AN EVALUATION OF HAHNEMANNIAN QUINQUAGENIMILLESIMAL POTENCIES USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

by

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I, Ashley Hilton Adrian Ross, do hereby declare that this dissertation represents my own work both in concept and execution.

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Dedicated to my wife,
Jennifer Anne,
in grateful acknowledgement
of her unfailing support and
encouragement.

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ABSTRACT

The purpose of this investigation was to analyse and compare the Nuclear Magnetic Resonance (NMR) spectra of samples of quinquagenimillesimal (LM) potencies of homoeopathic Sulphur and a lactose-based control produced according to Hahnemann, in order to evaluate homoeopathic medicines thus prepared. It was hypothesised that differences existed in the spectra of respective Sulphur samples, control samples, and between parallel samples of Sulphur and control. It was further hypothesised that these differences correlated proportionately with the degree of potency of samples.

The design of the investigation was that of a scientific experiment. Potencies of Sulphur and a lactose-based control were prepared (according to the directions of Hahnemann*) to the LM10 level. LM2, LM6 and LM10 liquid potencies (95% ethanol) of each group were then prepared in ≅20.8160 ml volumes and despatched for sampling and measurement.

NMR spectroscopy was conducted on fifteen (15) samples of each potency. These were prepared in coaxial sample tubes using deuterium oxide (D_2O) as an external lock and dioxane as a reference. Samples were drawn and measured in overlapping sequence by the Department of Chemistry, University of Cape Town. The spectrometer employed was a Varian VXR200 operating at a frequency of 200.057 MHz. Acquisition time for each sample was 3.727 seconds, using a pulse width of 6°. Measurement of each sample was repeated eight times, at a constant temperature of 298.1 K (25.0°C).

The data were recorded in the form of NMR spectra (listing chemical shift and integration values) and computer-linked tables. The chemical shift and integration (mean intensity) values of the CH_2, H_2O and OH signals were subjected to statistical analysis using two methods. The one-way analysis of variance (ANOVA) method was used to test differences between potencies within each group, followed, in the case of rejection of the null hypothesis, by two-way unpaired t-tests. This latter method was also used to test differences between the Sulphur and control groups. The level of significance used was $\alpha = 0.05$.

Significant differences were noted between the integration values of the CH_2 signals of Sulphur LM6 and LM10 potencies ($p = 0.0072204$), and the CH_2 signals of the control LM2 and LM10 ($p = 0.00142383$) and LM6 and LM10 potencies ($p = 0.00161819$). The chemical shift values of all three signals compared (CH_2:$p = 0.0352837$; H_2O:$p = 0.0480296$; and OH $p = 0.00256373$), and the CH_2 ($p = 7.06947 \times 10^{-8}$) and H_2O ($p = 0.0166579$) integration values were significantly different between LM10 potencies of Sulphur and the control. Standard deviations and variances were noted to be very small across all groups.

The investigation supported the hypothesis that differences exist between respective potencies of homoeopathic Sulphur and a lactose-based control, and that differences exist between parallel potencies of Sulphur and control. Additionally it served to support the employment of NMR spectroscopy as a method for the analysis of structure within homoeopathic potencies. The investigation was unable to provide conclusive evidence that differences correlate proportionately with the degree of potency.

While it was clear that the pharmaceutical processes employed in this study were valid and did yield distinct physico-chemical entities a rational explanation for the observed phenomena remains elusive. The various anomalies were noted and recommendations were made to address some of these. There is an urgent need for replication of observations, re-evaluation of assumptions and inter-disciplinary collaboration..

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TABLE OF ABBREVIATIONS

CH	-	Centesimal Hahnemannienne
δ	-	delta; represents the chemical shift
integ.	-	integration
LM	-	Quinquagenimillesimal potency [erroneously derived from Roman numerals 50 (L) and 1000 (M) = 50 000]
S.G.	-	specific gravity (see definition of terms)

THE DEFINITION OF TERMS

Analyse - For the purposes of this investigation, the term analyse refers to the statistical manipulation of the recorded δ-values and integration values of CH_2, H_2O and OH signals of ethanolic LM2, LM6 and LM10 potencies

Analysis of Variance - a method of statistical analysis used for the analysis of data relating to three main effects [See 3.5.1].

Batch - a specific quantity of a medicine which has a uniform characteristic and quantity within specified limits, and is produced according to a single preparation procedure during the same cycle of manufacture.

Chemical Shift - indicates the resonance frequency of nuclei subjected to an electromagnetic forcefield. The resonance frequency of individual nuclei is affected by the molecular environment and is an indicator of three-dimensional structure within molecules.

Drop - The original text for the manufacture of quinquagenimillesimal potencies (Hahnemann 1987) simply refers to fluid volumes as "drops". Due to the varying compositions of liquids involved in LM potency manufacture, and the need for standardisation and reproducibility, for the purposes of this investigation the volume of each "drop" of a each liquid has been calculated with reference to the hygrometrically measured specific gravity of each liquid. [see "specific gravity" below]

Integration - refers to the relative intensity of individual NMR peaks. It is a function of the ratio of protons within individual groups of a molecule.

Mean (x) - the most commonly used measure of central tendency, defined as the sum of the values in the sample divided by the sample size.

NMR-spectroscopy - an analytical method most frequently employed to obtain information about the structure of organic compounds, by measuring the interaction of protons within a magnetic field. Such interaction is recorded as a series of peaks known as a spectrum (see below).

Physical structure - the three-dimensional geometry existing between individual atoms and/or radicals, within molecules, and that existing between molecules of a compound, or within a mixture.

Potency - a state of altered remedial activity to which a drug is taken by means of a measured process of deconcentration and the introduction of kinetic energy through succussion or trituration (see below). Three rates of deconcentration are used in preparation of homoeopathic potencies.

Quinquagenimillesimal - a homoeopathic potency scale, introduced by Hahnemann, in which the rate of deconcentration at each potency stage is 1:50 000. Deconcentration is effected in two stages: the initial being 1:100 (with 100 succussions) and the second being 1:500 (without succussion) [a.k.a. LM].

Specific gravity - refers to the mass of a substance relative to its volume. Specific gravity is defined by the formula:

$$S.G. = \frac{\text{Mass (g)}}{\text{Volume (ml)}}$$

Specific gravity may be calculated from a known mass and volume, or measured by the use of a hygrometer.

Spectrum (NMR) - a display by a series of peaks, of the various resonant frequencies at which the protons of different types of hydrogen atoms within organic compounds absorb electromagnetic radiation [Ethanol ($CH_3\,CH_2\,OH$) has three types of hydrogen atoms: those of CH_3 -, those of - CH_2 - and those of - OH. Each type of proton has different resonant frequencies; the NMR-spectrum of ethanol therefore has three peaks.]

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 the data set.

Succussion - the action of shaking up vigorously a liquid dilution of a homoeopathic medicine in its vial/bottle, where each stroke ends with a jolt; usually effected by pounding the hand engaged in the shaking against the palm of the opposite hand.

Trituration - the act of prolonged grinding with a mortar and pestle to reduce an insoluble homoeopathic drug to a fine powder while amalgamating it thoroughly with lactose by rubbing together under the pestle.

T-tests - parametric hypothesis tests applied to the means of two normal distributions of differences. They are of two types: paired and unpaired.

CHAPTER ONE: INTRODUCTION

The quinquagenimillesimal (LM) potency scale is described by Hahnemann (1986:196) as "the most nearly perfect of them all". However, this potency is only rarely employed in present-day homoeopathic practice and even dismissed by some (Schore, 1990). Barthel (1991) believes that this circumspection is due to failure to reproduce the clinical results claimed by Hahnemann, due to remedies not having been prepared according to Hahnemann's directions.

Measurement of the phenomenon of homoeopathic potency poses particular problems due to the absence of any molecules of the base substance in solutions above the 12CH, 24DH and LM4 potency levels. In Smith (1989:113), Gaier (1991:446) and Bol (1997) Nuclear Magnetic Resonance (NMR) spectroscopy is cited as a valuable method of measurement of structural changes in homoeopathic solutions.

Barnard (1965) demonstrated the formation of giant water polymers by succussion. Smith and Boericke (1968) confirmed this research and demonstrated that changes increased as potency increased, and that there was increased interaction between water structures and the hydroxyl radical of ethanol in ethanolic homoeopathic solutions. More recently Sacks (1983) used NMR spectroscopy to demonstrate differences between various remedies and control. Weingärtner (1991) compared

CH_2, H_2O and OH signals in decimal potencies of Sulphur and a control. More recent still is the research of Demangeat et al. (1992) in which relaxation times in various centesimal Silicea potencies were compared. No NMR research has been conducted on quinqaugenimillesimal (LM) potencies.

It is within this context that an analysis and comparison of NMR-spectra of LM potencies of Sulphur and a control, (prepared according to Hahnemann) assumes great importance. The positive results of such an investigation will allow for greater understanding of the distinct physico-chemical identity of such potencies, and indirectly lead to more widespread use of this scale where indicated (Schore 1990) as a result of greater credibility brought about through scientific evaluation. Likewise a negative result will provide a more objective basis for non-inclusion of this potency in the practice of the individual homoeopath.

1.1 THE AIM OF THE STUDY

The aim of this study is to analyse and compare the Nuclear magnetic Resonance spectra of samples of quinquagenimillesimal (LM) potencies of homoeopathic Sulphur and a lactose-based control produced according to Hahnemann, in order to evaluate homoeopathic medicines thus prepared.

1.2 THE STATEMENT OF THE OBJECTIVES

1.2.1 The first objective

The first objective is to analyse and compare NMR-spectra of homoeopathic Sulphur with respect to the chemical shift (δ) and integration values of CH_2, H_2O and OH signals of liquid LM2, LM6 and LM10 potencies in order to determine the existence of differences in physical structures.

1.2.2 The second objective

The second objective is to analyse and compare NMR-spectra of a lactose-based control with respect to the chemical shift (δ) and integration values of liquid LM2, LM6 and LM10 potencies in order to determine the existence of differences in physical structures.

1.2.3 The third objective

The third objective is to statistically evaluate any similarities and/or differences existing between the analyses of spectra of homoeopathic Sulphur and control, in order to evaluate homoeopathic medicines produced according to the process outlined by Hahnemann.

1.3 THE HYPOTHESES

1.3.1 Hypothesis one

It is hypothesised that differences exist between the chemical shift (δ) and integration values of CH_2, H_2O and OH signals of respective LM2, LM6 and LM10 potencies of homoeopathic Sulphur, and that these indicate parallel differences in the physical structure of the respective potencies.

1.3.2 Hypothesis two

It is hypothesised that differences exist between the chemical shift (δ) and integration values of CH_2, H_2O and OH signals of respective LM2, LM6 and LM10 potencies of a lactose-based control, and that these indicate parallel differences in the physical structure of the respective potencies.

1.3.3 Hypothesis three

It is hypothesised that statistically significant differences exist between parallel potencies of Sulphur and control, and that these differences increase in significance as potency increases.

CHAPTER TWO: THE REVIEW OF THE RELATED LITERATURE

2.1 THE INTRODUCTION

If one assumes that homoeopathic remedies do have specific effects on the human body and mind, and further, that these effects are due to the very substance of which they consist then two questions demand an answer:

1. What is the physical or material difference between a homoeopathic remedy prepared in the prescribed way and the pure solvent or a mere dilution?
2. How are these physical differences transmitted to the human body?

The former of these questions is the foundation out of which this investigation is built. What is the nature of homoeopathic potency? Do all prescribed methods of preparation produce remedies distinctly different from a mere dilution, or the pure solvent? How does one best measure these differences if they do exist? Research has been conducted with a view to addressing these questions, a brief review of which follows:

2.2 THE PHENOMENON OF HOMOEOPATHIC POTENCY

Vithoulkas (1980 : 101) states that the technique of homoeopathic potentisation (producing a homoeopathic 'potency') is Samuel Hahnemann's second ingenious contribution to medicine (after 'The Law of Similars', a fundamental law routinely applied in the practice of Homoeopathy). This has its origin in Hahnemann's attempts at reducing the toxicity of homoeopathic substances by dilution. The result was decreased toxicity but also a proportionately reduced therapeutic efficacy. Somehow Hahnemann came to refine this technique by the introduction of kinetic energy through succussion and/or trituration. The resulting combination of serial dilution and serial succussion (and/or trituration) brought Hahnemann to the crucial observation that the more a substance is diluted and succussed the more effect it has therapeutically, while simultaneously nullifying any toxic effect. He describes this for the first time in his "Organon" (1831).

The initial scale of deconcentration employed by Hahnemann was 1:100 (Bärthel 1991) meaning that each potency is 10^{-2} times that of the previous potency. (designated 1CH, 2CH etc. as each successive potency is produced). Avogadro's Constant ($6,023 \times 10^{23}$) is therefore exceeded by the 12CH potency (a deconcentration of 10^{-24}), meaning that statistically there is no possibility of any molecule of the original substance existing in the 12CH 'potency'.

2.3 MEDIA OF HOMOEOPATHIC POTENTISATION

Homoeopathic potentisation is effected in two different media. The first medium of potentisation employed by Hahnemann was that of a mixture of ethanol and water (Barthel 1991). Potentisation of alcoholic plant extracts (mother tinctures) and soluble mineral substances was effected by serial dilution of the base substance in a determined percentage ethanol solution, followed by succussion of the liquid. This medium and method of potentisation was employed exclusively between 1796 and 1818 (Barthel 1991). The second medium, pure lactose powder, was introduced into homoeopathy by Hahnemann in 1818. Its use was predominantly as a vehicle for potentisation of insoluble substances (previously potentised by liquid-medium dilutions and succussions of alcoholic suspensions) especially metallic gold (Dellmour 1994).

The method of potentisation in this solid medium was by trituration, by mortar and pestle, of the insoluble base substance with lactose (as a diluent) for a determined period of time (one hour). The notion and technique of trituration was drawn from the writings of Arab physicians in the 12th Century (Barthel 1991).

The preparation of homoeopathic potencies by trituration became increasingly more routinely employed by Hahnemann. The result of this trend was that, in 1835, he abandoned entirely the use of mother tinctures, and prepared all substances

(soluble and insoluble) to the 3CH potency exclusively by trituration in lactose. Higher potencies continued to be prepared in liquid medium (Dellmour 1994). Dellmour (1994) convincingly discusses the advantages, from a purely pharmaceutical perspective, of trituration of plant and insoluble materials, and cites numerous references from Hahnemann's writings which indicate his clear preference for the use of lactose as a medium of lower level potentisation. The investigation of, and the formulation of models to explain, the mechanism of potentisation in the above-mentioned two media has been a scientific conundrum of considerable intrigue.

2.4 EXPERIMENTAL MODELS FOR POTENTISATION

Gaier (1991 : 436 - 438) discusses experimental evidence spanning one hundred years, which points to the existence of a physico-chemical forcefield in the potencies which is able to be carried forward to the 12CH potency and beyond (the 'ultramolecular' potencies). It therefore becomes clear that the ascribed efficacy of homoeopathic medicines (in which extremely few, if any, molecules of the original base substance exist) is more likely to be understood within the context of physics than within that of chemistry (Smith, C. 1989 : 112).

Drawing on frequency measures conducted on allergic patients, Dr Cyril Smith (1989 :112 - 113) proposed a helical structure in water which is capable of 'remembering' frequency. He cites as evidence of this the observation that a

sealed tube of water which produced no clinical effects on an electrically sensitive allergy patient would, after being exposed to a magnetic field at one of the patient's allergy neutralising frequencies, function as an allergy neutralising dilution. This induced 'potency' is furthermore, able to maintain the effect for a period of weeks or months.

An alternative model is provided by Anagnostatos et al. (1991) in which research suggesting that the therapeutic power of the remedy lies in the solvent (Barnard 1965) rather than the diluted substance, is explained by a three step hypothesis. The model is based on i) the formation of shells of organised hydrogen-bonded molecules of the solvent (clathrates) around aggregates of a small number of molecules of the base substance; ii) because of the force of succussion and the different inertial properties, clusters of base molecules move out of their clathrates, to have new clathrates form around them. The initial clathrate is now hollow (called a "core clathrate") and an additional "mantle" forms around this "core"; iii) at the point at which no base substance is present, the application of successive force causes core clathrates to move out of their mantle clathrates and stimulate the formation of new mantle clathrates. Old "mantle" clathrates become "core" clathrates and new "mantle" clathrates form around these.

The notion of hydration shells is supported by Antonchenko and Ilyin (1992) who elaborate that the structure of water may be viewed as a set of flickering clusters ("embryos") of possible hydration shell structures which, upon addition of a

dissolved substance, undergoes "(relative) fixation of a specific kind of hydration structure corresponding to the given dissolved substance" (Antonchenko and Ilyin 1992: 91).

They further propose the formation of more static water structures which serve to stabilise the overall structure within the liquid, and allow for high water molecule mobility. Evidence further suggests the existence of "one-dimensional chains" along these structures which allow for proton transfer in hydrogen-bonded systems (such as occur in homoeopathic potencies). The presumed mechanism of transfer is by the movement of extended areas of compression and rarefaction of the average proton density within the water system. This "soliton mechanism" (Antonchenko and Ilyin 1992: 92) of proton transfer, they argue, stabilises dissipative water microclusters, and allows for the existence of substance-specific (radioactive) characteristics in water.

These highly structured models are consistent with the findings of other researchers in the field. Resch and Gutmann (1991 : 231) propose a highly ordered "Super molecular system" for liquid water in which "normal" water is distinguished from "Solvation spheres" (around hydrophillic solutes), "Inner surface" molecules (around hydrophobic solutes) and molecules at the interface. Their research clearly points to a highly organised structure inherent in water which, as is the case in Anagnostatos et al., is able to be substance-specifically modified by interaction with a base substance/solute.

Models for potentisation in a lactose medium are rare. Resch and Gutmann (1987: 271 - 276) describe the structure of lactose monohydrate. They draw attention to the existence of a "loose hydrogen bond" (Resch and Gutmann 1987: 273) between the galactose and glucose components of the molecule, as well as a highly flexible three-dimensional network of lactose molecules interconnected by water molecules and ten flexible hydrogen bonds (of variable bond length). Similar to liquid water, where each H_2O molecule is tetrahedrally surrounded by four H_2O molecules, each H_2O molecule in the lactose framework is surrounded by four lactose molecules connected to them by hydrogen bonds. Within the overall structure water molecules demonstrate greater mobility than lactose molecules.

These researchers argue that the relatively higher, more dynamic, mobility of water molecules within the structure exerts a regulating effect upon the oscillating network. The differing mobilities, it is argued, further contribute to the formation of "voids" within the structure. These are able to take up other particles (solutes) resulting in substance-specific changes in system organisation. Following on from studies of changes in system organisation in solids induced by grinding (Gutlich (1981); Imamura and Senna (1982)) they contend that due to increased flexibility of hydrogen bonds due to trituration, the formation of the above-mentioned voids is increased during the process of trituration.

Lessell (1994 : 85) contends that the large number (8) of hydroxyl groups ("promotable moieties") accounts for its apparent ability to act as a medium of potentisation. This contention totally ignores the influence of H_2O molecules within the lactose structure and is largely speculative.

2.5 HAHNEMANN'S LM POTENCIES

In homoeopathic pharmacy, potencies of a base substance are produced by three scales of deconcentration: (Gaier, 447)

i) decimal - introduced by Constantin Hering, in which each potency contains 1/10 of the preceding potency;

ii) centesimal - as described in 2.2, in which each potency contains 1/100 of the preceding potency;

iii) quinquagenimillesimal (LM) - introduced by Hahnemann, in which each potency contains 1/50 000 of the preceding potency.

The last of these, the LM potency scale, is described in Aphorism 270 of the Sixth edition of Hahnemann's Organon (written 1842), which regrettably, Gaier asserts (1991 : 463), was only published seventy-eight years after Hahnemann's death (ie 1921). The consequence of this fact is that the potency scale which Hahnemann believed, at the very end of his life, to be "the most nearly perfect of them all" (Hahnemann, 1986 : 196) is only rarely employed in present-day homoeopathic practice (Vithoulkas, 1980 : 164) and even dismissed on the basis of conclusions

"couched in modern 'scientific' conjecture" (Schore,1990 : 13).

Bärthel (1991 : 112; 119) believes that this attitude of circumspection surrounding the LM potencies is in part due to failure of present-day homoeopaths to achieve the reactions claimed by Hahnemann. He attributes this reality to remedies not being prepared exactly as Hahnemann directs, and argues that remedies prepared and prescribed as Hahnemann directs do indeed produce the claimed results, as does Schmidt (1971 : 236). This assertion is borne out by Schore (1990 : 15; 19 – 21) who cites common errors in prescription which lead to undue reaction, or no reaction at all. The Indian champion of the LM potencies, Dr Harimohan Choudhary (1990 : 65 - 96), provides numerous case studies (both of his own and two of Hahnemann) in illustration of the efficacy of these potencies. In the concluding paragraphs of his book on the LM potencies (1990 : 97) he softens his aggressive argument for the LM potencies (and unnecessarily aggressive stance against all others) by stating that Hahnemann's claim of the sixth edition of the Organon being the "most nearly perfect" may be acceptable to the idealist but not to the scientist. Truth is relative, he says, and raises the challenge to 'prove' the truth of Hahnemann's assertion. A search for any literature on objective research of the LM potencies produced only two papers.

2.6 METHODS OF MEASUREMENT

As stated in 2.2, the absence of any molecules of the base substance in homoeopathic remedies above the 12CH, 24DH, LM4 potencies makes a 'chemical' analysis of homoeopathic remedies difficult. It therefore follows that most research 'proving' the distinct identity of homoeopathic potencies against mere dilutions or solvent falls into the realm of the physicist. The world-renowned authority on Biomedical Electronics, Dr Cyril Smith in his discussion of homoeopathy, (Smith 1989 : 112 - 116) quotes the research of various other physicists who have measured such parameters as electrochemical changes in the diluent (Brucato and Stephenson 1966) capacitance,pH, electrode potential, enzyme activity (Jusaal et al. 1984), frequency resonance measurements (Ludwig : 1986) and ultra-weak photon emission from living plant tissues (Slawinski and Slawinski : 1986). In addition to these, Gaier (1991 : 436) cites controlled clinical trials on human, plant and animal subjects. In both (Smith 1989 : 113; Gaier 1991 : 446) NMR-spectroscopy is quoted as a valuable method of measurement of structural changes in homoeopathic solutions (as opposed to biological action). This view is endorsed by Bol (1997).

2.7 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) is a process whereby interactions between nuclear magnetic dipoles and electromagnetic radiation are observed. A very strong magnetic field causes a split in the energy levels of the sample molecules, and thus enables resonant interaction with electromagnetic waves (Fremantle, 1987 : 652). The resonant frequencies are directly correlated to molecular components of the sample by a series of peaks, the area underneath these and the ppm-distances between the peaks, and represent a one-to-one picture of the molecular geometry.

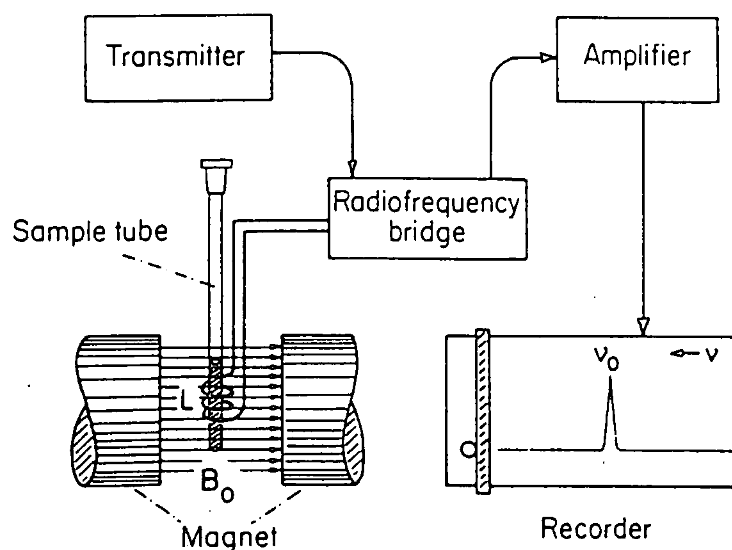

Figure 2.1 Diagram of a nuclear magnetic resonance spectrometer (Gunther 1980 ; 7)

The NMR experiment consists of the exposure of the sample, contained in a glass tube situated in the probehead of the spectrometer between the pole pieces of a

magnet [Figure 2.1 above], to electromagnetic radiation of variable frequency. The nuclei (either ^{1}H or ^{13}C, depending on the type of spectroscopy utilised) are excited when their particular resonance frequency is applied.

The source of exciting radiation is a radiofrequency oscillator (transmitter), and absorption of radiant energy by nuclei is detected by means of a radiofrquency bridge (see Fig. 2.1). The signal from the bridge is amplified and recorded on an x-y plotter (Gunther 1980 :7). Calibration of the resonance signal is achieved by the addition of a reference substance (most commonly tetramethylsilane (TMS)) (Fremantle, 1987 : 652). A solvent is added to the sample to serve as a 'lock' during spectroscopy.

The significance of NMR spectroscopy in chemistry is not based on its ability to differentiate between elements, but on its ability to distinguish a particular nucleus with respect to its molecular environment (Gunther 1980: 14). The resonance frequency of an individual nucleus is dependent upon its relative position within the molecular structure.

The proton (^{1}H) NMR spectrum consists of a series of peaks each representing the groups of protons within the molecule. This effect, produced by the different chemical environments of the protons in the molecule is referred to simply as the chemical shift. The magnetic shielding (during application of the magnetic field) of the individual nucleus (a function of its immediate chemical environment) by

electrons in its environment affects the field strength at which its resonance frequency is recorded. A relatively more shielded nucleus will be recorded at a higher chemical shift value. (Gunther 1980 : 16)

The peaks produced in the NMR experiment not only have placements upon a relative chemical shift reference, but also differing relative intensities (Appendix B) These intensities (represented by the area under the resonance signal) are proportional to the number of protons giving rise to the signal. These integrations, indicated on the spectrum by a step curve across each peak, are a valuable indicator of relative proton distribution across the respective molecule. (Gunther 1980 : 19)

From the foregoing it would be clearly that NMR would be a very appropriate method for measuring the differences existing between ethanol-water homoeopathic liquids.

2.8 NMR RESEARCH IN HOMOEOPATHY

In 1965, Barnard (1965 : 209) developed the idea that the solvent, rather than the original solute, carried the informational content of the solute. He used NMR to demonstrate the formation of giant water polymers formed by succussion. This research set the stage for later research by Smith and Boericke (1968 : 198 - 200) in which potencies of Bradykinin triacetate (up to 60DH) were prepared either with

or without succussion. In each case, diluents containing either water or deuterium oxide were used. The conclusions of their research confirmed Barnard's research, as well as demonstrating that solvent structure is changed by succussion and that these changes become more extreme as the dilutions approach and pass the negative function of Avogadro's Constant. They proposed a water structure having four hexagonal and twelve pentagonal faces, which was able to interact (proton exchange) with the OH of ethanol. They did not dismiss the possibility of other structures. (see Anagnostatos et al. 1991; Antonchenko and Ilyin 1994).

More recent NMR studies include those of Sacks and Weingärtner. Sacks (1983 174 - 175) used NMR-spectroscopy to demonstrate distinct differences between controls and several homoeopathic remedies in the H_2O and OH regions of the spectra. Otto Weingärtner, upon whose work, this investigation is based, has conducted a number of NMR studies on decimal Sulphur potencies. The most recent, of which I am aware (1991 : 61 - 65), compares the mean intensities of the CH_2, H_2O and OH peaks of D13 and D23 Sulphur potencies with 87% Ethanol (serving as a control). His argument in support of the use of ethanol (without succussion) as a 'control' rests on the belief that pouring of the ethanol from one bottle to another twenty times, with two shakings of the 'empty' bottle, is sufficiently analogous to the process of dilution and succussion applied in preparation of the experimental potencies. Consultation with Mr K. Reich (statistician) confirms that the extremely high significance level of 99.9% is in part due to a possible flaw in Weingärtner's belief. Parsch (1991:132) also contested the validity of Weingärtner's

assertion. (Smith and Boericke (1968) also found that unsuccussed serial dilutions were distinctly different from pure dilutions (Weingärtner 1991 : 66). This would indicate that 'assumptions' are perhaps uncertain in what is still an explorative field.) The most recent NMR study (unfortunately not available in English) is that of Demangeat et al. (1992). Bol (1997) describes the results of their research which compares the proton relaxation times of three high potencies of Silicea. They found that in each case relaxation time was raised in comparison to the pure diluent prepared and measured in the same way.

2.9 SUMMARY

The literature, as reviewed above, indicates that clear differences are able to be detected between homoeopathic potencies and dilutions, even beyond the inverse function of Avogadro's Constant. In the particular area of physical structure of homoeopathic solutions, it would seem that NMR-spectroscopy is the most effective method of detection and measurement of these differences.

Research has been conducted with the aim of demonstrating these physical differences where they exist in centesimal and decimal potencies, but no clear research has been directed at similar demonstration in LM potencies. As has been suggested, such investigation is all the more important because LM potencies are not universally accepted, either scientifically or clinically, despite claims of their

efficacy. Clearly an investigation into the possible existence of distinct structure within Hahnemannian LM potencies will contribute to both the scientific credibility of such potencies, and to more widespread use of these potencies where indicated. (see Schore 1990 : 16; Choudhary 1990 : 60 - 62).

The purpose of this investigation is therefore to analyse and compare the NMR spectra of LM potencies of Sulphur and a control, both prepared according to Hahnemann, in order to evaluate this method of potency in terms of the production of a distinct physico-chemical entity.

CHAPTER THREE: MATERIALS AND METHODS

3.1 THE PREPARATION OF POTENCIES

Both the LM potencies of homoeopathic Sulphur and those of the control were prepared by hand according to directions contained in Aphorism 270 of Hahnemann's "Organon of Medicine" [See Appendix A(i) and (ii)]. In the initial process (i.e. preparation of the 1CH trituration), one part flowers of Sulphur (99.5% purity) was triturated with 99 parts pure lactose powder to produce the Sulphur 1CH trituration, whereas the 'control' was produced by like trituration of one part pure lactose powder with 99 parts pure lactose powder. (Within the context of Homoeopharmaceutics, pure lactose is considered a medicinally inert substance (i.e. diluent.) Having produced a 1CH trituration of Sulphur and a '1CH' of diluent, all subsequent steps in the process were absolutely identical in all possible respects. The sole independent variable was therefore the presence, or absence, of Sulphur in the initial step in manufacture.

Rigorous steps were taken to ensure that other variables were not inadvertently introduced into the process of manufacture *viz.* different batches of diluent (i.e. lactose, distilled water or alcohol), varying atmospheric conditions and other environmental conditions and contaminants: 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 autoclaved (121°C for 15 minutes) before use and all potencies were prepared from a single container each of lactose powder, lactose granules (sifted three times through a double seive to ensure a 'standardised' granule size)[Appendix A(ii)], distilled water and alcohol (either 90% or 95%). At each stage of manufacture, the preparation of both experimental and control potencies was completed before progressing to the next stage. Furthermore, both experimental and control potencies were prepared to a parallel stage in a single process. This served to guard against / minimise differences in conditions surrounding the manufacture of parallel potencies.

For the purposes of this research, manufacture of LM potencies was only up to and including the tenth potency (LM10), the second, sixth and tenth of which would, upon completion of the entire manufacturing process, be produced in ≅20.8160 ml volumes. (See 3.2 below)

3.2 THE PREPARATION OF SAMPLE VOLUMES

Upon completion of the entire manufacturing process as outlined in Appendix A(i) and (ii), it was necessary to prepare the potencies required for sampling in volumes sufficient for the purpose.

In each respective case (SULPHUR/CONTROL LM´2, LM 6, and LM 10) 16 (sixteen) granules of the preceding potency (0/1, 0/5 or 0/9) were transferred to autoclaved 30 ml dark glass screwtop bottles. These granules were dissolved by the addition of 0.496 ml (16 x 31μl) distilled water. A volume of 20.3200 ml (16 x 1.2700 ml) 95 % ethanol was added and the liquid was succussed100 times by hand. Preparation was therefore a strictly proportional replica of the method employed in initial manufacture. Each resultant potency was labelled appropriately, individually wrapped in bubble plastic and securely packaged for priority postage to Cape Town.

3.3 THE MEASUREMENT OF SAMPLES

Samples were drawn from the respective sample bottles (fifteen of each) at the Department of Chemistry (UCT) and NMR spectra recorded for each sample by the resident NMR-technician.

Each sample (0.75 ml) was drawn into a coaxial tube with D_20 (deuterium oxide) (3 drops) as an external lock, and dioxane (1 drop) as a reference. No solvent was added to the samples. Fifteen (15) samples of each were drawn from the provided volumes in 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 micropipette, using an unused capillary tube for each sample.

The NMR-spectrometer employed was a Varian VXR200 operating at a frequency of 200.057 MHz. Acquisition time for each sample was 3.727 seconds, using a pulse width of 6°. Measurement of each sample was repeated eight times, at a thermostatically controlled temperature (probe head) of 298.1 K (25.0°C). The laboratory is maintained at a constant temperature of 21.0°C.

3.4 THE RECORDING OF THE DATA

Data were recorded in the form of NMR spectra (listing chemical shift and integration values) (See Appendix B) and computer-linked tables recorded on Microsoft Excel Version 5.0. The spectra and tables of the respective potencies of homoeopathic Sulphur and Control were recorded and printed by the NMR-Spectroscopy Laboratory of the Department of Chemistry, University of Cape Town.

3.5 STATISTICAL ANALYSIS

The chemical shift (δ, ppm) values (to four decimal places) of CH_3, CH_2, H_2O and OH signals of each sample were recorded as well as their respective integration values (to five decimal places). Only CH_2, H_2O and OH values were used for subsequent statistical analysis. In the case of respective CH_2 δ values, where four values are ordinarily available, an average was derived for the purposes of

statistical analysis. Where multiple values were provided for H_2O δ -values these too were averaged. Data thus manipulated, in each case underwent statistical evaluation according to the one-way Analysis of Variance (ANOVA) method for respective CH_2, OH and H_2O groups, and where necessary the two-sample unpaired t-test. The software package, Statgraphics 6.1, was used to facilitate statistical analysis.

3.5.1 THE ONE-WAY ANALYSIS OF VARIANCE (ANOVA) METHOD

The one-way analysis of variance (ANOVA) method is used for the analysis of data relating to three main effects. The resultant ANOVA table is constructed according to the mathematical model having the following equation:

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

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

μ is the overall (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 true differences between the three treatment groups + variation due to random error.

The ANOVA table resulting from this statistical manipulation is as follows:

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Significance Level
Between Treatments	K -1	SS (trt)	MS(trt)	$\frac{MS(trt)}{MS(err)}$	
Within Treatments (error)	N-K	SS (err)	MS(err)		
Total	N-1	SS (total)			

TABLE 3.1 - SAMPLE ANALYSIS OF VARIANCE (ANOVA) TABLE

where

$$SS(trt) = \sum_{j=1}^{k} \frac{T_{.j}^2}{n_j} - \frac{T_{..}^2}{N}$$

$$SS(tot) = \sum_{j=1}^{k} \sum_{i=1}^{n_j} Y_{ij}^2 - \frac{T_{..}^2}{N}$$

$$SS(err) = SS(tot) - SS(trt)$$

$$T_{..} = \sum_{j=1}^{k} \sum_{i=1}^{n_j} Y_{ij}$$

$$MS(trt) = \frac{SS(trt)}{K-1}$$

$$MS(err) = \frac{SS(err)}{N-K}$$

$$F_{cal} = \frac{MS(trts)}{MS(err)}$$

(Reich 1993 : 49 -52)

Only in the case of there having been a need for rejection of the null hypothesis (H_0: $\mu_1 = \mu_2 = \mu_3$) according to the decision rule (i.e. $F_{cal} > F_{tab}$), was further statistical analysis carried out, using the two-sample unpaired t-test.

3.5.2 THE TWO-SAMPLE UNPAIRED T-TEST

The two-sample unpaired t-test is employed to compare two unpaired or independent samples X and Y, where both X and Y are random samples drawn from respective parent populations having normal distributions and respective means μ_1 and μ_2 and variances σ_1^2 and σ_2^2. The two samples are independent of each other and have a common unknown variance σ^2.

These assumptions having been satisfied, the equality of the two population means may be tested as follows:

H_0: $\mu_1 = \mu_2$ (null hypothesis)

H_1: $\mu_1 \neq \mu_2$ (alternative hypothesis)

α = level of significance of test = 0.05

The H_0 is rejected if the absolute value of the calculated t-statistic (t_{cal}) is greater than the tabulated t-value (t_{tab}). If the absolute value of t_{cal} is less than or equal to t_{tab} the H_0 is accepted (i.e. $\mu_1 = \mu_2$).

The calculated and tabulated t-values are given as follows:

$$t_{cal} = \frac{\bar{X} - \bar{Y}}{S_p\,(1/n_1 + 1/n_2)^{1/2}}$$

$$\text{where } S_p^2 = \frac{(n_1 - 1)S_1^2 = (n_2 - 1)S_2^2}{n_1 + n_2 - 2}$$

= the pooled error variance;

t_{tab} = $t(df)_{\alpha/2}$ where

df = $n_1 + n_2 - 2$ = degrees of freedom of the t-statistic

α = level of significance of the test (Worku 1997 : 2)

The values of t_{tab} are read from the t-distribution table.

The unpaired t-tests were run at the 95% (α = 0.05) level of significance. Confidence intervals for $\mu_1 - \mu_2$ were calculated according to the formula:

$$\mu_1 - \mu_2 \in [(\bar{X} - \bar{Y}) - L , (\bar{X} - \bar{Y}) + L]$$

where L is the margin of error as given below:

$$L = t_{tab}.S_p.(1/n_1 + 1/n_2)^{1/2}$$ (Worku 1997: 1-2)

If the 95% confidence interval for $\mu_1 - \mu_2$ contained 0, and / or the p-value > α [0.05] the H_0 was accepted (i.e. $\mu_1 = \mu_2$). Otherwise H_0 was rejected at the same level. The two-way unpaired t-test was employed in all parallel potency comparisons, and in those cases where the ANOVA method indicated possible statistically significant differences existing within groups (See 4.2.1).

CHAPTER FOUR: THE RESULTS

4.1 THE CRITERIA GOVERNING THE ADMISSIBILITY OF THE DATA

Due to the sensitive nature of homoeopathic solutions, and the sensitivity of measurement of NMR-spectroscopy, it was imperative that the storage and drawing of samples, and their consequent measurement should be subject to the same degree of caution as is evident in the manufacture of the samples [See Appendix A(i) and (ii); 3.2 and 3.3]. Respective samples to be measured were taken from a single bottle of the respective potency (homoeopathic Sulphur and Control) and identified appropriately. All samples were drawn in an overlapping process. Pipetting equipment was not used across samples. All bottles from which samples were drawn were kept under the same conditions at all times.

Crude data obtained were subjected to statistical analysis as set out in 3.5. An initial comparison was made of the chemical shift (δ) values of CH_2, H_2O and OH signals of each respective sample. A subsequent comparison of integration values (mean intensity of the signal) of CH_2, H_2O and OH was made. In each case comparison was made both within respective experimental and control groups (e.g. SULPHUR LM2, LM6 and LM10), and between parallel experimental and control potencies (e.g. SULPHUR LM2 and CONTROL LM2). No data obtained was excluded from statistical analysis.

4.2 COMPARISON OF CHEMICAL SHIFT (δ) VALUES:

4.2.1 CHEMICAL SHIFT VALUES OF THE SULPHUR GROUPS

A comparison of CH_2 means of Sulphur LM2, LM6 and LM10 was made. The means of the three groups differ only in third and fourth decimal values. The very low standard deviation (S_x) and variance (S^2_x) values are notable and were found to be consistently low across all samples.

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	3.8315	3.8320	3.8371
S.D. (S_x)	0.003919	0.003154	0.01022
Variance (S_x^2)	0.000015	9.9497×10^{-6}	0.000104

Table 4.1 Summary statistics: CH_2 δ-values - SULPHUR GROUPS

A statistically significant difference between the three Sulphur groups was indicated after application of the Analysis of Variance(ANOVA) method (F_{cal} (3.359) > F_{tab} (3.23)) [Table 4.2].

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	0.0002906	1.45278×10^{-4}	3.359	0.0443
Within Treatments (error)	42	0.0018165	4.32489×10^{-5}		
Total	44	0.0021070			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.2 ANOVA Table: CH_2 δ-values - SULPHUR GROUPS

but subsequent unpaired t-tests [Tables 4.3 - 4.5]revealed that no statistically significant difference existed between LM2 and LM6 (p = 0.680762), LM2 and LM10 (p = 0.0557741) or LM6 and LM10 (p = 0.0753702).

CH2 δ-values - SULPHUR LM2 and SULPHUR LM6			
Sample Statistics	SULPHUR LM2	SULPHUR LM6	POOLED
Average	3.8315	3.83204	3.83177
Variance	1.53557×10^{-5}	9.94971×10^{-6}	1.26527×10^{-5}
Std. Deviation	0.003919	0.003154	0.0035570
Median	3.8309	3.8309	3.8309
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00320121 ; 0.00212121 (α = 0.05)	
Computed t-statistic	-0.41575	p-value:	0.680762

Table 4.3 Unpaired t-test: CH_2 δ-values - SULPHUR LM2 and SULPHUR LM6

CH2 δ-values - SULPHUR LM2 and SULPHUR LM10			
Sample Statistics	SULPHUR LM2	SULPHUR LM10	POOLED
Average	3.8315	3.83714	3.83432
Variance	1.53557×10^{-5}	1.04441×10^{-4}	5.98984×10^{-5}
Std. Deviation	0.003919	0.010220	0.007739
Median	3.8309	3.8336	3.8328
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.0114302 ; 0.00015023 (α = 0.05)	
Computed t-statistic	-1.99573	p-value:	0.0557741

Table 4.4 Unpaired t-test: CH_2 δ-values - SULPHUR LM2 and SULPHUR LM10

CH2 δ-values - SULPHUR LM6 and SULPHUR LM10			
Sample Statistics	SULPHUR LM6	SULPHUR LM10	POOLED
Average	3.83204	3.83714	3.83459
Variance	9.94971×10^{-6}	1.04441×10^{-4}	5.71954×10^{-5}
Std. Deviation	0.003154	0.010220	0.007563
Median	3.8309	3.8336	3.8325
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.0107581 ; 0.00055807 (α = 0.05)	
Computed t-statistic	-1.8468	p-value:	0.0753702

Table 4.5 Unpaired t-test: CH_2 δ-values - SULPHUR LM6 and SULPHUR LM10

Similar statistical evaluation of the Sulphur H_2O [Tables 4.6 and 4.7] and OH [Tables 4.8 and 4.9] chemical shift values yielded similar results. As was the case in the CH_2 values, mean values across the three groups differed only in the third and fourth decimal places, standard deviations and variances were very small, and

ANOVA revealed no statistically significant differences between groups [H2O: (F_{cal} $(0.304) < F_{tab} (3.23)$) and OH: ($F_{cal} (3.058) < F_{tab} (3.23)$)].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	4.7702	4.7711	4.7716
S.D. (S_x)	0.005329	0.004091	0.005065
Variance (S_x^2)	0.000028	0.000017	0.000026

Table 4.6 Summary statistics: H_2O δ-values - SULPHUR GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	1.43284×10^{-5}	7.16422×10^{-6}	0.304	0.7398
Within Treatments (error)	42	9.91169×10^{-4}	2.35993×10^{-5}		
Total	44	1.0055×10^{-3}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.7 ANOVA Table: H_2O δ-values - SULPHUR GROUPS

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	5.5534	5.5524	5.5559
S.D. (S_x)	0.004087	0.003843	0.004146
Variance (S_x^2)	0.000017	0.000015	0.000017

Table 4.8 Summary statistics: OH δ-values - SULPHUR GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	9.92218×10^{-5}	4.96109×10^{-5}	3.058	0.0575
Within Treatments (error)	42	6.91296×10^{-4}	1.62213×10^{-5}		
Total	44	7.80518×10^{-4}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.9 ANOVA Table: OH δ-values - SULPHUR GROUPS

4.2.2 CHEMICAL SHIFT VALUES OF THE CONTROL GROUPS

Statistical analysis of the chemical shift values of the CH_2, H_2O and OH signals of the control groups [Tables 4.10 - 4.15] revealed no statistically significant differences. As was noted in the case of the experimental group, means were extremely close between the three potencies within the group. Standard deviations and variances were consistently small.

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	3.8295	3.8308	3.8311
S.D. (S_x)	0.002433	0.003592	0.002671
Variance (S_x^2)	5.9207×10^{-6}	0.000013	7.1350×10^{-6}

Table 4.10 Summary statistics: CH_2 δ-values - CONTROL GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	2.11240×10^{-5}	1.05620×10^{-5}	1.221	0.3052
Within Treatments (error)	42	3.63368×10^{-4}	8.65162×10^{-6}		
Total	44	3.84492×10^{-4}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.11 ANOVA Table: CH_2 δ-values - CONTROL GROUPS

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	4.7713	4.7724	4.7757
S.D. (S_x)	0.004509	0.00548	0.005806
Variance (S_x^2)	0.000020	0.000030	0.000034

Table 4.12 Summary statistics: H_2O δ-values - CONTROL GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	0.0001539	7.69749×10^{-5}	2.738	0.0763
Within Treatments (error)	42	0.0011807	2.81125×10^{-5}		
Total	44	0.0013347			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.13 ANOVA Table: H_2O δ-values - CONTROL GROUPS

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	5.5529	5.5533	5.5500
S.D. (S_x)	0.003826	0.003155	0.005593
Variance (S_x^2)	0.000015	9.9541×10^{-6}	0.000031

Table 4.14 Summary statistics: OH δ-values - CONTROL GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	9.96298×10^{-5}	4.98149×10^{-5}	2.674	0.0807
Within Treatments (error)	42	7.82356×10^{-4}	1.86275×10^{-5}		
Total	44	8.81986×10^{-4}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.15 ANOVA Table: OH δ-values - CONTROL GROUPS

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

Although no statistically significant differences were noted within the respective experimental and control groups (i.e. between individual potencies within each group) the application of two-way unpaired t-tests to individual pairs of parallel potencies revealed the existence of significant differences between the chemical shift values of all three signals of parallel LM10 potencies.

4.2.3.1 THE CH_2 CHEMICAL SHIFTS

There is no statistically significant difference between the means of CH_2 chemical shift values ($p = 0.108704$) of parallel LM2 potencies [Table 4.16 below]. Comparison of parallel LM 6 potencies also revealed no statistically significant difference ($p = 0.326211$).

CH_2 δ-values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	3.8315	3.82953	3.83051
Variance	1.53557×10^{-5}	5.92067×10^{-6}	1.06382×10^{-5}
Std. Deviation	0.003919	0.002433	0.003262
Median	3.8309	3.8294	3.8303
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00046685 ; 0.00441351 ($\alpha = 0.05$)	
Computed t-statistic	1.6569	p-value:	0.108704

Table 4.16 Unpaired t-test: CH_2 δ-values - SULPHUR LM2 and CONTROL LM2

CH_2 δ-values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	3.83204	3.83081	3.83142
Variance	9.94971×10^{-6}	1.28992×10^{-5}	1.14245×10^{-5}
Std. Deviation	0.003154	0.003592	0.003380
Median	3.8309	3.8312	3.83105
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00129542 ; 0.00376208 ($\alpha = 0.05$)	
Computed t-statistic	0.999293	p-value:	0.326211

Table 4.17 Unpaired t-test: CH_2 δ-values - SULPHUR LM6 and CONTROL LM6

The outcome of the unpaired t-test applied to parallel LM10 potencies led to rejection of the null hypothesis ($\mu_1 = \mu_2$). A statistically significant difference was noted to exist between the means of the CH_2 chemical shift values of Sulphur LM10 and Control LM10 ($p = 0.0352837 < \alpha$). [Table 4.18]

CH_2 δ-values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	3.83714	3.83111	3.83459
Variance	1.04441×10^{-4}	7.13495×10^{-6}	5.5788×10^{-5}
Std. Deviation	0.010220	0.002671	0.007469
Median	3.8336	3.8310	3.8324
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		0.000445306 ; 0.0116214 (α = 0.05)	
Computed t-statistic	2.21216	p-value: 0.0352837	

Table 4.18 Unpaired t-test: CH_2 δ-values - SULPHUR LM10 and CONTROL LM10

4.2.3.2 THE H_2O CHEMICAL SHIFTS

`As was the case in comparison of the means of the CH_2 chemical shift values of parallel potencies, the null hypothesis was accepted at the 95% level of significance ($p > \alpha = 0.05$) for the H_2O chemical shift means of parallel LM2 and LM6 potencies. The p-values were, respectively, 0.551433 [Table 4.19] and 0.474521 [Table 4.20].

H_2O δ-values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	4.77022	4.77131	4.77076
Variance	2.84017×10^{-5}	2.03292×10^{-5}	2.43655×10^{-5}
Std. Deviation	0.005329	0.004509	0.004936
Median	4.7691	4.7708	4.77035
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00477963 ; 0.00260630 (α = 0.05)	
Computed t-statistic	-0.602892	p-value: 0.551433	

Table 4.19 Unpaired t-test: H_2O δ-values - SULPHUR LM2 and CONTROL LM2

H_2O δ-values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	4.77114	4.77242	4.77178
Variance	1.67397×10^{-5}	3.00274×10^{-5}	2.33836×10^{-5}
Std. Deviation	0.004091	0.005480	0.004836
Median	4.7714	4.7738	4.7717
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00489776 ; 0.00233779 (α = 0.05)	
Computed t-statistic	-0.724912	p-value: 0.474521	

Table 4.20 Unpaired t-test: H_2O δ-values - SULPHUR LM6 and CONTROL LM6

The null hypothesis was rejected, however, in the t-test applying to the H_2O means of the Sulphur LM10 and Control LM10 potencies. The p-value in this instance was 0.0480296 which, although marginally so, is less than α =0.05. One may therefore conclude that a significant difference exists between the two means compared [Table 4.21].

H_2O δ-values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	4.77157	4.77569	4.77363
Variance	2.56564×10^{-5}	3.37098×10^{-5}	2.96831×10^{-5}
Std. Deviation	0.005065	0.005806	0.005448
Median	4.7711	4.7787	4.77345
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00818941 ; -0.00003726 (α = 0.05)	
Computed t-statistic	-2.06762	p-value: 0.0480296	

Table 4.21 Unpaired t-test: H_2O δ-values - SULPHUR LM10 and CONTROL LM10

Bar graphs constructed by using the means of CH_2 and H_2O chemical shift values (Figures 4.1 and 4.2 overleaf) illustrate an apparent inverse relationship of CH_2 and H_2O signals between experimental and control groups. The CH_2 mean of Sulphur LM10 is significantly larger than that of the control, whereas the H_2O mean of the Control LM10 is proportionately greater than that of the Sulphur LM10. The means

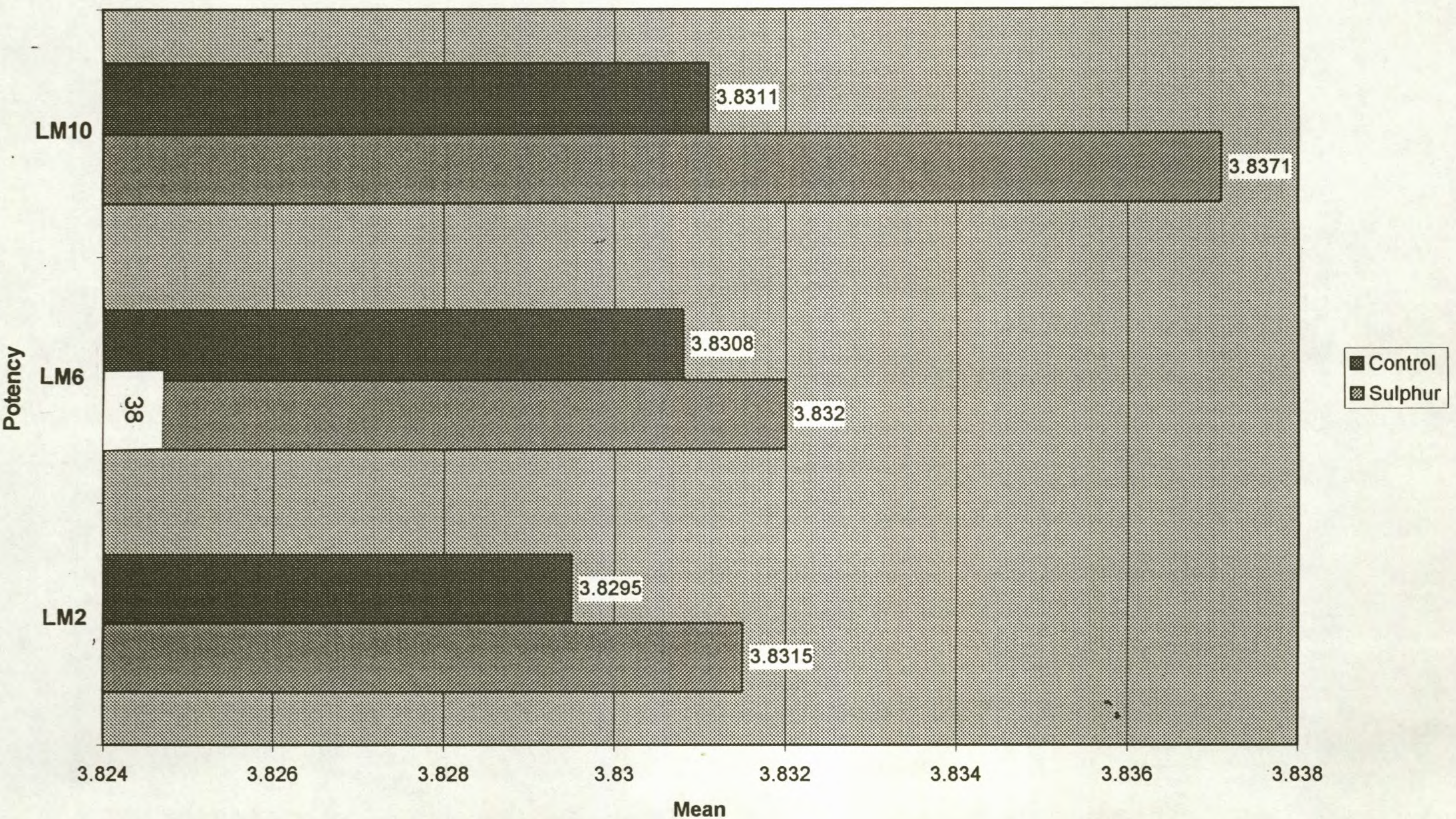

Figure 4.1 A Comparison of CH_2 Chemical Shift Values

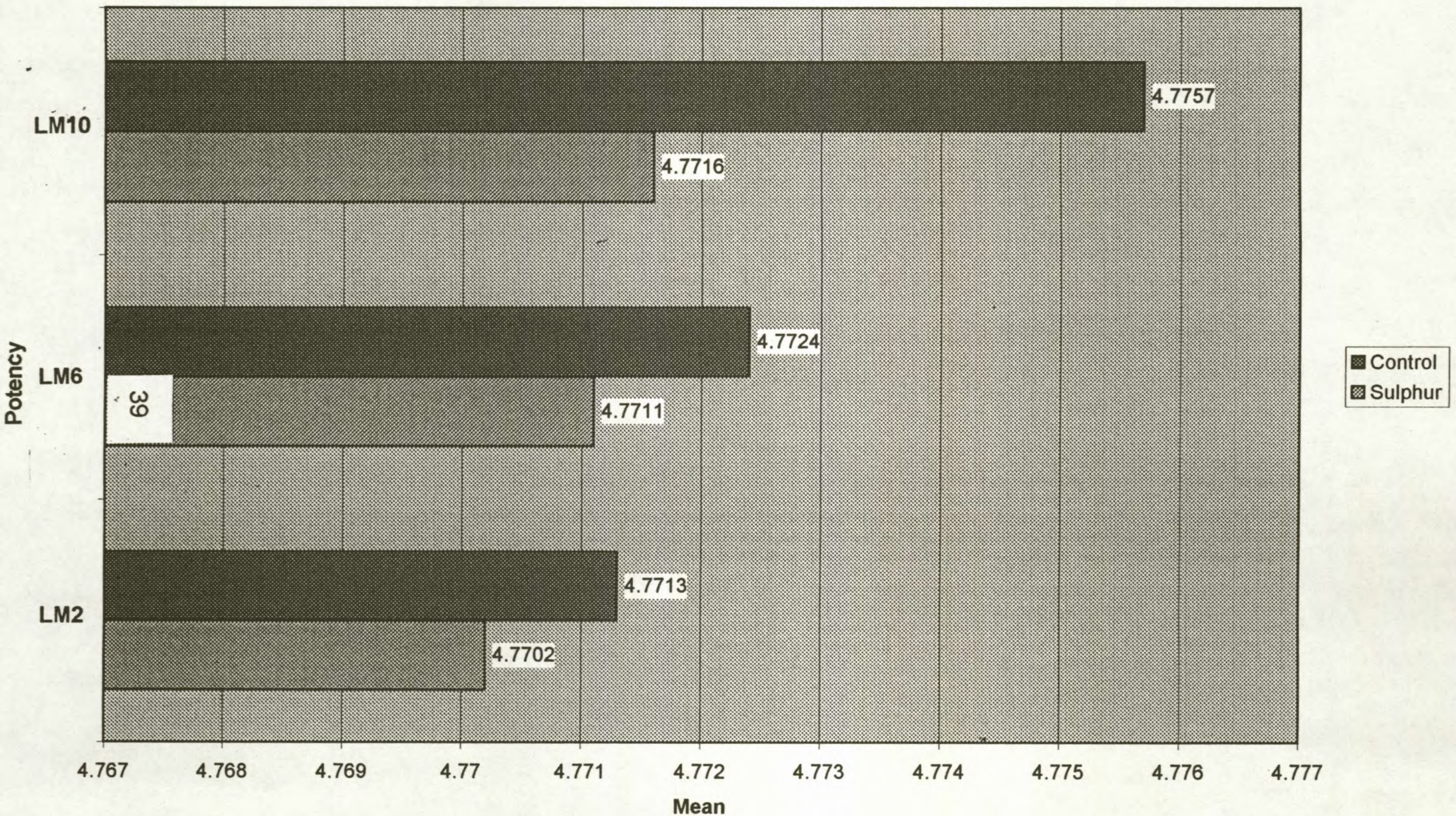

Figure 4.2 A Comparison of H_2O Chemical Shift Values

of the Sulphur CH_2 signals are also consistently greater than those of the control, whereas the inverse applies to the H_2O means.

4.2.3.3 THE OH CHEMICAL SHIFTS

The results of statistical analysis of the OH chemical shift values of parallel potencies were similar to those of preceding analyses. The null hypothesis was accepted (at the 95% level of significance) for parallel LM2 and LM6 potencies. Their respective p-values were 0.738891 [Table 4.22] and 0.479528 [Table 4.23], indicating that no statistically significant difference exists ($p > \alpha$).

OH δ-values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	5.55339	5.55291	5.55315
Variance	1.6705×10^{-5}	1.46421×10^{-5}	1.56735×10^{-5}
Std. Deviation	0.004087	0.003827	0.003959
Median	5.5517	5.5536	5.5520
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00247524 ; 0.00344857 ($\alpha = 0.05$)	
Computed t-statistic	0.33665	p-value:	0.738891

Table 4.22 Unpaired t-test: OH δ-values - SULPHUR LM2 and CONTROL LM2

OH δ-values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	5.55243	5.55335	5.55289
Variance	1.47664×10^{-5}	9.9541×10^{-6}	1.23602×10^{-5}
Std. Deviation	0.003843	0.003155	0.003516
Median	5.5517	5.5549	5.5525
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.00355028 ; 0.00171028 ($\alpha = 0.05$)	
Computed t-statistic	-0.716647	p-value:	0.479528

Table 4.23 Unpaired t-test: OH δ-values - SULPHUR LM6 and CONTROL LM6

The null hypothesis was rejected with reference to the parallel LM10 potencies. A statistically significant difference (p =0.00256373) was found between the means of the OH chemical shift values of Sulphur LM10 and Control LM10 samples [Table 4.24].

OH δ-values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	5.55595	5.54999	5.55297
Variance	1.71927×10^{-5}	3.12864×10^{-5}	2.42395×10^{-5}
Std. Deviation	0.004146	0.005593	0.004923
Median	5.5562	5.5500	5.55385
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		0.00226992 ; 0.00963674 (α = 0.05)	
Computed t-statistic	3.31153	p-value:	0.00256373

Table 4.24 Unpaired t-test: OH δ-values - SULPHUR LM10 and CONTROL LM10

A bar graph [Figure 4.3 overleaf] constructed of the means of OH chemical shift values of the six sample groups illustrates a notably significant difference in LM10 means compared to the differences existing between CH_2 and H_2O means [Figure 4.1 and 4.2].

4.3 COMPARISON OF INTEGRATION VALUES:

4.3.1 INTEGRATION VALUES OF THE SULPHUR GROUPS

A comparison of CH_2 means of Sulphur LM2, LM6 and LM10 was made. As was noted in the corresponding chemical shift value comparison the means are within a narrow range (1.53073) and the standard deviation (S_x) and variance (S^2_x) values

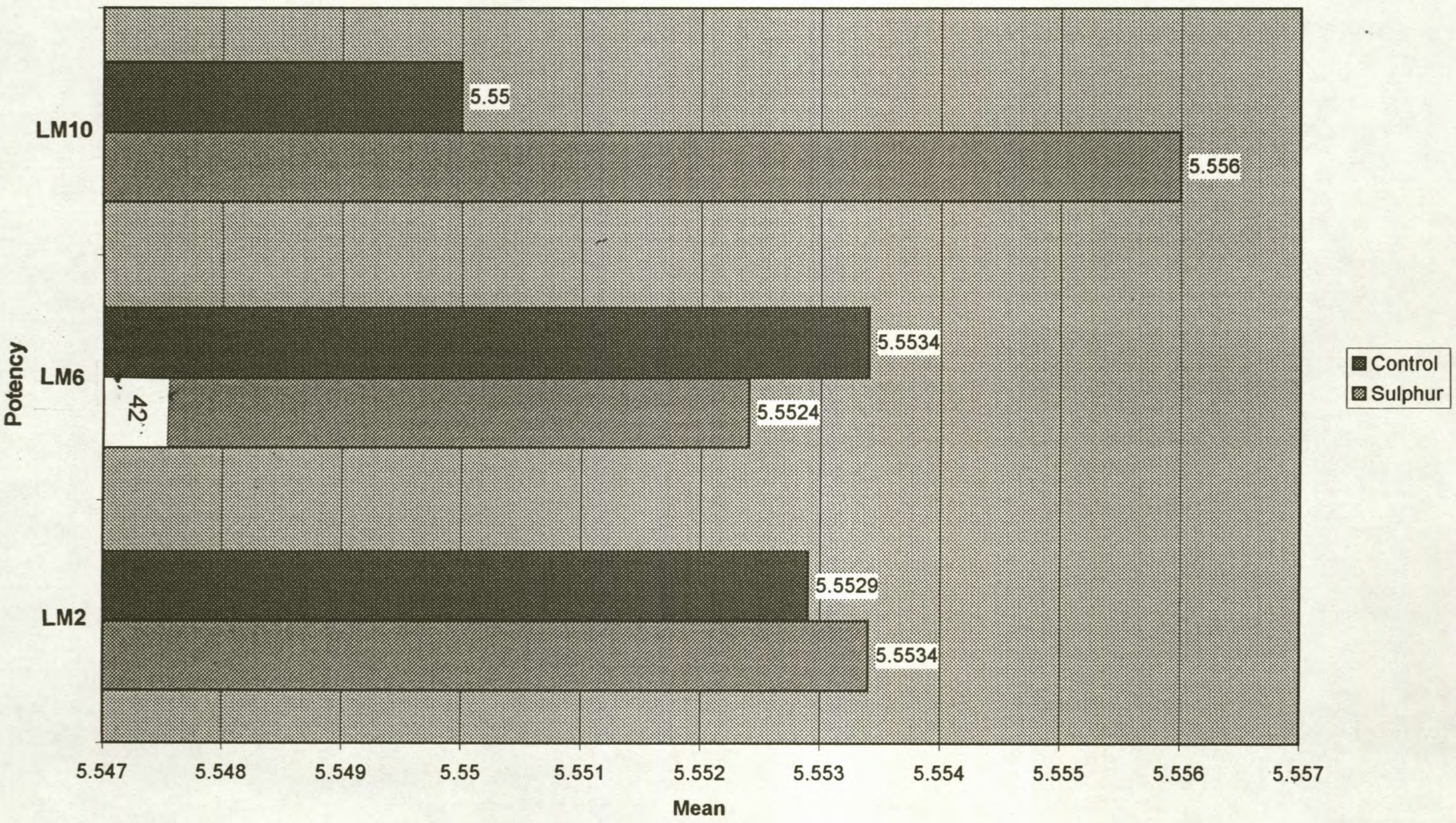

Figure 4.3 A Comparison of OH Chemical Shift Values

are proportionately low. Of particular interest is the relatively lower (compared to LM2 and LM6) LM10 S_x and S_x^2 values [Table 4.25].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	53.80223	54.36303	52.83230
S.D. (S_x)	1.75916	1.69430	1.14662
Variance (S_x^2)	3.094626	2.87064	1.31473

Table 4.25 Summary statistics: CH_2 integration values - SULPHUR GROUPS

ANOVA method applied to the three potencies in this group led to a rejection of the null hypothesis ($\mu_1 = \mu_2 = \mu_3$). F_{cal} (3.359) was calculated to be greater than F_{tab} (3.23) indicating that a statistically significant difference exists between at least two of the potencies involved [Table 4.26].

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	0.0002906	1.45278×10^{-4}	3.359	0.0443
Within Treatments (error)	42	0.0018165	4.32489×10^{-5}		
Total	44	0.0021070			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.26 ANOVA TABLE: CH_2 integration values - SULPHUR GROUPS

Subsequent unpaired t-tests [Tables 4.27 -4.29] led to the rejection of the null hypothesis with respect to the mean integration values of the LM6 and LM10 potencies (p = 0.0072204). No statistically significant difference was found between LM2 and LM6 (p = 0.38143) nor LM2 and LM10 (p = 0.0844452).

CH2 integration values - SULPHUR LM2 and SULPHUR LM6			
Sample Statistics	SULPHUR LM2	SULPHUR LM6	POOLED
Average	53.8022	54.3630	54.0826
Variance	3.09463	2.87064	2.98263
Std. Deviation	1.75915	1.69430	1.72703
Median	53.4979	54.6561	54.3777
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-1.85287 ; 0.731276 (α = 0.05)	
Computed t-statistic	-0.889279	p-value: 0.38143	

Table 4.27 Unpaired t-test: CH_2 integration values - SULPHUR LM2 and SULPHUR LM6

CH2 integration values - SULPHUR LM2 and SULPHUR LM10			
Sample Statistics	SULPHUR LM2	SULPHUR LM10	POOLED
Average	53.8022	52.8323	53.3173
Variance	3.09463	1.31473	2.20468
Std. Deviation	1.75915	1.14662	1.48482
Median	53.4979	53.1340	53.2542
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.140925 ; 2.0808 (α = 0.05)	
Computed t-statistic	1.78897	p-value: 0.0844452	

Table 4.28 Unpaired t-test: CH_2 integration values - SULPHUR LM2 and SULPHUR LM10

CH2 integration values - SULPHUR LM6 and SULPHUR LM10			
Sample Statistics	SULPHUR LM6	SULPHUR LM10	POOLED
Average	54.3630	52.8323	53.5977
Variance	2.87064	1.31473	2.09268
Std. Deviation	1.69430	1.14662	1.44661
Median	54.6561	53.1340	53.5159
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		0.448456 ; 2.61302 (α = 0.05)	
Computed t-statistic	2.89787	p-value: 0.0072204	

Table 4.29 Unpaired t-test: CH_2 integration values - SULPHUR LM6 and SULPHUR LM10

No statistical differences were found to exist between the mean H_2O integration values of respective potencies of Sulphur. Although it would appear, in the light of the relatively higher mean, standard deviation and variance values of the LM10 potency [Table 4.30], that some difference might exist within the group,

subsequent ANOVA method analysis indicated that the null hypothesis ought to be accepted

(F_{cal} (0.304) < F_{tab} (3.23)) [Table 4.31].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	70.92667	70.55116	72.25204
S.D. (S_x)	6.37361	6.31325	6.99341
Variance (S_x^2)	40.62295	39.85708	48.90783

Table 4.30 Summary statistics: H_2O integration values - SULPHUR GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	1.43284×10^{-5}	7.16422×10^{-6}	0.304	0.7398
Within Treatments (error)	42	9.91169×10^{-4}	2.35993×10^{-5}		
Total	44	1.0055×10^{-3}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.31 ANOVA TABLE: H_2O integration values - SULPHUR GROUPS

Statistical analysis (ANOVA) of the OH integration values of the Sulphur potencies revealed no significant difference (F_{cal} (3.058) < F_{tab} (3.23)) between individual LM potencies [Tables 4.32 and 4.33].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	15.16969	15.17349	15.18540
S.D. (S_x)	0.27468	0.38320	0.26894
Variance (S_x^2)	0.07545	0.14684	0.07233

Table 4.32 Summary statistics: OH integration values - SULPHUR GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	9.92218×10^{-5}	4.96109×10^{-5}	3.058	0.0575
Within Treatments (error)	42	6.91296×10^{-4}	1.62213×10^{-5}		
Total	44	7.80518×10^{-4}			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.33 ANOVA TABLE: OH integration values - SULPHUR GROUPS

4.3.2 INTEGRATION VALUES OF THE CONTROL GROUPS

The results obtained through statistical analysis of the integration data relating to the control potencies were not wholly different from that obtained in the experimental group.

Sample statistics derived from the CH_2 integration values [Table 4.34] are not in isolation remarkable. Of particular noteworthiness, however, are the relatively high S_x and S_{x2} values of the control group LM10 potencies when compared to the Sulphur group (S_x: 1.91955 vs. 1.14662 and ; S_x^2: 3.684507 vs. 1.31473).

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	54.62892	54.80787	57.00985
S.D. (S_x)	1.76192	1.51228	1.91951
Variance (S_x^2)	3.10436	2.286977	3.684507

Table 4.34 Summary statistics: CH_2 integration values - CONTROL GROUPS

As was found in the experimental group, statistically significant differences (F_{cal} (8.718) > F_{tab} (3.23)) exist between the mean CH_2 integration values of individual potencies within the control group [Table 4.35].

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	52.74819	26.374094	8.718	0.0007
Within Treatments (error)	42	127.06219	3.025290		
Total	44	179.81037			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.35 ANOVA TABLE: CH_2 integration values - CONTROL GROUPS

In contrast to the experimental group, however, these differences were found to exist between both LM2 and LM10 (p = 0.00142383 [Table 4.37] and LM6 and LM10 potencies (p = 0.00161819) [Table 4.38]. No statistically significant difference was found between LM2 and LM6 (p =0.767546) [Table 4.36].

CH_2 integration values - CONTROL LM2 and CONTROL LM6			
Sample Statistics	CONTROL LM2	CONTROL LM6	POOLED
Average	54.6289	54.8079	54.7184
Variance	3.10436	2.28697	2.69566
Std. Deviation	1.76192	1.51227	1.64185
Median	54.5328	54.6656	54.5992
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-1.40729 ; 1.04941 (α = 0.05)	
Computed t-statistic	-0.298473	p-value:	0.767546

Table 4.36 Unpaired t-test: CH_2 integration values - CONTROL LM2 and CONTROL LM6

CH_2 integration values - CONTROL LM2 and CONTROL LM10			
Sample Statistics	CONTROL LM2	CONTROL LM10	POOLED
Average	54.6289	57.0099	55.8194
Variance	3.10436	3.68451	3.39443
Std. Deviation	1.76192	1.91951	1.8424
Median	54.5328	57.0099	55.7237
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-3.75932 ; -1.00254 (α = 0.05)	
Computed t-statistic	-3.5391	p-value:	0.00142383

Table 4.37 Unpaired t-test: CH_2 integration values - CONTROL LM2 and CONTROL LM10

CH_2 integration values - CONTROL LM6 and CONTROL LM10			
Sample Statistics	CONTROL LM6	CONTROL LM10	POOLED
Average	54.8079	57.0099	55.9089
Variance	2.28697	3.68451	2.98574
Std. Deviation	1.51227	1.91951	1.72793
Median	54.6656	57.0099	55.5220
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-3.49473 ; -0.909239 (α = 0.05)	
Computed t-statistic	-3.48995	p-value:	0.00161819

Table 4.38 Unpaired t-test: CH_2 integration values - CONTROL LM6 and CONTROL LM10

No statistically significant differences were noted between the H_2O integration values of individual potencies in the control group (F_{cal} (1.442) < F_{tab} (3.23)). A relatively low variance (26.567518) was noted in the summary statistics [Table 4.39] of the LM10 control samples. This variance is low in respect of the other control potencies as well as those of the experimental group, in particular that of the corresponding potency (48.90783) [Table 4.30].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	69.77202	69.58843	66.53946
S.D. (S_x)	6.10772	6.24739	5.15437
Variance (S_x^2)	37.304226	39.029824	26.567518

Table 4.39 Summary statistics: H_2O integration values - CONTROL GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	98.8974	49.448676	1.442	0.2480
Within Treatments (error)	42	1440.6191	34.300454		
Total	44	1539.5164			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.40 ANOVA TABLE: H_2O integration values - CONTROL GROUPS

As was the case in the experimental analysis, the null hypothesis was accepted (F_{cal} (2.633) < F_{tab} (3.23)) for the OH integration value means of the control potencies [Table 4.42].

	LM2	LM6	LM10
Sample size	15	15	15
Mean (x)	15.19532	15.20101	15.02164
S.D. (S_x)	0.23623	0.22055	0.27045
Variance (S_x^2)	0.055806	0.048642	0.073143

Table 4.41 Summary statistics: OH integration values - CONTROL GROUPS

Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F_{cal}	Sig. level
Between Treatments	2	0.311769	0.155885	2.633	0.0837
Within Treatments (error)	42	2.486645	0.0592058		
Total	44	2.798414			

$F_{tab} = F_{0.05}(2,42) = 3.23$

Table 4.42 ANOVA TABLE: OH integration values - CONTROL GROUPS

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

In view of the number of statistically significant differences noted within the respective experimental and control groups, it was in some measure anticipated that statistically significant differences may exist between integration values of parallel experimental and control potencies. The application of two-way unpaired t-tests to individual pairs of parallel potencies revealed that such differences do indeed exist. As was noted in the comparison of chemical shift values (See 4.2.3.1 - 4.2.3.3), statistically significant differences exist between the experimental and control LM10 potencies.

4.3.3.1 THE CH_2 INTEGRATION VALUES

There is no statistically significant difference between the means of CH_2 integration values ($p = 0.20899$) of parallel LM2 potencies [Table 4.43 below]. Comparison of parallel LM6 potencies also revealed no statistically significant difference.
($p = 0.454428$)

CH_2 integration values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	53.8022	54.6289	54.2156
Variance	3.09463	3.10436	3.09949
Std. Deviation	1.75915	1.76192	1.76054
Median	53.4979	54.5328	54.3777
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-2.14383 ; 0.490454 ($\alpha = 0.05$)	
Computed t-statistic	-1.28596	p-value:	0.20899

Table 4.43 Unpaired t-test: CH_2 integration values - SULPHUR LM2 and CONTROL LM2

CH_2 integration values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	54.3630	54.8079	54.5854
Variance	2.87067	2.28698	2.57882
Std. Deviation	1.69431	1.51228	1.60587
Median	54.6561	54.6656	54.6609
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.756599 ; 1.64626 ($\alpha = 0.05$)	
Computed t-statistic	0.758604	p-value:	0.454428

Table 4.44 Unpaired t-test: CH_2 integration values - CONTROL LM6 and CONTROL LM6

A statistically significant difference ($p = 7.06947 \times 10^{-8}$) was found between CH_2 integration values of parallel LM10 potencies [Table 4.45 below].

CH_2 integration values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	52.8323	57.0099	54.9211
Variance	1.31473	3.68451	2.49962
Std. Deviation	1.14661	1.91951	1.58102
Median	53.1340	57.0099	54.5421
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-5.3604 ; -2.99473 ($\alpha = 0.05$)	
Computed t-statistic	-7.23631	p-value:	7.06947×10^{-8}

Table 4.45 Unpaired t-test: CH_2 integration values - SULPHUR LM10 and CONTROL LM10

4.3.3.2 THE H_2O INTEGRATION VALUES

As was the case in comparison of the means of the CH_2 integration values of parallel potencies, the null hypothesis was accepted at the 95% level of significance

($p > \alpha = 0.05$) for the H_2O integration means of parallel LM2 and LM6 potencies. The p-values were, respectively, 0.616411 [Table 4.46] and 0.677833 [Table 4.47].

H_2O integration values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	70.9267	69.7720	70.3493
Variance	40.623	37.3042	38.9636
Std. Deviation	6.37361	6.10772	6.24208
Median	71.7777	71.8016	71.7896
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-3.51535 ; 5.82466 ($\alpha = 0.05$)	
Computed t-statistic	0.506586	p-value:	0.616411

Table 4.46 Unpaired t-test: H_2O integration values - SULPHUR LM2 and CONTROL LM2

H_2O integration values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	70.5512	69.5884	70.0698
Variance	39.8571	39.0298	39.4435
Std. Deviation	6.31325	6.24739	6.2804
Median	72.0389	69.3096	71.283
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-3.73595 ; 5.6614 ($\alpha = 0.05$)	
Computed t-statistic	0.419803	p-value:	0.677833

Table 4.47 Unpaired t-test: H_2O integration values - SULPHUR LM6 and CONTROL LM6

Parallel LM 10 potencies were, however, significantly different ($p = 0.0166579$) with respect to mean H_2O integration values [Table 4.48].

H_2O integration values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	72.2521	66.5395	69.3958
Variance	48.9078	26.5675	37.7376
Std. Deviation	6.99341	5.15437	6.1431
Median	73.7135	66.5395	68.7039
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		1.11664 ; 10.3085 ($\alpha = 0.05$)	
Computed t-statistic	2.54669	p-value:	0.0166579

Table 4.48 Unpaired t-test: H_2O integration values - SULPHUR LM10 and CONTROL LM10

The apparent inverse relationship of means of CH_2 and H_20 values noted in reference to chemical shift values (See 4.2.3.2) is likewise evident with respect to corresponding integration value means. Figures 4.4 and 4.5 (overleaf) clearly illustrate this dovetailing relationship, as well as clarifying the extent of significant difference to be noted between parallel LM10 means.

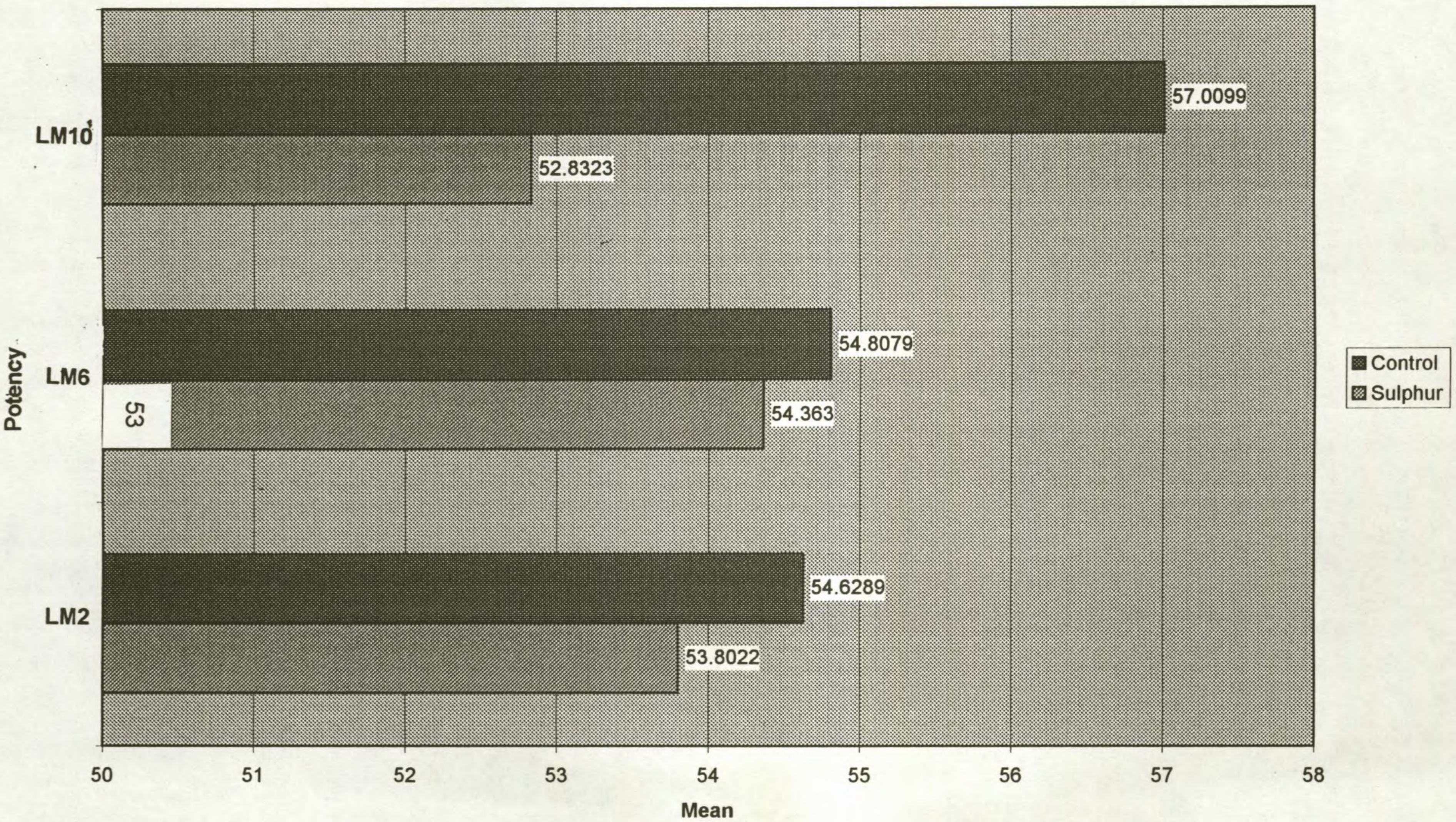

Figure 4.4 A Comparison of CH_2 Integration Values

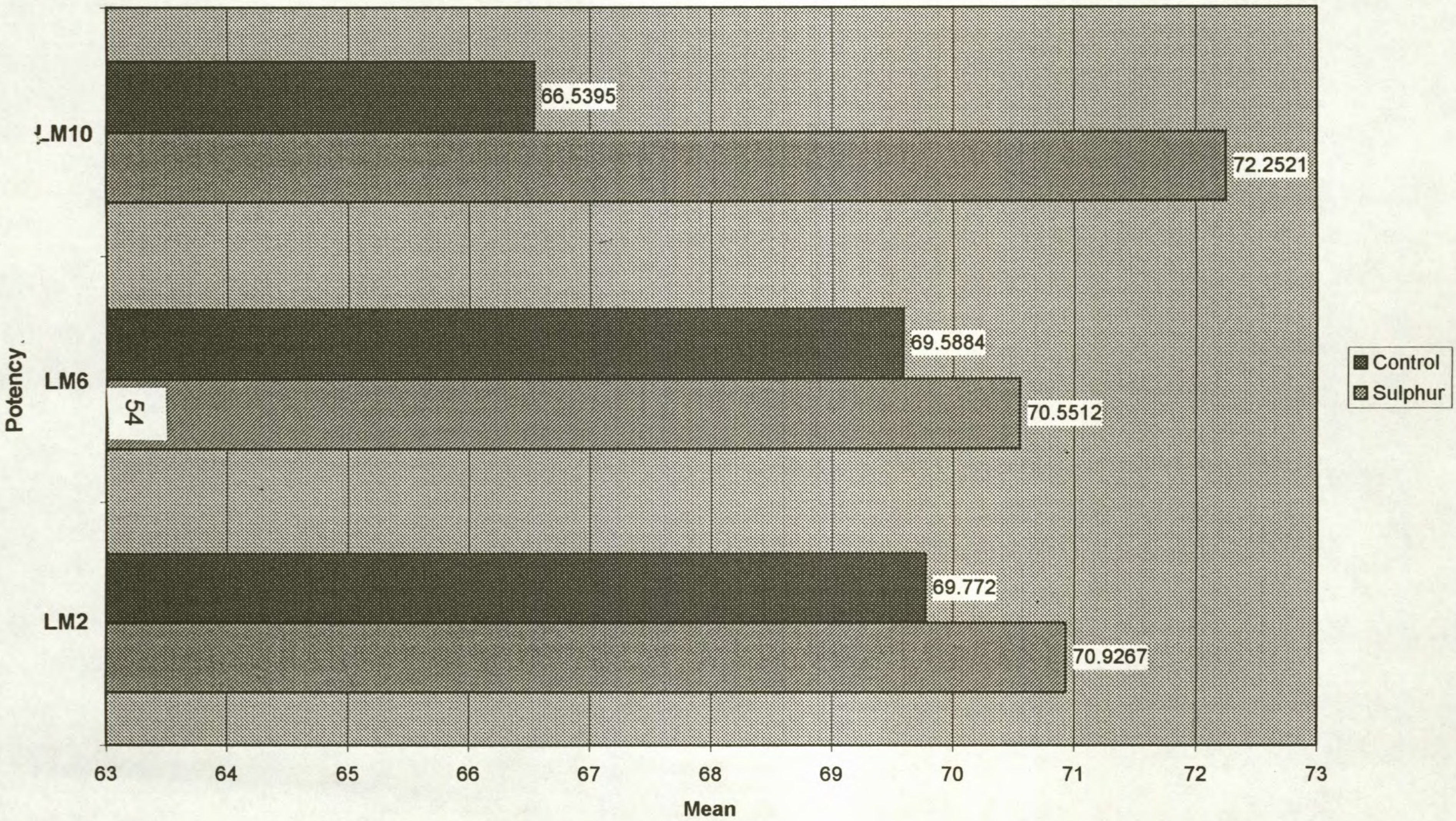

Figure 4.5 A Comparison of H_2O Integration Values

4.3.3.3 THE OH INTEGRATION VALUES

The null hypothesis was accepted for all unpaired t-tests applied to the OH integration values. No statistically significant difference exists between parallel LM2 (p = 0.786064) [Table 4.49], LM6 (p = 0.811286) [Table 4.50] or LM10 (p = 0.107492) [Table 4.51]potencies.

OH integration values - SULPHUR LM2 and CONTROL LM2			
Sample Statistics	SULPHUR LM2	CONTROL LM2	POOLED
Average	15.1697	15.1953	15.1825
Variance	0.075448	0.055806	0.065627
Std. Deviation	0.274677	0.236232	0.256177
Median	15.2036	15.1613	15.1769
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.217293 ; 0.166024 ($\alpha = 0.05$)	
Computed t-statistic	-0.274043	p-value: 0.786064	

Table 4.49 Unpaired t-test: OH integration values - SULPHUR LM2 and CONTROL LM2

OH integration values - SULPHUR LM6 and CONTROL LM6			
Sample Statistics	SULPHUR LM6	CONTROL LM6	POOLED
Average	15.1735	15.2010	15.1872
Variance	0.146842	0.048642	0.097742
Std. Deviation	0.383199	0.220549	0.312637
Median	15.1491	15.1090	15.1147
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.261415 ; 0.206383 ($\alpha = 0.05$)	
Computed t-statistic	-0.241032	p-value: 0.811286	

Table 4.50 Unpaired t-test: OH integration values - SULPHUR LM6 and CONTROL LM6

OH integration values - SULPHUR LM10 and CONTROL LM10			
Sample Statistics	SULPHUR LM10	CONTROL LM10	POOLED
Average	15.1854	15.0216	15.1035
Variance	0.072330	0.073143	0.072737
Std. Deviation	0.268943	0.270450	0.269698
Median	15.1706	15.0874	15.1247
CONFIDENCE INTERVAL (Difference of means at 95% Level of Significance)		-0.038014 ; 0.365534 ($\alpha = 0.05$)	
Computed t-statistic	1.66288	p-value:	0.107492

Table 4.51 Unpaired t-test: OH integration values - SULPHUR LM10 and CONTROL LM10

A bar graph [Figure 4.6 overleaf] constructed of the integration value means of the six sample groups indicates a suspiciously low Control LM10 value (15.0216) compared to the other five values (ranging between 15.1697 and 15.201). This is however not of any statistical significance.

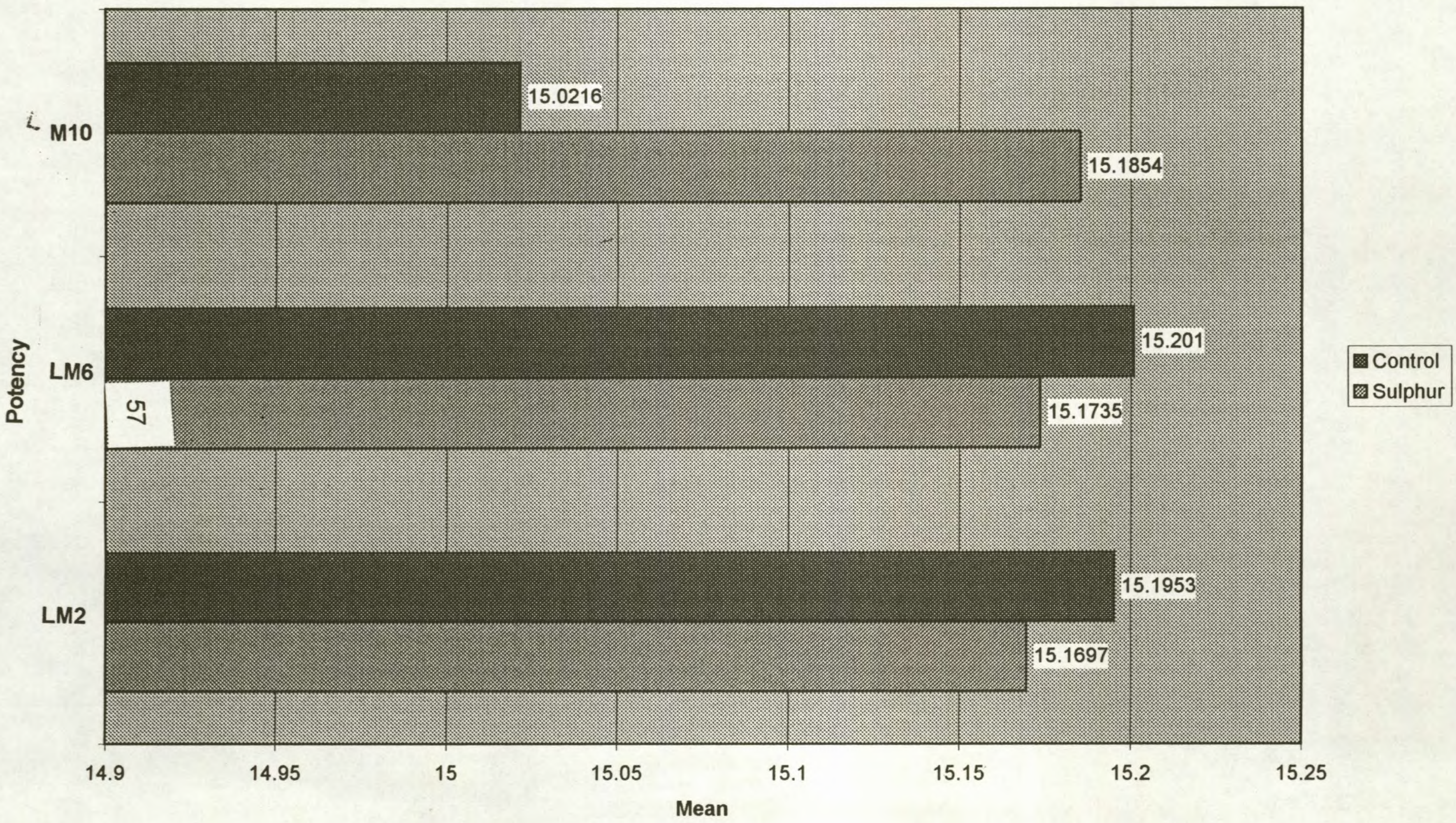

Figure 4.6 A Comparison of OH Integration Values

CHAPTER FIVE: DISCUSSION

The results of this investigation clearly indicate that differences exist between LM10 potencies of homoeopathic Sulphur and a lactose-based control. No statistically significant differences were found to exist between respective LM2 and LM6 potencies.

Statistical analyses (unpaired t-tests) indicate that LM10 potencies of homoeopathic Sulphur and a lactose control differ in terms of the chemical shift values of all three signals evaluated (i.e. signals), as well as the integration values of the CH_2 and H_2O signals. Only the OH integration values yield no significant difference. Although no clear model exists (Bol 1997) for the interpretation of the observed differences certain observations need elaboration.

The statistically significant differences in chemical shift value would serve to indicate that the molecular environment in which parallel LM10 proton groups (i.e. CH_2, H_2O and OH) find themselves is different. The relatively higher values found in CH_2 Sulphur chemical shifts, statistically significant in LM10, but noted throughout the three potencies investigated [Figure 4.1], would suggest that protons in this group are relatively more 'shielded' (see 2.8) than those in the control potencies. This in turn would indicate increased electron density at this point in the molecule. In contrast the consistently lower, and again statistically significant at the LM10

potency level, chemical shift values of the H_2O signals (Figure 4.2) would suggest relative 'unshielding' of protons (and hence decreased electron density) in this group, relative to those of the control. The markedly greater OH chemical shift value of Sulphur LM10 likewise would suggest a greater degree of shielding than is evident in the control LM10 potency.

The significant differences and integration values (in LM10 potencies) suggest differences in the relative proton densities at the respective poles. Figure 4.4 clearly illustrates a notably stronger CH_2 signal for the control potency over the Sulphur, whereas the inverse applies to the H_2O signal. This would suggest that relative proton distribution within the respective LM10 potencies is distinctly different, and possibly in inverse relationship. A sinusoidal trend is observed in the OH integration values of both potencies [Figure 4.6]. Furthermore, manipulation of integration values in the manner of Weingärtner (1991) (i.e. the derivation of ratios of integration values such that $Q_1 = CH_2/H_2O$; $Q_2 = CH_2/OH$ and $Q_3 = H_2O/OH$) yields results [see Appendix D] suggesting that an increased number of protons is associated with the H_2O signal <u>across all six sample groups</u>. This may be due to "interference" from the presence of lactose molecules within the sample (not significant enough to produce any peaks), but in the author's opinion is more suggestive of structural change.

There is however insufficient evidence to relate the above findings to Antonchenko and Ilyin's (1994) "proton transfer" model, or to existing models of possible helical

(Smith 1989 ; Antonchenko and Ilyin 1994) and clathrate structures (Anagnostatos et al. (1991) in water. The results above however do support the third hypothesis of this investigation (see 1.3.3) which states that differences exist between respective signals of Sulphur and control potencies, and that these increase with increasing potency. The latter statement (drawn from the research of Barnard (1965)) is not conclusively proven by this study, but the fact that differences are only statistically significant at the highest potency measured does not refute the validity of this hypothesis.

Although no significant differences in chemical shift values were found to exist within respective experimental and control groups, significant differences were noted with reference to integration values. The CH_2 integration value of Sulphur LM10 is significantly less than that of the Sulphur LM6 suggesting relative sparsity of protons, while the proton distribution at the CH_2 pole of the Control LM10 potency is significantly greater than that of both the LM2 and LM6 potencies. Again the significance is elusive.

Attention has been drawn in the previous chapter to the consistently small standard deviations (S_x) and variances (S_x^2) across all sample groups investigated. Since measurement of NMR samples is taken over a relatively long time chemically speaking (I millisecond), the fine differences existing within each sample group suggest that a great degree of consistency exists in the measurement of individual samples and/or a clear structure exists within the liquids so as to remain within

narrow limits over a relatively long time. Both these conclusions relate to earlier research suggesting that NMR spectroscopy is a sensitive and worthwhile method of measurement of homoeopathic potencies (Barnard 1965; Smith and Boericke 1968; Sacks 1983; Weingärtner 1991; Bol 1997) and that ordinarily dissipative structures are stabilised through potentisation (Antonchenko and Ilyin 1994). Attention was further drawn (see 4.3.2) to the relatively larger S_x and S_x^2 values of the control LM10 H_2O values over Sulphur LM10 values. In the light of the apparent role of water in the development and maintenance of potency (refer to 2.4 and 2.8) this suggests a need for further investigation.

The results as discussed above represent another of the "dissonant entities" referred to by Rubik (1994 : 157). Since the deconcentrations of the base substance in the three potencies investigated are, respectively, $4x10^{-16}$, $6.4x10^{-35}$ and $1.024x10^{-53}$, from the perspective of chemistry LM6 and LM10 potencies, whether "Sulphur" or "Control", ought to be similar. There is sufficient evidence (2.4, 2.8 and the above) to suggest that this assumption needs to re-evaluated. Indeed even within the "field of the frontier scientist" (Rubik 1994: 156), of which this research is part, assumptions need re-evaluation.

The results of this research proffer re-evaluation of certain aspects. Resch and Gutmann (1987: 273-276;278) explain the changes induced in lactose by trituration. Central to their argument is the introduction of either a different substance, or a "more structured" lactose into the "unstructured" lactose medium. However, the

results above suggest that even when "unstructured" lactose is triturated with a larger quantity of equally "unstructured" lactose (from the same batch and bottle) some change is induced by trituration, and that this change is continued through the subsequent stages of potentisation. The use of lactose as an adequate control in this investigation therefore bodes questioning. It is indeed a question whether the distinction of "experimental" and "control" should be "sulphur / no sulphur" or "potentisation / no potentisation".

The observation that, although change is observable across both the Sulphur and Control groups (refer to Figures 4.1 - 4.6 and CH_2 integration values discussed above) these changes are not always in parallel, supports the argument for substance-specific alteration of structure in media consequent to potentisation. Further investigations involving two or more substances against a control would certainly clarify this issue. The possibility of two control sample (the one potentised, the other not) will also shed light on the question of the effect of succussion and trituration as processes.

The NMR experiments, in this investigation, were conducted in liquid medium at 200.057 MHz using D_2O and dioxane as external lock and reference respectively. The use of an external lock in ^1H spectroscopy yields generally less accurate results than does an internal lock (Abraham and Loftus 1980 : 9). The use of an external lock was to obviate possible contamination of the sample. Other avenues need to be explored with respect to solvents and scanning frequencies. In view of a

loss of accuracy due to the employment of an external lock, it would be more appropriate to run the spectra at a higher frequency than the 200.057 MHz employed. This would also increase the overall sensitivity of the experiment, and may clarify trends across potencies which are, in the context of this evaluation, not statistically significant.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

It is evident from the results of this evaluation of Hahnemannian quinquagenimillesimal potencies using nuclear magnetic resonance spectroscopy that distinct physico-chemical entities are produced by the processes of homoeopathic potentisation employed. Statistically significant differences were noted between individual potencies within the experimental Sulphur group (supporting hypothesis one), within the control (lactose) group (supporting hypothesis two), and most notably between respective LM10 potencies of homoeopathic Sulphur and control (supporting hypothesis three).

The evidence serves to support the employment of NMR spectroscopy as a method for the analysis of structure within homoeopathic potencies, and further lends credence to the claimed efficacy of quinquagenimillesimal potencies in clinical practice. While it is clear that the pharmaceutical processes employed in this study do yield distinct "potencies" many questions remain unanswered. Many questions, indeed, have arisen out of this study. Further research must be conducted with a view to clarification, and ultimately the explanation, of the many anomalies observed by this researcher and others in the field.

6.2 RECOMMENDATIONS

Rubik (1994) in her paper dealing with the challenge of "anomalies" within the framework of 'Science' suggests various strategies for combating obstacles. Among these she emphasises the need for finely honed experimental design, replication of phenomena, interaction between scientific disciplines (collective scientific effort) and communication of observed phenomena (and the cases where anomalies are not observed) in the scientific literature. These collectively drive towards the ultimate theoretical explanation of the observed effects. All of these apply to this study. Most notable are the need for replication and refinement of the experimental design. The collaboration of chemists and physicists in the re-evaluation of the observed phenomena is welcomed, and indeed essential. There are many questions and few answers.

Recommendations for further NMR research involving Hahnemannian quinquagenimillesimal potencies are:

1. An increase in potency, and potency interval;

Extending the range of potencies measured (e.g. LM6 - LM14 - LM22) will allow for observed trends (i.e. differences increase with potency) to be more accurately evaluated. The failure to find statistically significant differences in the lower potencies does not exclude the possibility that these do exist. A retrospective

extrapolation of a clearly observed progression between respective potencies, in a readily observable range, may be possible as an alternative to actual measurement. The ability to measure is also affected by the capacity of the instrument.

2. An increase in the number of sample groups;

The employment of different base substances would provide valuable insight into the question of substance-specific modification of structure within homoeopathic potencies. The increased total sample size (45, 60 or 75) would also allow for multifactorial statistical analysis of the data.

3. The employment of more than one control;

As stated in Chapter five, questions relating to the role of potentising processes exist. The employment of two controls, with and without the processes of potentisation, would clarify the issue of an "adequate control".

4. The re-evaluation of the medium and frequency of NMR spectroscopy.

An increased accuracy may be achieved by conducting the NMR experiment at a higher frequency. A higher frequency of measurement would allow for more comfortable employment of fourth decimal place chemical shift values. The benefits of liquid-state measurement over solid state measurement need to be determined.

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APPENDICES

APPENDIX A

i) **AIM:** To produce LM tinctures of flowers of sulphur and a lactose 'control'

APPARATUS: Glazed porcelain mortar and pestle

Spatula

95% Alcohol (for flaming)

Cigarette lighter

Distilled water (Aq.dist.) ***[S.G. 0.9737]***

Chemical balance (accurate to four decimal places)

Paper (20 10x15cm sheets)

Flowers of Sulphur (B.P.)

Pure lactose powder

90% Ethanol (10% H_2O) ***[S.G. 0.8139]***

20ml dropper bottles

METHOD:

1) Thoroughly clean mortar, pestle and spatula before commencing preparation viz.:

   i) wash well in hot water, rinse with Aq.dist., and dry carefully

ii) flame all implements using 96% alcohol;

iii) allow to cool completely

2) Place a single sheet of paper on chemical balance, and zero.

3) Accurately mass **2,1000g** pure lactose powder.

4) Repeat steps 2 - 3 twice more (i.e. three times in total).

5) Transfer one of these quantities to the mortar.

6) Place a single sheet of paper on balance, and zero.

7) Accurately mass **0,0630g** flowers of sulphur/pure lactose powder.

8) Add this quantity of base substance (i.e. either flowers of sulphur OR lactose powder) to mortar, and mix well using a spatula.

9) Triturate the mixture for twenty minutes, scraping the mass from the bottom of the mortar and the pestle at regular intervals to ensure homogeneity.

10) After the first twenty minutes, add the second quantity of lactose powder, stir briefly, and triturate, as in step 9, for twenty minutes.

11) Finally, add the third 2,1000g lactose powder, stir briefly, and repeat step 9.

12) The powder thus prepared is stored in a tightly closed glass vial, protected from light, and labelled: SULPHUR 1CH or CONTROL 1CH.

13) The above process (steps 1-12) is to be repeated twice more (i.e. three times in total), replacing step 7, as the case may be, with the identical mass of SULPHUR/ CONTROL 1CH/2CH.

14) Accurately mass 0,0630g SULPHUR 3CH or CONTROL 3CH triturate (powder) and transfer to a 20ml dropper bottle.

15) Add 500 drops ***[9,5805 ml]****(S.G. 0.9655; mass of 10 drops: 0.1850g)* of a mixture of one part 90% ethanol and four parts Aq. dist. by volume and mix well, allowing powder to dissolve.

16) Label: SULPHUR LM∅ or CONTROL LM∅, as the case may be.

ii) **AIM:** To produce LM10 potencies of SULPHUR and CONTROL from their respective LM tinctures.

APPARATUS:
Storage box with tray insert
5ml glass screwtop bottles
Micropipette (accurate to 0.1 μl)
Capillary tubes
95% Ethanol ***[S.G. 0.7871]***
Distilled water (Aq.dist.) ***[S.G. 0.9737]***
Lactose granules (#10 preseived through double seive three times)
50ml glass beaker
95% Alcohol (for flaming)
Cigarette lighter

METHOD:

1) Clean and flame 50ml beaker (as in APPENDIX A(i). Allow to cool.

2) Place one drop ***[19,2 μl]*** of the respective LM∅ (either SULPHUR LM∅ or CONTROL LM∅) into a clean 5ml screwtop bottle.

3) Add 100 drops ***[1.2700 ml]*** *(S.G. 0.7871; mass of 10 drops: 0.1000g)* 95% ethanol, cap and succuss by hand 100 times.

4) Label this bottle: LM1

5) Place 500 granules ***[0.5940g]*** of lactose (accurately massed according to the average of ten samples accurately counted) into the cooled glass beaker.

6) Use a clean (unused) capillary tube to micropipette one drop ***[12.7μl]*** of LM1 onto granules.

7) Swirl beaker to ensure that all granules are evenly moistened. Continue swirling until all granules are dry.

8) Transfer dry granules to a clean 5ml screwtop bottle and label: O/1 (signifying LM1 granule)

9) Place one granule of O/1 in a clean 5ml screwtop bottle and cap.

10) Clean, flame and cool 50ml beaker as before.

11) Place the "LM1" and "O/1" bottles next to each other in storage box, and discard used capillary tube.

12) Add one drop ***[31 μl]*** *(S.G. 0.9737; mass of 10 drops: 0.3018g)* Aq. dist. to bottle containing a single granule of O/1, cap, and rotate slowly to encourage solution of the granule.

13) Repeat steps 3 - 11, labelling each successive potency appropriately, until O/10 (LM10 granule) is reached.

14) Clean all equipment.

15) Store both potency banks (i.e. SULPHUR LM1 - LM10 and CONTROL LM1 - LM10) in a cool, dry place away from sunlight, strong odours and electromagnetic disturbances.

APPENDIX B: Sample NMR-spectra

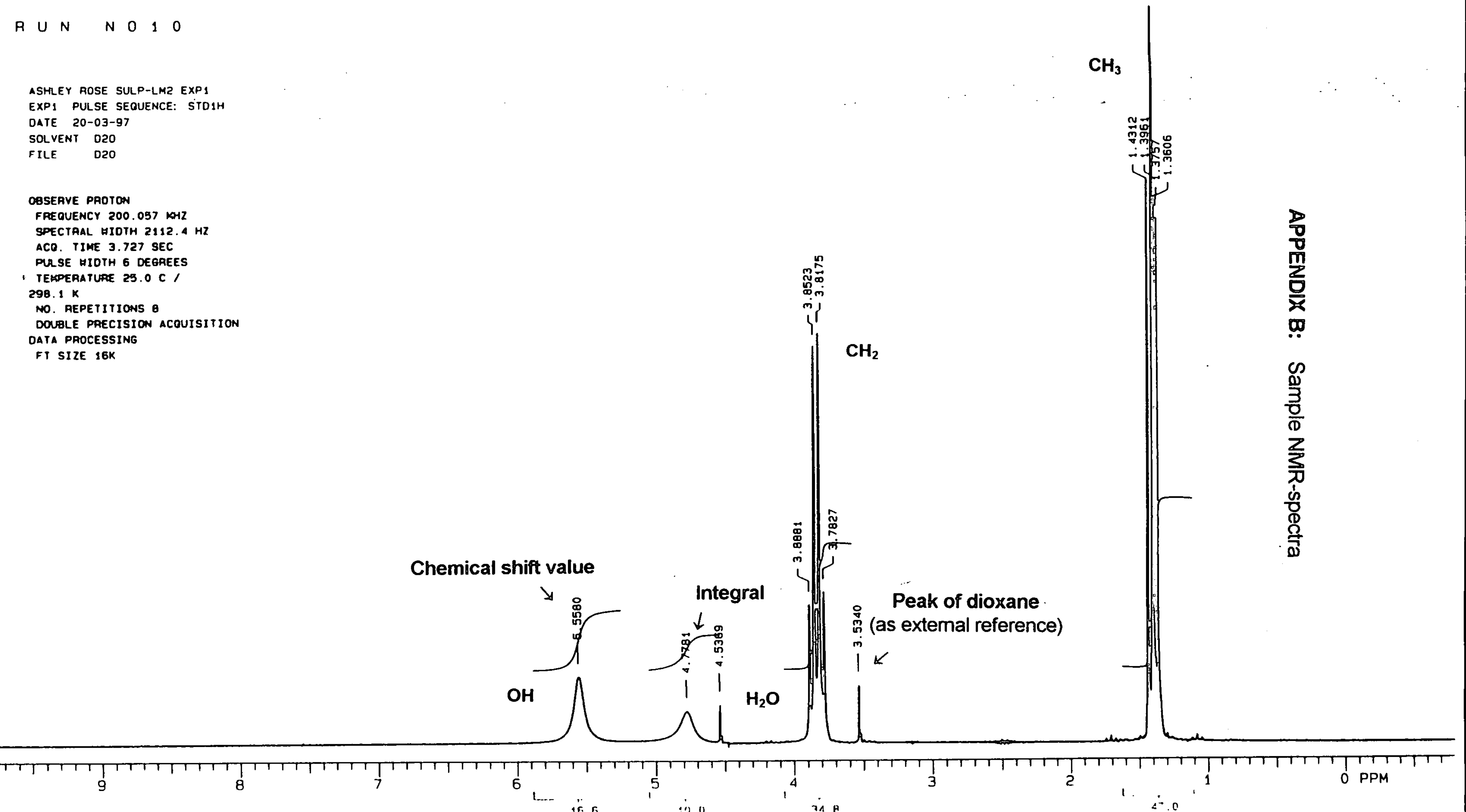

Figure 1: Specimen NMR spectrum of Sulphur LM2

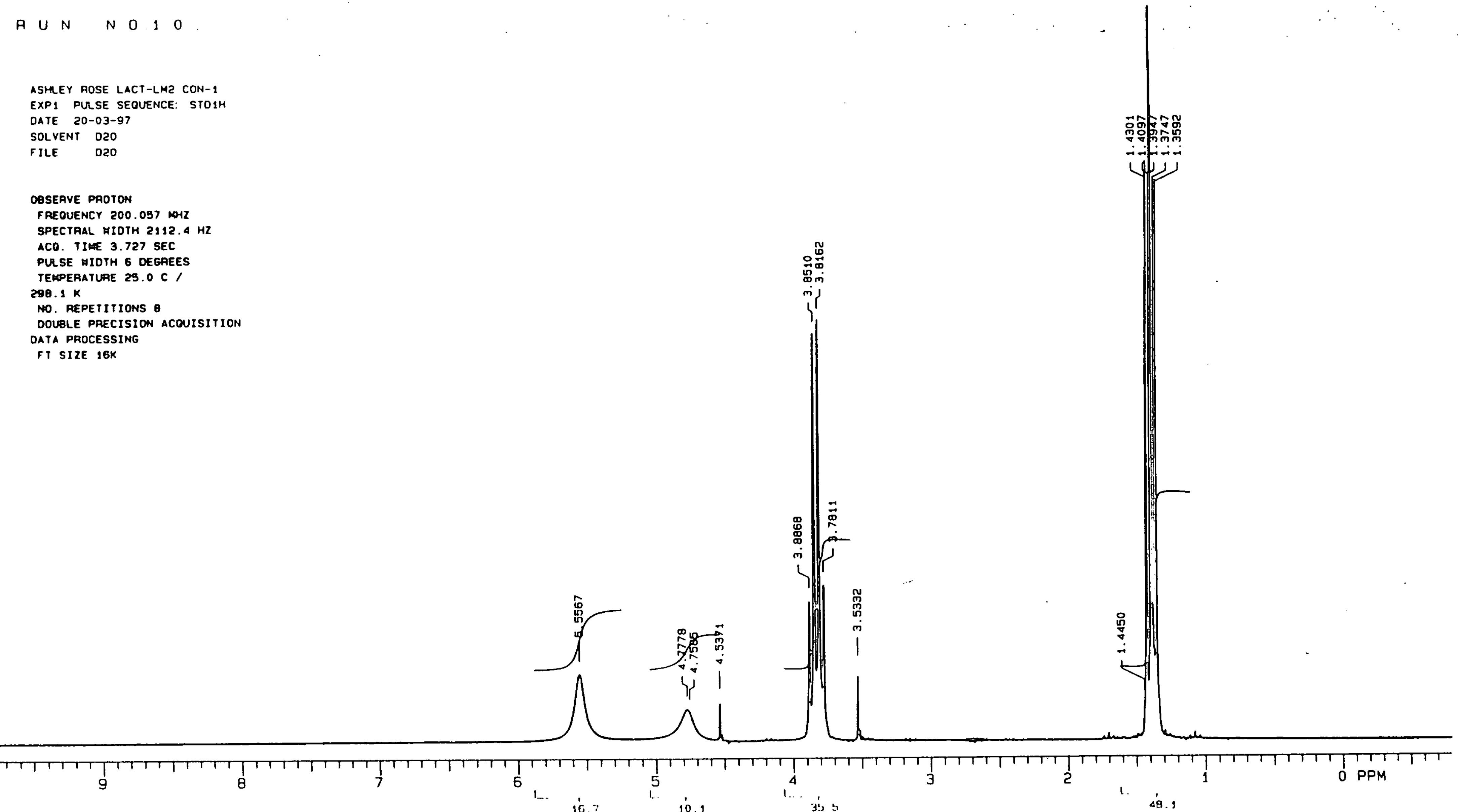

Figure 2: Specimen NMR spectrum of Control LM2

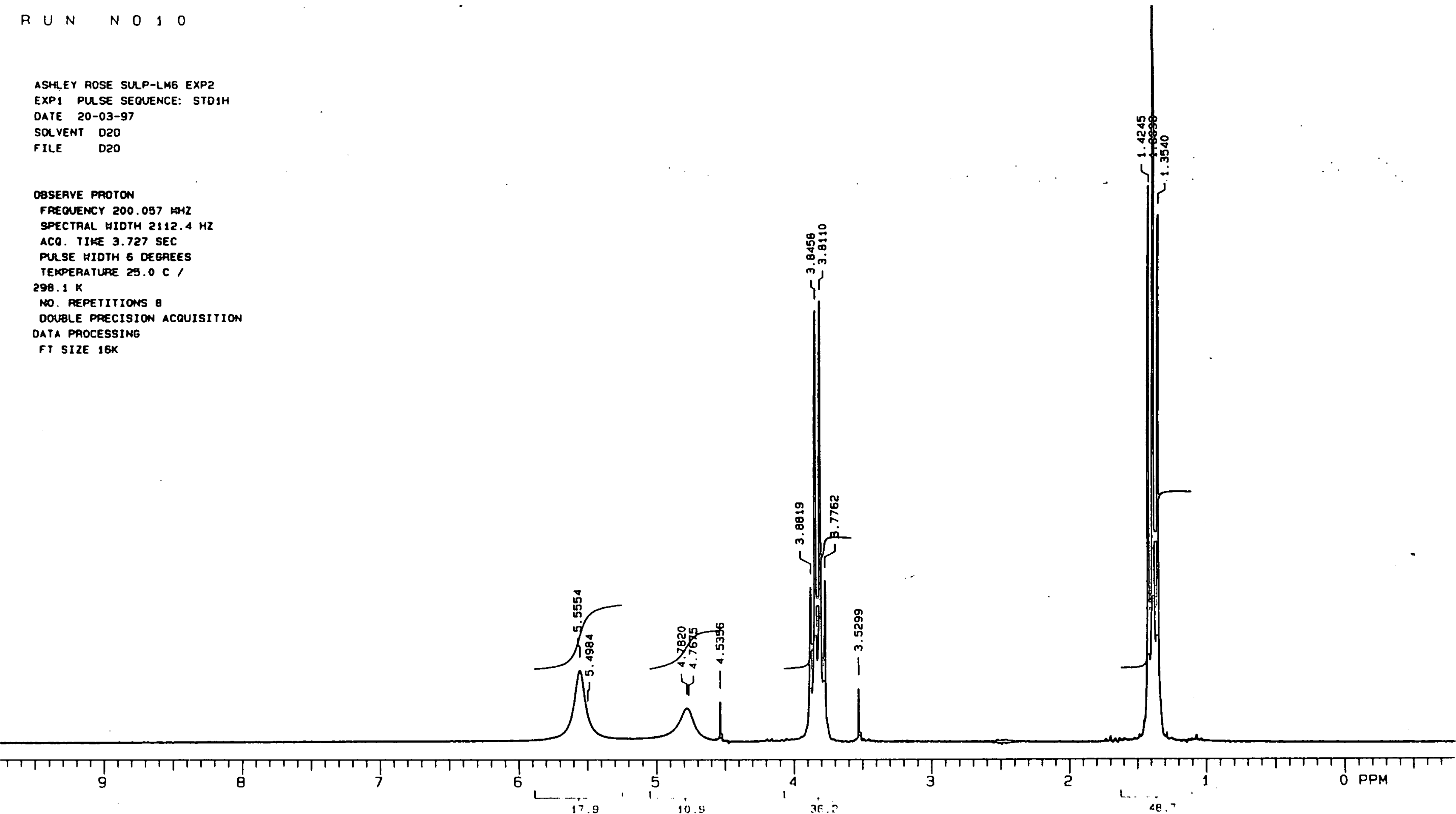

Figure 3: Specimen NMR spectrum of Sulphur LM6

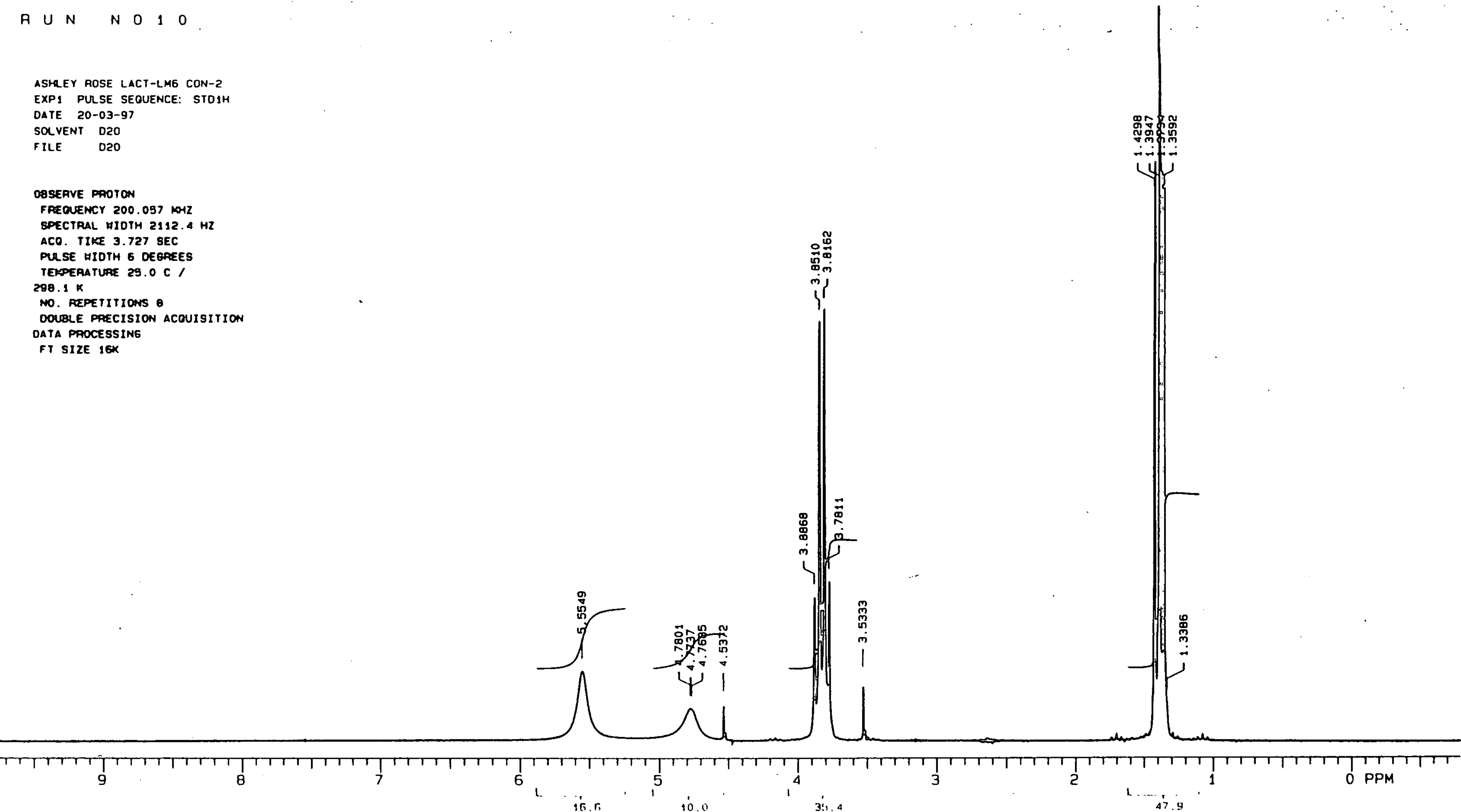

***Figure 4*:** Specimen NMR spectrum of Control LM6

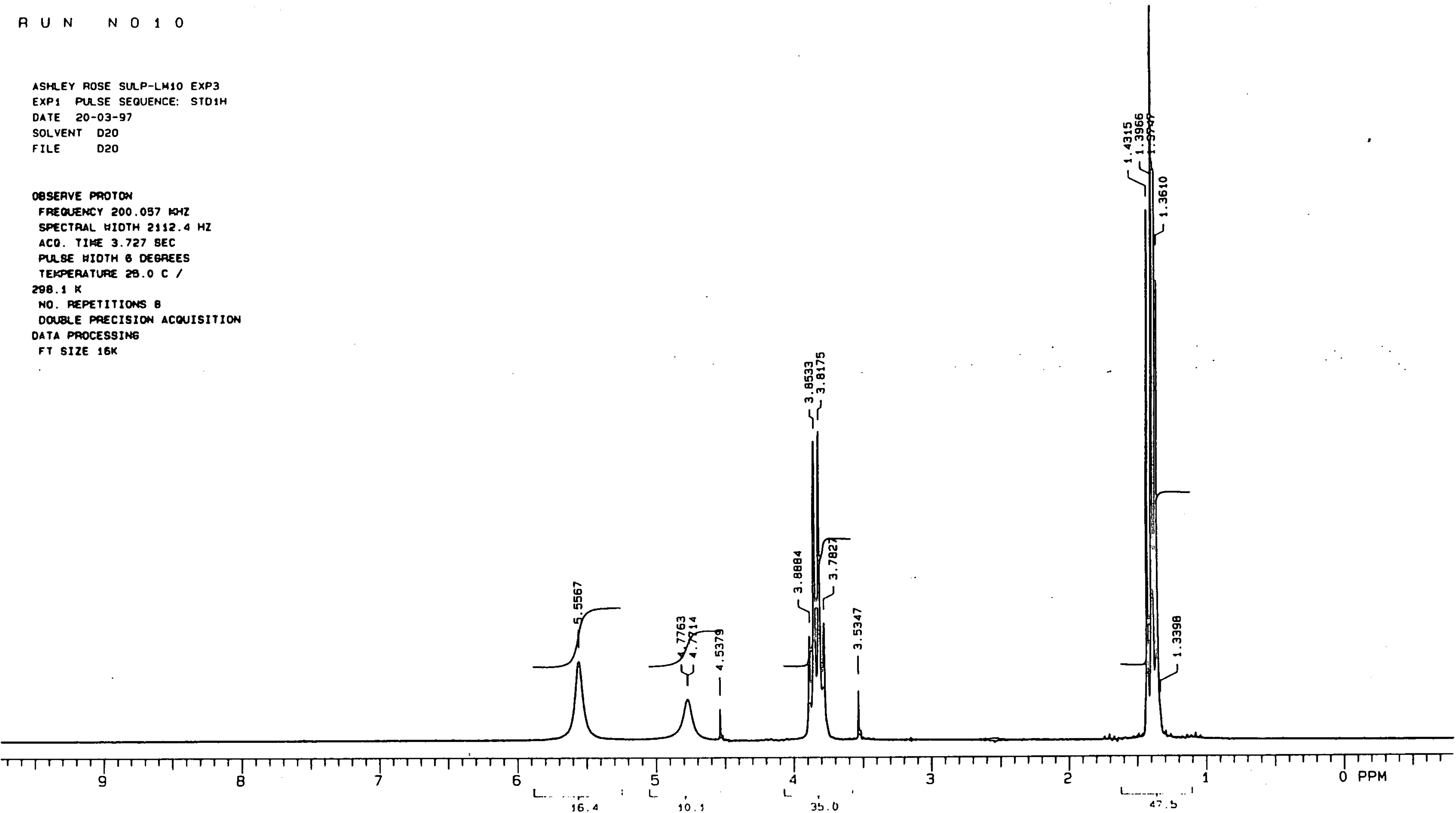

Figure 5: Specimen NMR spectrum of Sulphur LM10

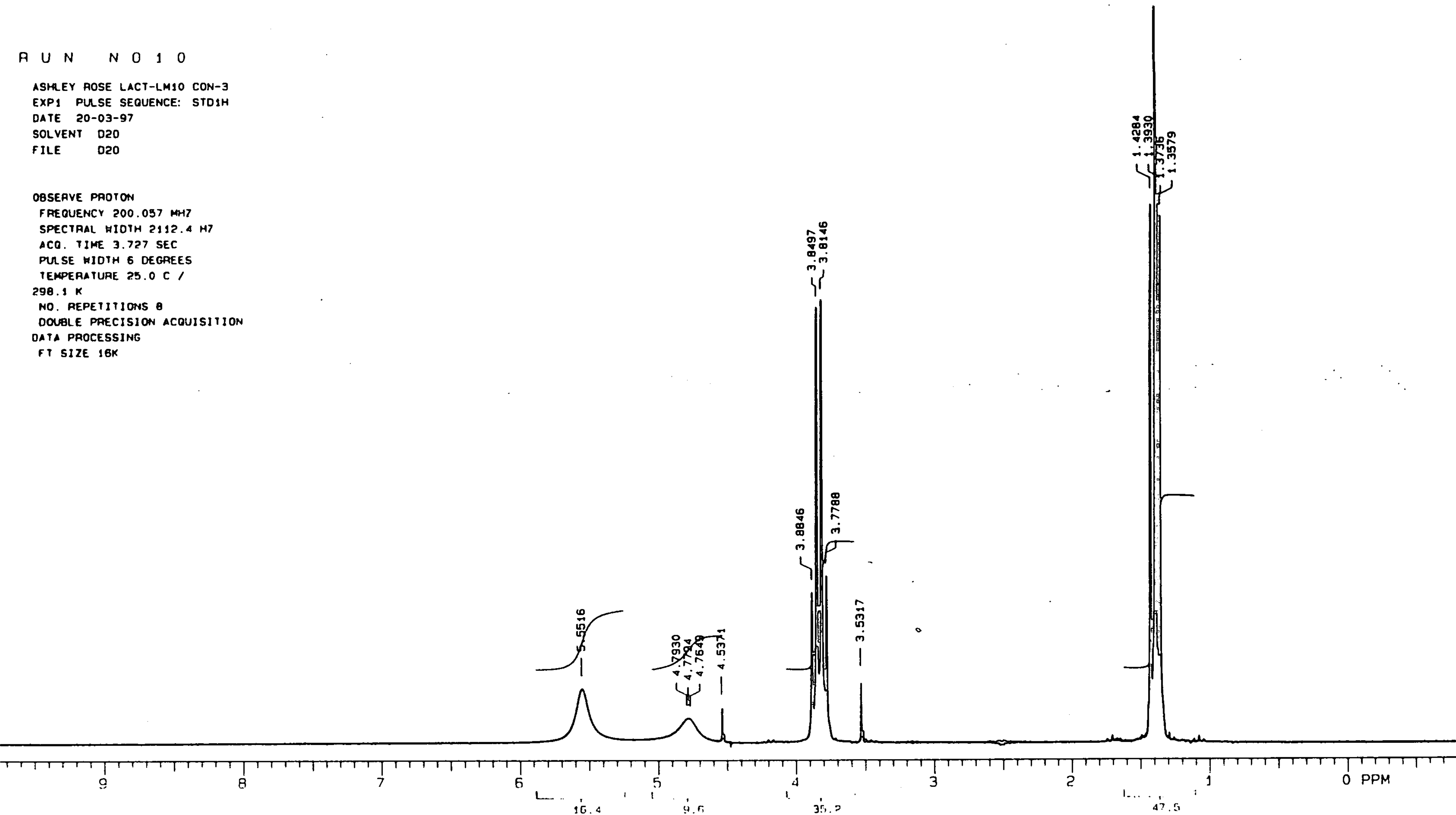

Figure 6: Specimen NMR spectrum of Control LM10

APPENDIX C:

Crude data relating to NMR-spectra of samples drawn

Sample	CH_2	H_2O	OH
1	3.8271	4.7627	5.5497
2	3.8276	4.7635	5.5464
3	3.8351	4.7685	5.5523
4	3.8374	4.7703	5.5574
5	3.8347	4.7655	5.5516
6	3.8302	4.7682	5.5503
7	3.8284	4.7691	5.5516
8	3.8252	4.7674	5.5499
9	3.8304	4.7647	5.5516
10	3.8349	4.7781	5.5580
11	3.8330	4.7706	5.5562
12	3.8309	4.7794	5.5517
13	3.8380	4.7735	5.5574
14	3.8278	4.7775	5.5619
15	3.8318	4.7743	5.5549

Table 1: **SULPHUR LM2 (δ values)**

Sample	CH_2	H_2O	OH
1	3.8284	4.7648	5.5510
2	3.8249	4.7625	5.5427
3	3.8330	4.7700	5.5536
4	3.8273	4.7693	5.5510
5	3.8304	4.7750	5.5497
6	3.8286	4.7659	5.5510
7	3.8325	4.7771	5.5541
8	3.8286	4.7750	5.5516
9	3.8310	4.7708	5.5562
10	3.8336	4.7778	5.5567
11	3.8294	4.7704	5.5510
12	3.8271	4.7700	5.5567
13	3.8310	4.7738	5.5567
14	3.8273	4.7717	5.5549
15	3.8298	4.7755	5.5567

Table 2: **CONTROL LM2 (δ values)**

Sample	CH_2	H_2O	OH
1	3.8307	4.7753	5.5464
2	3.8291	4.7619	5.5464
3	3.8351	4.7736	5.5523
4	3.8392	4.7714	5.5554
5	3.8312	4.7714	5.5503
6	3.8299	4.7714	5.5503
7	3.8297	4.7706	5.5549
8	3.8299	4.7713	5.5503
9	3.8297	4.7641	5.5503
10	3.8284	4.7748	5.5554
11	3.8329	4.7696	5.5516
12	3.8309	4.7774	5.5517
13	3.8373	4.7720	5.5549
14	3.8341	4.7743	5.5613
15	3.8325	4.7680	5.5549

Table 3: **SULPHUR LM6 (δ values)**

Sample	CH_2	H_2O	OH
1	3.8291	4.7678	5.5516
2	3.8381	4.7786	5.5562
3	3.8297	4.7682	5.5473
4	3.8289	4.7695	5.5477
5	3.8328	4.7765	5.5525
6	3.8252	4.7607	5.5510
7	3.8312	4.7794	5.5562
8	3.8291	4.7711	5.5510
9	3.8336	4.7775	5.5574
10	3.8286	4.7741	5.5549
11	3.8330	4.7777	5.5549
12	3.8322	4.7728	5.5567
13	3.8348	4.7738	5.5554
14	3.8317	4.7744	5.5525
15	3.8241	4.7642	5.5549

Table 4: **CONTROL LM6 (δ values)**

Sample	CH_2	H_2O	OH
1	3.8315	4.7665	5.5503
2	3.8713	4.7624	5.5484
3	3.8304	4.7685	5.5541
4	3.8336	4.7685	5.5536
5	3.8315	4.7704	5.5562
6	3.8318	4.7688	5.5529
7	3.8343	4.7743	5.5554
8	3.8323	4.7711	5.5541
9	3.8428	4.7131	5.4887
10	3.8354	4.7738	5.5567
11	3.8325	4.7682	5.5564
12	3.8393	4.7756	5.5593
13	3.8330	4.7765	5.5587
14	3.8426	4.7840	5.5657
15	3.8348	4.7719	5.5587

Table 5: **SULPHUR LM10 (δ values)**

Sample	CH_2	H_2O	OH
1	3.8325	4.7649	5.5471
2	3.8320	4.7692	5.5500
3	3.8273	4.7656	5.5397
4	3.8310	4.7720	5.5447
5	3.8310	4.7794	5.5594
6	3.8276	4.7712	5.5473
7	3.8300	4.7802	5.5590
8	3.8270	4.7787	5.5446
9	3.8349	4.7842	5.5549
10	3.8322	4.7791	5.5516
11	3.8305	4.7799	5.5449
12	3.8353	4.7800	5.5489
13	3.8339	4.7792	5.5558
14	3.8330	4.7740	5.5507
15	3.8284	4.7774	5.5513

Table 6: **CONTROL LM10 (δ values)**

Sample	CH_2	H_2O	OH
1	52.11781	78.09231	15.36643
2	55.86697	71.77766	15.18578
3	54.32797	75.28351	15.27559
4	54.46188	75.46563	15.10622
5	56.98148	70.37374	14.63614
6	54.42752	75.69700	15.58846
7	55.07013	74.45053	15.04355
8	53.49794	77.76000	15.43210
9	55.76634	69.93466	14.72803
10	53.29151	65.30120	15.31365
11	52.91456	67.08929	15.20362
12	52.33599	69.35954	15.28269
13	52.82117	66.82927	14.96350
14	49.93539	73.49497	15.51127
15	53.21682	52.99077	14.90826

Table 7: **SULPHUR LM2 (integ. values)**

Sample	CH_2	H_2O	OH
1	54.14747	73.87234	15.16129
2	56.12689	71.80159	15.18072
3	56.06422	72.77368	15.11535
4	56.31450	72.45026	15.18283
5	54.08404	77.28713	15.52652
6	55.35997	74.06073	15.12272
7	56.38024	72.36578	14.92418
8	56.98235	70.72366	14.98791
9	50.20501	73.69783	15.73995
10	53.16850	66.76886	15.12681
11	54.53276	66.5655	15.15303
12	52.58696	70.16949	15.39130
13	53.89128	66.80116	15.41889
14	54.50741	66.04606	14.83813
15	55.08223	51.19618	15.04018

Table 8: **CONTROL LM2 (integ. values)**

Sample	CH_2	H_2O	OH
1	55.43043	71.44091	14.55749
2	55.66436	72.03892	14.99190
3	55.36298	72.61170	15.14907
4	55.47016	73.73333	15.18987
5	54.65608	75.19749	15.55903
6	55.74646	74.08543	15.52262
7	56.11583	73.06316	15.05547
8	53.03134	79.38702	15.74565
9	56.89497	72.41413	14.63803
10	52.28470	69.23631	15.74318
11	53.30390	67.53728	15.10277
12	52.33599	69.35954	15.28269
13	53.81481	66.15280	14.66302
14	50.89631	71.12500	15.46573
15	54.43714	50.88438	14.93582

Table 9: **SULPHUR LM6 (integ. values)**

Sample	CH_2	H_2O	OH
1	55.22401	72.43226	15.04854
2	56.53752	71.10323	15.04854
3	56.37704	72.19251	14.96000
4	56.63985	72.21064	14.95625
5	54.66651	76.46364	15.30139
6	55.13472	75.45155	15.10903
7	54.16022	78.47087	15.54717
8	57.86296	68.61038	15.01231
9	52.95020	68.17727	15.54770
10	53.47043	66.20482	15.10464
11	54.14111	67.60112	15.53229
12	52.51797	68.73837	15.27531
13	54.38618	65.27394	15.01365
14	53.38370	69.30955	15.43799
15	54.66561	51.58636	15.12027

Table 10: **CONTROL LM6 (integ. values)**

Sample	CH_2	H_2O	OH
1	53.13399	74.52857	14.89362
2	53.14044	75.46042	15.37230
3	51.57713	80.07424	15.36074
4	53.72794	75.93814	15.27559
5	52.20704	81.02356	15.67445
6	53.29569	76.92929	15.46875
7	54.05387	76.77526	15.10903
8	54.23821	73.38000	14.71791
9	54.80681	73.71347	15.32963
10	52.13916	67.12805	15.04587
11	51.72004	69.79886	15.47303
12	51.17700	69.56250	15.09434
13	52.07585	68.55385	15.17056
14	51.42781	68.83436	14.81818
15	53.76344	52.08000	14.97696

Table 11: **SULPHUR LM10 (integ. values)**

Sample	CH_2	H_2O	OH
1	59.04176	67.74866	15.20325
2	58.47760	68.57326	15.16626
3	59.88440	65.62644	14.47587
4	59.78102	67.74725	14.76075
5	57.09802	73.03231	15.19875
6	57.09490	71.98542	15.14196
7	56.71565	73.70100	15.46790
8	59.42954	67.47486	14.67213
9	54.61538	65.00000	15.38462
10	55.32045	63.62927	15.08740
11	55.72338	64.06647	15.14053
12	54.46866	66.27665	14.93739
13	54.76299	65.00741	14.76755
14	55.72396	51.68333	14.89842
15	57.00990	66.53950	15.02160

Table 12: **CONTROL LM10 (integ. values)**

APPENDIX D: Bar graphs in the manner of Weingärtner

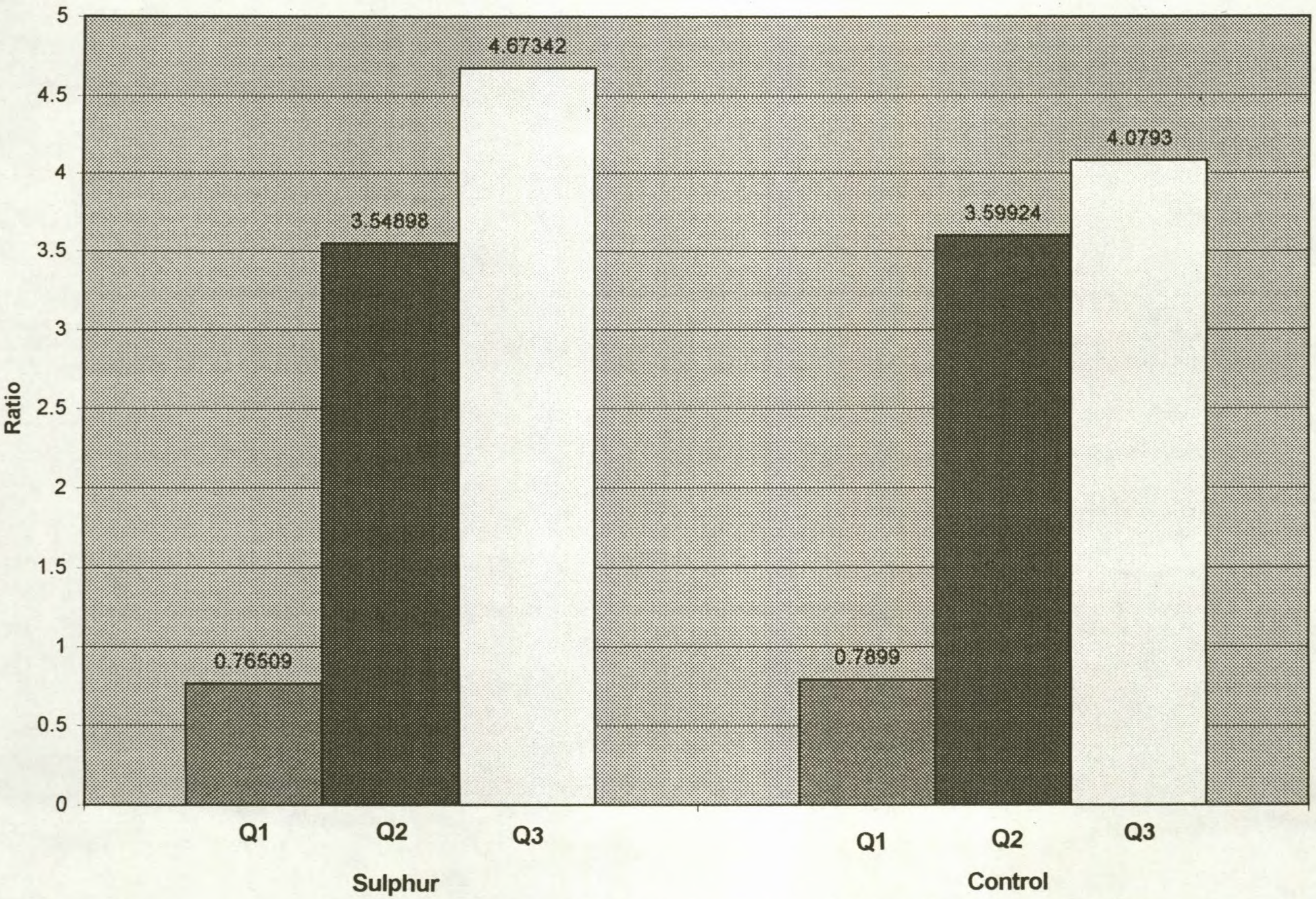

Figure 7: Comparison of Ratios (in the manner of Weingärtner) for LM2 Potencies

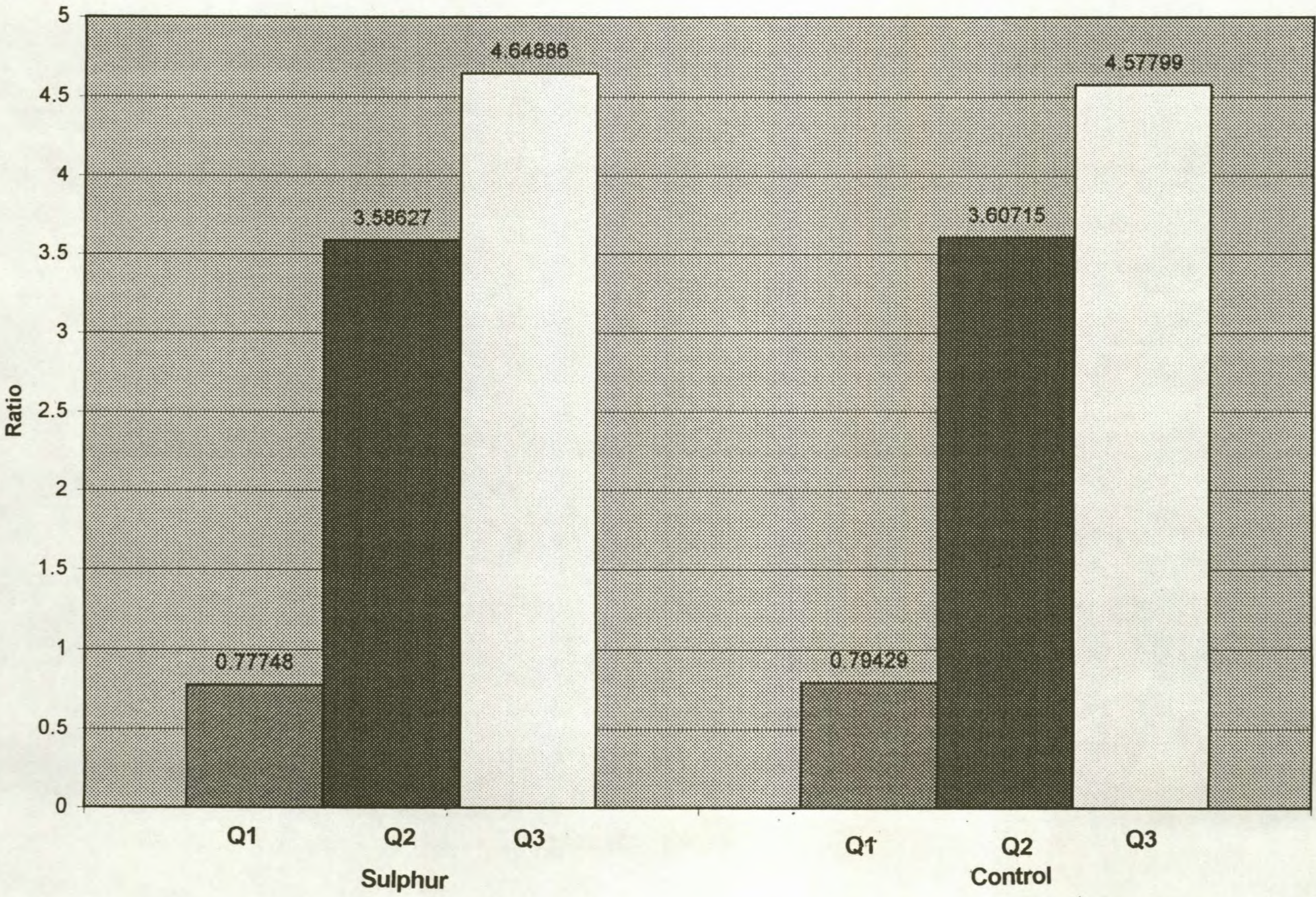

Figure 8: Comparison of Ratios (in the manner of Weingärtner) for LM6 Potencies

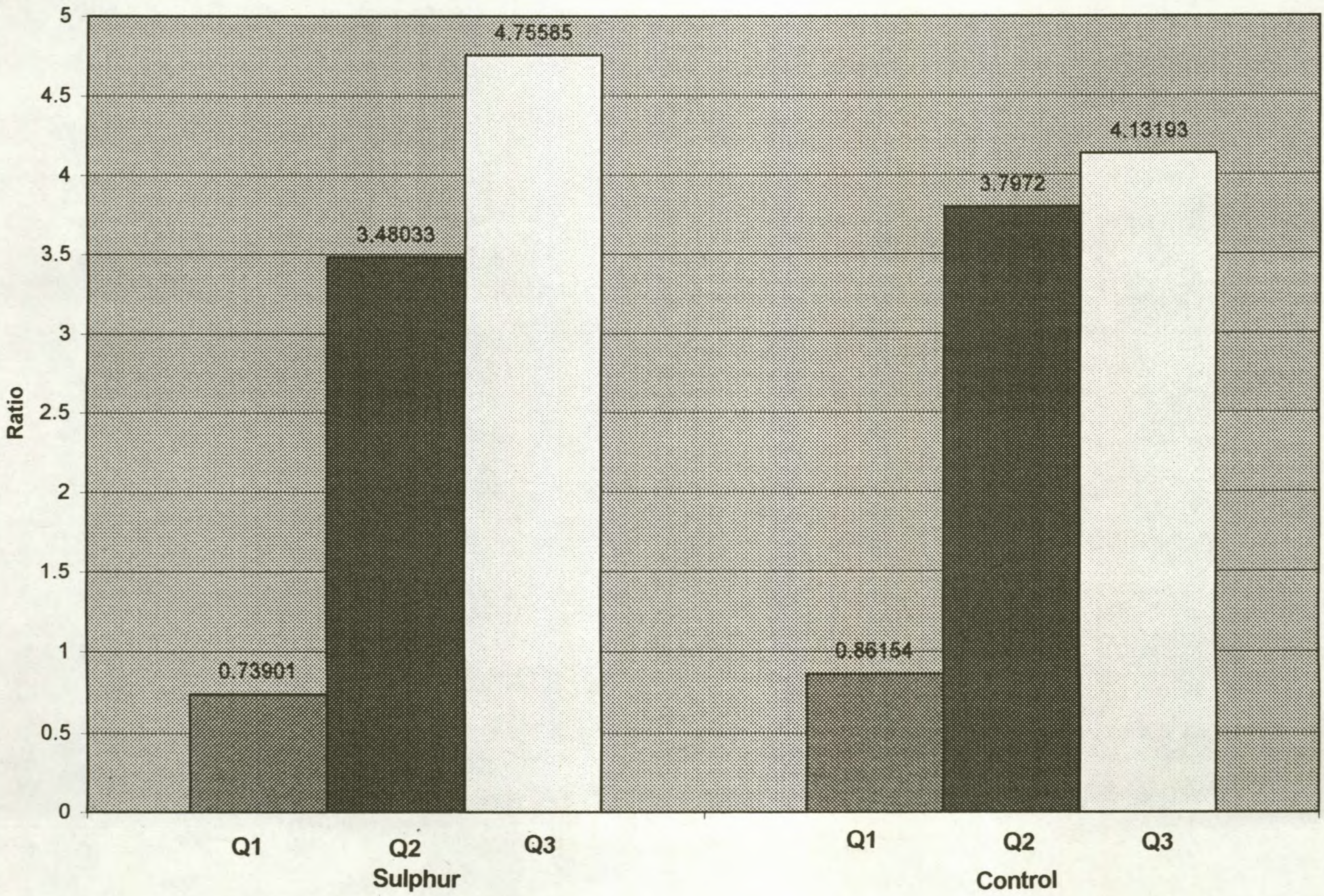

Figure 9: Comparison of Ratios (in the manner of Weingärtner) for LM10 Potencies