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ORIGINAL PAPER

A nuclear magnetic resonance spectroscopy comparison of 3C trituration derived and 4C trituration derived remedies

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Background: **Trituration of base substances, commonly to the 3cH level, is the cornerstone of the homeopathic pharmaceutical process or insoluble solutions.[1] Becker and Ehrler claim that trituration to 4cH gives a new, spiritual dimension to the homoeopathic medicine picture.[2]**
Aim and method: **This study sought to establish whether the claim that C4-derived potencies possess different physicochemical qualities to the homoeopathic medicines derived from a 3cH trituration is valid. All potencies were produced by hand according to the German Homoeopathic Pharmacopoeia (GHP). Five different samples were analysed using Nuclear Magnetic Resonance (NMR) Spectroscopy.**
Results: **The results indicated a significant difference between the 12cH samples of potassium dichromate (*Kalium bichromicum*) produced from 3cH and 4cH triturations. This was especially prominent in the chemical shift values of all four peaks and the relative integration levels of the H_2O, OH and CH_3 peaks when comparing two sample groups.**
Conclusion: **Trituration plays a part in the development of physicochemical properties specific to homoeopathic medicines. The higher the level of trituration, the more pronounced is the alteration of the physical structure of the active ingredient. The study concludes that 4cH potencies are physicochemically distinct from 3cH-derived potencies (as currently employed).** *Homeopathy* (2008) **97**, 196–201.

Keywords: Nuclear Magnetic Resonance spectra; NMR; *Kalium bichromicum*; potentised; Trituration; 4cH; Potassium dichromate

Introduction

Hahnemann, throughout his life, experimented clinically with different methods of preparation of his potencies. Early in the 19th century, he started employing trituration as a method to render insoluble source material soluble. Initially he triturated to 2cH but his further experiments led him to look into triturations up to a 12cH level. Finally he decided that 3cH was the optimum level up to which a source material should be triturated before converting into a liquid potency. This was also the level he chose for the preparation of his quinquagemillenesimal potencies.[1,4] This standard method of preparing homoeopathic potencies was documented in various pharmacopoeias, for example the German Homoeopathic Pharmacopoeia (GHP) followed in this study.

In 1998, Becker and Ehrler claimed that the C4 (or 4cH) trituration level gives ''a new, spiritual dimension to the homoeopathic medicine picture, thus giving a deeper knowledge and understanding as to the homoeopathic potentisation.''[2] This was met with mixed responses from homoeopathic circles. Homoeopaths, like Timmerman, were intrigued by the suggestions. She started doing further work on the subject and lecturing on her findings.[5] Others, such as Dellmour, have objected to both the notion itself, and the scientific basis of the studies conducted by Timmerman and Becker. They further questioned the quality of the resulting homoeopathic medicines and homoeopathic medicine pictures obtained using the C4 method of proving.[6]

C4 provings are conducted during the trituration process. After every level of trituration is completed, the prover records all feelings and impressions gathered during the

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trituration.[5] The trituration of one substance up to the C4 level is usually done over a period of five to six days. It is claimed that at the C1 level, physical symptoms are experienced both during and after the trituration process; the C2 level expresses the sensational and mental information of the substance, whereas the C3 level contains the delusions and dreams as an expression of the psychic/mental level. The C4 level is said to expose the essence of the homoeopathic medicine, expressing the spiritual, unconscious level of the individual. Usually, symptoms experienced on one level apparently disappear on going to the next trituration level.[7] This proving method contrasts to the traditional method where the proving substance is administered, usually orally, to the prover. The prover then records all symptoms experienced during the duration of the proving. This process usually runs over the period of a few weeks.[8]

This study is a step towards providing objective evidence around the validity of the claims that C4 potencies possess different qualities to the homoeopathic medicines currently employed. The researcher endeavoured to investigate the physical and chemical properties of the potencies in question, reasoning that if statistically reliable differences were detected Nuclear Magnetic Resonance (NMR) spectroscopy might provide an objective assertion of difference.

The aim of this study was to compare the NMR spectra of *Kalium bichromicum* 12cH, which have been potentised using Hahnemannian methods, from initial 3cH trituration or 4cH trituration.

Material and methods

We hypothesised that there is a significant differences between the chemical shift (δ) and relative integration values of the CH_2, CH_3, H_2O and OH signals of the 12cH potencies of the various samples of *Kalium bichromicum*. Also hypothesised, is that the trituration level plays an important part in the development of distinct physicochemical properties specific to homoeopathic medicines. All potencies were produced by hand according to the GHP.[3] The starting materials – potassium dichromate and lactose monohydrate - were procured from Merck Chemicals (Pty) Ltd., South Africa. The same 3cH triturate used for preparing the sample for analysis was utilised to as the starting material for the manufacture of the 4cH triturate. In manufacturing the 6cH to 12cH potencies, 87% alcohol was used. Although the GHP states the use of 30% alcohol, this ethanol percentage was used to correlate with ethanol/water concentrations used in other NMR experiments.[9–11]

Samples were produced in a volume large enough to accommodate the drawing of three samples from each bottle. The potencies were thus produced in 25 ml amber glass screw top bottles. The amber glass was selected for it protects the homoeopathic medicines from destruction by light. Boro-silicate glass bottles were used as suggested by Milgrom *et al.*[12] To ensure that the sample had space to be succussed to produce the final potency, the 25 ml bottles were only filled two-thirds, thus containing 16,000 ml. The samples were manufactured in the 12cH potency, for at this level of deconcentration, the theoretical concentration of the original substance is 10^{-24}, which is beyond Avogadro's number (6.022×10^{23}). Thus at this dilution level not one molecule of the original substance is theoretically present.

After manufacture the samples were stored in a temperature-controlled environment, away from any magnetic influences, until analysis. Three samples were drawn from each of the provided volumes using a micropipette and a clean capillary tube. Only one bottle of each sample was prepared to standardise the method of preparation. This eliminated the additional variables introduced by multiple samples. The three samples of 600 μl were drawn from each bottle in a linear fashion, so as to exclude the possibility of contamination. Deuterated acetone (acetone-d_6) was used as both the external lock and the reference substance, as it provides a very reliable chemical shift value outside the range of other peaks. The deuterated acetone was placed inside a separate capillary tube within the NMR tube so as not to come in contact with the sample and cause contamination.

The instrument used was the Varian Unity Inova 500 MHz Spectrometer. The magnet was shimmed before every run to ensure a homogenous field around each sample before it was tested. The sixteen transients per sample were used to generate the NMR spectra. This raw data was used to obtain the chemical shift (δ) values and to calculate the relative integration values and subjected to the statistical methods. The data was recorded in the form of NMR spectra listing the chemical shifts (in Hertz) and integration values. These were then transferred onto Excel spreadsheets.

Analysis

Both the chemical shift and integration values were recorded from the printout of the NMR spectrometer. For the CH_2 and CH_3 chemical shift values, where the values comprise of more than one peak, the average value was calculated and a single value used. The relative integration value for each peak was calculated by dividing the integration values of each peak by the sum of all integration values for the run.

The data was then entered into an Excel spreadsheet and from there transferred into the SPSS software package for statistical analysis. For analysis, all the samples were compared with each other to determine whether a significant difference existed between any of the samples. Since the sample size per group was small, the comparison was made between the five unpaired groups using the non-parametric Kruskal–Wallis test. If a significant difference existed between any of the groups, individual comparisons between groups were made using the non-parametric Mann–Whitney test. The following comparisons were made:

- *Kalium bichromicum* soluble derived 12cH and *Kalium bichromicum* 3cH derived 12cH;
- *Kalium bichromicum* 3cH derived 12cH and *Kalium bichromicum* 4cH derived 12cH;
- Lactose 3cH derived 12cH and Lactose 4cH derived 12cH;