

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 homeopathic medicine picture.²

Aim and method: This study sought to establish whether the claim that C4-derived potencies possess different physicochemical qualities to the homeopathic 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 H₂O, OH and CH₃ peaks when comparing two sample groups.

Conclusion: Trituration plays a part in the development of physicochemical properties specific to homeopathic 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 quinquagemillesimal potencies.^{1,4} This standard method of preparing homeopathic potencies was docu-

mented 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 homeopathic medicine picture, thus giving a deeper knowledge and understanding as to the homeopathic potentisation."² This was met with mixed responses from homeopathic 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 homeopathic medicines and homeopathic 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 CH₂, CH₃, H₂O 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 μ l were drawn from each bottle in a linear fashion, so as to exclude the possibility of contamination. Deuterated acetone (acetone-d₆) 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 CH₂ and CH₃ 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;

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

Results

Table 1 shows the values for the 5 samples and 4 peaks. For this study a value of $p < 0.05$ was considered significant. As illustrated in Table 2, all comparisons showed a significant differences when in chemical shift values. The relative integration values only reflected a significant difference when comparing the H₂O and CH₂ peaks. For a more in-depth analysis of the data, individual results were compared with each other using the Mann-Whitney test. The results are shown in Tables 3 and 4. This shows that a significant difference exists between the NMR spectra of *Kalium bichromicum*, which have been potentised to 12cH potency, from initial 3cH and 4cH triturations. This difference is observable between all the peaks of the respective samples in both the chemical shift and relative integration values. A significant difference is especially noticeable between the chemical shift values for the CH₂ and CH₃ peaks. The exception is the CH₂ peak when comparing the relative integration values. This peak shows no significant difference.

Discussion

This data, shows that the trituration process causes physical alterations of the substance. These alterations persist through the serial dilution to the 12th centesimal deconcentration level. These results agree with the findings of Resch and Gutman.¹³ They found that the act of grinding has a profound influence on the physical properties of the system. It

Table 2 Kruskal-Wallis Test: Comparison of Chemical shift (δ) and Relative integration values of all samples * $p < 0.05$

	H ₂ O	OH	CH ₂	CH ₃
Chemical shift (δ)	0.033*	0.028*	0.017*	0.025*
p values				
Relative integration p values	0.028*	0.102	0.022*	0.464

produces a highly developed system organisation, whereby the energy of the grinding process is absorbed by the system. This strengthens the system organisation and integrates the solute into the lactose solvent. The characteristics of the solvent is preserved even beyond the level at which any trace of the original molecular substance is present.

When comparing the NMR spectra of the lactose (control) groups (Figures 4 and 5), potentised to 12cH potency, from initial 3cH and 4cH trituration, a similar trend is observable. The level of significance of the differences observed is less, however, and the only significant differences are found in the chemical shift values of the CH₂ and CH₃ peaks, and the relative integration values of the CH₂ peak.

We hypothesise, that the trituration process does causes structural changes in the lactose carrier as well, but that the changes are not as marked as those alterations imprinted by a medicinal source. The structural changes are attributable to the strengthening of the systems organisation. If another solute is mixed in, e.g. potassium dichromate, it modifies the structural framework of the lactose, which accepts and preserves the structural information of. This results in the establishment of a new systems organisation with dynamically better developed static aspects of order than the pure lactose, conserving the aspects of order in the highly potentised drug.¹³

Table 1 Data obtained from the NMR spectroscopy

Run		Chemical shift			Relative integration		
		1	2	3	1	2	3
H ₂ O	<i>Kalium bichromicum</i> soluble	4.729	4.729	4.729	0.128900	0.128700	0.128700
	<i>Kalium bichromicum</i> from 3cH	4.729	4.729	4.729	0.129000	0.129300	0.129413
	<i>Kalium bichromicum</i> from 4cH	4.731	4.731	4.731	0.128887	0.128887	0.128913
	Lactose from 3cH	4.727	4.727	4.730	0.128800	0.128600	0.128300
	Lactose from 4cH	4.727	4.727	4.728	0.128500	0.128700	0.128700
OH	<i>Kalium bichromicum</i> soluble	4.002	4.002	4.002	0.220300	0.220400	0.220400
	<i>Kalium bichromicum</i> from 3cH	4.001	4.001	4.001	0.220200	0.220000	0.220022
	<i>Kalium bichromicum</i> from 4cH	4.002	4.002	4.002	0.220278	0.220278	0.220322
	Lactose from 3cH	3.999	3.999	4.002	0.220300	0.220000	0.220200
	Lactose from 4cH	3.999	3.999	3.999	0.220000	0.220300	0.220300
CH ₂	<i>Kalium bichromicum</i> soluble	3.0085	3.0085	3.0085	0.260000	0.260000	0.260100
	<i>Kalium bichromicum</i> from 3cH	3.0063	3.0063	3.0063	0.260100	0.259900	0.259826
	<i>Kalium bichromicum</i> from 4cH	3.0080	3.0080	3.0080	0.259974	0.259974	0.259926
	Lactose from 3cH	3.0065	3.0065	3.0095	0.260300	0.260300	0.260100
	Lactose from 4cH	3.0050	3.0050	3.0050	0.260600	0.260400	0.260400
CH ₃	<i>Kalium bichromicum</i> soluble	0.566	0.566	0.566	0.390800	0.390900	0.390800
	<i>Kalium bichromicum</i> from 3cH	0.564	0.564	0.564	0.390700	0.390800	0.390739
	<i>Kalium bichromicum</i> from 4cH	0.565	0.565	0.565	0.390861	0.390861	0.390839
	Lactose from 3cH	0.564	0.564	0.567	0.390600	0.391100	0.391400
	Lactose from 4cH	0.562	0.562	0.562	0.390900	0.390600	0.390600

Table 3 Mann-Whitney Test – Chemical shift (δ) Values (p Value) * $p < 0.05$

Relative integration	H ₂ O	OH	CH ₂	CH ₃
<i>Kalium bichromicum</i> soluble derived 12cH	1.000	0.025*	0.025*	0.025*
<i>Kalium bichromicum</i> 3cH derived 12cH				
<i>Kalium bichromicum</i> 3cH derived 12cH	0.025*	0.025*	0.025*	0.025*
<i>Kalium bichromicum</i> 4cH derived 12cH				
Lactose 3cH derived 12cH	0.796	0.317	0.034*	0.034*
Lactose 4cH derived 12cH				
<i>Kalium bichromicum</i> 3cH derived 12cH	0.480	0.480	0.034*	0.371
Lactose 3cH derived 12cH				
<i>Kalium bichromicum</i> 4cH derived 12cH	0.034*	0.025*	0.025*	0.025*
Lactose 4cH derived 12cH				

The NMR spectra of *Kalium bichromicum* prepared respectively from an initial soluble form and from a 3cH trituration (Figures 1 and 2) show a difference in both the chemical shift and the relative integration values of the four peaks. A significant difference is observable in the chemical shift values of the OH, CH₂ and CH₃ peaks. The H₂O peaks are however identical. The relative integration values show a significant difference present between the H₂O and OH peaks, and a statistically insignificant difference present in the CH₂ and CH₃ peaks.

This lead us to assume that the trituration process alters the structure of the medicinal source and that the two methods of homeopathic medicine manufacture, i.e. from a soluble substance and through trituration, give rise to two different substances even when they are potentised to the exact same level. The manufacturing method alters the physical properties of the substance, giving rise to different NMR spectra. At the 12cH level, a deconcentration level beyond Avogadro's number is reached. There is thus no molecular trace of the original substance present in the solution. The structural changes are thus present in the systems organisation of the water-ethanol mixture.¹²

When comparing the NMR spectra of parallel potencies of *Kalium bichromicum* and lactose (control) manufactured from initial 3cH and 4cH triturate respectively (Figures 2

and 3), a difference is observed for all the chemical shift values, but the difference is more marked between those trituated to the 4cH level, where all the differences are statistically significant. The relative integration values show similar trends for both the samples manufactured from an initial 3cH and 4cH level.

The assumption can thus be made that the higher the level of trituration, the more pronounced the difference in the physical structure of respective active ingredients becomes.

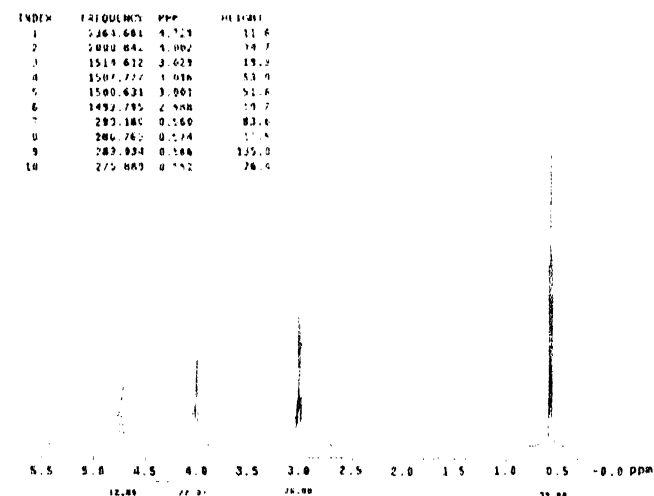


Figure 1 NMR Spectra of Kalium Bichromicum 12cH from Soluble Potassium Dichromate.

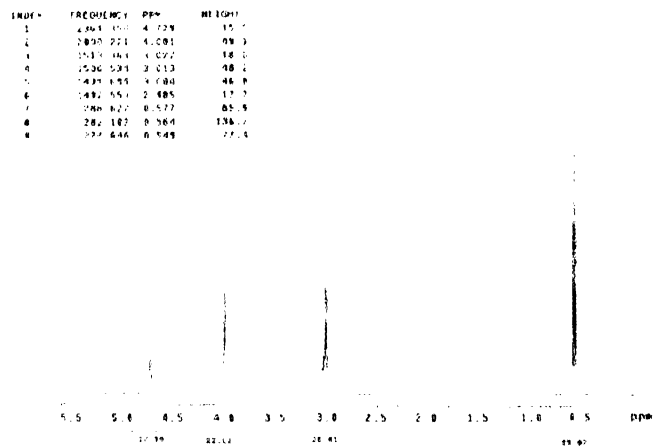


Figure 2 NMR Spectra of Kalium Bichromicum 12cH from 3cH trituration.

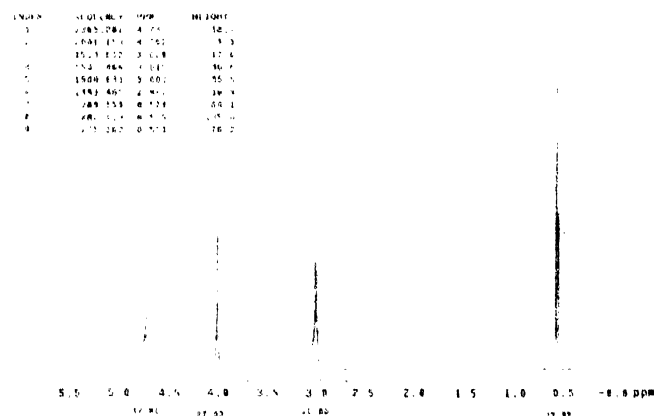


Figure 3 NMR Spectra of Kalium Bichromicum 12cH from 4cH trituration.

Table 4 - Mann-Whitney Test – Relative integration values (*p* Values) * *p* < 0.05

	H ₂ O	OH	CH ₂	CH ₃
<i>Kalium bichromicum</i> soluble derived 12cH	0.046*	0.046*	0.369	0.105
<i>Kalium bichromicum</i> 3cH derived 12cH	0.046*	0.046*	0.507	0.046*
<i>Kalium bichromicum</i> 4cH derived 12cH	0.043*	0.822	0.043*	0.500
Lactose 3cH derived 12cH	0.825	0.637	0.043*	0.246
Lactose 4cH derived 12cH	0.050*	0.500	0.072	0.513
<i>Kalium bichromicum</i> 3cH derived 12cH	0.046*	0.046*	0.507	0.046*
Lactose 3cH derived 12cH	0.825	0.637	0.043*	0.246
<i>Kalium bichromicum</i> 4cH derived 12cH	0.043*	0.822	0.043*	0.500
Lactose 4cH derived 12cH	0.825	0.637	0.043*	0.246

INDEX	FREQUENCY	PPM	HEIGHT
1	2253.014	4.227	9.5
2	1459.402	3.999	39.0
3	1513.058	3.826	15.5
4	1505.312	3.812	16.1
5	1459.067	3.989	95.2
6	1491.951	2.985	17.1
7	280.216	0.577	68.8
8	281.176	0.562	140.2
9	274.024	0.506	78.8

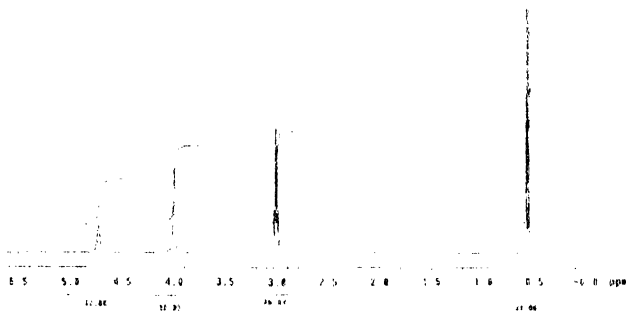


Figure 4 NMR Spectra of Lactose 12cH from 3cH trituration.

INDEX	FREQUENCY	PPM	HEIGHT
1	2253.014	4.227	9.5
2	1459.402	3.999	39.0
3	1513.058	3.826	15.5
4	1505.312	3.812	16.1
5	1459.067	3.989	95.2
6	1491.951	2.985	17.1
7	280.216	0.577	68.8
8	281.176	0.562	140.2
9	274.024	0.506	78.8

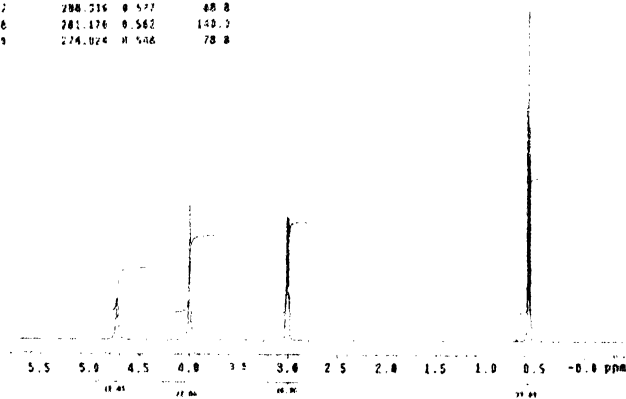


Figure 5 NMR Spectra of Lactose 12cH from 4cH trituration.

i.e. a difference present between the lactose and the *Kalium bichromicum* at a 3cH trituration level will be greater at the 4cH trituration level.

Conclusion

The hypothesis that significant differences exist between the chemical shift (δ) and relative integration values of the CH₂, CH₃, H₂O and OH signals of the 12cH potency level (which have been manufactured from a soluble form, a 3cH and a 4cH triturate) indicate differences inherent to each trituration level is supported by this experiment.

It also supports the hypothesis that the level of trituration plays an important part in the development of distinct physicochemical properties specific to homeopathic medicines. There exist statistically significant differences between parallel potencies in terms of substance versus substance, substance versus control and control versus control, with regard to chemical shift (δ) and relative integration values of the CH₂, CH₃, H₂O and OH signals of respective trituration levels.

We feel that C4 potencies are an avenue that requires further exploration to obtain scientific validation of the claims made.

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