An NMR study of the effect of succussion on parallel potencies of Natrum muriaticum.

By

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I, Daphne Lyell do hereby declare that this dissertation is representative of my own work.

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Dedication

This work is dedicated to my parents, Buks and Magda Lyell, my family and my friends for their ineffable love and support in whatever life brought my way.

It is also dedicated to a greater understanding of Homoeopathy.

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ABSTRACT

The purpose of this study was to analyse and compare the NMR spectra of multiple parallel potencies of the homoeopathic remedy Natrum muriaticum (chosen for its easy solubility and purity) using 0, 10 and 100 succussions. The remedies were prepared using the classical single vial Hahnemannian method with the same potency and the varying numbers of succussion. A comparison was made in terms of the chemical shift and relative integration values of the OH, CH_2, CH_3 and H_2O signals.

The control employed during the experiment was the preparation of Natrum muriaticum with 0 succussions. The only difference between the control and the test remedies was the exclusion of succussion during the manufacture process.

A comparison was made between the control (0 succussions) and the 10 succussions, the control and 100 succussions as well as the 10 and 100 succussions. It was hypothesised that succussion plays an important part in the preparation process of homoeopathic remedies and thus becomes part of the remedy's information content.

The design of the experiment was within the limitations of the scientific method. A volume of16ml of each remedy was made up using distilled water and ethanol. The Natrum muriaticum was taken up to the 12CH potency using serial dilution and the respective amounts of succussion. After

preparation the remedies were transported to the University Kwazulu-Natal Chemistry Department, where they were subjected to NMR spectroscopic analysis.

The three sample groups (0, 10 and 100 succussions), with three of the same remedies in each group, were then run in a Valerian 500MHz INOVA spectrometer a total of 16 times for each sample. The pulse angle was set at 90º with an acquisition time of 1.9 seconds per run. The temperature was held constant at 25ºC during the NMR analysis. A new sample was drawn from the original 16ml volume with a new, unused micropipette in 1.75ml volumes and then inserted into a coaxial tube, using acetone both as the external lock as well as the internal reference.

The data was recorded in the form of NMR spectra, listing chemical shift values and indicating integration values. The data was then transferred to a Microsoft Excel© 2000 spreadsheet and from there it was transferred to the SPSS© Base 10.0 software package for statistical analysis. The analysis was performed using Repeated Measures Analysis of Variance. From the statistical analysis it was apparent that the succussion *did* have an effect on the physicochemical structure of the samples that were examined. When the samples were compared with regards to chemical shift, the CH_2 peak was the one most affected. When the relative integration values were compared, all four peaks were affected, but more so in the comparison of 10 and 100 succussions and 0 and 100 succussions than in the comparison of 0 and 10 succussions.

The results of this study were in keeping with previous research obtained by international studies of the NMR spectra of homoeopathic substances. As in this study, significant differences were noted in the CH_2 peaks during the investigation of the effect of succussion.

The results of this study confirmed that significant differences existed between potencies of 0, 10 and 100 succussions, however the results did not serve to hypothesize a specific model on the energies responsible for these differences. It also confirmed that NMR spectroscopy remained a valuable tool in the investigation of the properties of homoeopathic remedies.

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TABLE OF ABBREVIATIONS

ANOVA	-	Analysis of variance
B_0	-	Static magnetic field
CH	-	Centesimal Hahnemanniene
CH_2	-	Methylene group
CH_3	-	Methyl group
δ	-	Delta, represents the chemical shift
Hz	-	Hertz, represents the frequency
I	-	Nuclear spin
RF	-	Radio Frequency

DEFINITIONS OF TERMS

Avogadro's number: the number of units contained in one mole of a substance, normally represented as $N_A = 6.022 \times 10^{23}$. One molecule of a remedy substance in a mole is considered to be equivalent to the 12C dilution level, i.e not one molecule of the original substance or material can be found at this dilution level.

Centesimal: 1:100 deconcentration scale originally introduced by Hahnemann.

Chemical shift (δ): the amount by which the proton resonance shifted relative to a set reference standard (eg. Acetone). It is measured in parts per million (ppm) and it is directly proportional to the field strength or the oscillating frequency of the NMR spectrometer.

Clathrate: A compound formed when the small molecules of the substance fill in the holes in the structural lattice of another. Therefore, clathrates are intermediate between mixtures and true compounds.

Deconcentration: the level of concentration reached by serial dilution of the original substance in a diluent. The level is dependent on the potency scale that is employed e.g. the centesimal scale diluted a substance one hundredfold at each successive step.

Electromagnetic waves: the effects of oscillating electric and magnetic fields that are capable of travelling across space, thus requiring no medium through which to be transmitted.

LM: Quinquagenimillisimal - a homoeopathic potency scale in which the rate of deconcentration at each potency stage is 1:50 000. This deconcentration, introduced by Hahnemann, is achieved in two stages; 1:100 and then 1:500.

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

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

NMR spectroscopy: it is an experimental tool for chemical analysis whereby a test substance is placed in a magnetic field and exposed to a radio frequency in order to achieve resonance. The energy absorption and emission results in a spectrum of the resonant protons.

Pharmacopoeia: a reference text wherein the uses, preparation and contents of medicines are laid out.

Potentisation (dynamisation): it is the process of preparation of a homoeopathic remedy whereby a specific substance is taken up to a certain

level of deconcentration through serial dilution and succussion or trituration. The scope, strength and refinement of a substance are believed to increase through this process.

Radio frequency (RF): an oscillating electromagnetic field (see electromagnetic waves).

Succussion: a vigorous mechanical process employed during the preparation of a homoeopathic remedy whereby the bottle containing the remedy is held in the hand and pounded against a hard, but elastic body such as a leather-bound book or the surface of the palm of the other hand.

Standard deviation: a measure of dispersion using all of the data points in a sample to indicate the variation existing within a data set.

CHAPTER ONE: INTRODUCTION

The is an investigation of the Nuclear Magnetic Resonance (NMR) spectra of samples of the 12CH homoeopathic potency of Natrum muriaticum, in terms of recorded OH, CH_2, CH_3 and H_2O chemical shift and mean integration values, in order to evaluate the differences in parallel homoeopathic potencies produced by different numbers of succussions.

The two fundamental processes of manufacture of homoeopathic remedies are dilution and succussion respectively. The contribution of each process is unclear and research into this area is crucial.

Furthermore, a clearly defined model, which can be scientifically tested and serve as an observation of the process that takes place during the manufacture of a homoeopathic remedy, remains elusive (Demangeat et al., 2001).

NMR spectroscopy has been shown to be a valuable tool in the assessment of the differences of the physico-chemical properties of homoeopathic substances. Although it is not yet known how the difference in chemical shift and mean integration values of the recorded NMR spectra of various homoeopathic remedies can be translated into an absolute indicator of specific structural differences, it does indicate that there is a structural difference (Demangeat et al., 2001).

NMR spectra of three different samples, in which 0, 10 and 100 succussions were employed in manufacture, were produced. The effect of succussion, if

any, was hypothesised to correlate with observed changes in respective spectra.

This study attempted to observe the differences between remedies manufactured according to the same basic methodology (the “Hahnemannian” method) with regards to deconcentration (1:99), but different numbers of succussion.

1.1 THE AIM OF THE STUDY

The purpose of the study was to compare centesimal (CH) potencies of Natrum muriaticum (Sodium chloride) that had been prepared with the same levels of deconcentration (dilution), and different numbers of succussions, using Nuclear Magnetic Resonance spectroscopy.

1.2 STATEMENT OF OBJECTIVES

1.2.1 The first objective

To compare and evaluate the NMR spectra of Natrum muriaticum with respect to the chemical shift and integration values of the OH, CH_2, CH_3 and H_2O signals, which had been potentised to the 12CH potency level using the Hahnemannian method with no succussions (i.e only serial dilutions) versus the same potency with 10 succussions in terms of structural similarities and differences.

1.2.2 The second objective

To compare and evaluate the NMR spectra of Natrum muriaticum with respect to the chemical shift and integration values of the OH, CH_2, CH_3 and H_2O signals which had been potentised to the 12CH potency level using the Hahnemannian method with no succussions versus the same potency with 100 succussions in terms of structural similarities and differences.

1.2.3 The third objective

To compare and evaluate the NMR spectra of Natrum muriaticum with respect to the chemical shift and integration values of the OH, CH_2, CH_3 and H_2O signals which had been potentised to the 12CH potency level using the Hahnemannian method with 10 succussions versus the same potency with 100 succussions in terms of structural similarities and differences.

1.3 THE HYPOTHESES

1.3.1 The first hypothesis

It was hypothesized that significant differences existed between the chemical shift (δ) and relative integration values of the OH, CH_2, CH_3 and H_2O signals of the 12CH potencies of Natrum muriaticum that had been prepared using 0, 10 and 100 succussions respectively.

1.3.2 The second hypothesis

It was hypothesized that the effect of succussion in the respective groups would correlate with the changes visible in the NMR spectra.

1.3.3 The third hypothesis

It was hypothesized that statistically significant differences existed between parallel potencies of Natrum muriaticum in terms of 0 succussions versus 10 succussions, 10 succussions versus 100 succussions and 0 succussions versus 100 succussions and that these comparisons would highlight differences inherent to the different numbers of succussions.

CHAPTER TWO: THE REVIEW OF RELATED LITERATURE

2.1 Introduction

Homoeopathy is a system of medicine that is based on two main principles known as the law of similars and treating with the infinitesimal dose. The law of similars simply means that a substance capable of producing certain effects when taken by a healthy human being is capable of curing any illness that displays the same symptoms. Treating with the infinitesimal dose entails administering the smallest dose possible of the homoeopathic remedy in order to stimulate a reaction towards cure. The method of 'potentisation' was developed by the founder of homoeopathy, Samuel Hahnemann, as a vehicle of administration of the homoeopathic substance at infinitesimal dose. Later on another method of potentisation was developed by the Russian, General Simeon, Nicolaevich Korsakov (or von Korsakov).

Potentisation is broken up into two complementary phases, dilution and succussion, that are both employed at each phase of the manufacturing process of a homoeopathic remedy.

The guidelines for the scales of dilution are clear-cut, however, succussion has always been an area of debate. Different schools of thought advocate different numbers of succussion without any substantial evidence as to why such statements are made.

This study aims to shed light on the investigation of the role of the number of succussions necessary by looking at the differences in remedies of parallel potencies with the number of succussions employed in preparation being the primary dynamic variable.

2.2 The Process of Potentisation

Potentisation is a process specific to homoeopathy whereby a ratio of crude substance to lactose is mechanically ground in a mortar and pestle (trituration) followed by the serial dilution and succussion in water or an ethanol-water solution according to a specific scale (Hahnemann, 1997: 235-242).

Hahnemann describes the potentisation process, also referred to as dynamisation, as taking one part of the triturated substance and placing it in a bottle, then adding the relevant amount of solvent to the same bottle (the dynamisation bottle should be two-thirds full). The tightly closed bottle should then be slammed 100 times against a hard, but elastic, body (Hahnemann, 1997: 239).

Hahnemann's dilution of the drug substances is seen as the practical result of a method required to lessen the toxicity of drug substances, and is a common dilution method still used in chemistry and microbiology today (Dellmour, 1994). It requires less work and material, and the smaller volumes make homogenisation, through strong steady stirring or shaking, easier. Apart from decreasing the toxicity of the substances, the process has exposed the medicinal properties of substances previously thought of as inert. In the same way, it also disclosed more important therapeutic properties of crude

substances already known to have an effect on the living being.

2.2.1 Dilution

There are three standard scales of dilution routinely employed in homoeopathy *viz.* Centesimal, C-scale, with a 1:99 (substance: solvent) ratio; the Decimal or D-scale, with a 1:9 ratio; and the Quinquagenimillesimal-scale with a 1:50 000 ratio. The C-scale will be used in this study.

Within the centesimal scale, two methods are currently employed. The Hahnemannian method entails serial dilution and succussion with the one part of the previous dilution being placed into a clean vial for the successive dilution. By contrast, the Korsakovian method employs the same vial with vigorous emptying between successive potencies. However because of the lack of standardisation of the Korsakovian method, it has been omitted in numerous countries or used only for production of the higher Hahnemannian potencies as a cost and time saving strategy (Gaier 1991:460-462). The Hahnemannian potentisation technique will be used in this study to limit additional variables.

2.2.2 The Phenomenon of Succussion

The process of succussion entails holding the two-thirds full dynamisation bottle in one hand. This hand, with elbow bent, is then raised up to the height of the succussor's ear and then slammed down on a hard, elastic surface.

Barnard (1965) developed the idea that the solvent is the carrier of the remedy information instead of the original crude substance. Hence the solvent, rather than the crude substance, will act as the remedy. He believed that water polymers - spatial changes in the solvent's structure – were formed by succussing the dissolved substance in the solvent. He believed that these polymers were induced to grow and split by the energy from the succussion.

One of the most popular theories of the molecular events during potentisation is that proposed by Resch and Gutmann (1991). They hypothesized that the whole system reacts as a unity and responds to changes in such a manner so as to preserve the main characteristics of the system by counteracting external forces acting on it. They postulated that there is the so-called supermolecular system organisation of liquid water, which is composed of different hierarchical levels of bonds with gases in between the bonds that vibrate with the structure to keep it stable. When a soluble solute is diluted into a pure solvent, two similar systems come into contact, namely the more differentiated solution with its better-developed static structural aspects and the less differentiated and dynamically more active solvent. By mixing them – e.g. by the process of dilution – a new system, with a system organisation which differs from those of the component liquids are produced. With each step of dilution, the concentration of molecules of the solute is decreased, but the information is not lost, rather spread over the whole of the more dilute solution. During the process of succussion, the existential conflict between the two system organisations is intensified, the energy is redistributed and the concentration of

dissolved gas molecules is increased. The solute molecules are subordinated to the dissolved gas molecules, the latter will consume a greater amount of the additional energy provided by the shaking procedure and the additional transfer of structural information form the original solute molecules to the gas molecules is accomplished. The original remedy information is integrated and dynamically maintained in the more dilute solution. The more the remedy is succussed, the more refined these structures become. Hence the improvement of the system organisation as well as the precision of the remedy information as a result.

Auerbach (1994) looks at the different fluid dynamics that occur in the liquid during the mechanical mixing process of potentisation. In brief, the fluid undergoes three main dynamical stages. Firstly, there is *flow*, which is broken down into saddle and vortex flow respectively. Then, secondly, there is the *shock wave motion*, which is a result of the container that is slammed onto the hard surface and the fluid that impinges on the bottom and moves upwards at the velocity of sound. Thirdly there is *diffusion*, which is always present, since there is no such thing as absolutely quiescent water. At a microscopic scale it is in restless chaotic Brownian motion. This Brownian motion brings the substance to an equilibrium distribution. Thus, although flow carries and stirs substances, the most dissolving and mixing is due to diffusion. Hence diffusion is really the mechanism that activates the all-penetrating quality of water, since this is where the bulk mixture of the dilution takes place. This does not exclude the other two components, but all three components operating in unison is what renders the process of dynamisation effective.

Building on models by Anagnostatos et al. (1991), Antonchenko and Ilyin (1992) and Smith and Best (1989), Lessel (1994) proposes a dynamic field to explain what happens during the succussion process.

This model proposes the following:

The solid has an intrinsic electromagnetic field, with a unique frequency related to its components and their relative molecular presence. The various components of the diluent become attuned to this frequency and collectively resonate in accordance with it. The vibrations of the characteristic electromagnetic field and the resonantly vibrating components of the diluent maintain each other mutually, so that the vibrations persist even after the removal of the intended solute by serial dilution.

The number of succussions has always been an area of controversy. This has been highlighted by Bärthel (1993), in which he discusses the numerous recommendations put forward by a number of physicians over a 180-year period. The most commonly utilised numbers of succussion are 10 and 100 respectively. Since the level of dilution of the solute at 12 CH (10^{-24}), is in excess of Avogadro's number ($6{,}023 \times 10^{23}$), it is assumed that solute-solvent interactions will be eliminated at this potency.

Hahnemann noticed very soon that the shaking of the remedy seemed to impart some form of therapeutic power. Their efficacy increased as they were diluted further (Dellmour, 1994).

2.3 Variables in Succussion

Succussion is probably the most widely used mechanism in homoeopathic pharmacy. Even where potency scales differ, succussion is usually employed, although there may be disagreement as to how many strokes to apply. Succussion can vary from 2 strokes to 100, or many more.

Even when following the standard guidelines for succussion, the following variables are present:

- Variation in the rate of leaching sodium ion into the preparation, depending on the type and shape of soda glass bottle and the force of succussion. According to Lessel (1994) most potencies are prepared in soda glass bottles and that this may be an advantage due to the protective layer formed by the leaching sodium.

- The speed of succussion with respective people may vary as each person tires or become hurried or lazy.

- The time lapse between succussions may vary.

- The movement of the hand, its course during acceleration, the force applied and impact and rebound will vary from one person to the next.

- The method of holding the bottle, and therefore the temperature

conducted from the hand to the bottle, and the impact created by succussion because of the way the bottle is held, is likely to vary form person to person.

- The height to which the hand is raised before it is brought down onto the elastic surface will vary with each person.

- The consistency of the surface onto which the bottle is slammed is not adequately prescribed and creates variance.

2.4 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance is an analytical technique where a sample is immersed in a magnetic field and irradiated with radio waves. Using this technique to study a molecule enables the recording of differences in the magnetic properties of the various nuclei, protons, electrons and neutrons present as well as what positions these hold within the molecule. This is directly influenced by the environment of, for example, a proton, and thus enables predictions concerning the structure of the liquid.

2.4.1 The Principles of Nuclear Magnetic Resonance

NMR spectra arise from a property that is characteristic to some nuclei, known as spin(I). This is a quantum effect determined by the nuclear spin quantum number. The only nuclei that exhibit the NMR phenomenon are those for

which the spin quantum number (I) is greater than 0: the spin quantum number (I) is associated with the mass number and atomic number of the nuclei. Spin refers to the actual rotation of the positively charged nucleus about its own axis. Examples of nuclei with spin are hydrogen protons and carbon-13. This moving charge generates a magnetic dipole along the spin axis, called the nuclear magnetic moment (u). This magnetic moment can align with an externally applied magnetic field of strength B_o in only (2I + 1) ways, either re-enforcing (lower energy state) or opposing (higher energy state) B_o. The proton is behaving as a spinning magnet. This moves in a characteristic way under the influence of the externally applied magnetic field, this is called precessional motion. Precession is the result of the interaction of spin with the earth's gravitational force acting vertically downwards. In an applied magnetic field, magnetic nuclei like the proton precess at a frequency (v), which is proportional to the strength of the applied field. The amount of energy required is this applied field is minimal and it obeys the equation (a random small field strength of 1mT is assumed)

$$\Delta E = \frac{h\gamma B_o}{2\pi}$$

where: B_o is the applied field = 1 x 10^{34} T

h is Planck's constant (6.626 x 10^{-34}J s),

γ is the magnetogyric ratio = 26.75 x 10^{7} rad $T^{-1}s^{-1}$

A diagrammatical presentation of what has been mentioned thus far looks like this:

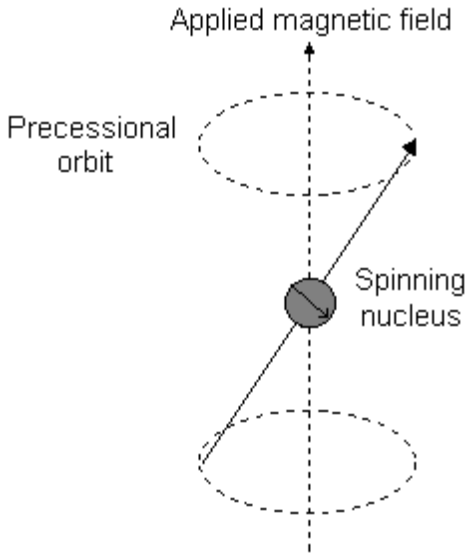


Figure 2.1 A diagrammatical presentation of the movements of a nucleus

As mentioned above, the proton that re-enforces the applied magnetic field is of a lower energy state and that opposing it is of a higher energy state. If a proton is precessing in the lower energy, or aligned state, it can absorb energy and pass into the opposed, higher energy, state; subsequently it can lose the extra energy and relax back into the lower energy state.

The resonance frequency depends on the intensity of the magnetic field. The precessing proton will only absorb energy from the radiofrequency source if the precessing frequency is the same as the frequency of the radiofrequency beam; when this occurs, the nucleus and the radiofrequency beam are said to be in resonance; hence the term nuclear magnetic resonance. These

precessing protons are irradiated with radiofrequency energy of the appropriate frequencies, and promote protons from the low-energy (aligned) state to the high-energy (opposed) state. This absorption of energy is recorded in the form of an NMR spectrum.

The strength of the signal, and hence the sensitivity of the NMR experiment for a particular nucleus is related to the magnitude of the magnetic moment (μ). The magnetic moments of ^{1}H and ^{19}F are relatively large, and detection of NMR with these nuclei is fairly sensitive. The magnetic moment for ^{13}C is about one quarter that of ^{1}H, and thus these nuclei are less sensitively detected in NMR.

The movement of protons from a low to a high energy state and *vice versa,* due to the absorption of radiofrequency, can lead to the eventual equal distribution of the populations in the respective states. As soon as this arises the observed resonance signal fades out due to the absence of further net absorption. This is termed the saturation signal. In the recording of a normal NMR spectrum, however, the populations in the two spin states do not become equal, because higher-energy nuclei are constantly returning to the lower energy spin state. The high-energy nucleus can undergo energy loss (or *relaxation*) by transferring energy difference to some electromagnetic vector present in the surrounding environment.

2.4.2 Chemical Shift

As cited by Kemp (1987), Packard first observed it in 1951 that the

precessional frequency of all protons in the same external applied field is not, however, the same, and the precise value for any one proton depends on a number of factors. He was able to detect three different values for the precessional frequencies of the protons in ethanol, and the realization that these corresponded to the three different chemical environments for the protons in ethanol (CH_3, CH_2 and OH) marked the beginning of NMR as a tool of the organic chemist. The term *chemical shift* was given because the shift in frequency depended on the chemical environment.

2.4.3 The NMR Spectrometer

The majority of the machine is just a big "cooler" filled with two very cold liquids, liquid helium and liquid nitrogen. Liquid nitrogen has a temperature of -195^{o}C and liquid helium is -269^{o}C. The superconducting coil, that creates the magnetic field, is cooled to its critical temperature of –269ºC by immersing it in liquid helium. This is surrounded by liquid nitrogen to keep the helium from evaporating too fast.

The sample is put inside the spectrometer. Once inside the machine, an air jet spins the sample tube to give a more uniform sample to scan.

When a sample is made for solution NMR spectroscopy, the solvent or part of the solvent used should be deuterated. This means that there are deuterium atoms in the place of the hydrogens of the solvent molecule. Hydrogen has one proton while deuterium consists of a proton and a neutron. Thus it is necessary to "lock" the NMR on a specific frequency so the spectrum will not

drift around during acquisition.

The sample is placed in an external magnetic field and is then subjected to a short burst of radio waves. The radio waves encourage the nuclei of the molecule to respond in a way can only be picked up on a radio receiver. The nuclei respond in a ‘language’ that only a decoder, called the Fourier Transform algorithm can ‘translate’. The signal of the nuclei is then analysed to determine various properties of the molecule and it's surroundings**.** If the nuclei in the sample do not resonate with the source, the detector will only record a weak signal coming directly from the source coil to the detector coil. An increased signal will be detected if the nuclei in the sample resonate with the source, since energy will be transferred from the source, via the nuclei, to the detector coil.

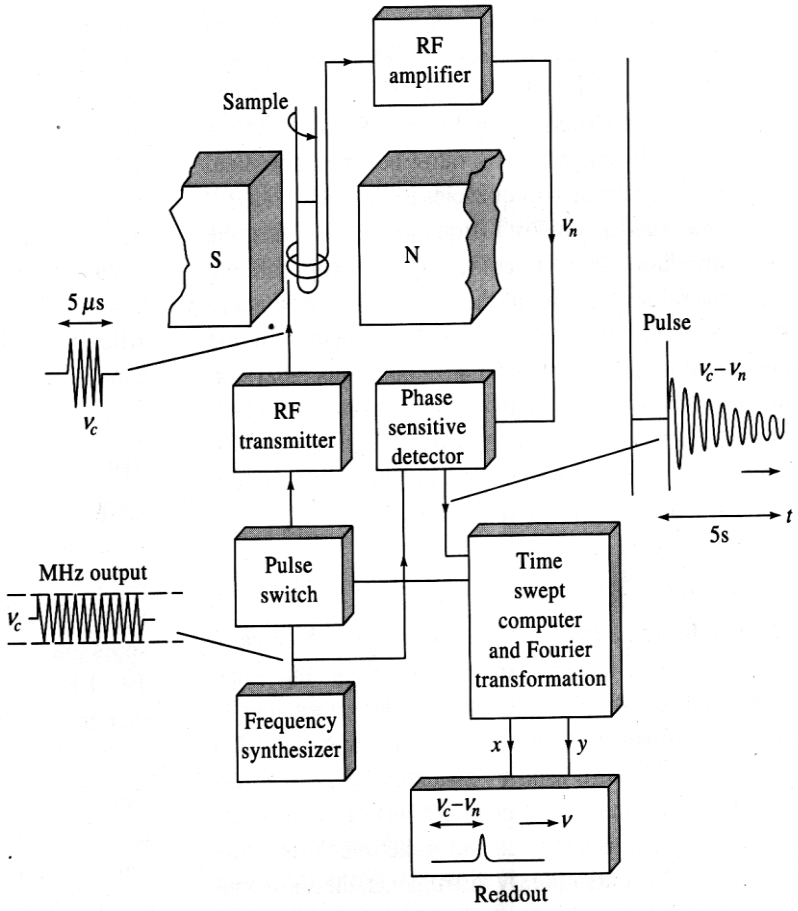


Figure 2.2 Block diagram of a Fourier NMR Spectrometer (Williams and Fleming, 1980).

2.5 NMR Research in Homoeopathy

A variety of different tools have been used to investigate homoeopathic remedies, such as infrared spectra, electronic spectra and NMR spectroscopy (Sukul et al. 2001), diffraction, Raman spectroscopy (Berezin 1994). Ives (2000) has stated that she believes the most promising line of research has come with the use of NMR spectroscopy. This is due to the fact that NMR

spectroscopy can record energy transitions of protons, which are reliant on their precession rates and electronic environments. Nuclear Magnetic Resonance spectroscopy is a powerful tool used to determine the structure of molecules. The chemical environment of specific nuclei is deduced from information obtained about the nuclei. NMR takes advantage of the fact that the nuclei of these molecules have an intrinsic spin. The process of NMR entails placing the sample in a simple magnetic field and irradiating it with radio waves. NMR spectra then arise from the so-called spin property. The spin is a quantum effect where spin is quantised, i.e. having two directions, up or down.

Earlier research was conducted by Smith and Boericke (1966) on Sulphur potencies to evaluate homoeopathic drug structure. Distinct changes were noted in the hydroxyl part of the spectrum. They concluded that the solvent structure is changed in unsuccussed serial dilutions as compared to undiluted solvent. They established further differences in succussed serial dilutions, and the changes became more extreme as the potencies passed Avogadro's limit. This led them to believe that there is a physical rearrangement in the solvent, most likely in the form of self-replicating polymers. A further study by Smith and Boericke (1968) with higher potency levels up to 60X level with bradykinin-triacetate, only compounded the evidence that the act of succussion increased the area of the hydroxyl spectrum as opposed to identical unsuccussed dilutions.

The employment of Nuclear Magnetic Resonance spectroscopy as an experimental technique on homoeopathic potencies, has proven very useful

(Schulte, 1999), however problems have been reported by various investigators assessing this tool [Bol (1997), Demangeat and Poitevin (2001)].

Sukul et al. (2001) conducted an experiment comparing the effects of Nux vomica 30c succussed and unsuccussed on adult toads as well as the NMR spectra of the above mentioned compared to a range of variations. In the preparation of their samples they used 10 succussions except with the samples that were unsuccussed. They concluded that the ethanol-water mixture has the capability to imbibe some specific properties of drug molecules or particles during the dynamisation process, but that succussion is not an essential factor in producing an effective homoeopathic potency. The possibility remains that there is a threshold in the number of succussions that need to be used before its effect on the remedy will be evident.

The employment of NMR Spectroscopy as a method for the analysis of structure within homoeopathic potencies and as a method for the analysis of the differences between the respective homoeopathic potencies and a lactose-based control and differences between parallel potencies and a control has been well substantiated (Ross 1997).

Significant differences have been demonstrated between Hahnemannian and Korsakovian methods of preparation of homoeopathic remedies with parallel potencies using NMR spectroscopy (Davies, 2001). Natrum muriaticum, as well as the controls, were prepared to the 9C, 30C and 200C potencies using the Hahnemannian and Korsakovian potentising methods and employing a fixed number (ten) succussions. In an intra-potency comparison for each

respective method and its control, it was found that differences exist regarding the chemical shift values for all peak types and potency levels. However, the relative integration levels show no significant differences for any of the peaks or potency levels.

Differences exist between centesimal (CH) and decimal (DH) Hahnemannian potencies for equal levels of deconcentration and equal number of succussions (Malan 2002). In order to assess similarities and differences between the two potency scales, remedies with the same levels of deconcentration, and remedies with fixed numbers of succussions were tested. The remedies, as well as controls, were prepared to the 6CH, 12CH, 24CH, 48CH and 12DH, 24DH, 48DH respectively. The investigation suggested that differences exist between centesimal and decimal Hahnemannian potencies for Sulphur and the control with equal deconcentration and equal number of succussions regarding the relative integration values, but the chemical shift values did not show significant differences. No statistically significant differences were found between homoeopathic Sulphur and the potentised water-ethanol control.

2.6 Summary

Studies cited above have indicated that the function of succussion still remains very uncertain. The fact that some researchers claim to find differences in results due to succussion and others claim that succussion is not essential certainly creates a question that needs to be looked at. Up to now all the research done employed a fixed number of succussions, hence this study will

investigate the effect of succussion in the process of manufacture of the homoeopathic remedy by varying the numbers of succussion. This may not only prove beneficial as a means to find some form of standardisation, but also continue the work of these researchers to form a consolidated theory on how homoeopathic remedies work. In order to give homoeopathy the opportunity to function as a medical science and a primary health care tool, irrefutable proof is a necessity. This will also allow for the boundaries of science to be extended and create a better understanding of the world around us.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Production of Sample Potencies

The nine samples (3 groups with 3 samples each) of Natrum muriaticum 12CH potencies were prepared by hand under laminar flow in the department of Homoeopathy, Durban Institute of Technology (DIT), according to Method 5a in the German Homoeopathic Pharmacopoeia (GHP, 1978). The specific details on the method and materials used for preparation are given in **Appendix A.**

3.1.1. The Base Substance

According to the GHP (1978), the Sodium chloride used for the preparation of the remedies should not contain less than 99.5% of sodium chloride (NaCl), calculated with reference to the dried substance. Sodium chloride, that fulfils these criteria, was obtained from the DIT, Chemistry Department. From here it was taken to the Homoeopathic pharmaceutical laboratory at DIT.

3.1.2. Precautionary Measures during Manufacture

Precautions were taken to eliminate the possibility of any contaminants in the samples, which may alter NMR spectra readings. This was done according to the standards in the British pharmacopoeia for sterilisation of

glass apparatus (BP: A208). All the equipment to be used was autoclaved, sterilised and allowed to cool down before preparation commenced. All potencies were prepared in the Labaire laminar flow room to avoid unnecessary contamination. The potencies were prepared from single containers of the same batch of ethanol and distilled water. This was to avoid the introduction of extraneous variables during the manufacture of the remedies.

3.1.3. Method of Preparation

The remedies were prepared in accordance with Method 5a (GHP, 1978), which is specific for solutions produced from basic materials and a liquid vehicle. According to this method, 1 part of the basic drug material is dissolved in 99 parts of the liquid vehicle and then succussed. For the remedies to be prepared, 1 part (0.03g) Natrum muriaticum was weighed out on a mass balance and then placed in a 5ml screw top bottle. 99 parts (2.97g or 3.0455ml) 15% ethanol was then added to that screw top bottle using a 5 ml pipette. The Natrum muriaticum was then given 30 seconds to dissolve and then succussed however many times each group required. This bottle was then labelled 1CH (Sukul et. al., 2001). From this bottle of 1CH, 1 part (0.03g or 0.0307ml) was then placed in a second, clean 5ml screw top bottle using a micropipette. 99 parts (3.595ml) 87% ethanol was then added to that. Again the mixture is given 30 seconds to diffuse and then succussed appropriately. This bottle was then labelled 2CH. This process was repeated up to 11CH. For the final potency place 15.84ml

87% ethanol into 25ml amber bottle using 2ml pipette, 5ml pipette and 10ml measuring cylinder. Add 0.16ml of 11CH to that. After the 30 seconds diffusion time the remedy was succussed and labelled 12CH.

3.2. Preparation of Sample Potencies for Transport and Analysis

The sample potencies for this study were produced as detailed above. The samples of 12CH Natrum Muriaticum were produced using 0, 10 and 100 succussions for each group respectively. The final potencies of each were produced in an amber bottle to prevent any harmful influence of the sun. The samples were then wrapped in tissue paper and placed in a secure cardboard box before it was transported to the Chemistry Department of the University of Kwazulu-Natal, Pietermaritzburg.

3.3. NMR measurement of Samples

The samples were submitted to Mr Craig Grimmer (the laboratory technician, Chemistry Department, UKZN) who drew a volume of 1,75ml to each sample into a new, unused, coaxial tube by means of a micropipette. The NMR spectra of the samples were then run using acetone as both the external lock and reference substance, as it provided a very reliable chemical shift value outside the range of the other peaks (Grimmer, 2004).

The most accurate NMR spectra were obtained from one sample with sufficient transients per run (in this instance 16). Therefore each spectrum was the result of 16 measurements (Grimmer, 2004).

The instrument used was a Varian 500 MHz INOVA Spectrometer that operated at a frequency of 499.9832268 MHz. The spectrometer had a 5mm broadband 'switchable' probe and a 5mm inverse detection probe. To ensure a homogenous field around each sample, the magnet was shimmed before each sample was tested. The acquisition time was 1.9 seconds multiplied by 16 transients per run. The pulse angle was set at 90˚. The temperature was maintained at 25˚C (Grimmer, 2004). Spectra of the OH, H_2O, CH_2 and CH_3 signals were recorded and printed out by the spectrometer. The values were expressed in terms of chemical shifts and integration values.

3.4. Statistical Analysis

Chemical shift and relative integration values of H_2O, OH, CH_2 and CH_3 peaks of the NMR spectra were recorded and calculated to six decimal places. Three and four peaks arose for CH_3 and CH_2 chemical shift values respectively; these were averaged to find a single peak that was then used for further calculations. Relative integration values were calculated by dividing the integration values of each peak by the sum of all the integration values for that specific run. The data was then entered into a Microsoft Excel© 2000 spreadsheet and from there it was transferred to the SPSS© Base 10.0 software package for statistical analysis. All the samples were then compared

with each other to determine whether there was a significant difference between any of them. Since the sample group was small and the values for each run so similar, it was impossible to run multivariate tests. On attempting to run multivariate tests only errors were obtained, hence the statistical evaluation was performed using Repeated Measures Analysis of Variance (Singh, 2004).

3.4.1. Repeated Measures Analysis of Variance

A straightforward application of the Analysis of Variance (ANOVA) test could not be applied, because for some of the results obtained, statistical tests for normality were violated. As the comparison groups had the same number of samples, the assumption of homogeneity could however be made.

3.4.1.1. Common assumptions between Repeated Measures Analysis of Variance and the standard ANOVA

Firstly, that the model was correctly specified and additive. Secondly, that the errors would follow a normal distribution and were independent of the effects in the model (Singh, 2004).

3.4.1.2. Special Repeated Measures Analysis of Variance features

In addition to standard ANOVA assumptions, there was one specific to repeated measures when there were more than two levels to a repeated

measures factor. If a repeated measures factor contained only two levels, there was only one difference variable that could be calculated, and the assumption could be ignored. However, if a repeated measures factor had more than two levels, an overall test of differences (main effect) was needed. Pooling the results of the contrasts between conditions creates the test statistic (F). The assumption called sphericity dealt with these values (Singh, 2004).

3.4.1.3. Sphericity

The assumption called sphericity dealt with this pooled data when such pooling was appropriate. The basic idea was that if the results of two or more contrasts (the sums of squares) were to be pooled, then they should be equally weighted and uncorrelated.

3.4.1.4. F-test, Epsilon or Multivariate test depending on Sphericity

The ability to perform the F-statistic calculation depended on whether the condition of sphericity was met or not. In the event of this condition being violated, then an epsilon corrected test might have been used, such as Greenhouse-Geisser. If violations did occur at this stage, then further multivariate tests would have been needed. These multivariate tests considered the differences in the contrast variable to zero, that is, claiming that there were no differences in terms of the methods used. If sphericity was satisfied, there was no need to consider the multivariate tests.

3.4.1.3. Pairwise Comparison

Unlike the standard ANOVA tests, repeated measures offered a Pairwise Comparison of results indicating the actual pairing of similar methods and the strength of this in terms of a significance value. Hence decisions could be made regarding the effects of one method as compared to another.

3.5 An example of the statistical analysis with regards to the chemical shift values of OH:

General Linear Model - OH

Within-Subjects Factors

Measure: oh

method	Dependent Variable
1	zero_succ
2	ten_succ
3	hund_succ

The table above indicated that there was only a single within-subject factor and no between-subject factors (Singh, 2004).

Descriptive Statistics

	Mean	Std. Deviation	N
zero_succ	22.5667	.25166	3
ten_succ	21.4333	.50332	3
hund_succ	34.8333	17.38285	3

Means, standard deviations and sample sizes were given for each factor level (Singh, 2004).

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
method	Pillai's Trace	.994	81.350[a]	2.000	1.000	.078
	Wilks' Lambda	.006	81.350[a]	2.000	1.000	.078
	Hotelling's Trace	162.700	81.350[a]	2.000	1.000	.078
	Roy's Largest Root	162.700	81.350[a]	2.000	1.000	.078

a. Exact statistic

b.
Design: Intercept
Within Subjects Design: method

The multivariate tests indicated that there was no differences in the experimentation method used as all the values were more than the specified alpha-value (α = 0.05). This test was only considered if the sphericity test did not hold (Singh, 2004).

Mauchly's Test of Sphericity[b]

Measure: oh

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
method	.000	9.407	2	.009	.500	.500	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.
Design: Intercept
Within Subjects Design: method

It was observed that the significance value was less than 0.05, thereby indicated that sphericity was not maintained. However, the Greenhouse-

Geisser epsilon correction gave an acceptable value. Hence, the testing procedure could be conducted (Singh, 2004).

Tests of Within-Subjects Effects

Measure: oh

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
method	Sphericity Assumed	331.316	2	165.658	1.580	.312
	Greenhouse-Geisser	331.316	1.000	331.302	1.580	.336
	Huynh-Feldt	331.316	1.000	331.261	1.580	.336
	Lower-bound	331.316	1.000	331.316	1.580	.336
Error(method)	Sphericity Assumed	419.351	4	104.838		
	Greenhouse-Geisser	419.351	2.000	209.667		
	Huynh-Feldt	419.351	2.000	209.641		
	Lower-bound	419.351	2.000	209.676		

The test result was highly significant in the event of the sphericity condition holding. The conclusion from these results indicated that there were no differences in the type of method used.

The aim was to test for differences in methods. After checking the sphericity assumption, it was observed that there were no differences in the method used. The next step was to analyse these (differences) more closely.

The Bonferroni adjustments with pairwise tests were performed. The Bonferroni multiple comparison test was a conservative test, that is, the FEW (family-wise error rate) was not exactly equal to α, but was less than α in most situations. It was easy to apply and could be used for any set of comparisons. To get the Bonferroni adjusted p-values, the ordinary, not adjusted pairwise p-

values (for example, t-test p-values for comparing two means) were multiplied by the number of comparisons in the family and the minimum of the obtained number and 1 was then chosen.

The results are as follows:

Transformation Coefficients (M Matrix) using the Estimated Marginal Means method:

Transformation Coefficients (M Matrix)

Measure: oh

	method		
Dependent Variable	1	2	3
zero_succ	1	0	0
ten_succ	0	1	0
hund_succ	0	0	1

The diagonal elements represented the variance of each contrast and the off-diagonal values represented the covariances. The sphericity assumptions held in this instance as the diagonal values were all the same and the off diagonal values were all zero.

Pairwise Comparisons

Measure: oh

		Mean Difference			95% Confidence Interval for Difference[a]	
(I) method	(J) method	(I-J)	Std. Error	Sig.[a]	Lower Bound	Upper Bound
1	2	1.133*	.145	.048	.022	2.245
	3	-12.267	10.171	1.000	-90.061	65.528
2	1	-1.133*	.145	.048	-2.245	-.022
	3	-13.400	10.306	.970	-92.227	65.427
3	1	12.267	10.171	1.000	-65.528	90.061
	2	13.400	10.306	.970	-65.427	92.227

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Each method mean was then tested against every other. It was observed that there was perfect correlation between methods 1 and 3, with a very significant relation between methods between 2 and 3. Methods 1 and 2 were fairly comparable, but not to the degree of the pairings above. The significance value of 0.048 indicated that if the null hypothesis was true, then there was about a 5% chance of obtaining sample means as far or further apart as we observed in the data. This was not significant and we concluded that there was no evidence of a difference in the methods used to obtain the results.

The same test was run for all four peaks for both the chemical shifts and relative integration values of all the samples. To this end the hypotheses were stated as follows:

H_0: The two independent population groups had identical medians, i.e. succussion *did not* have an effect.

H_1: The two independent population group have different medians, i.e. succussion *did* have an effect.

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

Accept H_0: if $p \geq \alpha$

Accept H_1: if $p \leq \alpha$

$\mu_1 = \mu_2$ [accept nul hypothesis (H_0)]

$\mu_1 \neq \mu_2$ (reject H_0 i.e. alternative hypothesis)

α = level of significance = 0.05

CHAPTER FOUR: STATISTICAL ANALYSIS OF THE RESULTS

4.1 Criteria Governing the Admissibility of Data:

The electromagnetic nature of homoeopathic remedies combined with the sensitive nature of the experiment was the cause for the absolute care that was taken to eliminate extraneous variables as far as possible. A high degree of caution was exercised right from the very beginning while sourcing the base substance according to GHP (1978). From there the manufacturing process was very meticulous as seen in 3.1.2 and 3.1.3. All samples were stored under the same conditions. During transportation all external influences were avoided as far as possible. During the NMR experiment at the University of Kwazulu Natal the samples were handled according to the methods detailed in 3.3 to avoid contamination. Each sample was analysed sixteen times through a process of sixteen transient runs per sample from which the NMR spectra was then generated. The initial chemical shift values of the four peaks (OH, CH_2, CH_3, H_2O) were recorded and the subsequent relative integration values of the data determined. Crude data was then subjected to statistical analysis detailed in 3.4.

4.2 The Results

The parameters of the statistical analysis was stated as follows:

- H_0: The two independent population groups have identical medians.
- H_1: The two independent population groups have different medians.
- $\mu_1 = \mu_2$ [accept null hypothesis (H_0)]

- $\mu_1 \neq \mu_2$ (reject H_0 i.e. alternative hypothesis)
- α = level of Significance = 0.050

Method 1 = 12CH Natrum muriaticum manufactured using **0** succussions

Method 2 = 12CH Natrum muriaticum manufactured using **10** succussions

Method 3 = 12CH Natrum muriaticum manufactured using **100** succussions

			Chemical Shift Value		**Relative Integration Value**	
Peak	**Method**	**Nr of Samples**	**Mean**	**Std Deviation**	**Mean**	**Standard Deviation**
OH	Method 1	3	22.5667	0.25166	19.0667	0.01528
OH	Method 2	3	21.4333	0.50332	19.3400	0.39850
OH	Method 3	3	34.8333	17.38285	19.7967	0.02082
CH_2	Method 1	3	37.5167	18.02013	27.0100	0.01732
CH_2	Method 2	3	35.9500	17.19828	26.9933	0.01528
CH_2	Method 3	3	32.9083	15.73077	26.7833	0.00577
CH_3	Method 1	3	100.4333	25.85928	40.5133	0.01528
CH_3	Method 2	3	108.1778	41.22853	40.5033	0.03512
CH_3	Method 3	3	99.4000	26.19165	40.1233	0.00577
H_2O	Method 1	3	10.7000	0.10000	13.4100	0.01000
H_2O	Method 2	3	13.9000	6.58483	13.4033	0.01528
H_2O	Method 3	3	10.7000	0.10000	13.3000	0.01732

Table 4.1 Initial values obtained from the NMR spectra

In the above table the means, standard deviation and samples sizes were given for each method respectively.

	Chemical Shift			Relative Integration Value		
Peak	**Significance of Multivariate Test**	**Significance of Mauchly's Sphericity Test**	**Greenhouse-Geisser Correction (if necessary)**	**Significance of Multivariate Test**	**Significance of Mauchly's Sphericity Test**	**Greenhouse-Geisser correction (if necessary)**
OH	0.078	**0.009**	0.500	0.001	**0.006**	0.500
CH_2	0.000	**0.000**	0.533	0.064	0.391	0.541
CH_3	0.103	**0.000**	0.504	0.018	0.300	0.524
H_2O	0.488	**0.000**	0.500	0.153	0.960	0.927

Table 4.2 The significant values for the Multivariate Test and Mauchly's Sphericity Test

The table above gave the values of significance for the Multivariate test and Mauchly's Sphericity Test. For the final comparison to be done, the sphericity value of 0.05 had to be met. In the table above, all the highlighted values violated the sphericity test. The Greenhouse-Geisser correction adjusted these values and made them acceptable for the subsequent comparison. These values were then used to determine the Pairwise Comparison after the p-values were adjusted as set out in 3.5.

	Chemical Shift (δ)				Relative Integration Values			
	OH	**CH_2**	**CH_3**	**H_2O**	**OH**	**CH_2**	**CH_3**	**H_2O**
Method 1 vs. **Method 2**	0.050	**0.000**	0.880	1.000	1.000	0.113	1.000	1.000
Method 2 vs. **Method 3**	0.970	**0.000**	0.692	1.000	0.515	**0.007**	**0.009**	**0.040**
Method 1 vs. **Method 3**	1.000	**0.000**	0.226	0.000	**0.002**	**0.008**	**0.002**	0.056

Table 4.3 Pairwise Comparison of the different Methods using adjusted p-values

In the above table, all the values with a level of significance of **$p < \alpha$,** were highlighted. These were the values where the null hypothesis was rejected.

For all the **Chemical Shift** values of the samples of **CH_2** the null hypothesis was **rejected** because p= 0.00, i.e. less than 0.05. This indicated that there was a statistically significant difference in these peaks when using the respective numbers of succussion in preparation of the remedy. This indicated that succussion had a significant effect on the chemical shifts of these CH_2 samples.

On the other hand, for the **Relative Integration Values**, the values of all four peaks seem to be affected, but more so in the groups comparing Method 1 vs.

Method 3 and Method 2 vs. Method 3. For these values the null hypothesis was again rejected and this indicated that succussion *did* have an effect on these peaks.

The conclusion that was drawn from the results portrayed above was that succussion did have and effect on the NMR spectra of the samples tested. The Pairwise Comparison of Table 4.3 showed that the areas most affected were that of the CH_2 peak in the chemical shift values. On the other hand, all four peaks were affected with regards to the relative integration values, but more so in the comparison of 0 and 100 succussions and 10 and 100 succussion as opposed to that in 0 and 10 succussions.

CHAPTHER FIVE: DISCUSSION

The results of this investigation suggested that significant differences existed between samples of Natrum muriaticum of **equal deconcentration** and **different numbers of succussion.**

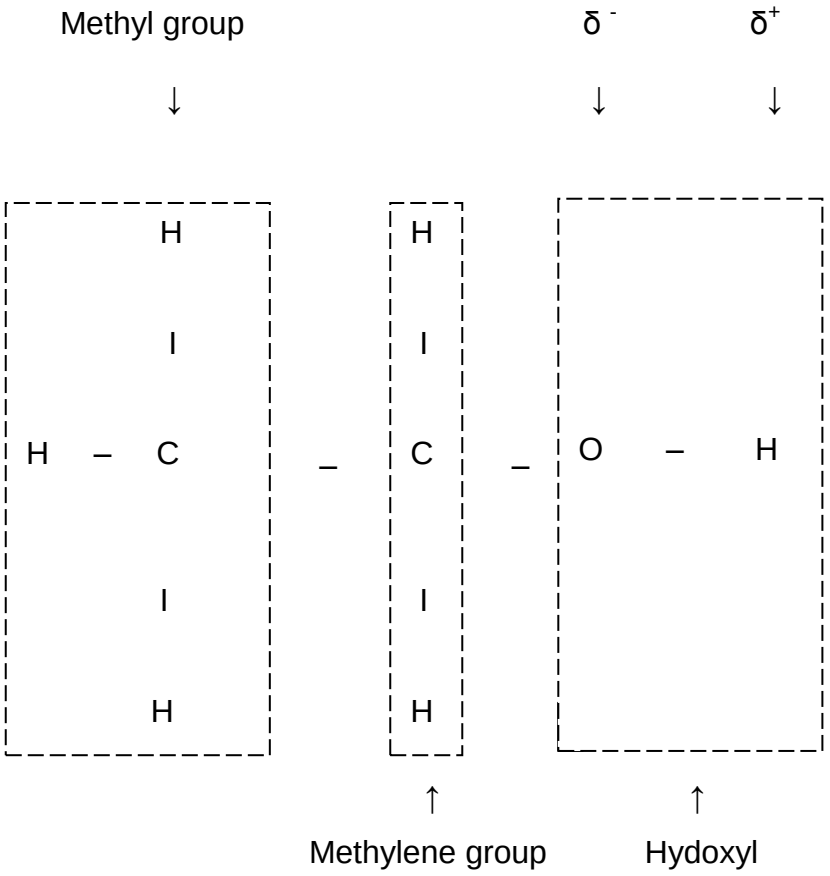
In order to explain our findings we adopted the model proposed by Anagnostatos et al. (1991), where it was suggested that the effect of succussion was to redistribute the energy within a higher order structure based on the concept of clathrates. Here characteristic small clusters (aggregates of a small number of molecules) of the diluted base substance were formed. Shells of the organised hydrogen-bonded molecules of the solvent (called clathrates) were then formed around these clusters.

A combination of succussion and the different inertial properties, forced the small clusters of base molecules to move out of their original clathrates. A new clathrate was then formed around each relocated cluster, and an additional clathrate (mantle clathrate) was formed around the initial clathrate (now called the core clathrate), which became hollow, having lost its small cluster. The dilution got up to a point beyond, where no effective amount of the base substance remained.

From this point onwards the role of small clusters in dilutions and succussions to follow was totally influenced by the compact structure of the core clathrate, which possessed an interior void, characteristic of the properties of the initial substance. Again due to forcefully applied succussions and different inertial

properties, core clathrates moved out of their mantle clathrates and stimulated the formation of new mantle clathrates. Likewise, old mantle clathrates became new core clathrates and stimulated formation of their own mantle clathrates. As succussion and dilution continued, the process was repeated. An informational imprint of the original substance on the solvent was the result of the continual application of the succussive force.

The depiction of the NMR spectra was influenced by the spin-spin coupling phenomenon that occurred due to the interaction between groups of protons in a molecule. In terms of the chemical shifts for the OH, CH_2, CH_3 and H_2O signals, CH_2 showed statistically different results for inter-group comparisons. This was to be expected when the ethanol structure was considered more closely:



group

Ethanol had a polar OH - group as shown above. The ethanol molecule lacked symmetry of individual atoms; the electronic cloud was determined by the localisation of specific nuclei as well as the nature of the bonds present. The chemical shifts for the OH, CH_2, CH_3 and H_2O peaks were also subjected to local diamagnetic effects, local paramagnetic effects, anisotropic effects from neighbouring groups and electric field effects. The presence of the strong *polar* OH–group influenced the molecular electron configurations most notably at the location of the methylene group. Anisotropy due to neighbouring groups in the molecule would generate local magnetic field effects and these would in turn affect chemical shifts. For the ethanol molecule it was the combination of the local group anisotropy and the electric field effects due to the polar OH–group that made the CH_2 chemical shifts most susceptible to external influences – in this case, succussion (Shaw, 1976). This was exactly what seemed to predominate in the chemical shift findings, where the CH_2 results of **Table 4.3** displayed the most significant statistical difference.

When looking at the NMR spectrum, the area under the peak was proportional to the number of protons generating that peak. These relative values were called integral values. From the investigations it was found that statistically significant differences existed for all four peaks between the comparison of 10 and 100 and 0 and 100 succussions, however not for the comparison of 0 and

10 succussions. This was to be expected on the basis of the clathrate model. The effect of the succussion was to dynamically redistribute the energy within the higher order structures. The effect of succussion was a linear, additive effect, which meant that the effect would be greater for a larger number of succussions.

These findings were congruent with earlier research conducted on various aspects of potentisation by Ross (1997), Davies (2002) and Malan (2003). It also served to confirm that NMR spectroscopy was an invaluable tool in the field of research of homoeopathy.

CHAPTER SIX – CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of this study served to confirm the hypothesis that different numbers of succussion would have an effect on the physicochemical structure of samples of 12CH Natrum muriaticum. These correlated with the changes visible on the NMR spectra. The pairwise comparison that was done in Repeated Measures Analysis of Variance revealed statistically significant differences among the three groups - 0 and 10 succussions, 10 and 100 succussions and 0 and 100 succussions.

When the **chemical shift values** were looked at, CH_2 seemed to be the most notably affected of the peaks. This was to be expected when the ethanol molecule was considered more closely. This revealed that the electric field effects of the bonds surrounding the methylene group combined with the strong polar OH – group would render the electron configuration at CH_2 the most susceptible of all four peaks to the influences of an external mechanical force such as succussion.

When the **relative integration values** were considered it was clear that all four peaks were affected. The most significant differences were to be seen among the groups comparing 10 and 100 succussions and 0 and 100 succussions. This was explained by adopting the theory proposed by Anagnostatos et al. (1991) that was based on the clathrate model as detailed

in chapter five. The bottom line with regards to this is that the effect of succussion on the relative integration values is that the mechanical force is linear, cumulative and the larger the number of succussions applied, the greater the effect.

In conclusion, this study confirmed that succussion *did* have a statistically significant effect on the samples that were examined as compared to those not succussed. It thus contributed to the scientific data available for the assessment of homoeopathy and added to the standardisation of homoeopathic practice and manufacture.

6.2 Recommendations

With the growing awareness of the holistic approach of treatment, an explanation of how exactly homoeopathy works is crucial. Every research conducted properly brings us one step closer to a better understanding. Considering all the unquantified and unspecified areas in homoeopathy, it is very obvious that a sound scientific basis needs to be established and explained in order for scientists and laymen alike to receive this discipline without hesitation. The following aspects, amongst others, need to be addressed in order to assist in achieving this:

1. A wider variety in number of succussions

Now that it has been observed that the number of succussions during the manufacturing process play a role, a wider variety in the number of succussions used should be tested. This will entail an increased number of samples tested compared to this research as well as not only three different numbers, but a substantially higher number.

2. Compare the difference between different people succussing similar potencies

External factors such as the person succussing the remedy can have a great influence on the quality of remedy produced. In order to observe these differences, for example, 10 different people should produce ten similar samples in the same way, of the same remedy, at the same time, under the

same conditions to a specific potency. This will highlight the effect of seemingly unimportant variables.

3. Repetition of experiments

Since the scientific method requires a certain amount of reproducibility to be present in experiments, it is necessary for all experiments to be repeated several times. The main drawback regarding this aspect is the low availability of time and funding.

4. Control of external factors

There are a variety of external factors that need to be excluded. Some of the grosser examples are transportation of the samples from the laboratory of manufacture to the NMR spectrometer, then there's also a change in temperature and a pressure variation that should be taken into account. In order to standardise the research that is to follow, definite standards need to be set to limit the amount of variables present.

5. Use of a larger sample size

In order to draw significant conclusions regarding the effect of succussion on homoeopathy it is suggested that a larger sample size is used in future.

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APPENDIX A: The Preparation of Sample Potencies

a) AIM: To produce 3 groups of 12CH potencies of Natrum muriaticum according to the Hahnemannian method using serial dilutions and 0, 10 and 100 succussions respectively with 3 samples in each batch respectively.

APPARATUS: 96% alcohol (for flaming)

Cigarette lighter

5 ml screw top bottles

25ml amber bottles

Mass balance

Spatula

Labels and pens

Pure crushed Natrum muriaticum

Paper toweling

Distilled water

15% Ethanol

87% Ethanol

5ml and 2ml pipettes and 10ml measuring cylinder

Micropipettes

METHOD:

1. Rinse and autoclave all utensils and allow to cool.
2. Place a single sheet of paper on the mass balance and tare.
3. Weigh out 1 part (0.03g) of Natrum muriaticum using mass balance and place into the first 5ml screw top bottle.
4. Place 99 parts (2.97g or 3.0455ml) 15% ethanol into first screw top bottle using a 5ml pipette.
5. Wait 30 seconds and succuss 10 times and label as Natrum muriaticum 1CH.
6. Place 2.97g (3.595ml) 87% ethanol into a second, new 5ml screw top bottle.
7. Add 0.03g (0.0307ml) of 1CH into a second screw top bottle using a clean micropipette.
8. Wait 30 seconds.
9. Succuss 10 times and label as Natrum muriaticum 2CH.
10. Repeat the above procedure (steps 6 to 9) adding 0.03ml of the previous potency to 2.97ml 87% ethanol at each dilution level up to 11CH, accepting that a volumetric ratio of 1: 99 (0.03ml: 2.97ml) may now be used as ethanol concentrations are the same.
11. Place 15.84ml 87% ethanol into 25ml amber bottle using 2ml pipette, 5ml pipette and 10ml measuring cylinder.
12. Add 0.16ml of 11CH to that.
13. Succuss 10 times and label as Natrum muriaticum 12CH, 10 succussions.

14. Repeat the above procedure (steps 1 – 11) using 100 succussions at each dilution level instead of 10.
15. Label the product of step 14 as Natrum muriaticum 12CH, 100 succussions.
16. For the preparation of the 0 succussion batch, steps 1-13 must be repeated as above with succussion omitted at every step.
17. Label the product of step 16 as Natrum muriaticum 12CH, 0 succussions. With 0, 10 and 100 succussions forming 3 separate groups, 3 similar samples will be produced for each group respectively. The sample size will consist of 9 remedies.