING FREQUENCY
Smith and Boericke (1968)	Varian 60HR	unknown*
Sacks (1983)	Hitachi Perkin Elmer R-24	unknown*
Weingartner (1990)	Bruker AM300	unknown*
Demangeat *et al.*(1992)	Minispec Bruker PC104	4 MHz
Ross (1997)	Varian VXR200	200.057 MHz
Aabel *et al.* (2001)	Varian XL300	299.944 MHz
Aabel *et al.* (2001)	Bruker Advance DRX 500	500.13 MHz
Aabel *et al.* (2001)	Minispec PC 120-b	20 MHz
Troy (2001)	Varian Inova 500	499.982 MHz

* Operating frequency not stated in journal article

APPENDIX B – THE PREPARATION OF POTENCIES

i) AIM: To produce a 3CH trituration by hand of flowers of Sulphur

APPARATUS:

Unglazed pestle and mortar
96% alcohol (for flaming)
cigarette lighter
steel spatula
pure lactose powder
flowers of Sulphur
mass balance (accurate and calibrated)
filter paper
sterilized 20ml amber glass bottles
labels

METHOD:

1) Clean the working surface with 73% ethanol.
2) Clean the mortar and pestle with distilled water, flame with 96% alcohol.
3) Flame the spatula.
4) Allow mortar and pestle to cool sufficiently before use.
5) Place a piece of filter paper on the scale and tare it. Mass 0.1g of flowers of Sulphur.
6) Place a new piece of filter paper on the scale and tare it. Mass 3,3g of pure lactose powder.
7) Repeat step 6 twice more. (Total lactose powder mass: 3 x 3,3g = 9.9g, therefore drug substance to vehicle ratio = 0,1g : 9,9g = 1: 99)
8) Place 3,3g lactose into mortar and triturate for a short period.
9) Add the 0,1g crude Sulphur into the mortar.
10) Triturate for 6 minutes and scrape down for 4 minutes with a porcelain spatula. Triturate for six minutes and scrape down for

4 minutes again. (Total time: 2 x 10min = 20min)

11) Add the second portion of 3,3g of lactose powder and continue as in step (10) above.

12) Finally add the third portion of 3,3g of lactose and proceed as in step (10) above. (Total trituration time: 20min x 3 = 60min [minimum]). Place triturate in a 20ml amber bottle, secure the lid tightly, and label as Sulphur 1CH.

13) Repeat steps 1-12 when preparing Sulphur 2CH and 3CH replacing crude Sulphur with Sulphur 1CH and 2CH respectively at each dilution level.

B ii) AIM: To produce liquid dilutions of Sulphur 30CH and from the 4CH trituration.

APPARATUS:

50ml amber glass screw top bottle
20ml amber glass screw top bottles
30%, 43% and 87% ethanol
distilled water
pasteur pipettes
rubber dropper bulbs
Sulphur 3CH triturate
mass balance (accurate and calibrated)
filter paper
micropipette
pipette tips

METHOD:

1) Clean all apparatus.
2) Place a piece of filter paper on the scale and tare it. Mass 0,1g of Sulphur 3CH and place it in a 20 ml amber glass bottle.

3) Add 9,9ml of aqua distillata and succuss 10 times without stopping. Label as Sulphur 4CH.
4) Place 14,85ml 30% ethanol in a 20ml amber glass bottle and add 0,15ml Sulphur 4CH. Succuss 10 times without stopping. Label as Sulphur 5CH.
5) Repeat step (4) to prepare Sulphur 6CH – Sulphur 9CH from the Sulphur 5CH.
6) Place 14,85ml 87% ethanol in a 20ml amber glass bottle and add 0,15ml Sulphur 9CH. Succuss 10 times without stopping. Label as Sulphur 10CH.
7) To prepare Sulphur 11CH - 29CH repeat step (6), adding 0,15ml of the previous potency to 14,85ml 87%ethanol at each dilution level.
8) 30CH place 35,64ml 87% ethanol in a 50ml amber glass reagent bottle. Add 0,36ml Sulphur 29CH. Succuss 10 times without stopping. Label as Sulphur 30CH.
9) Divide the final amount into three equal volumes of 10ml each and place in three separate 20ml amber bottles.
10) Have the appointed person place the appropriate labels on the samples.

B iii) AIM: To prepare the control, Lactose 30CH

APPARATUS:
50ml amber glass screw top bottle
20ml amber glass screw top bottles
30%, 43% and 87% ethanol
distilled water
pasteur pipettes
rubber dropper bulbs
Lactose 3CH triturate
mass balance (accurate and calibrated)
filter paper
micropipette
pipette tips

METHOD:

1) Clean all apparatus.
2) Place a piece of filter paper on the scale and tare it. Mass 0,1g of Lactose 3CH and place it in a 20 ml amber glass bottle.
3) Add 9,9ml of aqua distillata and succuss 10 times without stopping. Label as Lactose 4CH.
4) Place 14,85ml 30% ethanol in a 20ml amber glass bottle and add 0,15ml Lactose 4CH. Succuss 10 times without stopping. Label as Lactose 5CH.
5) Repeat step (4) to prepare Lactose 6CH – Lactose 9CH from the Lactose 5CH.
6) Place 14,85ml 87% ethanol in a 20ml amber glass bottle and add 0,15ml Sulphur 9CH. Succuss 10 times without stopping. Label as Lactose 10CH.
7) To prepare Lactose 11CH - 29CH repeat step (6), adding 0,15ml of the previous potency to 14,85ml 87%ethanol at each dilution level.
8) 30CH place 35,64ml 87% ethanol in a 50ml amber glass reagent bottle. Add 0,36ml Lactose 29CH. Succuss 10 times without stopping. Label as Lactose 30CH.
9) Divide the final amount into three equal volumes of 10ml each and place in three separate 20ml amber bottles.
10) Have the appointed person place the appropriate labels on the samples.

APPENDIX C : NMR PARAMETERS EMPLOYED FOR ANALYSES OF SAMPLES

PARAMETERS		INSTRUMENT		
		80MHz	**200MHz**	**500MHz**
sfrq	spectrometer frequency	79.542MHz	199.975MHz	499.983MHz
tn	transmitter nucleus	H1	H1	H1
at	acquisition time	4.095s	2.968s	3.000s
np	number of data points collected	8 192	65 536	131 072
sw	spectral width	1000Hz	1595.7Hz	3 590.7 Hz
fb	filter bandwidth	1000	1500	not used
bs	blocksize	-	64	32
ss	steady state	not used	not used	not used
tpwr	transmitter power	fixed	26	54
pw	pulse width	17 deg. (3 us)	14 deg. (4 us)	16deg. (2us)
p1	second pulse in sequence	not used	not used	not used
d1	first delay in sequence	4s	4s	4s
d2	second delay in sequence	not used	not used	not used
tof	transmitter offset	50Hz	572.3Hz	1211.1Hz
nt	number of transients (scans)	4	4	4
ct	number of transients collected	4	4	0

APPENDIX D: BAR GRAPHS

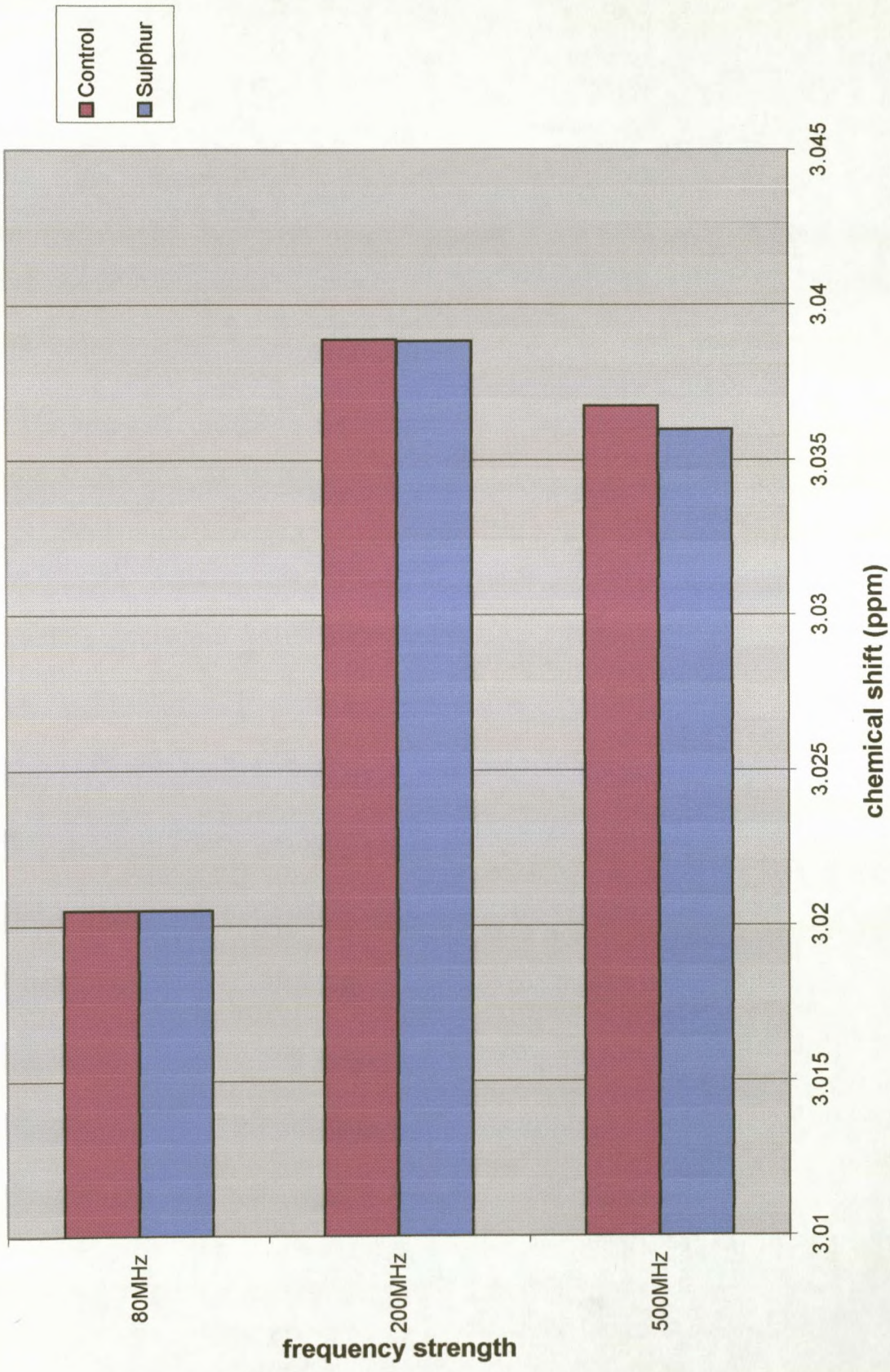


figure 6. CH_2 chemical shift values

figure 7. **H_2O chemical shift values**

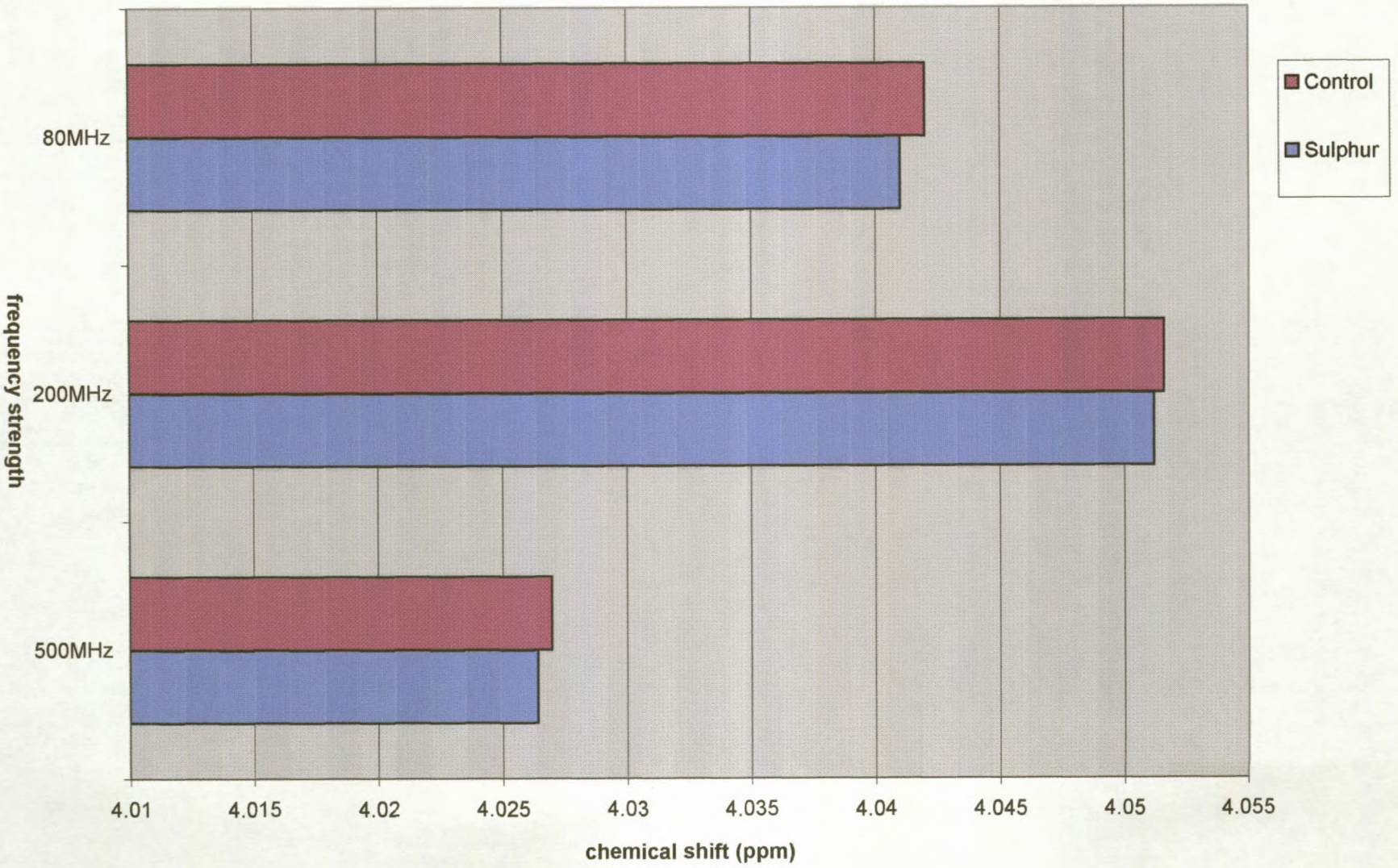


figure 8. **OH chemical shift values**

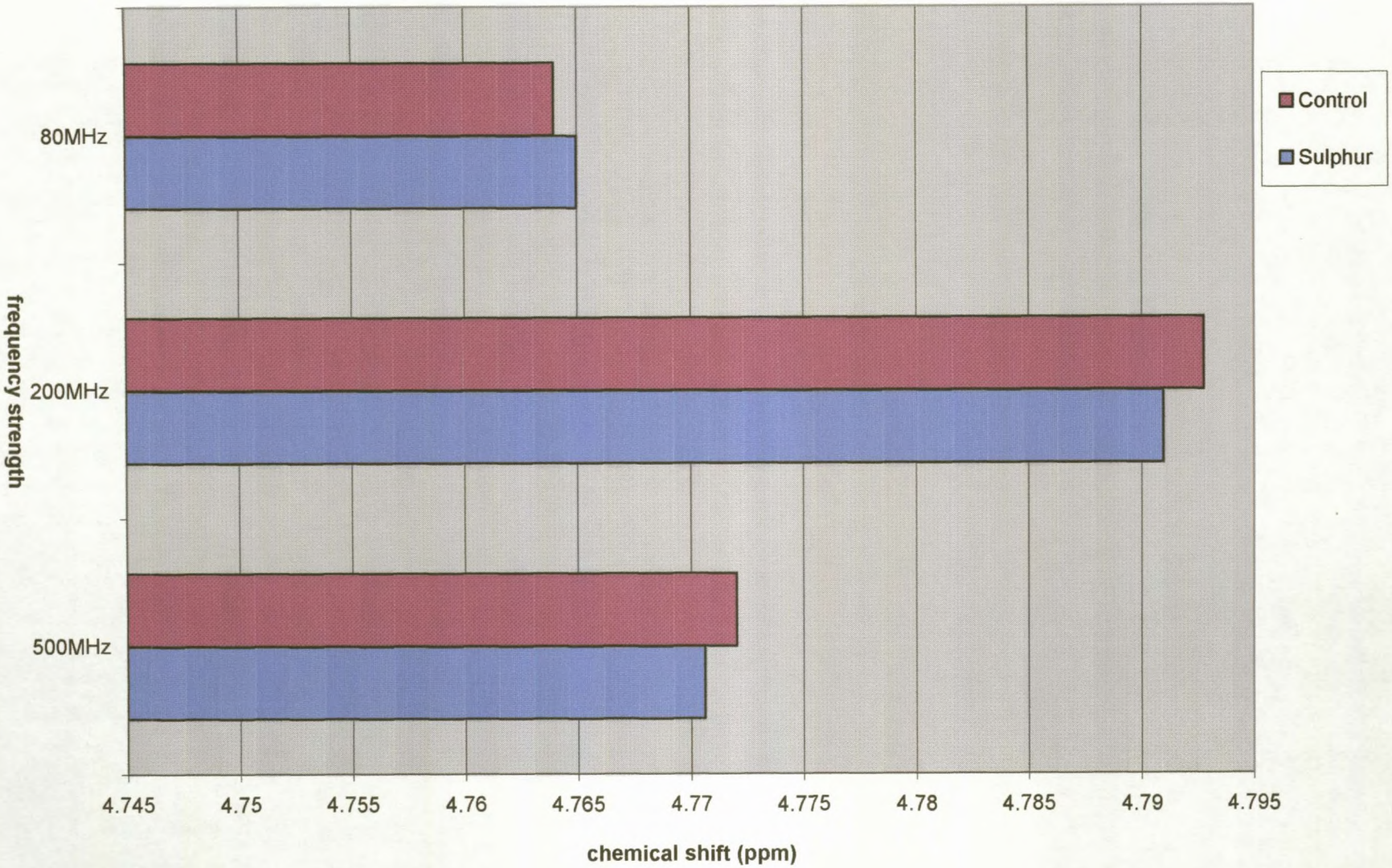


figure 9. **CH_2 Integration Values**

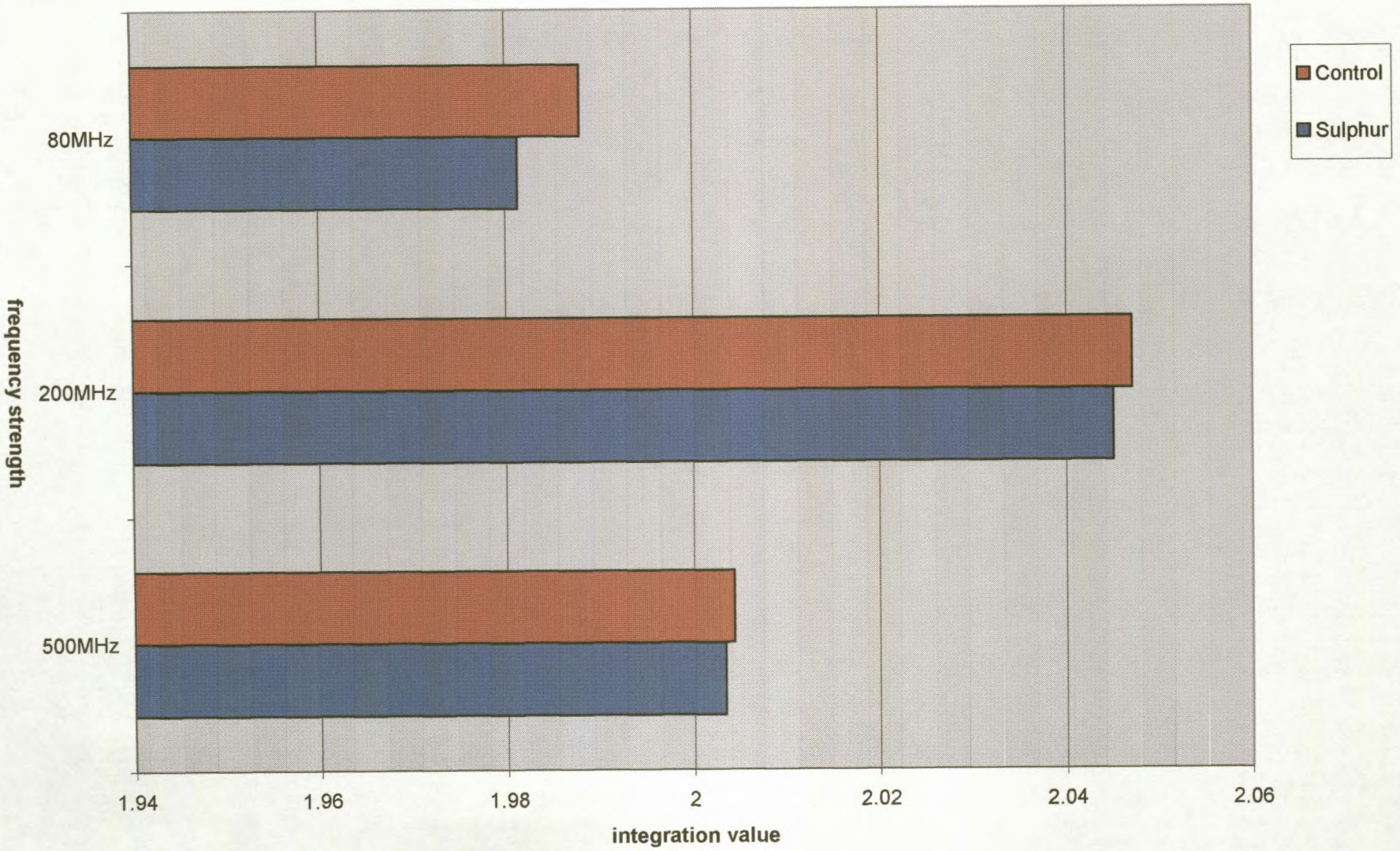


figure 10. **H_2O integration values**

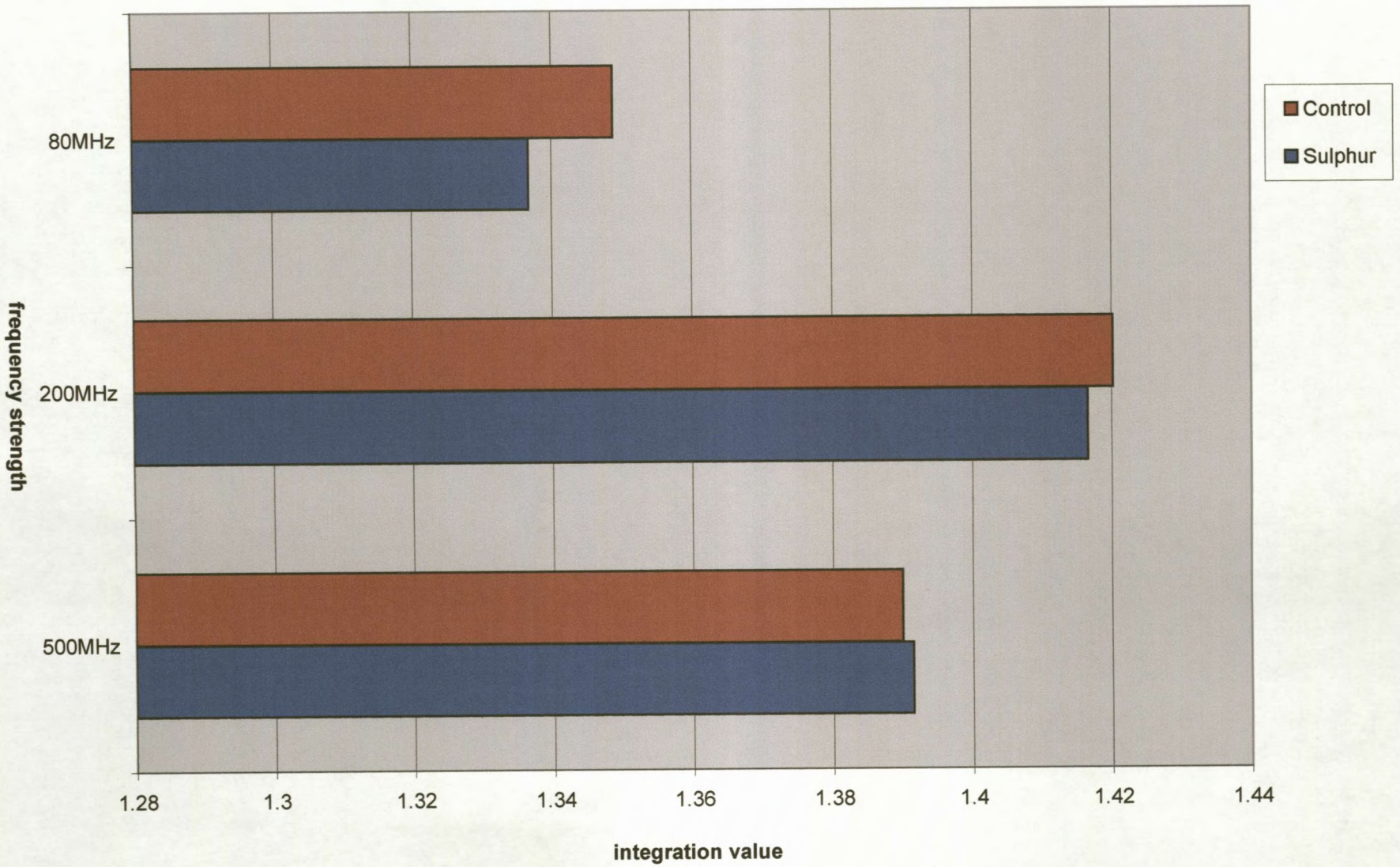
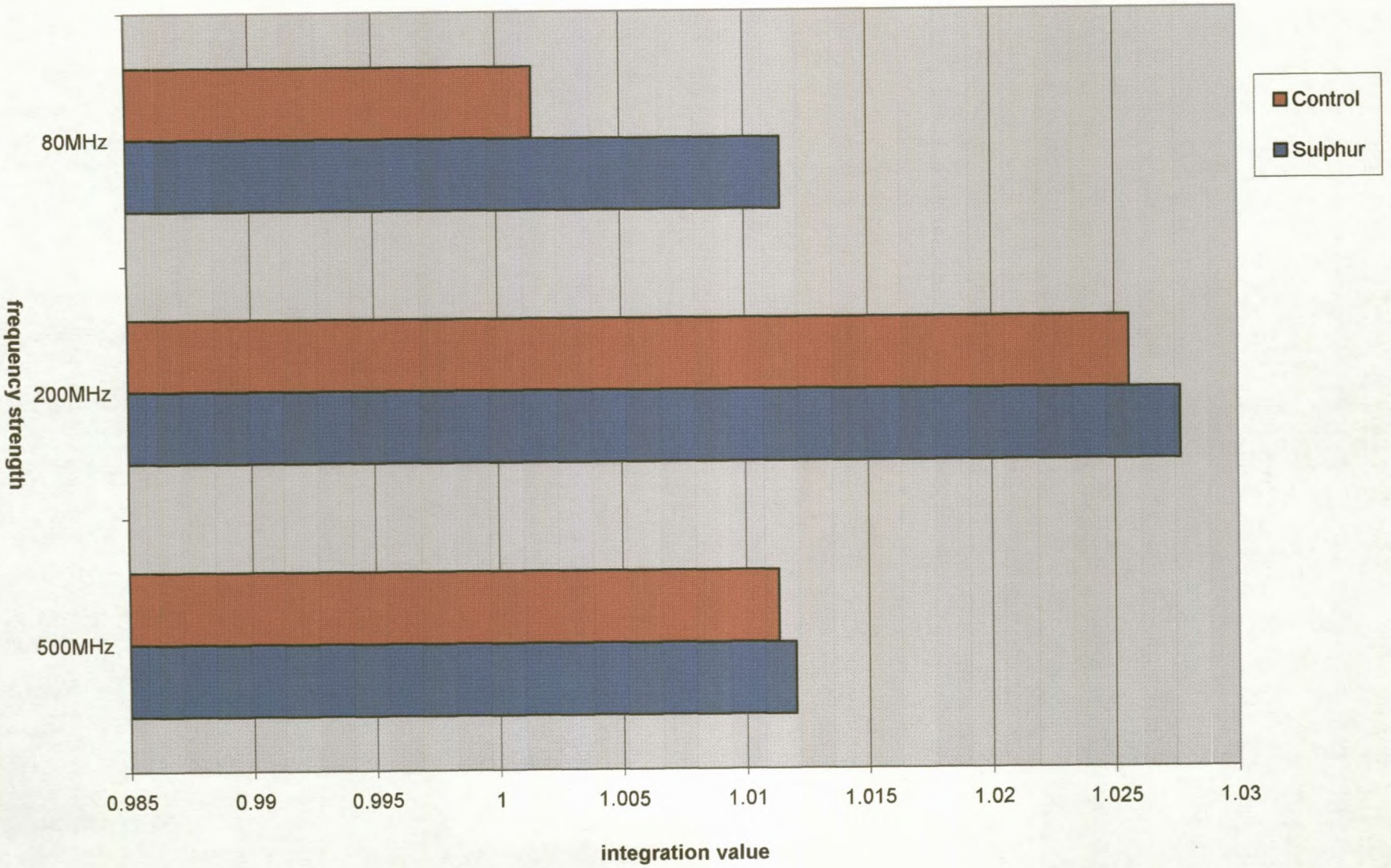


figure 11. **OH integration values**



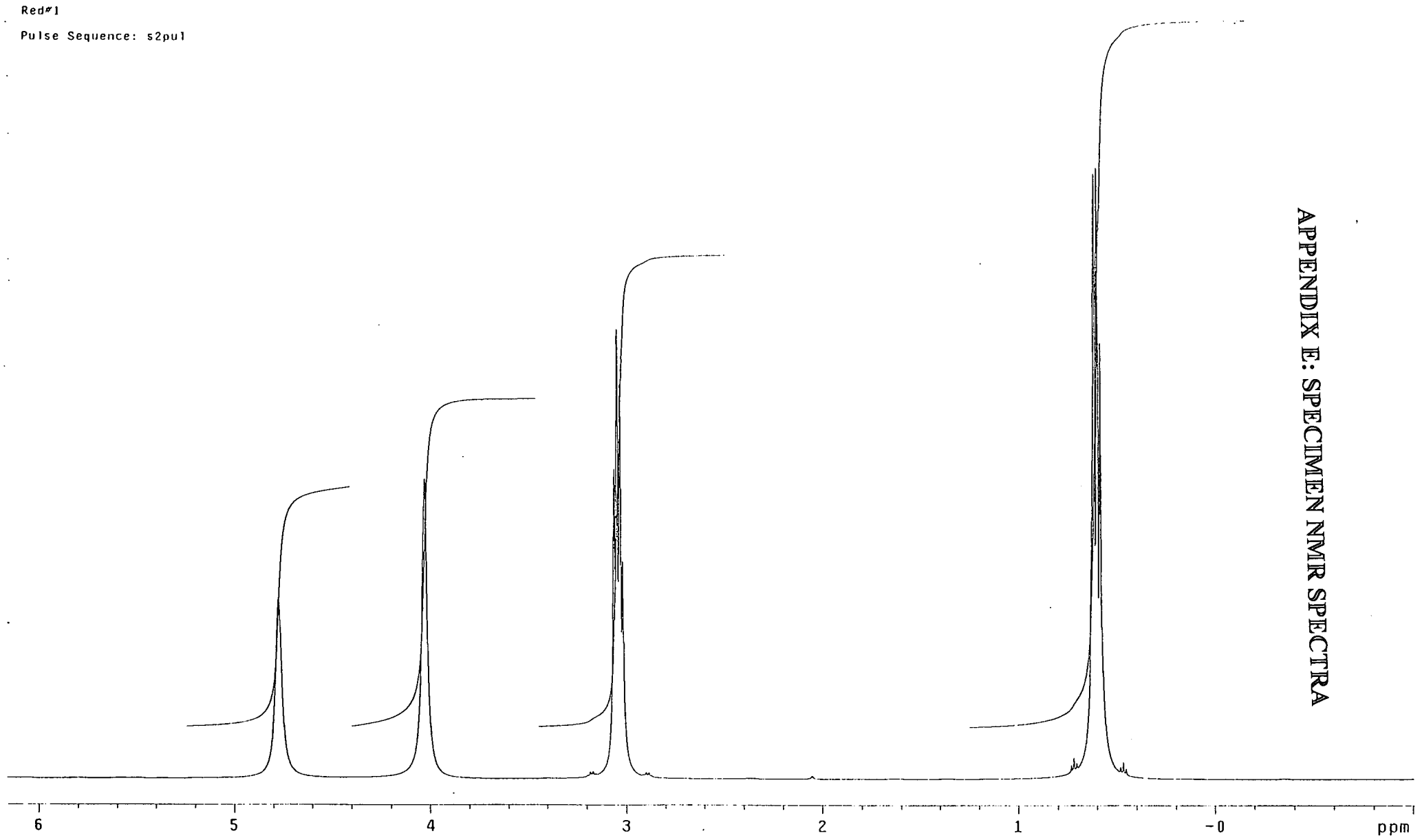


figure 12. Specimen NMR spectrum of Sulphur 30CH at 500MHz

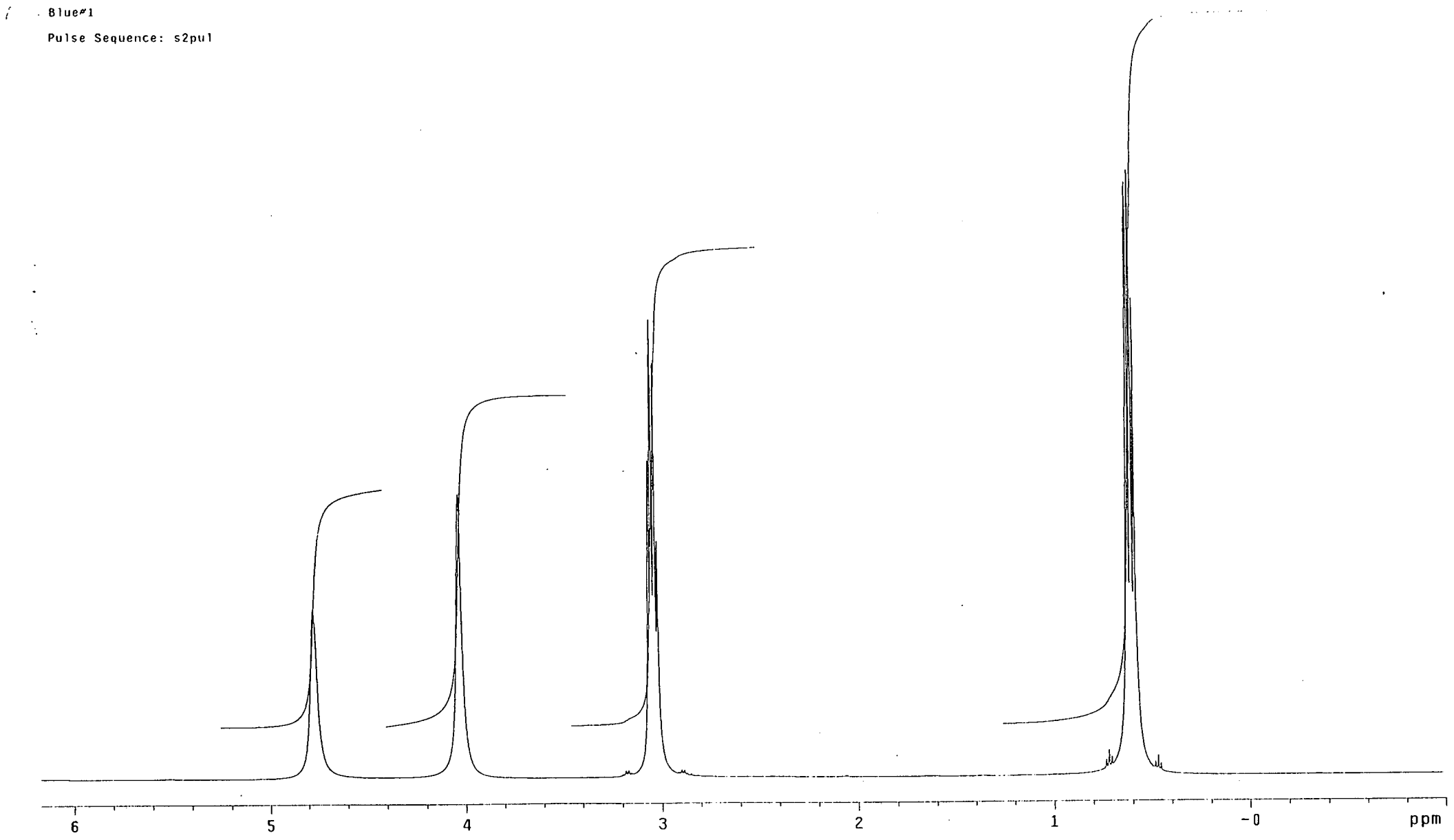


figure 13. Specimen NMR spectrum of Control 30CH at 500MHz

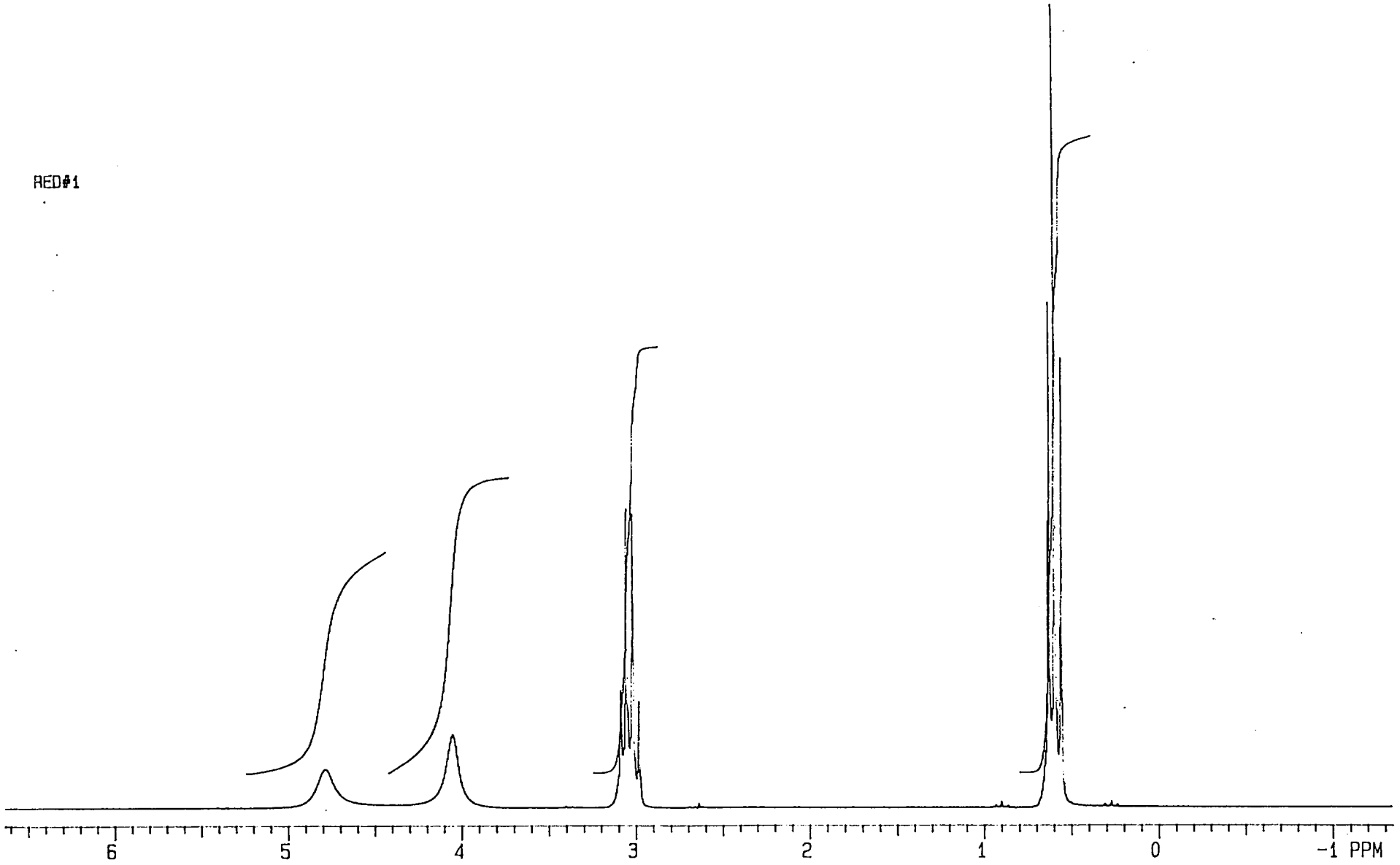


figure 14. Specimen NMR spectrum of Sulphur 30CH at 200MHz

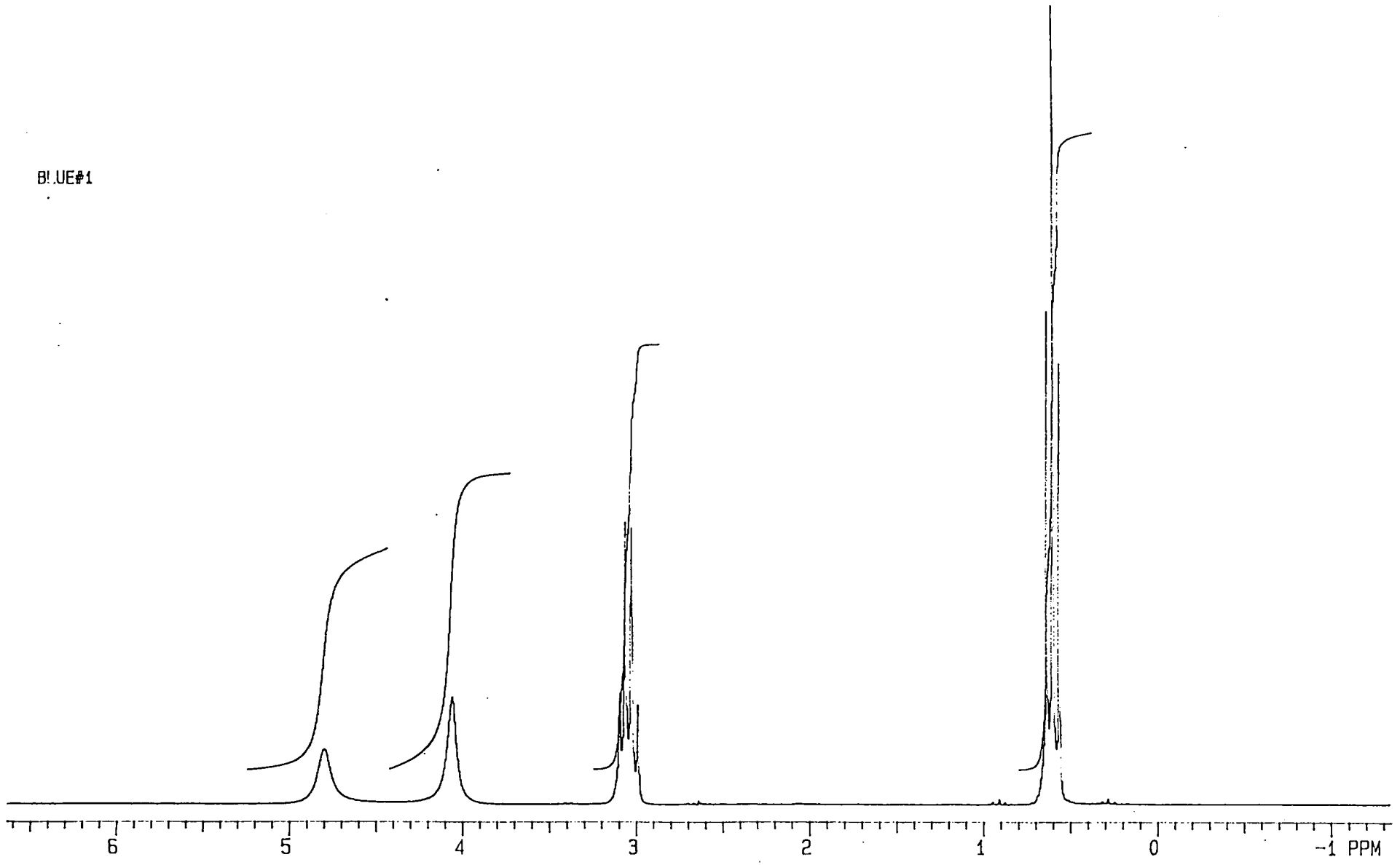


figure 15. Specimen NMR spectrum of Control 30CH at 200MHz

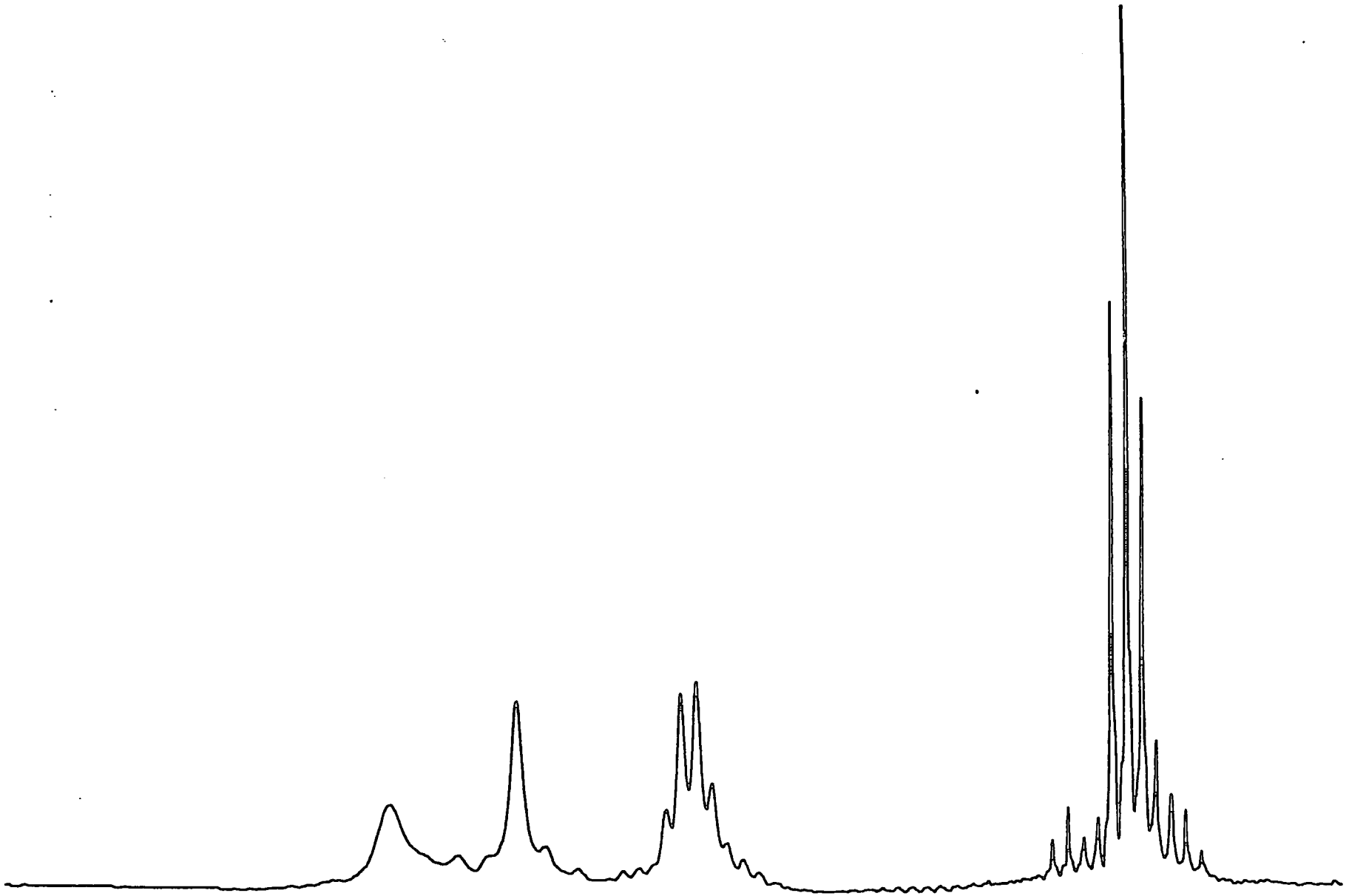


figure 16. Specimen NMR spectrum of Sulphur 30CH at 80MHz

APPENDIX F: CRUDE DATA RELATING TO NMR SPECTRA

A. CHEMICAL SHIFT VALUES - SULPHUR GROUPS

500MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.5951303	3.0359147	4.02509	4.76983
2	0.5952073	3.036175	4.02524	4.77042
3	0.5953828	3.0360375	4.02636	4.77067
4	0.5955619	3.0363525	4.02618	4.77071
5	0.595883	3.0367375	4.02635	4.77022
6	0.595857	3.0366925	4.02779	4.77209
7	0.5954333	3.03609	4.02641	4.7705
8	0.5953753	3.03603	4.02657	4.77066
9	0.5956486	3.03645	4.02721	4.77086
10	0.5956066	3.03608	4.02695	4.77038
MEAN	0.59550861	3.03625597	4.026415	4.770634

200MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.596	3.03875	4.055	4.79
2	0.596	3.03775	4.052	4.791
3	0.596	3.03935	4.052	4.791
4	0.596	3.039	4.051	4.789
5	0.596	3.039	4.051	4.79
6	0.596	3.039	4.051	4.792
7	0.59567	3.039	4.05	4.791
8	0.596	3.03925	4.05	4.791
9	0.596	3.03875	4.05	4.793
10	0.596	3.0385	4.05	4.792
MEAN	0.595967	3.038835	4.0512	4.791

80MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.58	3.025	4.05	4.77
2	0.573333	3.02	4.04	4.76
3	0.57	3.015	4.04	4.76
4	0.576666	3.02	4.04	4.77
5	0.57	3.015	4.04	4.77
6	0.576666	3.02	4.04	4.76
7	0.58	3.025	4.04	4.76
8	0.576666	3.02	4.04	4.76
9	0.576666	3.02	4.04	4.77
10	0.58	3.0225	4.04	4.77
MEAN	0.5759997	3.02025	4.041	4.765

B. CHEMICAL SHIFT VALUES: CONTROL GROUPS

500MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.595931	3.036805	4.02713	4.77144
2	0.595437	3.036463	4.02673	4.77148
3	0.595488	3.036253	4.02625	4.77056
4	0.595285	3.035813	4.02641	4.77028
5	0.595623	3.036388	4.02682	4.77069
6	0.595868	3.036633	4.02685	4.77094
7	0.595968	3.037445	4.02892	4.77345
8	0.595358	3.036178	4.02678	4.77986
9	0.595438	3.036058	4.02671	4.77058
10	0.595582	3.036328	4.02715	4.77101
MEAN	0.595598	3.036436	4.026975	4.772029

200MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.596	3.03875	4.054	4.793
2	0.596	3.03875	4.052	4.794
3	0.596	3.0385	4.051	4.791
4	0.596	3.039	4.051	4.791
5	0.596	3.03925	4.051	4.792
6	0.596	3.039	4.051	4.794
7	0.596	3.03875	4.051	4.794
8	0.59567	3.0385	4.052	4.794
9	0.596	3.03925	4.052	4.792
10	0.596	3.03875	4.051	4.793
MEAN	0.595967	3.03885	4.0516	4.7928

80MHz				
Sample	CH_3	CH_2	H_2O	OH
1	0.576666	3.0175	4.05	4.77
2	0.576666	3.02	4.05	4.77
3	0.573333	3.02	4.05	4.78
4	0.576666	3.02	4.04	4.77
5	0.576666	3.0175	4.04	4.76
6	0.58	3.025	4.04	4.78
7	0.576666	3.0175	4.03	4.74
8	0.576666	3.02	4.04	4.76
9	0.58	3.02	4.04	4.75
10	0.576666	3.0225	4.04	4.76
MEAN	0.577	3.02	4.042	4.764

C. INTEGRATION VALUES: SULPHUR GROUPS

500MHz				
Sample	CH_3	CH_2	H_2O	OH
1	3	2.0015	1.38797	1.01317
2	3	2.00838	1.39758	1.01331
3	3	2.00101	1.38801	1.01389
4	3	2.00282	1.39201	1.01321
5	3	2.00474	1.39336	1.01234
6	3	1.99566	1.38346	1.00625
7	3	2.00203	1.38638	1.01271
8	3	2.0043	1.391	1.01117
9	3	2.00828	1.39741	1.01284
10	3	2.00531	1.39748	1.01127
MEAN	3	2.003403	1.391466	1.012016

200MHz				
Sample	CH_3	CH_2	H_2O	OH
1	3	2.0379	1.4043	1.0215
2	3	2.042	1.4079	1.0253
3	3	2.0487	1.4012	1.0151
4	3	2.0476	1.4072	1.0135
5	3	2.0545	1.445	1.0347
6	3	2.0447	1.4171	1.02
7	3	2.0496	1.4203	1.0443
8	3	2.0441	1.4198	1.0445
9	3	2.0436	1.4239	1.0307
10	3	2.038	1.4188	1.0271
MEAN	3	2.04507	1.41655	1.02767

80MHz				
Sample	CH_3	CH_2	H_2O	OH
1	3	1.968244	1.310234	0.996657
2	3	1.965916	1.34701	1.008232
3	3	1.99831	1.332337	1.008318
4	3	1.991282	1.340924	1.023943
5	3	1.982511	1.309755	1.030315
6	3	1.972837	1.358168	0.985642
7	3	1.979821	1.348855	1.011253
8	3	1.995842	1.291021	1.039631
9	3	1.986026	1.353663	1.01515
10	3	1.973141	1.375243	0.995718
MEAN	3	1.981393	1.336721	1.011486

D. INTEGRATION VALUES: CONTROL GROUPS

500MHz				
Sample	CH_3	CH_2	H_20	OH
1	3	2.00631	1.3961	1.01129
2	3	2.00214	1.38692	1.012
3	3	2.00359	1.38933	1.01152
4	3	2.00687	1.391162	1.01174
5	3	2.00195	1.38575	1.00834
6	3	2.00075	1.38775	1.00728
7	3	2.00386	1.38786	1.01328
8	3	2.00309	1.38757	1.01154
9	3	2.00537	1.39307	1.01161
10	3	2.00972	1.39398	1.01466
MEAN	3	2.004365	1.3899492	1.011326

200MHz				
Sample	CH_3	CH_2	H_2O	OH
1	3	2.04088	1.4088	1.0277
2	3	2.0488	1.4126	1.0274
3	3	2.0406	1.4311	1.04
4	3	2.0445	1.4201	1.0293
5	3	2.0456	1.4184	1.0284
6	3	2.0484	1.4183	1.0225
7	3	2.0436	1.4162	1.0153
8	3	2.0592	1.4262	1.0316
9	3	2.0461	1.4122	1.0171
10	3	2.0534	1.4377	1.0166
MEAN	3	2.047108	1.42016	1.02559

80MHz				
Sample	CH_3	CH_2	H_2O	OH
1	3	1.9695989	1.3397153	1.001682
2	3	1.9889953	1.3392024	0.999353
3	3	1.9914062	1.3503906	1.022266
4	3	1.9911779	1.3598858	0.981578
5	3	1.986518	1.3129375	1.005315
6	3	1.9850649	1.3542857	1.005195
7	3	1.998043	1.369863	0.998043
8	3	1.974792	1.3768191	1.005327
9	3	1.9803819	1.3349356	1.025464
10	3	2.0143696	1.350489	0.970346
MEAN	3	1.98803477	1.3488524	1.001457