A comparative study of the NMR spectra of parallel potencies of Pulsatilla pratensis, prepared according to Hahnemannian and Anthroposophical Extended Medicine methods respectively.

By

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Durban Institute of Technology.

I, Fourie Erasmus do hereby declare that this dissertation is representative of my own work.

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Dedicated with love to my parents.

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ABSTRACT

The purpose of this study was to analyse and compare the NMR spectra of three analogous ultra-high dilutions, prepared according to the classical Hahnemannian method and two Anthroposophical methods viz; Wala and Weleda.

Comparison was made in terms of the chemical shift and relative integration values of the CH_2, CH_3, H_2O and OH signals obtained via NMR spectroscopic methods. All three samples analysed were theoretically identical, except for the method of dynamization specific to each method.

Comparison was drawn between all three methods. It was hypothesized that the method of dynamization plays a significant role in the potentization process, and therefore in the establishing of the physico-chemical identity of each individual sample. This in turn could indicate a pharmacological uniqueness in each sample.

The experiment was conducted as per the limitations of the scientific method. All samples were diluted and potentized to D12 potency level. Samples were produced in 16ml volumes and transported to the NMR spectroscopy laboratory at the University of Kwa Zulu Natal, Pietermaritzburg.

A Varian 500MHz INOVA spectrometer was used in the experiment, having a 5mm broadband switchable probe and a 5mm inverse detection probe. The

pulse angle was set at 90 degrees and the temperature maintained at 25 degrees Celsius. A volume of 1.75 ml of each sample was drawn into a coaxial tube by means of a new micropipette. Acetone was used as an external lock and ethanol as the reference. NMR spectroscopic analysis was conducted on three samples of each group.

The data was recorded and represented in the form of NMR spectra, providing chemical shift and integration values for the CH_2, CH_3, H_2O and OH peaks. All three sample groups were statistically compared by application of the Pairwise Comparison test to the chemical shifts and the relative integration values. The level of significance was set at $\alpha = 0.05$ for all test comparisons.

The results of this study showed that statistically significant differences were observed on NMR spectroscopy between Hahnemannian and Anthroposophical potentising methodologies, most notably in the chemical shift of the CH_2 and CH_3 signals. These differences would make their equivalent use in the pharmacological industry questionable. This study also served to validate the use of NMR spectroscopy as a valuable tool in researching ultra-high dilutions.

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TABLE OF ABBREVIATIONS

CH	-	Centesimal Hahnemannienne. Interchangeably used with C
CH_2	-	Methylene group
CH_3	-	Methyl group
DH	-	Decimal after Hahnemann. Used interchangeably with D
OH	-	Hydroxyl group
B_0	-	Applied or static magnetic field
µl	-	Microlitre

DEFINITION OF TERMS

Analyse: For the purpose of this project, the term analyse refers to the statistical manipulation of the recorded chemical shift-values and integration values of CH_2, CH_3, H_2O and OH signals.

Analysis of Variance: a method of statistical analysis used for the analysis of data.

Batch: a specific quantity of a medicinal substance 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.

Centesimal: The concentration scale originally introduced by Hahnemann. Dilution steps are 1:100. It may be indicated as CH (centesimal Hahnemannian) or C, or it is assumed as the potency scale when no scale is indicated.

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.

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

Decimal: The concentration scale primarily used in Germanic countries. The dilution steps are 1:10. It may be indicated as DH (decimal Hahnemannian) or D. Homoeopaths often use it interchangeably with the centesimal scale based on equal deconcentrations.

Electromagnetic waves: the effects of oscillating electric and magnetic fields that are capable of travelling across space, i.e. not requiring a medium for propagation.

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

Heisenberg Uncertainty Principle: the uncertainty of a simultaneous measurement concerning both the momentum and position of a subatomic particle.

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

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

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 succession or trituration (see below). Three rates of deconcentration are used in preparation of homoeopathic potencies.

Potentization: The process of preparing a homoeopathic remedy by repeated dilution and succussion or trituration. It is believed to involve the transfer of information from the original substance into the carrier. The scope and strength of the effected of the substance is believed to increase through this process.

Relative integration: The process of finding the area under the respective peaks on a graph. Relative integration is calculated by dividing the integration values of each peak by the sum of all integration values of the run. The value is proportional to the number of protons generating each of the peaks.

Remedy: As opposed to a drug, a homoeopathic remedy remedies a situation, causing symptomatic expression of disease to be cured.

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

CHAPTER ONE: INTRODUCTION

1.1 THE AIM OF THE STUDY

The purpose of this study was to compare the NMR spectra of parallel potencies of Pulsatilla pratensis, prepared according to Hahnemannian, Wala and Weleda methodologies, in order to evaluate their similarity demonstrable through NMR spectroscopy.

1.2 THE OBJECTIVE

To compare and evaluate the NMR spectra of Pulsatilla pratensis D12, prepared according to Hahnemannian, Wala and Weleda methodologies, with respect to the chemical shifts and relative integration values of the CH_2, CH_3, H_2O and OH signals, by determining possible differences in their respective NMR spectra.

1.3 THE HYPOTHESES

1.3.1 The first hypothesis

It was hypothesized that significant differences exist between the chemical shift and relative integration values of CH_2, CH_3, H_2O and OH signals of Pulsatilla pratensis in ultra-high dilution of D12, which have been produced according to Hahnemannian, Wala and Weleda methodology respectively.

1.3.2 The second hypothesis

It was hypothesized that the method of dynamization plays a significant part in the development of a distinct physico-chemical identity of homoeopathic and anthroposophical remedies. It was therefore hypothesized that statistically significant differences exist between parallel potencies of ultra-high dilutions and that these indicate potentially significant differences inherent to each method and product.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

During the last forty years, NMR spectroscopy has been utilized to an increasing degree in researching the physico-chemical composition of homoeopathic preparations. In particular, NMR appears to be a useful experimental technique in a continuing attempt to examine proposed theories as to the mechanisms present in the production of homoeopathic remedies (Weingartner:1990).

2.2 HOMOEOPATHIC POTENTIZATION

The homoeopathic process of preparing medicine was introduced by Hahnemann in the fifth edition of the Organon, in 1831. It is characterized by four distinguishing features (Gaier 1981:456):

1. It is a purely mechanical and mathematico-physical process.
2. The procedure involves neither uncertain, unreliable nor immeasurable factors.
3. The resultant product is stable and can readily be maintained that way.
4. The process is theoretically illimitable, though it becomes laboriously time consuming in the higher range of potencies.

Potentized substances possess certain attributes:

1. Quantitative (chemical) reduction linked to qualitative increment of therapeutic (reactive) property.
2. Physical solubility (even of substances, like metals, believed to have been insoluble).
3. Physiological assimilability and bioavailability.
4. Altered therapeutic activity (suppression of primary (direct), and enhancement of secondary (reactive) effect of drugs.

In homoeopathic potentization three scales are used:

1. The decimal scale where the first potency contains one tenth part of the crude substance and each succeeding potency contains one tenth part of the potency immediately preceding. The decimal potency is indicated by the numerals denoting the deconcentration with the suffix D or X.
2. The centesimal scale where the first potency contains one hundredth part of the crude substance and each succeeding potency contains one one-hundredth part of the potency immediately preceding. The decimal potency is indicated by the numerals denoting the deconcentration with the suffix C.
3. The quinquagenimillesimal (50-millesimal) scale, involving a different method of preparation altogether, resulting in each potency level containing one fifty thousandth of the preceding level.

For the purposes of this study the Hahnemannian sample was serially diluted on a 1:10 scale with 100 succussions between each dilution.

2.3 ANTHROPOSOPHICAL EXTENDED MEDICINE

Anthroposophical extended medicine is one of a number of practical applications of the work of the Austrian scientist and philosopher, Rudolf Steiner (1861-1925), the founder of Anthroposophy.

Anthroposophy seeks to extend the understanding of man in a perspective exceeding purely material or mechanical means. It is a philosophical and scientific approach to creation, emerging from, but extending beyond the foundations of natural science. In particular, it rejects the reductionist approach and recognizes man as consisting of not only the physical body, but also the soul and spirit (Evans & Rodger 1992:10).

The application of the principles of Anthroposophy has reached far beyond the purely philosophical. Anthroposophical principles have been successfully applied to various fields, resulting in an innovative approach to not only medicine, but also new forms of education, art, architecture, caring for the handicapped, agriculture and economics (Evans & Rodger 1992:10).

Anthroposophical medicine came about as a result of a group of medical doctors recognizing that this extended physiology, of regarding man not only

as a physical body, but as an integrated four-fold being, had remarkable implications for medical treatment.

Through Steiner's collaboration with a Dutch doctor, Ita Wegman, the foundation for a new approach to medicine was laid. Their collaboration resulted in a book for the medical profession, 'Fundamentals of Therapy', and the first Anthroposophical clinic was opened at Arlesheim, Switzerland.

As Steiner was not a medical doctor, he worked with qualified practitioners in the development of anthroposophical medicine. He insisted that it should *extend* orthodox medicine rather than become an alternative. Thus all anthroposophical practitioners qualify first in conventional medicine, thereafter do further study in the understanding of man in health and illness from the anthroposophical perspective (Evans & Rodger 1992:10).

The aim of anthroposophical medicine is to stimulate the natural healing forces in the patient. These are the life forces which maintain the physical body and oppose decay. Steiner describes man in terms of this life force, as a four-fold being (Evans & Rodger 1992:21). Besides the physical body, he identifies

- The etheric body. Comprised of non-physical formative forces, particularly active in growth and nutrition.
- The astral body. Expressing itself particularly in the nervous system.
- The ego. Representing man's spiritual core and self-consciousness. This is expressed in the muscular activity and the blood.

Anthroposophical medicine thus seeks to understand illness in holistic terms, based on the way these four aspects of man interrelate to form a whole.

Anthroposophical medicine consists of two distinct productions: Wala and Weleda. Potencies most often employed are produced in a 1:10 dilution ratio and lie within the decimal scale.

Dynamization however, differs significantly from the Hahnemannian method. The Weleda succussion technique requires that the container be moved in an oscillatory motion of a figure eight (the sign of infinity)[1]. Wala preparations undergo dynamization by a swift, horizontal movement of the arm from back to front, resulting in a vortex being created within the container[2].

Anthroposophical potentising continues for a period of two and a half minutes for plant substances and four minutes for metals, whereafter the liquid is allowed to settle until all movement ceases. Each respective action; the completion of one period oscillation and one period vortex creation, is considered to be equivalent to one period of dynamization in the Hahnemannian method.

2.4 THE ROLE OF POTENTIZATION IN HOMOEOPATHY

By experimental evidence, the effect of homoeopathic preparations in succussed high dilutions on a living organism is no longer anecdotal. Positive

results in studies on cellular elements, plants and animals disprove the possibility of a simple placebo effect (Smith & Boericke:1968).

Still, the mechanism of succussed high dilutions and its action on an organic system remains undecided. Most theories endorse a scheme of some physical restructuring of the solvent, as a result of both serial dilution and succussion of the substance (Anagnostatos:1991; Barnard:1965; Smith & Boericke:1968). Suggested theories commonly focus on complex organised hydrogen bonded molecules in ethanol-water mixtures, or electromagnetic coherence and resonance phenomena.

In an attempt to understand the mechanism of succussed high dilutions, the divergence from a causal, biochemical model is necessary, as much of succussed high dilution medicinal substances fall beyond Avogadro's limit, where theoretically no original solute is present in the substance. Even below this limit (D24), the chemical bio-availability is usually too insignificant to produce a biochemical effect on the physical body, or in fact, to easily justify a causal effect within an orthodox scientific paradigm.

Although investigations within current scientific paradigms are essential, a non-reductionist approach as suggested by Wallach (2000) may afford a better opportunity to understanding the phenomenon.

Barnard and Stephenson first hypothesized that it is not the solute but the structure of the solvent that is the active participant, and thus the phenomena

of interest in succussed high dilutions, since many remedies are diluted to such an extent that there is theoretically none of the original solute remaining in the remedy. They postulated the hypothesis of stereospecific solvent molecule polymers formed by association with the original solute (Sacks:1983*).*

These polymers would self-replicate during the process of serial dilution and succussion. Addition of monomers in a specific pattern occurs until a certain length is reached, whereafter it is broken by the shearing force of the applied succussion. New, shorter polymers lengthen in the same manner until maximum dimensions are reached. The process would repeat itself throughout the dilution and succussion process. These polymers are deemed to be the informational molecules which are “recognized” by biological systems.

Anagnostatos et al.’s (1991) model of succussed dilutions centred on the concept of clathrates. He hypothesised the specific organization of molecules of the solvent in homoeopathic microdilutions which can maintain the properties of an initial substance not effectively present (Anagnostatos et al.:1991).

This is based on the idea of the formation of shells of organised hydrogen-bonded molecules of the solvent (clathrates) around aggregates of a small number of molecules of base substance. Together with different inertial properties, the succussion forces clusters of base molecules out of their

clathrates, with new clathrates forming around them. The displaced clathrate leaves a hollow in the matrix, a “core clathrate”, and a “mantle” forms around this core. At the point where no base substance is present, the application of succussive force results in core clathrates moving out of mantle clathrates and stimulate the formation of new clathrates. This process is perpetuated to result in a specific molecular matrix, bearing the informational imprint of the original substance (Ross 1997:8).

The work of Resch and Gutmann (1991) pointed to a highly organised structure inherent in water which is able to be substance-specifically modified by interaction with an added substance or solute.

It was proposed that a “super molecular system” forms within succussed solutions. This is distinguishable from normal liquid water through “solvation spheres” or “hydration shells” forming around hydrophilic molecules, and a network of “inner surface” molecules at the interface of hydrophobic molecules. Hydrophobic molecules within a liquid may adopt structural information from the added substance. This would be preserved within its oscillating expression and, in turn, exhibit a strong influence on the oscillating pattern of the liquid as a whole.

The dilution process results in an interface between the solute and the solvent, which allows for the transfer and integration of the structural information content into the new dilution.

Berezin (1991) presented a model based on isotopic diversity. He proposes a model of homoeopathic action centred on the patterning of stable isotopes in water.

This argument is based on the notion that the succussion process results in a non-equilibrium state within the liquid, with an excess of free energy. This would make for a system vulnerable to pattern formation. Dissolved molecules would be able to cause re-ordering and positional arrangement of isotopes within water, water having three isotopic degrees of freedom; H to D and ^{17}O or ^{18}O to ^{16}O.

As a change of a singular neutron in a substance with atomic mass 200 could cause a variation of 0,5%, it would follow that such isotopic change could cause substantial variations in atomic vibrational frequencies, bond strengths and changes in chemical activity.

Isotopic combinations provide immense information storage capability. Fragments would be sufficient to provide the structural information requirements to a next stage dilution. An example of such a degenerate system is that of crystallisation where a ‘micro-change’ in the lattice structure will result in an ordered structure formation conducive to that change throughout the rest of the crystal.

Current theories on the mechanism of homoeopathy more and more demand the ability of lateral thinking. Within quantum theory the opportunity may have appeared whereby the link between consciousness and physical matter may move from a pseudo-scientific regard into the domain of true science. The development of this notion has been expanded from the works of Bohm, Schrödinger and Bohr amongst others (Davies & Brown 1986:32).

In spite of rigorous care and precision, scientific research in homoeopathy tends to show unrepeatable and anomalous results. It has been suggested that this may not be completely independent of, and un-influenced by the researcher. The 'Pauli – effect' is a simple example of the observer as unintentional participant in a scientific experiment (McEvoy & Zarate 1991:96). Robert Oppenheimer stated that "the physical world is not completely determinate. There are predictions you can make about it but they are statistical; and any event has in it the nature of a surprise, of the miracle, of something that you could not figure out. Physics is predictable - but within limits; its world is ordered but not completely causal". He also remarks that "every atomic event is individual. It is not, in its essentials, reproducible" (Whitmont 1991:4).

Wallach (2000) proposed a non-local interpretation of the homoeopathic phenomenon. He suggests that a more precise explanation of the mechanism of homoeopathy is more likely to be found in conjectures made around concepts based on quantum theory, rather than theorizing within a purely physico-chemical paradigm.

The concept on non-locality is perhaps best illustrated in the EPR paradox. According to the Copenhagen interpretation presented by Bohr, the existence of an external world independent of an observer is problematic. One is in effect, unable to solve the problem of how the universe exists without an observer looking at it. Dealing with phenomena, appearances and regularities in phenomena, he essentially claims that reality is ultimately ambiguous and unspecifiable, as affirmed in the EPR paradox. Herein Bohr refuted Einstein's locality principle of separateness of phenomena. Bohr basically states that quantum mechanics does not permit a separation between the observer and the observed. Any observed phenomenon (in the case of the EPR thought experiment; the two electrons) and the observer are part of a single system – independent of distance and the speed of light, and therefore, time. It has thus been stated that the EPR experiment does not demonstrate the incompleteness of quantum theory, but the naiveté of assuming local conditions in atomic systems. Once they have been connected, atomic systems are *never* separate (McEvoy & Zarate 1991:166).

Walach developed this concept of non-locality to be applied to the mechanism of homoeopathy. This has also been demonstrated in the works of Edward Whitmont, who emphasises that the homoeopathic approach is finalistic and phenomenalistic, rather than causalistic – thus a symbol, representational of a whole.

It was Bohm that suggested that in the *implicate order*, mind and matter can be looked at in a similar way, that quantum mechanics may see mind and matter as *enfolded*. He has further stated that within the framework of quantum mechanics, phenomenal reality comes about from a deeper order in which it is enfolded or implied. In order to extrapolate on the meaning of this innate property of implication in the physical universe, he uses the example of the hologram; each part of the photographic plate contains information about the whole. The whole is unfolding from each region on the photographic plate (Davies & Brown 1986:118).

Wallach (2000) also suggests that the effect of the homoeopathic remedy is not a causal one as would be explained in an orthodox sense, but rather through a system of “signs” or concepts. Thus a universal non-local and acausal means are present within the substance. This universal interconnectedness of all creation may be the mechanism whereby homoeopathy acts through consciousness.

The work of Carl Jung would underline this very strongly. The occurrence of archetypal symbols and the universal meaning contained therein is very appropriate in the scientific domain – from physics and psychology to homoeopathy and philosophy (Jung 1993:384). This was also a conclusion reached in a discussion between Jung and Pauli; that psychological states and physical events could be acausally connected through an element of meaning (Davies 2001:38).

The homoeopathic remedy thus becomes a symbol with a specific element of *meaning*. The meaning that is so contained in the remedy as symbol, may also serve as a deeper understanding of the fundamental principle in homoeopathy: "*Similia Similibus Curentur*" – let like be cured by like.

It is important to also extend this concept to Anthroposophy, which is based ultimately, on a foundation of "spiritual science" where the *unseen* is in fact the template for physical matter and its behaviour. Speaking on the nature of man, Rudolf Steiner had remarked that "(the understanding of man) rests upon the recognition of a hidden *something* behind that which is manifest to the outer senses and to the intellect brought to bear upon their perceptions. These senses and this intellect can apprehend only a part of all that (is) the total human entity…" (Wilson 1985:10).

The words of Oppenheimer, to some degree, are echoed in Steiner's insistence that total material fails to account for the complexities of the universe and of human existence.

2.5 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is a characterization technique whereby the physico-chemical environment of specific nuclei is deduced from information obtained about the nuclei within an analysed sample. This in turn provides a means whereby the molecular identity of a substance may be expressed and described in scientific terms.

Subatomic particles possess a quality of intrinsic spin; a rotation around its own axis. During the experiment, the analysed sample is subjected to a static magnetic field B_o which establishes the z axis of the coordinate system, relative to which the proton spin can be parallel or antiparallel, that is

m_I = +/- ½.

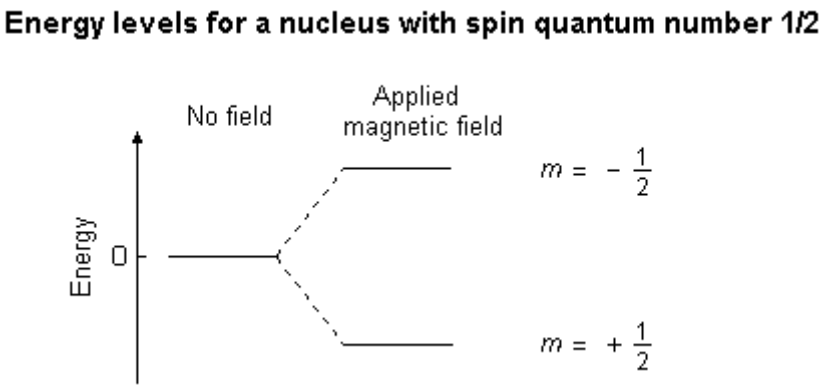

Figure 2.5.1 Energy levels of a nucleus with spin quantum number 1/2

Two distinct spin populations, with different energy states, are so established: parallel (with energy value $-\mu_p B_o$), and antiparallel (with energy value $+\mu_p B_o$). The difference in energy can be determined through the equation:

$$\Delta E = 2\mu_p B_o$$

Within the magnetic field, the number of protons in either spin orientation is determined by the Boltzmann factor ($e^{-\Delta E/kT}$). At room temperature, kT = 0.025 eV and $\Delta E = 1.8 \times 10^{-7}$ eV at B_o = 1T. There is thus a relatively small

imbalance of protons in the lower energy (parallel) state, of the order of $1 - e^{-\Delta E/kT} = 7x10^{-6}$.

The application of an external energy input will result in the excitation of the lower energy nuclei into the higher energy level. This may be achieved through irradiating the sample with radio waves.

The application of a sinusoidal varying signal at a frequency ($v = \Delta E/h$) thus causes the protons in the sample to absorb energy and "flips" the magnetic moment of the proton into opposing the applied field. The protons find themselves in a different orientation; that of the antiparallel population.

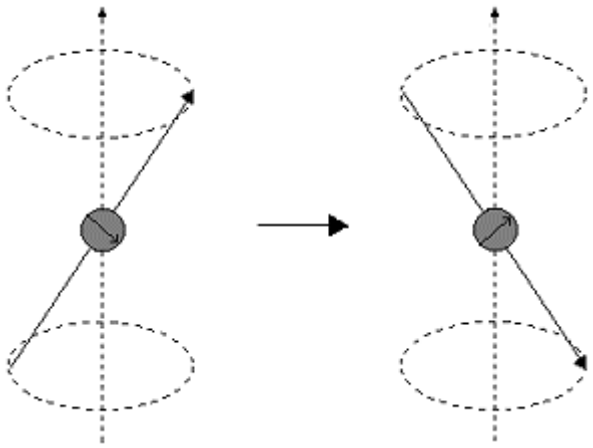

Figure 2.5.2 Change in the magnetic moment of a proton

At the particular value of *v*, it is seen that energy is absorbed and protons flip back and forth. Protons in the upper state can also lose their energy and "fall

back" to the lower state by transferring energy directly to the surrounding material through the process of spin-lattice relaxation (Krane 1988:630).

The resonant frequency can be obtained by simple substitution from the equation:

$v = 2\mu_p B/h$

- μ = magnetic moment
- h = Planck's constant
- E = energy at a particular level
- B_o = strength of the magnetic field *at the nucleus*. An increased magnetic field results in an increased transitional energy (ΔE).
- ΔE = Transition Energy: The energy difference between levels.
- *v* = Frequency of the applied electromagnetic wave.

This process continues until the system is saturated. Saturation occurs when populations of the higher and lower energy levels become equal and no further absorption of radiation occurs. After saturation, a relaxation process occur which return nuclei back to the lower energy stated.

The magnetic field at the nucleus however, is not equal to the applied magnetic field, due to the shielding effect of s-orbital electrons around the nucleus. Nuclear shielding is thus the difference between the applied magnetic field and the field at the nucleus. This implies that the applied field strength must be increased to overcome nuclear shielding and so allow the nucleus to absorb applied energy at its transition frequency. This is upfield

shift (diamagnetic shift). Similarly, p-orbital electrons produce their own magnetic fields at the nucleus, producing a downfield (paramagnetic) shift, and a de-shielding effect occurs.

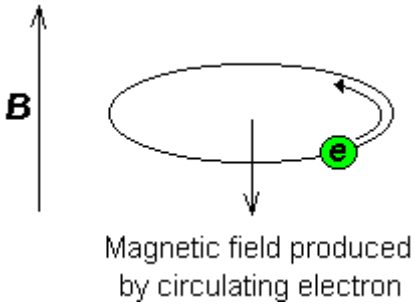

Figure 2.5.3 Magnetic field produced by the p-orbital of an electron

Chemical shift is a function of the nucleus and its environment and measured relative to a reference compound. In this experiment ethanol was chosen as the reference compound.

The graphic depiction of the NMR spectrum is influenced by the phenomenon of spin-spin coupling. Spin-spin coupling occurs due to the interaction between groups of protons in a molecule. In ethanol:

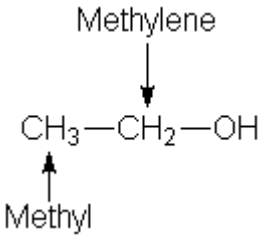

Figure 2.5.4 Biochemical structure of ethanol

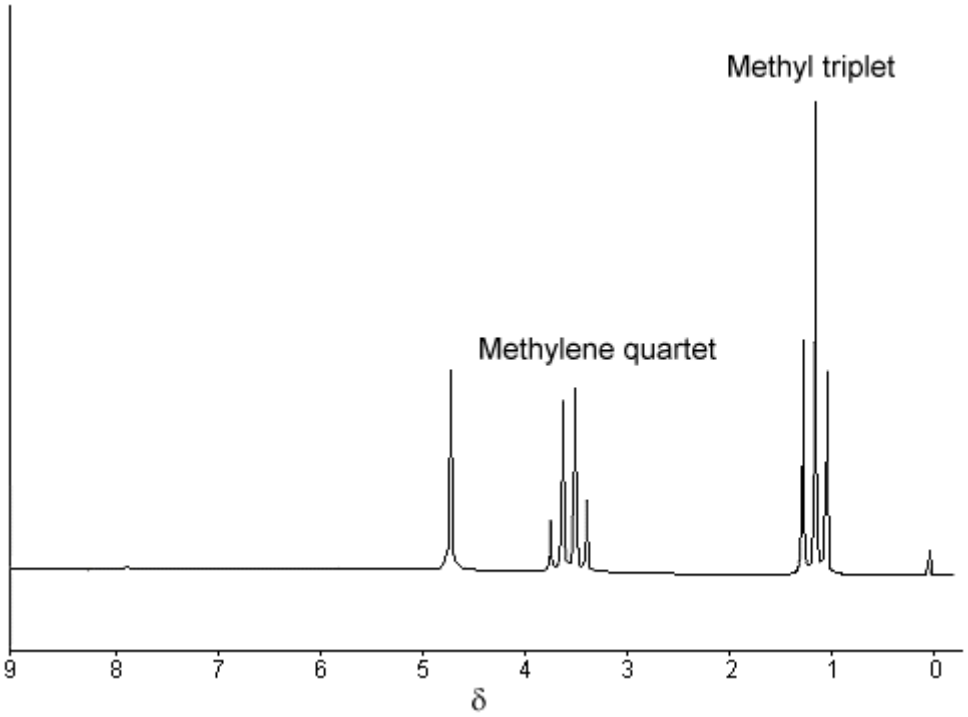

Figure 2.5.5 Graphic depiction of the NMR spectrum of ethanol

The methyl peak contains a triplet and the methylene peak a quartet. This is due to the coupling of the two groups of protons, each influencing the other. Methylene contains two protons, each having one of two possible orientations, aligned or opposed to the applied field. This means methylene has four possible states.

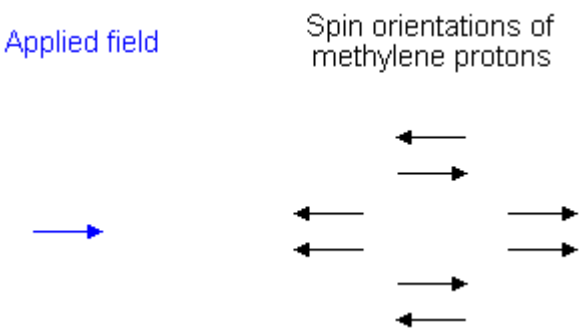

Figure 2.5.6 Methylene orientation states

Different combinations of the methylene will thus influence the field experienced by the methyl, and vice versa. Methylene spins paired and opposed to the applied field reduces the field experienced by the methyl protons, and a higher field is needed to bring them to resonance, resulting in an upfield shift. In the same manner, paired spins aligned with the direction of the applied field has a positive influence on the methyl protons and produce a downfield shift. The two spin combinations opposed to each other do not influence the field experienced by the methyl protons. There is thus no up or down shift and no effect on the methyl peak. Hence the methyl peak is split into three with ratio of areas 1:2:1. Similarly the methyl protons affecting the methylene protons, allow for eight possible spin combinations for the three methyl protons.

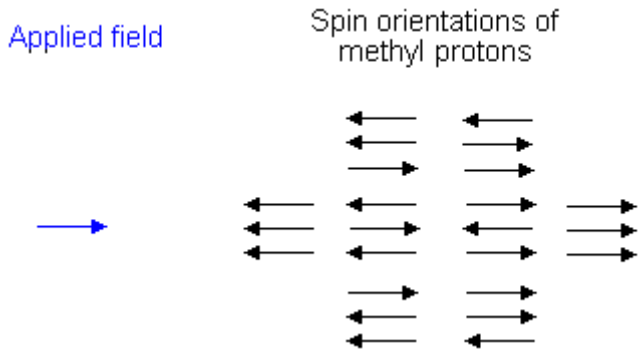

Figure 2.5.7 Methyl orientation states

Applying the same principle reveals the methylene quartet peak ratios as 1:3:3:1.The multiplicity of a multiplet can thus easily be established by the number of equivalent protons in the neighbouring atom plus one; the

(n + 1) rule. Equivalent nuclei do not interact with each other. Protons do not cause splitting amongst themselves, only on neighbouring protons.

On the NMR spectrum, the area under the peak is proportional to the number of protons generating that peak. These *relative values* are the integral values. Calculating the ratio of protons at specific chemical shift values can thus reveal very accurately the structure of the sample being tested. Thus the potential for NMR spectroscopy to discover the physical and chemical structure of a sample can easily be realized (Krane:1988).

2.6 NMR RESEARCH IN HOMOEOPATHY

In 1965 Barnard developed the idea of the potentization process resulting in the transfer of informational content from the solute to the solvent. He proposed the formation of stereospecific solvent molecule polymers in water (solvent). These polymers would be structurally determined by the original solute and the spatial changes it caused in the solvent and were theorised to be induced to self-replicate and split by the energy provided by succussion (Sacks:1983).

Smith and Boericke further investigated Barnard's theory by method of NMR spectroscopy. Their experiment with various Sulphur potencies, lead to the following conclusions with regard to the H_2O and OH sections of the spectra:

- Solvent structure is changed in unsuccussed serial solutions when compared to undiluted solvents.
- Solvent structure is further changed by succussion of serial dilutions when compared to unsuccussed dilutions and undiluted solvent.
- These changes become more extreme as the dilutions approach and pass the negative function of Avogadro's number.

These results were extended to the hypothesis that catalysis of exchange between C_2H_5OH and H_2O protons of ethanol-water mixtures is very likely to take place in homoeopathic preparations (Weingartner:1990).

Additional proof of the effect of succussion, compared to identical dilution, was carried out by studying the effect of serial succussions and dilutions of bradykinin triacetate (BKTA). It was established, that there is a definite, recurring, reproducible change in NMR patterns by succussion and that these patterns are reproducible and may be caused by water polymers
(Smith & Boericke:1968).

Sacks (1983) conducted an experiment on 32 different potencies of high and ultra high dilutions; Sulphur in mother tincture, 6x, 12x, 22x, 23x, 24x, 25x, 30x, 200x, 1M, 10M, 50M, CM and seven other remedies in 30C. It was graphically shown that, whereas the ethanol-water control spectra did not vary throughout the experiment, the hydroxyl region changed in a variety of ways.

Practically all of the succussed high dilution samples were distinctly different from ethanol, and many were different from each other. However, no clear pattern of peak shape was discernible from Sulphur 6x through CM, nor among the seven remedies of 30C potency.

Different quantities of ethanol-water control caused very minor variations in size, but no variation in shape of hydroxyl peaks. Different quantities of succussed high dilutions distinctly altered the shape of the curve or the hydroxyl peaks. Clear differences emerged on the NMR spectra of succussed high dilutions and ethanol-water control in this study. The differences lie in the hydroxyl regions. In ethanol-water controls two peaks are visible, one belonging to ethanol and the other to water. In the succussed samples the peaks become less distinct and even merge. Sacks concluded that this may indicate differences in relationship of ethanol and water hydroxyl groups. It may also be some manner of artefact.

In a statistical trial conducted by Weingartner (1990), the ^{1}H NMR spectra of homoeopathic Sulphur-potencies and their solvent were compared with each other. At a significance level of 99.9% the recorded spectra could be distinguished with respect to the relative intensities of the H_2O and the OH signals.

It was hypothesised that the ^{1}H NMR spectra of Sulphur D23 and its solvent have statistically different relative intensities of the H_2O and OH signals with respect to the mean intensity of the CH_2 signal.

It was confirmed in three separate runs that the quantities in the ^{1}H NMR-spectra of Sulphur D23 differed systematically from those of ethanol 87% spectra and that the same quantities of Sulphur D13 do not.

No distinction between the Sulphur D23 and the ethanol 87% group of samples could be made in the shifts relative to 1.186 ppm, in the integrals and in the coupling constants. The intensities of the H_2O and the OH signals relative to CH_2 however, were drastically different in the Sulphur D23 and in the ethanol group. Sulphur D13 spectra did not differ so obviously from the solvent-spectra. In the experiment, different spectrometers were utilized to exclude the possibility of machine artefact (Weingartner:1990).

Lasne et al. showed that in higher degrees of dilution, significant differences exist in spin-spin relaxation times between potentized substances and their solvents. This result was also obtained independently by a co-worker of Resh and Gutmann (Weingartner:1990).

In 1992 Demangeat performed a study comparing vortexed potencies of Silicea (in 0.9% saline solution) and versus pure saline solvent prepared according to the French Homoeopathic Pharmacopoeia. Dilution levels were measured at levels ranging from 1.66×10^{-5} to 1.66×10^{-29} mol/l.

The relaxation times T1 as well as the T1/T2 values of the pure saline solution were found to be lower than the values of the Silicea potencies. The differences were statistically significant at $p<0.034$ and $p<0.018$, respectively.

Comparison was also made with agitated dilutions of solvent (0.9 % NaCl), and distilled water. T1/T2 values were observed to increase with the addition of a solute in all solvent comparisons.

Theories given for the observed differences were the interaction of solutes with water molecules through hydrogen bonding, and perhaps also electrostatic forces linked to their dipolar moment. Isotope effects similar to the theories of Berezin (1991) were also considered, as was the structure breaking effect of the solute first introduced by Resch and Guttman
(Davies 2001:30).

More recently, in comparing Hahnemannian and Korsakovian potentising methods in analogous samples, Davies (2001) confirmed that statistically significant differences was found in almost all intra-potency comparisons for each method and its respective control. This confirmed a hypothesis that changes occur during potentizations which are specific to each method employed. He further draws the conclusion that the method of dilution is an integral part of the remedy's physico-chemical information content, and therefore not equivalent in homoeopathic practice.

2.7 SUMMARY

It is clear that the principle of dynamization is of critical importance, both in establishing a medically active preparation, as well as in establishing a particular physico-chemical identity in a sample. The effect of succussion is arguably investigable through NMR and creates the possibility of further understanding of homoeopathy as a whole, in particular the potentization process and its effects.

A comparison between Hahnemannian and Anthroposophical potentization thus warrants investigation. Not only are Anthroposophical remedies analogous to homoeopathic preparations, save for the succussion method, but they are also commonly utilized interchangeably, especially in the OTC industry. A comparative analysis would thus be informative as to its actual similarity.

With various NMR studies done analysing homoeopathic preparations, Anthroposophical preparations have not been subjected to the same scrutiny. With the only variable in this experiment being the succussion technique, any result, positive or negative, will reflect on the process of succussion and associated theories and allow greater enquiry with regards to their appropriateness and accuracy.

CHAPTER THREE: MATERIALS AND METHODS

3.1 PRODUCTION OF SAMPLE POTENCIES

All preparation and investigation of samples was conducted within a laboratorial setting. Thus the only variables that may successfully be controlled are those within the laboratory. The researcher made allowance for uncontrollable variables, for example cosmological influences, as purported to be of significance in Anthroposophical thought.

All samples were prepared by hand under laminar flow at Pharma Natura laboratories, according to standardized GHP, as per method 5a and Anthroposophical Extended Medicine methodology respectively.

Serial dilution was maintained in 1:10 with 62%, 43%, 30% and 15% ethanol successively up to the 4^{th} level of dilution (D4). Thereafter a concentration of 15% ethanol was maintained in all samples. Succussion of the Hahnemannian (homoeopathic) sample was constant at 100 succussions per dilution level. Both anthroposophical samples underwent succussion for a duration of two and a half minutes per dilution level.

All potencies were prepared from the same source of Pulsatilla pratensis mother-tincture, in glassware of identical nature and batch. Care was also taken to ensure that all ethanol used in deconcentration was of the same source.

A parallel process of production was employed to minimize differences in the conditions surrounding the manufacture of the potencies. Production of all samples was conducted by a singular individual, within the same time period (one day).

On completion all preparations were stored in amber glass bottles to reduce the possible influence of sunlight. Completed samples were packaged in insulated containers to minimize movement within the samples and to maintain a constant temperature during transportation.

Precautions were taken to eliminate the possibility of any contaminants in the samples, which may influence NMR spectra readings. This was done according to the standards in the British Pharmacopoeia for sterilisation of glass apparatus. All glass bottles used in the preparation of samples were rinsed in distilled water and underwent dry heat sterilization at 180^{o}C for 35 minutes. All the bottles were left to cool before the manufacturing commenced.

3.2 NMR MEASUREMENT OF SAMPLES

NMR analysis of the samples was conducted at the University of Kwa-Zulu Natal, department of Chemistry. The operation and running of the machine was performed by Mr. Craig Grimmer, resident NMR technician at the university. The spectrometer used was a Varian 500MHz INOVA

Spectrometer, having a 5mm broadband switchable probe and a 5mm inverse detection probe. The pulse angle was set at 90° and the temperature was thermostatically controlled, remaining constant at 25°C.

1.75 ml of each sample was drawn into a coaxial tube by means of a micropipette. The experiment was conducted using acetone as an external lock and ethanol as the reference.

NMR-spectra were recorded of the CH_3, CH_2, H_2O and OH signals of three separate samples of each respective preparation and expressed in the chemical shift and relative integration values. Readings were repeated sixteen times for every sample to eliminate inconsistencies and inaccuracies.

Spectra of the OH, H_2O, CH_2, and CH_3 signals were recorded and expressed in terms of chemical shifts and relative integration values. All data were transferred into Microsoft Word and printed.

3.3 STATISTICAL ANALYSIS

Chemical shift and relative integration values of CH_2, CH_3, OH and H_2O signals were recorded and utilized in statistical analysis. Chemical shift values were then measured for reliability using Cronbach's alpha. Tests for normality were done by means of the Kolmogorov-Smirnov and Shapiro-Wilk tests.

3.3.1 CRONBACH'S ALPHA

Cronbach's alpha is a coefficient of reliability or consistency. It measures how well a set of items or variables measures a single, unidimensional latent construct. Thus for data of a multidimensional structure, Cronbach's alpha will usually be low.

Cronbach's alpha can be represented as a function of the number of tests items and the average inter-correlation among the items.

$$\alpha = \frac{N.r}{1+(N-1).r}$$

- N = the number of items
- r-bar = the average inter-item correlation among the items.

From this formula it is clear that an increase in the number of items (N) would increase Cronbach's alpha. Similarly, a low average inter-item correlation (r-bar) results in a low Cronbach's alpha and vice versa.

If the inter-item correlations are high, then there is evidence that the items are measuring the same underlying construct. This makes for a 'high' or a 'good' reliability.

In multi-dimensional data, Cronbach's alpha will generally be low for all items. Here a factor analysis can be run to determine which items load highest on which dimensions, and then the alpha of each subset of items can be taken separately.

3.3.2 TESTS FOR NORMALITY OF A DISTRIBUTION

Normal distribution is a theoretical frequency distribution for a set of variable data, usually represented by a bell curve symmetrical about the mean. The test for the sampling distribution for normality is an appropriate test in spite of its limitations of use.

A significant outcome implies the conclusion that the distribution is not normal, whereas a non-significant outcome implies only that no deviation from normality has been demonstrated - not that it doesn't exist. A negative result however, is some evidence of normality and all the evidence we have, which leads us to carry out the analysis as if normality had been demonstrated.

The choice of the test to use depends on both the sample size and whether the user would rather err on the side of being too conservative or the opposite. The two tests of normality used for this statistical analysis was the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The Shapiro-Wilk test tends to reject the null hypothesis more readily than one would wish; whereas the Kolmogorov-Smirnov test is too conservative, retaining the null hypothesis too often.

3.3.3 THE GENERAL LINEAR MODEL (GLM)

When results of the statistical tests for normality are violated, a straight forward application of the ANOVA test cannot be applied.

Repeated Measures analysis of variance has several assumptions common to all ANOVA. Firstly, that the model is correctly specified and additive. Secondly, that the errors follow a normal distribution and are independent of the effects in the model. In addition to standard ANOVA assumptions, there is one specific to repeated measures when there are more than two levels to a repeated measures factor. If a repeated measures factor contains only two levels, there is only one difference variable that can be calculated, and the assumption can be ignored. However, if a repeated measures factor has more than two levels, an overall test of differences (main effect) is needed. Pooling the results of the sphericity deals with when such pooling is appropriate. The basic idea is that if the results of two or more contrasts (the sums of squares) are to be pooled, then they should be equally weighted and uncorrelated.

To be able to perform the F-statistic calculation, the condition of sphericity has to be met. In the event of this condition being violated, then an epsilon corrected test may be used, such as Greenhouse-Geisser. If violations occur at this stage, then further multivariate tests would be needed. These multivariate tests consider the differences in the contrast variable to zero, that is, claiming that there are no differences in terms of the methods used (or

effects observed). If sphericity is satisfied, there is no need to consider the multivariate tests.

Unlike the standard ANOVA tests, repeated measures offers 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 can be made regarding the effects of one method as compared to another (Singh:2004).

The hypothesis therefore appears as follows:

- H_0: There is no difference between the preparation methods.
- H_1: There is a difference between the preparation methods.

The decision rule follows accordingly:

- Reject H_0 at the a level of significance if $p<\alpha$
- Accept H_0 at the a level of significance if $p\geq\alpha$

The software package SPSS© Base 10.0 was used for statistical analysis.

CHAPTER FOUR: THE RESULTS

4.1 CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

The sensitive nature of the experiment demands great care and precision during every stage of the experiment. Care was taken to exclude external factors inasmuch was possible that may affect the integrity of the samples. The appropriate precautions were taken in manufacturing, storage, transport and analysis. The effect of electromagnetic radiation on the samples is uncertain.

Great effort was made in the prevention of variability in samples and analysis, as already explained in 3.1 and 3.2. Samples were constantly maintained under the same conditions and atmospheric exposure was kept to a minimum. Similarly, contamination was avoided by the singular use of glassware throughout.

Data containing chemical shift and relative integration values of the CH_3, CH_2, H_2O and OH peaks were subjected to statistical analysis as set out in 3.3.

4.2 STATISTICAL RELIABILITY OF DATA

Cronbach's alpha was used in testing the reliability of data (see 3.3.1).

Reliability Statistics

Cronbach's alpha	**N** of items
0.874	9

Table 4.2.1 Reliability Statistics of data

The alpha value (0.874) indicates that the reliability of the relevant data is acceptable.

4.3 COMPARISON OF CHEMICAL SHIFTS

4.3.1 TESTS FOR NORMALITY

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wala 1	0.284	3	0.000	0.933	3	0.501
Wala 2	0.385	3	0.000	0.750	3	0.000
Wala 3	0.385	3	0.000	0.750	3	0.000
Weleda 1	0.299	3	0.000	0.915	3	0.433
Weleda 2	0.375	3	0.000	0.775	3	0.056
Weleda 3	0.355	3	0.000	0.819	3	0.159
Homeo 1	0.182	3	0.000	0.999	3	0.935
Homeo 2	0.383	3	0.000	0.754	3	0.008
Homeo 3	0.377	3	0.000	0.768	3	0.041

[a]. Lilliefors Significance Correction

Table 4.3.1 Tests for Normality

The tests for normality reveal that 4 of the 9 distributions are not normal. The Kolmogorov-Smirnov statistic, with a Lilliefors significance level for testing normality, is displayed. If non-integer weights are specified, the Shapiro-Wilk statistic is calculated when the weighted sample size lies between 3 and 50. For no weights or integer weights, the statistic is calculated when the weighted sample size lies between 3 and 5000.

4.3.2 SAMPLE RESULTS FOR OH, CH_2 and CH_3

Within-Subjects Factors

Measure: sample

Method	Dependent Variable		
	OH	CH_2	CH_3
1	Wala 1	Wala 2	Wala 3
2	Weleda 1	Weleda 2	Weleda 3
3	Homeo 1	Homeo 2	Homeo 3

Table 4.3.2.1 Within-Subjects Factors for OH, CH_2 and CH_3

The table above indicates there is only a single within-subject factor and no between subject factors.

Descriptive Statistics

		Mean	Std. Deviation	N
OH	Wala 1	143.5667	2.12211	3
	Weleda 1	138.3	0.88882	3
	Homeo 1	136.8333	0.85049	3
CH2	Wala 2	19.2917	10.09766	12
	Weleda 2	17.6833	8.88091	12
	Homeo 2	21.7583	11.40594	12
CH3	Wala 3	38.4889	13.84833	9
	Weleda 3	36.4556	13.04637	9
	Homeo 3	44.3778	16.43311	9

Table 4.3.2.2 Descriptive Statistics for OH, CH_2 and CH_3

Means, standard deviations and sample sizes are given for each factor level.

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
OH	Pillai's Trace	1.000	6700.750[a]	2.000	1.000	0.009
	Wilks' Lambda	0.000	6700.750[a]	2.000	1.000	0.009
	Hotelling's Trace	13404.500	6700.750[a]	2.000	1.000	0.009
	Roy's Largest Root	13401.500	6700.750[a]	2.000	1.000	0.009
CH_2	Pillai's Trace	0.709	12.180[a]	2.000	10.000	0.002
	Wilks' Lambda	0.291	12.180[a]	2.000	10.000	0.002
	Hotelling's Trace	2.436	12.180[a]	2.000	10.000	0.002
	Roy's Largest Root	2.436	12.180[a]	2.000	10.000	0.002
CH_3	Pillai's Trace	0.828	16.799[a]	2.000	7.000	0.002
	Wilks' Lambda	0.172	16.799[a]	2.000	7.000	0.002
	Hotelling's Trace	4.800	16.799[a]	2.000	7.000	0.002
	Roy's Largest Root	4.800	16.799[a]	2.000	7.000	0.002

[a]. Exact statistic

[b]. Design:
Intercept
Within Subjects Design: method

Table 4.3.2.3 Multivariate Tests[b] for OH, CH_2 and CH_3

The multivariate tests indicate that there is no differences in the experimentation method used as all the values are more than the specified alpha-value α= 0.05. This test is only considered if the sphericity test does not hold.

Mauchly's Test of Sphericity[b]

Measure: sample

Within Subjects Effect		Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
						Greenhouse - Geisser	Huynh - Feldt	Lower-bound
Method	OH	0.001	7.087	2	0.29	0.500	0.501	0.500
	CH_2	0.347	10.575	2	0.005	0.605	0.640	0.500
	CH_3	0.291	8.638	2	0.013	0.585	0.625	0.500

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.

d. Design: Intercept
Within Subjects Design: method

Table 4.3.2.4 Mauchly's Test of Sphericity[b] for OH, CH_2 and CH_3

It is observed that the significance value is less than 0.05, thereby indicating that sphericity is not maintained. However, the Greenhouse-Geisser epsilon correction gives an acceptable value. Hence, the testing procedure can be conducted.

Tests of Within-Subjects Effects

	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
OH	method	Sphericity Assumed	75.227	2	37.613	27.790	0.005
		Greenhouse-Geisser	75.227	1.000	75.195	27.190	0.035
		Huynh-Feldt	75.227	1.002	75.101	27.190	0.035
		Lower-bound	75.227	1.000	75.227	27.190	0.035
	Error (method)	Sphericity Assumed	5.533	4	1.383		
		Greenhouse-Geisser	5.533	2.001	2.766		
		Huynh-Feldt	5.533	2.003	2.762		
		Lower-bound	5.533	2.000	2.767		
CH_2	method	Sphericity Assumed	101.107	2	50.554	24.217	0.000
		Greenhouse-Geisser	101.107	1.210	83.548	24.217	0.000
		Huynh-Feldt	101.107	1.279	79.046	24.217	0.000
		Lower-bound	101.107	1.000	101.107	24.217	0.000
	Error (method)	Sphericity Assumed	45.926	22	2.088		
		Greenhouse-Geisser	45.926	13.312	3.450		
		Huynh-Feldt	45.926	14.070	3.264		
		Lower-bound	45.926	11.000	4.175		
CH_3	Method	Sphericity Assumed	304.725	2	152.363	33.415	0.000
		Greenhouse-Geisser	304.725	1.170	260.369	33.415	0.000
		Huynh-Feldt	304.725	1.249	243.890	33.415	0.000
		Lower-bound	304.725	1.000	304.725	33.415	0.000
	Error (method)	Sphericity Assumed	72.955	16	4.560		
		Greenhouse-Geisser	72.955	9.363	7.792		
		Huynh-Feldt	72.955	9.996	7.299		
		Lower-bound	72.955	8.000	9.119		

Table 4.3.2.5 Tests of Within-Subjects Effects for OH, CH_2 and CH_3

The test result is highly significant in the event of the sphericity condition holding. The conclusion from these results indicates that there are 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 is to analyse these differences more closely.

The Bonferroni adjustments with pairwise tests were performed. The Bonferroni multiple comparison test is a conservative test, that is, the FEW (familywise error rate) is not exactly equal to ALPHA, but is less than ALPHA in most situations. It is easy to apply and can 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) are multiplied by the number of comparisons in the family and the minimum of the obtained number and 1 is then chosen.

The results are as follows:

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

Transformation Coefficients (M Matrix)

Measure: Samples

		method		
	Dependent Variable	1	2	3
OH	Wala 1	1	0	0
	Weleda 1	0	1	0
	Homeo 1	0	0	1
CH_2	Wala 2	1	0	0
	Weleda 2	0	1	0
	Homeo 2	0	0	1
CH_3	Wala 3	1	0	0
	Weleda 3	0	1	0
	Homeo 3	0	0	1

Table 4.3.2.6 Transformation Coefficients (M Matrix) for OH, CH_2 and CH_3

The diagonal elements represent the variance of each contrast and the off-diagonal values represent the covariances. The sphericity assumptions hold

in this instance as the diagonal values are all the same and the off diagonal values are all zero.

Pairwise Comparisons

Measure: sample

	(I) method	(J) Method	Mean Difference (I-J)	St. Error	Sig.[a]	95% Confidence interval for Difference [a]	
						Lower Bound	Upper Bound
OH	1	2	5.267	1.312	0.170	-4.768	15.301
		3	6.733	0.960	0.590	-0.608	14.074
	2	1	-5.267	1.312	0.170	-15.301	4.768
		3	1.467	0.353	0.160	-1.232	4.165
	3	1	-6.733	0.960	0.059	-14.074	0.608
		2	-1.467	0.353	0.160	-4.165	1.232
CH_2	1	2	1.608*	0.394	0.005	0.497	2.720
		3	-2.467*	0.517	0.002	-3.923	-1.010
	2	1	-1.608*	0.394	0.005	-2.720	-0.497
		3	-4.075*	0.788	0.001	-6.298	-1.852
	3	1	2.467*	0.517	0.002	1.010	3.923
		2	4.075*	0.788	0.001	1.852	6.298
CH_3	1	2	2.033*	0.577	0.023	0.294	3.773
		3	-5.889*	0.953	0.001	-8.762	-3.015
	2	1	-2.033*	0.577	0.023	-3.773	-0.294
		3	-7.922*	1.341	0.001	-11.967	-3.877
	3	1	5.889*	0.953	0.001	3.015	8.762
		2	7.922*	1.341	0.001	3.877	11.967

Based on Estimated Marginal Means

a. Adjustment for multiple comparisons: Bonferroni

* The mean difference is significant at the 0.05 level

Table 4.3.2.7 Pairwise Comparisons for OH, CH_2 and CH_3 Chemical Shift values

From the Pairwise Comparisons the following observations are made:

- For the OH values all the significance values are above 0.05 (>α). H_0 is accepted; therefore a high degree of correlation exists between the different methods.

- For the CH_2 values all the significance values are below 0.05 (<α). H_0 is rejected; therefore a low degree of correlation exists between the different methods.

- For the CH_3 values all the significance values are below 0.05 (<α). H_0 is rejected; therefore a low degree of correlation exists between the different methods.

4.3.3 SAMPLE RESULTS FOR H_2O

Due to the NMR signal's extreme sensitivity to concentration effects, a superpositioning occurs between the H2O and the ethanol peaks. This is due to a concentration effect between the water and ethanol on the spectra. (Grimmer, 2004)

Furthermore, the band-width of the OH and H_2O signals are influenced by the proton attached to the oxygen of the hydroxyl ion and water molecule. With the low concentration of water, this leads to a very broad band signal for H_2O, which is not as effective in revealing structural changes in the local

environment of the test substance as the OH-group. For this reason, focus is placed on the OH, CH_2 and CH_3 groups for analysis.

4.4 COMPARISON OF RELATIVE INTEGRATION VALUES

Within-Subjects Factors

Measure: OH, CH_2 and CH_3

Method	Dependent Variable		
	OH	CH_2	CH_3
1	Weleda	Weleda	Weleda
2	Wala	Wala	Wala
3	Homeo	Homeo	Homeo

Table 4.4.1 Within-Subjects Factors for OH, CH_2 and CH_3

The table above indicates there is only a single within-subject factor and no between subject factors.

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
OH	Pillai's Trace	0.972	17.612[a]	2.000	1.000	0.166
	Wilks' Lambda	0.028	17.612[a]	2.000	1.000	0.166
	Hotelling's Trace	35.224	17.612[a]	2.000	1.000	0.166
	Roy's Largest Root	35.224	17.612[a]	2.000	1.000	0.166
CH_2	Pillai's Trace	0.978	22.352[a]	2.000	1.000	0.148
	Wilks' Lambda	0.022	22.352[a]	2.000	1.000	0.148
	Hotelling's Trace	44.704	22.352[a]	2.000	1.000	0.148
	Roy's Largest Root	44.704	22.352[a]	2.000	1.000	0.148
CH_3	Pillai's Trace	0.948	36.571[a]	1.000	2.000	0.026
	Wilks' Lambda	0.052	36.571[a]	1.000	2.000	0.026
	Hotelling's Trace	18.286	36.571[a]	1.000	2.000	0.026
	Roy's Largest Root	18.286	36.571[a]	1.000	2.000	0.026

[a]. Exact statistic

[b]. Design:
Intercept
Within Subjects Design: method

Table 4.4.2 Multivariate Tests[b] for OH, CH_2 and CH_3

As all values in the multivariate tests are more than the specified alpha-value ($\alpha = 0.05$), it is indicated that there is no differences in the experimentation method utilized. This test is only considered if the sphericity test does not hold.

Mauchly's Test of Sphericity[b]

Measure: sample

	Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
						Greenhouse - Geisser	Huynh - Feldt	Lower-bound
OH	Method	0.084	2.482	2	0.289	0.522	0.591	0.500
CH_2		0.167	1.789	2	0.409	0.546	0.701	0.500
CH_3		0.000	0.000	2	0.000	0.500	0.500	0.500

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

b. 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.

e. Design: Intercept
Within Subjects Design: method

Table 4.4.3 Mauchly's Test of Sphericity[b] for OH, CH_2 and CH_3

It is noted that the significance value is greater than 0.05 (i.e. 0.289 for OH, and 0.409 for CH_2), thereby indicating that sphericity is maintained in these instances.

The significance value for CH_3 is less than 0.05 though, thereby indicating that sphericity is not maintained. However, the Greenhouse-Geisser epsilon correction gives an acceptable value. Hence, the testing procedure can be conducted.

With the aim to test for differences in methods, based on the sphericity assumption, it was observed that there were no differences in the method used. Closer analysis of these differences follows.

The Bonferroni adjustments with pairwise tests were performed. The Bonferroni multiple comparison test is a conservative test, that is, the FEW (familywise error rate) is not exactly equal to ALPHA, but is less than ALPHA in most situations. To get the Bonferroni adjusted p-values, the ordinary, not adjusted pairwise p-values (for example, t-test p-values for comparing two means) are multiplied by the number of comparisons in the family and the minimum of the obtained number and 1 is then chosen.

The results are as follows:

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

Transformation Coefficients (M Matrix)

Measure: samples

	Dependent Variable	method		
		1	2	3
OH	Weleda	1	0	0
	Wala	0	1	0
	Homeo	0	0	1
CH_2	Weleda	1	0	0
	Wala	0	1	0
	Homeo	0	0	1
CH_3	Weleda	1	0	0
	Wala	0	1	0
	Homeo	0	0	1

Table 4.4.4 Transformation Coefficients (M Matrix) for OH, CH_2 and CH_3

The variance of each contrast is represented by the diagonal elements and the covariances are represented by the off-diagonal values. As the diagonal values are all the same and the off-diagonal values are all zero, sphericity assumptions are maintained.

Pairwise Comparisons

Measure: Sample

	(I)	(J)	Mean Difference			95% Confidence interval for Difference [a]	
	method	Method	(I-J)	St. Error	Sig.[a]	Lower Bound	Upper Bound
OH	1	2	-0.533	0.274	0.574	-2.631	1.565
		3	-0.140	0.264	1.000	-2.155	1.875
	2	1	0.533	0.274	0.574	-1.565	2.631
		3	0.393*	0.047	0.042	0.034	0.753
	3	1	0.140	0.264	1.000	-1.875	2.155
		2	-0.393*	0.047	0.042	-0.753	-0.034
CH_2	1	2	0.113	0.027	0.160	-0.095	0.322
		3	-0.073	0.074	1.000	-0.643	0.496
	2	1	-0.113	0.027	0.160	-0.322	0.095
		3	-0.187	0.053	0.218	-0.595	0.221
	3	1	0.073	0.074	1.000	-0.496	0.643
		2	0.187	0.053	0.218	-0.221	0.595
Ch_3	1	2	0.150	0.000	0.000	0.150	0.150
		3	-0.053	0.009	0.079	-0.121	0.014
	2	1	-0.150	0.000	0.000	-0.150	-0.150
		3	-0.203*	0.009	0.006	-0.271	-0.136
	3	1	0.053	0.009	0.079	-0.014	0.121
		2	0.203*	0.009	0.006	0.136	0.271

Based on Estimated Marginal Means

[a]. Adjustment for Multiple Comparisons: Bonferroni

Table 4.4.5 Pairwise Comparisons of OH, CH_2 and CH_3

From the Pairwise Comparisons the following observations are made:

- For the OH values, the significance between:
    - Methods 1 and 2 > 0.05 (>α). H_0 is accepted; therefore a high degree of correlation exists between these methods.
    - Methods 1 and 3 > 0.05 (>α). H_0 is accepted; therefore a high degree of correlation exists between these methods.
    - Methods 2 and 3 < 0.05 (<α). H_0 is rejected; therefore a low degree of correlation exists between these methods.

- For the CH_2 values all the significance values are above 0.05 (>α). H_0 is accepted; therefore a high degree of correlation exists between the different methods.

- For the CH_3 values, the significance between:
    - Methods 1 and 2 < 0.05 (< α). H_0 is rejected; therefore a low degree of correlation exists between these methods.
    - Methods 1 and 3 > 0.05 (> α). H_0 is accepted; therefore a high degree of correlation exists between these methods.
    - Methods 2 and 3 < 0.05 (< α). H_0 is rejected; therefore a low degree of correlation exists between these methods.

4.5 SUMMARY OF STATISTICAL ANALYSIS

From the above results it is noted that there are *no* statistically significant differences between Wala, Weleda or Hahnemannian methods of potentization reflected in the chemical shift values of the OH peaks.

There are, however, statistically significant differences in the chemical shift values of both the CH_2 and CH_3 peaks between Wala, Weleda and Hahnemannian methods of potentization, interchangeably.

In terms of the relative integration values, statistically significant differences were noted between Wala and Hahnemannian methods of potentization for both the OH and CH3 peaks.

Wala and Weleda methods of potentization revealed statistically significant differences only in the CH3 peaks of the relative integration values.

CHAPTER FIVE: DISCUSSION

Of all the components in the NMR study, the chemical shift is more sensitive and indicative of any changes in the local environment than the relative integration values. Thus the results of this study can best be explained in considering the chemical shift values, as it is most susceptible in reflecting structural changes in the sample being investigated.

In the inter-group comparisons, CH_2 and CH_3 showed statistically different results in terms of chemical shift. Due to the molecular structure of ethanol, the polar hydroxyl group exerts an influence on the molecular electron configuration of ethanol – most notably at the location of the methylene group.

The chemical shift peaks are also subject to local diamagnetic effects, local paramagnetic effects and electric field effects. Anisotropic effects due to neighbouring groups in the molecule would generate local magnetic field effects, and these would in turn affect chemical shifts. In the case of ethanol, the combination of the local group anisotropy and the electric field effects due to the polar hydroxyl group makes the CH_2 chemical shift most susceptible to external influences like dynamization. This is affirmed in statistically significant differences found in the CH_2 chemical shift values of *all three* methods analyzed.

A theory on the influence of serial dilution and succussion on the specific organization of molecules within homoeopathic microdilutions, as proposed by

Anagnostatos et al. (1991), is adopted as base wherefrom the findings of this project may find support.

The clathrate-model states that through grinding and dilution into a solvent, the formation of small clusters of an original substance occurs in a solution. These clusters exhibit substantial stability and possess the characteristic and highly symmetrical shape of the specific substance.

Water molecules surrounding each cluster forms hydrogen bonds with one another, resulting in a shell with a shape similar to that of the cluster. These formations of water molecules are termed clathrates.

Subsequent succussion allows the clusters to overcome the water cohesion forces and relocate to a new position, with resultant new clathrates forming around it.

After the cluster relocation, the broken original clathrate shell repairs itself, possessing a void in the interior, similar to the relocated cluster. This now becomes a “core-clathrate.” Another “mantle-clathrate” is now formed around the core clathrate.

The role of substance clusters is now taken over by the core clathrates. Their symmetric and compact structure allows for extra stability and, thus, behaviour like large complex molecules, i.e., molecules with a much larger mass and different inertial properties than regular water molecules.

With serial dilution and succussion this process is perpetuated. It is thus put forward that the properties of the initial substance can be traced by the properties of the *shaped voids* in the solvent. Thus the specific homoeopathic remedy, resulting from serial dilution and dynamization may have the characteristic properties of an original substance not physically present.

The results of this study can be interpreted on the basis of the clathrate model.

The potentization methods in all three methods analyzed serve to dynamically redistribute the energy within higher order structures.

Hahnemannian potentization provides a linear, additive effect on the remedy solution, resulting in the effects as described by the standardized clathrate model.

The Wala method applies a non-linear energy distribution to clathrate structures, thus resulting in, and re-enforcing the formation of higher order clathrate structures.

In order to explain differences in the results associated with the Weleda method one would need to take a quantum mechanical approach. According to Heisenberg's Uncertainty Principle, the clathrates are non-static, which means they possess an inherent natural frequency associated with them. The Weleda method employs a harmonic distribution of energy at a fixed

frequency. This may result in resonance between the input energy and the clathrate oscillations. In turn, this may serve to break up the clathrates and serve to create higher order structures within the remedy, via a dynamic re-distribution of energy.

It may thus be concluded that the effect of vorticity (Wala), non-linearity (Weleda) and linearity (Hahnemannian) in the applied force of dynamization, all serve to alter the remedy to varying extents.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The results of this study showed that statistically significant differences were observed in the chemical shift values of the CH_2 and CH_3 signals for all three methods investigated. Relative integration values showed significant differences between the Wala and Weleda method for the CH_3 signal, and between the Wala and Hahnemannian method for both OH and CH_3 signals.

Based on the results of this investigation, it may thus be reasoned that the method of dynamization does indeed play a significant and crucial role in establishing a particular molecular environment within a succussed dilution, and therefore, in the development of a distinct physico-chemical identity of a remedy. It may therefore also follow that the hypothesis that different methods of dynamization exert an individualizing effect on a remedy and thus may imply inherent differences, is satisfied.

6.2 RECOMMENDATIONS

1. Standardization of the pharmaceutical process

Strict standardisation of the potentization process is necessary to allow scientific evaluation and reproducibility. As potential human error is unavoidable in any procedure, succussion may therefore require control with calibrated machinery.

2. Magnetic field strength

The effect of the strong magnetic field used in high resolution NMR spectroscopy may have an uncertain effect on the samples analysed. The utilization of spectrometers of different field strengths, ranging from very low to very high may be advisable in analysing ultra high dilution samples.

3. External factors

Although external factors in this study are controlled as far as possible, concerns may still exist regarding the exact nature of the potentising process, particularly in the production of Anthroposophical samples. Within Anthroposophy, certain prohibitions are placed on the production process, e.g. potentising may only be carried out between 02h30 and 10h30 and between 14h30 and 22h00. Also, when potentising metals, the day of the month must be indicated as suitable according to the Wala and Weleda Potentising Calendars; potentising is prohibited during certain celestial occurrences, e.g. sun and moon eclipses.

The extent of these theoretical influences can not easily be verified scientifically. Still, strict observations of these parameters establish guidelines in standardizing samples.

Chemical influences, e.g. the absorption of moisture during the potentising process and variations in factors like at atmospheric oxygen may play a role, one may also question excessive control as unnatural to the potentization process. One must therefore attempt to replicate the potentization process as

is done in practise as closely as possible, with as few variations as possible (Davies 2001).

4. Analysis of a wider variety of substances and samples

The use of a wide variety of substances prepared according to standardised methods would allow a better comparison of trends that arise within NMR spectroscopic analysis. Large amounts of anomalous results instead of definite trends could indicate that the use of NMR spectroscopy should be re-evaluated as a tool for analysing homoeopathic substances.

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APPENDIX A: METHODOLOGY OF REMEDY PREPARATION

Method 1:

Aim: To prepare Hahnemannian 12D potency.

Apparatus: Pulsatilla pratensis mother tincture

Purified water

62 % ethanol

43% ethanol

30% ethanol

15% ethanol

Paper towelling

25ml amber glass bottles

Labels and pens

5ml and 2ml pipettes, and 10 ml measuring cylinder

Micropipettes

Paper

Method:

1. Rinse and autoclave equipment, allow cooling.
2. Measure out 3ml Pulsatilla pratensis using 5ml micropipette and place in first 25ml amber glass bottle. Add 7ml 62% ethanol into first 25ml amber glass bottle using 5ml pipette.
3. Succuss 100 times and label “Pulsatilla pratensis 1D”.

4. Place 9ml 62% ethanol into second 25 ml amber glass bottle.
5. Add 1ml of Pulsatilla pratensis 1D.
6. Succuss 100 times and label “Pulsatilla pratensis 2D”.
7. Place 9ml 43% ethanol into third 25 ml amber glass bottle.
8. Add 1ml of Pulsatilla pratensis 2D.
9. Succuss 100 times and label “Pulsatilla pratensis 3D”.
10. Place 9ml 30% ethanol into fourth 25 ml amber glass bottle.
11. Add 1ml of Pulsatilla pratensis 3D.
12. Succuss 100 times and label “Pulsatilla pratensis 4D”.
13. Place 9ml 15% ethanol into fifth 25 ml amber glass bottle.
14. Add 1ml of Pulsatilla pratensis 4D.
15. Succuss 100 times and label “Pulsatilla pratensis 5D”.
16. Repeat the above procedure (13-15) up to 12D
17. Label all required potencies for NMR spectroscopy appropriately, and store in cool environment free from any electromagnetic disturbance.

Method 2:

Aim: To prepare Wala 12D potency.

Apparatus: Pulsatilla pratensis mother tincture

Purified water

62 % ethanol

43% ethanol

30% ethanol

15% ethanol

Paper towelling

25ml amber glass bottles

Glass potentising flask

Labels and pens

5ml and 2ml pipettes, and 10 ml measuring cylinder

Micropipettes

Paper

Method:

1. Rinse and autoclave equipment, allowing cooling.
2. Measure out 3ml Pulsatilla pratensis using 5ml micropipette and place in first glass potentising flask.
3. Add 7ml 62% ethanol into first glass potentising flask using 5ml pipette.

4. Potentise for two and a half minutes (plant substances) according to Wala principles:

   Take the flask and close cap. Stand in front of the window overlooking the garden.

   - Take the closed potentising flask loosely in the hand with fingers around the top of the bottle
   - Let the arm hang loosely but straight next to the body.
   - Bring the arm up with a quick flick of the wrist and hand to form a vortex. The liquid must move upwards before forming a vortex.
   - After two and a half minutes (for plant substances) or four minutes (for metal substances) stop potentising and allow settling until all movement in the liquid has ceased.

5. Bottles containing potentized liquid must be placed gently onto the working surface.
6. Label “Pulsatilla pratensis 1D.
7. Repeat the above procedure (2-6) up to 12D. A 43% and 30% ethanol concentration is used respectively in production of 2D and 3D. Thereafter, 15% ethanol is maintained until 12D potency.
8. Label all required potencies for NMR spectroscopy appropriately and store in cool environment free from any electromagnetic disturbance.

Method 3:

Aim: To prepare Weleda 12D potency.

Apparatus: Pulsatilla pratensis mother tincture

Purified water

62 % ethanol

43% ethanol

30% ethanol

15% ethanol

Paper towelling

25ml potentising glass bottles

Labels and pens

5ml and 2ml pipettes, and 10 ml measuring cylinder

Micropipettes

Paper

Method:

1. Rinse and autoclave equipment, allowing cooling.
2. Measure out 3ml Pulsatilla pratensis using 5ml micropipette and place in first potentising glass bottle.
3. Add 7ml 62% ethanol into first potentising glass bottle using 5ml pipette. Care is taken that bottles are not more than two thirds full.
4. Potentise for two and a half minutes (plant substances) according to Weleda

principles:

Take the flask and close cap. Stand in front of the window overlooking the garden.

- Bottle sizes less than 2kg can be shaken vertically.
- Take the bottle between thumbs and fingers at the top of the bottle. The cap is pointing away from the body. Hold the base in the other hand against the body.
- Gently rock the bottle backwards and forwards. The liquid contents will start making a figure eight motion.
- Bottles of more than 2kg are placed on a potentising stand and are moved from left to right.

5. After two and a half minutes (plant substances) or four minutes (metal substances) stop potentising and allow settling until all movement in the liquid has ceased.
6. Bottles containing potentized liquid must be place gently onto the working surface.
7. Label “Pulsatilla pratensis 1D.
8. Repeat the above procedure (2-6) up to 12D. A 43% and 30% ethanol concentration is used respectively in production of 2D and 3D. Thereafter, 15% ethanol is maintained until 12D potency.
9. Label all required potencies for NMR spectroscopy appropriately and store in cool environment free from any electromagnetic disturbance.