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ORIGINAL PAPER

High-field ^{1}H T_1 and T_2 NMR relaxation time measurements of H_2O in homeopathic preparations of quartz, sulfur, and copper sulfate

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Abstract Quantitative meta-analyses of randomized clinical trials investigating the specific therapeutic efficacy of homeopathic remedies yielded statistically significant differences compared to placebo. Since the remedies used contained mostly only very low concentrations of pharmacologically active compounds, these effects cannot be accounted for within the framework of current pharmacology. Theories to explain clinical effects of homeopathic remedies are partially based upon changes in diluent structure. To investigate the latter, we measured for the first time high-field (600/500 MHz) ^{1}H T_1 and T_2 nuclear magnetic resonance relaxation times of H_2O in homeopathic preparations with concurrent contamination control by inductively coupled plasma mass spectrometry (ICP-MS). Homeopathic preparations of quartz (10*c*–30*c*, $n=21$, corresponding to iterative dilutions of 100^{-10}–100^{-30}), sulfur (13*x*–30*x*, $n=18$, 10^{-13}–10^{-30}), and copper sulfate (11*c*–30*c*, $n=20$, 100^{-11}–100^{-30}) were compared to $n=10$ independent controls each (analogously agitated dilution medium) in randomized and blinded experiments. In none of the samples, the concentration of any element analyzed by ICP-MS exceeded 10 ppb. In the first measurement series (600 MHz), there was a significant increase in T_1 for all samples as a function of time, and there were no significant differences between homeopathic potencies and controls. In the second measurement series (500 MHz) 1 year after preparation, we observed statistically significant increased T_1 relaxation times for homeopathic sulfur preparations compared to controls. Fifteen out of 18 correlations between sample triplicates were higher for controls than for homeopathic preparations. No conclusive explanation for these phenomena can be given at present. Possible hypotheses involve differential leaching from the measurement vessel walls or a change in water molecule dynamics, i.e., in rotational correlation time and/or diffusion. Homeopathic preparations thus may exhibit specific physicochemical properties that need to be determined in detail in future investigations.

Keywords Homeopathy · NMR relaxation · Homeopathic remedies · Potentization

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Introduction

Homeopathy and anthroposophic medicine are complementary medical systems, which therapeutically use high or

ultrahigh dilutions, also known as homeopathic potencies. These are prepared by serial logarithmic dilution and shaking of a mother tincture, typically in water or water–ethanol mixtures. Depending on the dilution level, the dilution process may exceed the Avogadro limit, resulting in a virtually zero probability for even a single molecule of the mother tincture to be present in the dilutions. For homeopathic preparations of simple inorganic compounds such as SiO_2, S_8, or $CuSO_4$, ubiquity of the constituting elements in the environment poses an analogous limit of dilution at a concentration of about 10^{-12} since the dilution medium cannot be purified completely. Based on these considerations, it is often argued that the therapeutic effects of homeopathic dilutions are either unspecific or placebo since common pharmacological models cannot account for any specific effects of homeopathic dilutions.

Several meta-analyses of randomized clinical trials investigating the specific efficacy of homeopathic remedies were published in the last years. Interestingly, to the best of our knowledge all quantitative meta-analyses published so far that analyzed the specific therapeutic efficacy for specified medical conditions reported statistically significant effects of homeopathic preparations superior to placebo (Barnes et al. 1997; Jacobs et al. 2003; Jonas et al. 2000; Lüdtke and Wiesenauer 1997; Taylor et al. 2000; Vickers and Smith 2006). Thus, one may hypothesize that the dilution medium (e.g., water or water–ethanol mixtures) adopts specific properties related to the initial mother tincture even though no molecules of the latter are present. In the last years, various working hypotheses concerning a possible mode of action of homeopathic preparations have been put forward, e.g., by Atmanspacher et al. (2002), Bastide and Lagache (1997), Kratky (2004), Milgrom (2002), and Weingärtner (2003).

Recent investigations of physicochemical properties of homeopathic preparations involved mainly nuclear magnetic resonance (NMR) as method of measurement, either as low field ^1H relaxation time (T_1 and T_2) studies (Aabel et al. 2001; Demangeat et al. 1992; Demangeat et al. 2004; Lasne 1986) or as high-field ^1H spectroscopy (Aabel et al. 2001; Anick 2004; Weingärtner 1990). Thermodynamic properties (Elia and Niccoli 2000) or thermoluminescence (Rey 2003) were also investigated. The studies of Demangeat et al. (1992), Demangeat et al. (2004), and Lasne (1986) found statistically significant differences between homeopathic preparations and analogously agitated potentization (dilution) medium as control. Thus, trivial physicochemical artifacts such as dissolved oxygen, paramagnetic impurities, radicals, etc. can most probably be excluded. Aabel et al. (2001) and Anick (2004) could not identify stable cluster configurations. The latter result is in disagreement with many theoretical considerations, e.g., by Kratky (2004) or Anick (2004).

These findings need further corroboration. In addition, it is not yet clear which measurement methods are best suited to determine specific physicochemical properties of homeopathic preparations (in case there are any). The aim of this study was to explore the potential of two methods that have not been used for investigations of homeopathic preparations so far: high-field ^1H T_1 and T_2 NMR relaxation times and ultraviolet (UV) spectroscopy.

NMR relaxation times (T_1, spin–lattice relaxation, and T_2, spin–spin relaxation) are indicative for various intra- and intermolecular spin couplings (Abragam 1961). After the pioneering work of Bloembergen et al. (1948) who first used relaxation times to calculate correlation times τ_c (characterizing the time scale of water molecule reorientation), NMR T_1, and T_2 relaxation studies have been extensively used in water structure research, as can be seen in the substantial contributions to the treatise on water edited by Franks (1972).

In order to complement earlier investigations, we decided to measure T_1 and T_2 relaxation times of ^1H in H_2O in homeopathic preparations of quartz (SiO_2), sulfur (S_8), and copper sulfate ($CuSO_4$) at 600 and 500 MHz. Quartz and copper sulfate were prepared as so-called *c*-potencies (centesimal potencies, 100-fold dilution) in order to allow a comparison with the investigations of Demangeat et al. (1992) and Demangeat et al. (2004) who also used centesimal dilution steps. Sulfur was diluted in decimal steps (10-fold dilution, so-called *x*-potencies) for comparison to the investigation of Weingärtner (1990). All samples were also analyzed by UV spectroscopy; the results achieved with this method will be reported elsewhere.

Following the considerations of Demangeat et al. (1992) and Demangeat et al. (2004), we aimed at minimizing experimental artifacts, e.g., by use of a clean room for all sample preparations, by NMR sample closure within few hours, and by randomization and blinding of all samples during the course of the experiments. According to Cazin et al. (1991), nitrogen blanketing leads to inefficient homeopathic remedies; therefore, samples were not degassed. In addition, the concentrations of the most relevant inorganic compounds were determined for all samples using inductively coupled plasma mass spectroscopy (ICP-MS) in order to assess a potential contamination during the preparation of homeopathic preparations, e.g., by leaching of vessels.

Materials and methods

The text in this chapter is an abridged version only. Full details concerning materials and methods are given in the Electronic supplementary material S1.

Chemicals

Deionized water (DI-water) was prepared from tap water using two ion exchange columns (Culligan, Northbrook, IL, USA) for a first deionization and a subsequent Millipore system (Super-Q), resulting in water of 18 MΩ cm. Quartz distilled water (QD-water) was prepared by subsequent subboiling distillation of the DI-water (Seastar Chemicals, Sidney, BC, Canada). Hydrochloric acid (HCl) was subboiling double-distilled HCl, prepared from reagent grade HCl (certified ACS PLUS, normality 12.1, from Fisher Scientific, Fairlawn, NJ, USA). Nitric acid (HNO_3) was twice two-bottle distilled HNO_3, prepared from reagent grade HNO_3 (certified ACS PLUS, normality 15.8, from Fisher Scientific). Ethanol used was Ethyl Alcohol USP, Absolute-200 Proof (Aaper Alcohol and Chemical, Shelbyville, USA). Lactose was ordered from Dixa AG (St. Gallen, Switzerland), quartz powder (SiO_2) from Weleda AG (Schwäbisch Gmünd, Germany), copper sulfate ($CuSO_4 \cdot 5H_2O$) from Weleda AG (Arlesheim, Switzerland), sublimed sulfur (S_8) from Phytomed AG, Hasle/Rüegsau, Switzerland. ICP-MS standards were obtained from High-Purity-Standards, Charleston, SC, USA.

Preparation of homeopathic preparations and controls

Homeopathic samples and controls were prepared in a metal-free class 100 HEPA clean room using standard trace analytical procedures and equipment, as well as standard homeopathic procedures (Anonymous 2004). This includes careful vessel pretreatment and cleaning procedures (details see Electronic supplementary material S1 and S2, which contains a flow chart of sample preparations and measurements for the entire study).

All samples (homeopathic preparations and controls) were prepared in 500-ml narrow-necked bottles with conical shoulder, made from boro-silicate glass (DURAN, Schott, from VWR International, Dietikon, Switzerland). Homeopathic quartz and copper sulfate samples were "potentized" (diluted 1:99 and vigorously shaken) to 30*c* (100^{-30}), and sulfur samples were "potentized" 1:9 to 30*x* (10^{-30}) in quartz distilled water (QD-water) with 1% ethanol. The first three dilution steps of quartz (1:99) and the first six dilution steps of sulfur (1:9) were performed by solid mixing and grinding in lactose with mortar and pestle (homeopathic "Trituratio"). Correspondingly, concentrations of the first dilution step were 1% for quartz and copper sulfate (1 g pure quartz powder was triturated in 99 g lactose to obtain quartz 1*c*, and 2 g of pure copper sulfate was dissolved in 200 ml QD-water with 1% ethanol to obtain dilution level 1*c*) and 10% for sulfur (10 g pure sulfur powder was triturated with 90 g lactose to obtain sulfur 1*x*).

For each set of homeopathic preparations (quartz, sulfur, or copper sulfate), 10 independent controls were prepared as follows: one glass bottle was filled with 200 ml QD-water with 1% ethanol (without lactose) and shaken equally to the homeopathic preparations, without any dilution from one control to another. This type of control accounts for all the unspecific physicochemical effects associated with agitation, e.g., ion release from the vessel walls, air suspension and dissolution with subsequent pH alteration, and radical formation, as discussed by Baumgartner et al. (1998). Five controls were prepared before and five after the homeopathic preparations in order to control for a possible cross-contamination and other interference in the course of the production process. All homeopathic preparations and controls of a given set (quartz, sulfur, or copper sulfate) were prepared from the same batch of QD-water with 1% ethanol.

Randomization was effectuated through random allocation of the numbered potentization vessels to the dilution levels or controls to be produced. Codes were kept secret until the end of the measurements and data reduction, with the exception of one control sample (for each series) that was measured several times.

ICP-MS measurements

Samples of each homeopathic preparation and control were pipetted from the preparation vessels directly into ICP-MS vials to which internal standard (^{45}Sc, ^{74}Ge, ^{115}In, ^{205}Tl, 1 ppb each) and HNO_3 were added. For analysis, a Sector ICP-MS Finnigan MAT Element (Thermo Electron, Karlsruhe, Germany) with PFA inlet system, Teflon spray chamber, and PFA nebulizer was used. The system was run with guard electrode in operational mode. Analyzed elements were ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{27}Al, ^{28}Si, ^{44}Ca, ^{48}Ti, ^{56}Fe, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{133}Cs, ^{137}Ba, and ^{208}Pb. Samples were measured in random order in runs of 10 samples. Blank and external standard samples (all analyzed elements in a concentration of about 1 ppb) were measured at the beginning, in the middle, and at the end of each run. After measurement, data reduction was performed as follows. For each run, the slope of the corresponding calibration curve was calculated. The inverted calibration curve (according to Funk et al. 1985, p. 38) was used to calculate effective concentrations [parts per billion] for all samples. Errors (95% confidence limits) were calculated according to Funk et al. (1985, p. 43). Detection limit determination was based upon the standard deviation of the blank (n=3) for alpha=beta=5% according to Funk et al. (1992, p. 25).

NMR measurements

For each sample, four to six micropipettes tubes (Wiretrol II, 1–5 µl, boro-silicate glass, Drummond Scientific, Broomall PA, USA) were filled with about 10 µl fluid by capillary action in the clean room. Capillaries were flame sealed at one end, optically checked by binocular for tight closure, centrifuged at 1,400 rpm for 75 s, flame-sealed at the other end, and again checked by binocular. All samples were sealed within few hours in order to minimize environmental influences (changing air pressure, etc.). Filling level was measured for each capillary with a millimeter ruler.

The NMR measurements at the NHMFL Florida were performed with an Oxford superconducting magnet at 600 MHz (14.1 T) using a 5-mm Varian NMR PFG probe and a Varian console (Varian unity plus). Data processing was done with a Sun Ultra 5 Sparc PC workstation, equipped with Varian VNMR 6.1 software (Version C). For each sample, three capillaries out of a total of four to six were randomly selected. Capillaries were measured individually and placed in a standard 5-mm NMR tube without centering. All capillaries were measured at 20±0.1°C after 10 min of temperature equilibration within the magnet. Shimming for all samples was checked and adjusted individually by gradient shimming and by hand (minimizing the line width, which was <3 Hz). PW_{90} was measured for each sample and was in the order of 10 µs. Samples were measured in random order. For every sample, T_1 was measured by an inversion-recovery sequence with 18 spectra, T_2 immediately thereafter with the Carr–Purcell–Meiboom–Gill sequence using 19 spectra. For both sequences, we used the following parameters: D_1=10.6 s, NT=4 (cycling through all phases); LB=0.318 Hz, AT=4.098 s, NP=40,960. Data were Fourier-transformed, baseline-corrected, and phased. Integrals were calculated over the area of the peak to increase the signal-to-noise ratio. Relaxation times were obtained from the exponential data analysis provided by the VNMR 6.1 software.

The NMR measurements at the ETH Zurich were performed with a Bruker AVANCE 500 spectrometer (500.133 MHz, 11.7467 T) equipped with a 5-mm broad-band probe with an actively shielded *z*-gradient coil. Individual capillaries were centered by means of Teflon rings in high precession 5-mm NMR tubes. The temperature was 293 K, controlled with a nitrogen flow of 400 l h^{-1} to avoid temperature fluctuations of more than 0.1 K. T_1 times have been obtained with the standard inversion-recovery [rd–p180H–id1–p90H–acquisition]*n* (relaxation delay>5T_1) pulse sequence. In each experiment, a series of eight to 16 spectra were collected with an FID resolution of 0.8 Hz. After Fourier transformation and baseline correction, data processing by the SimFit algorithm in XWinNMR 3.5 was done. T_1 was measured three times in series and averaged.

All further data analysis was performed with the statistics software "Statistica 4.1" (Statsoft, Tulsa, OK, USA). Analysis of variance was calculated with the MANCOVA module, and correlations with the main module of the software.

Results

Chemical analysis by ICP-MS

Detection limits for all analyzed elements according to the performed data reduction are given in Table 1.

All 40 bottles used for liquid sample preparation were analyzed to determine their ion release before the first use (data set 0, pure water control investigation; see ESM S2). Measurable quantities of ^{11}B, ^{24}Mg, ^{28}Si, and ^{44}Ca were found (Table 1: data set 0). Since no outlier was observed, all bottles were used for sample preparation.

With the exception of ^{23}Na, the amount of all trace elements decreased in the subsequent quartz, sulfur, and copper sulfate sample series (Table 1: data sets I–III). No outliers or contamination were observed; none of the samples exceeded the concentration of 10 ppb for any of the elements. In addition, no systematic differences between homeopathic preparations and controls were observed.

T_1 and T_2 relaxation time measurements at 600 MHz

Three sample sets were prepared for measurement: (1) set I, quartz (21 dilution levels of quartz and 10 controls); (2) set II, sulfur (18 dilution levels of sulfur and 10 controls); and (3) set III, copper sulfate (20 dilution levels of copper sulfate and 10 controls).

For each sample, three capillaries (nos. 1, 2, 3) were included for T_1 and T_2 measurements. Within one sample set, all capillaries no. 1 were measured first, then all capillaries no. 2, and finally all capillaries no. 3. All data of capillaries no. 1 form the data subset no. 1; data subsets no. 2 and no. 3 are defined analogously. Within the subsets, samples were measured in randomized order. Measurements of an entire set (18–21 homeopathic preparations and 10 controls) were accomplished within 210–310 h after sealing, with about 100 h of net measurement time. Average sample volume was 9.8±1.1 µl (mean ± SD). There were no statistically significant differences in sample volume between homeopathic preparations and corresponding controls (set I, quartz preparations: $t_{Df=87}=-1.59$, $p=0.115$; set II, sulfur preparations: $t_{Df=81}=-1.25$, $p=0.215$; set III, copper sulfate preparations: $t_{Df=86}=1.01$, $p=0.316$).

Table 1 Elements analyzed by ICP-MS for all four data sets of the study

Element	Detection limit [ppb]	Set 0: bottle control	Set I: quartz		Set II: sulfur		Set III: copper sulfate	
		Controls (n=40)	Controls (n=10)	Potencies (10c–30c, n=21)	Controls (n=10)	Potencies (13x–30x, n=18)	Controls (n=10)	Potencies (11c–30c, n=20)
^{7}Li	0.12 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{11}B	0.95 ± 0.95	1.54 ± 1.05	1.31 ± 0.69	1.57 ± 0.39	1.16 ± 0.41	1.23 ± 0.28	n.d.	n.d.
^{23}Na	1.06 ± 0.86	n.d.	3.09 ± 0.52	3.19 ± 0.31	2.37 ± 0.36	2.62 ± 0.59	1.76 ± 0.22	1.90 ± 0.35
^{24}Mg	0.52 ± 0.35	0.62 ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{27}Al	1.16 ± 1.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{28}Si	1.24 ± 1.18	2.26 ± 1.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{44}Ca	0.61 ± 0.40	2.06 ± 0.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{48}Ti	0.20 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{56}Fe	0.16 ± 0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{65}Cu	0.21 ± 0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{66}Zn	0.28 ± 0.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{85}Rb	0.09 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{88}Sr	0.09 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{133}Cs	0.08 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{137}Ba	0.09 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{208}Pb	0.08 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Detection limit is given as mean of all ICP-MS runs (± standard deviation). Elementary composition was calculated for the controls and homeopathic preparations as mean of all corresponding samples (± standard deviation). All values are given in ppb=10^{-9}

n.d. below detection limit

The experimental procedure and the relevant NMR parameters were optimized with respect to the measurement error (given by the exponential fit function from the Varian software), which could be reduced to average values of (1.2±0.7)‰ (mean ± SD, n=259) for T_1 and (2.2±0.5)‰ (mean±SD, n=260) for T_2. Test–retest variability of an identical sample in place was 0.35‰ (standard deviation, n=5) for T_1.

An overview about all data measured as a function of time after sealing of the NMR samples (capillaries) is given in Fig. 1. T_1 was between 2.85 and 3.20 s, T_2 between 0.45 and 0.55 s. T_1 and T_2 relaxation times generally increased in course of time. In all three data sets, a single capillary from a control sample was measured several times. The mean increase of T_1 and T_2 of all three repeatedly measured control samples was 14±6 ms/day for T_1 and 1.3±1.3 ms/day for T_2 (mean ± SD).

T_1 and T_2 data from each sample set (I quartz, II sulfur, III copper sulfate) were statistically analyzed with a two-way analysis of variance (ANOVA) with two independent factors: (2) preparation (control, homeopathic preparation) and (b) capillary subset (nos. 1, 2, 3). Results are given in Table 2 and Fig. 2. The increase in T_1 as a function of time is reflected in the statistically significant F tests for the factor "capillary subset" (Table 2) and in the differences between the group means for the capillary subsets (nos. 1, 2, and 3 in Fig. 2a–c). The general increase of T_2 as a function of time was not as strong (Fig. 2d–f) and statistically significant in the sulfur data set only (Table 2). We did not observe statistically significant differences between homeopathic preparations and the controls (factor "preparation" in Table 2). T_1 and T_2 relaxation times as a function of the dilution step are given in the Electronic supplementary material S3 and S4.

The correlation coefficients between the relaxation time data for the three different capillary subsets are given in the Electronic supplementary material S5; corresponding graphics can be found in ESM S6 and S7. In 15 out of 18 comparisons, the correlation was stronger for the control samples (compared to the homeopathic preparations).

T_1 and T_2 were either negatively correlated or not correlated (table in ESM S8 and graphics in ESM S9). No clear pattern emerged with respect to a difference between homeopathic preparations and controls or between homeopathic preparations.

T_1 relaxation time measurement at 500 MHz

The samples of set II (sulfur preparations and controls, three capillaries for each sample) were measured again at a

different frequency of 500 MHz 1 year after preparation (T_1 only). Each capillary was measured three times. The average measurement error was 2.6‰ (calculated as the mean of the standard deviation of the three measurements of all 80 capillaries).

Data were statistically analyzed with a two-way ANOVA with two independent factors: (a) preparation (control, homeopathic preparation) and (b) capillary subset (nos. 1, 2, and 3) and the dependent variable T_1. Results are given in Table 3 ("all data") and Fig. 3. The difference between the three capillary subsets was statistically significant, but smaller than in the first measurement series at 600 MHz (cf., Table 2 and Fig. 2b). There were statistically significant differences between the homeopathic sulfur preparations and the controls (p=0.0396, Table 3). T_1 of the homeopathic preparations was on average 0.88% higher than T_1 of the controls. The interaction between the factors preparation and capillary was not significant (p=0.71), i.e., the difference between homeopathic preparations and controls seemed to be consistent over all three subsets of capillaries. Three samples had a measurement error larger than 1% (cf., Fig. 3a). One of the three single measurements was clearly deviating from the other two in all cases. Elimination of the outliers did not change the results substantially (Table 3 "without outliers").

The mean correlation coefficient between the relaxation time data for the three different capillary subsets was 0.339 for the homeopathic preparations and 0.357 for the controls. Detailed calculations and graphics can be found in the Electronic supplementary material S10.

The correlation of the T_1 mean values of the measurements at 600 and 500 MHz is r=0.385 (p=0.043, n=28). The correlation is better for the controls (r=0.407, p=0.244, n=10) than for the homeopathic preparations (r=

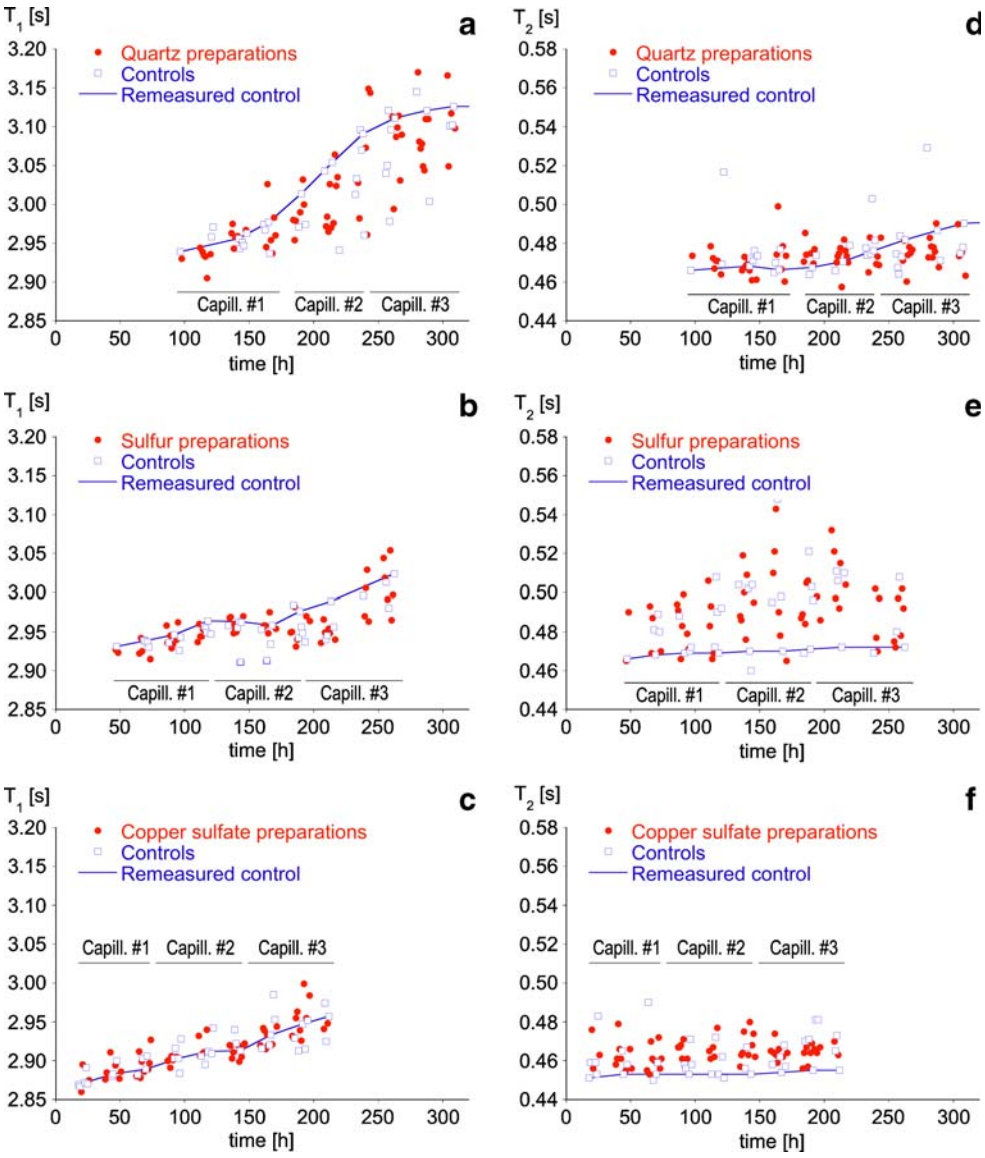

Fig. 1 Relaxation times T_1 (seconds; *left*) and T_2 (seconds; *right*) at 600 MHz for homeopathic preparations of quartz (**a**, **d**), sulfur (**b**, **e**), and copper sulfate (**c**, **f**) and corresponding controls (independent samples of analogously agitated potentization medium) as a function of time after capillary sealing (hours). Measurement error is on average 1‰ for T_1 and 2‰ for T_2 (smaller than the icons used). For each sample, three independent capillaries were measured. All capillaries of subset no. 1 were measured first, then those of subset no. 2, and finally subset no. 3. One capillary of a control sample was measured several times ("remeasured control")

Table 2 Results of analysis of variance (ANOVA) with two independent factors: (a) capillary subset (nos. 1, 2, and 3) and (b) preparation (control, homeopathic preparation)

Sample set	Effect	Df effect	Df error	*F*	*p* level
I: Quartz T_1	Capillary subset	2	83	61.9969	<0.0001
	Preparation	1	83	0.1992	0.6565
	Interaction	2	83	0.6940	0.5024
I: Quartz T_2	Capillary subset	2	83	1.2494	0.2920
	Preparation	1	83	2.8882	0.0930
	Interaction	2	83	0.0400	0.9608
II: Sulfur T_1	Capillary subset	2	77	13.5757	<0.0001
	Preparation	1	77	2.9909	0.0877
	Interaction	2	77	1.2059	0.3050
II: Sulfur T_2	Capillary subset	2	77	7.6398	0.0009
	Preparation	1	77	0.7523	0.3884
	Interaction	2	77	0.4501	0.6393
III: Copper sulfate T_1	Capillary subset	2	82	59.6562	<0.0001
	Preparation	1	82	0.0247	0.8754
	Interaction	2	82	0.2772	0.7586
III: Copper sulfate T_2	Capillary subset	2	82	1.6831	0.1922
	Preparation	1	82	0.1018	0.7505
	Interaction	2	82	3.1155	0.0496

Dependent variables are T_1 or T_2 obtained from measurements in Tallahassee at 600 MHz of the three data sets investigated (I: quartz, II: sulfur, III: copper sulfate)

0.253, p=0.312, n=18). Detailed calculations can be found in the Electronic supplementary material S11.

Discussion

The main focus of this investigation was the question, whether any difference in T_1 and/or T_2 relaxation times of homeopathic preparations and corresponding controls can be observed. If this were the case, the next question would be how these differences may be explained: Can the latter be reduced to trivial physicochemical artifacts or do they point to specific properties of homeopathic preparations (i.e., properties relating to the substance diluted even though none of its molecules can be expected to be present)?

With regard to the first question, we observed two major features of our data sets:

1. There was a small (< 1%) increase in T_1 relaxation times for homeopathic sulfur preparations (compared to the controls) in the second measurement series (at 500 MHz in Zurich) 1 year after sample preparation (p=0.040). In the first series of measurements (at 600 MHz in Tallahassee), there was no significant effect (p=0.088). The difference between homeopathic sulfur preparations and controls seemed to increase in the course of time.
2. Correlations were in many cases higher for the control samples than for the homeopathic preparations. This concerns the following correlations: (a) between T_1 data of different capillaries (NMR measurement vessels) of the same sample measured within one series (in Tallahassee or in Zurich), (b) between T_2 data of different capillaries of the same sample measured within one series (Tallahassee only), (c) between T_1 data of the same samples measured in Tallahassee and in Zurich.

An important cofactor that needs to be discussed in any interpretation of these features is the general increase of T_1 (and partially T_2) relaxation times as a function of time.

What are factors that influence T_1 and T_2 relaxation times and that might furnish an explanation of the phenomena observed? First, one has to carefully consider all types of unintended side effects such as: (1) contamination with dust, (2) leached substances from the dilution vessel walls, (3) varying ethanol content, (4) leached substances from the measurement vessel walls (capillaries), (5) microorganisms growing in the solutions, (6) contamination with flame gases, (7) differences in pH, (8) traces of the substance diluted, (9) paramagnetic oxygen (O_2), and (10) other paramagnetic substances. If all these factors can be ruled out, one may discuss specific physicochemical explanations involving (11) dipolar ^{1}H spin coupling and (12) scalar spin–spin coupling (quadrupole relaxation can be ruled out since ^{1}H has no electric quadrupole moment). All these possible explanations are discussed in detail in the Electronic supplementary material S12. The relevant results can be summarized below.

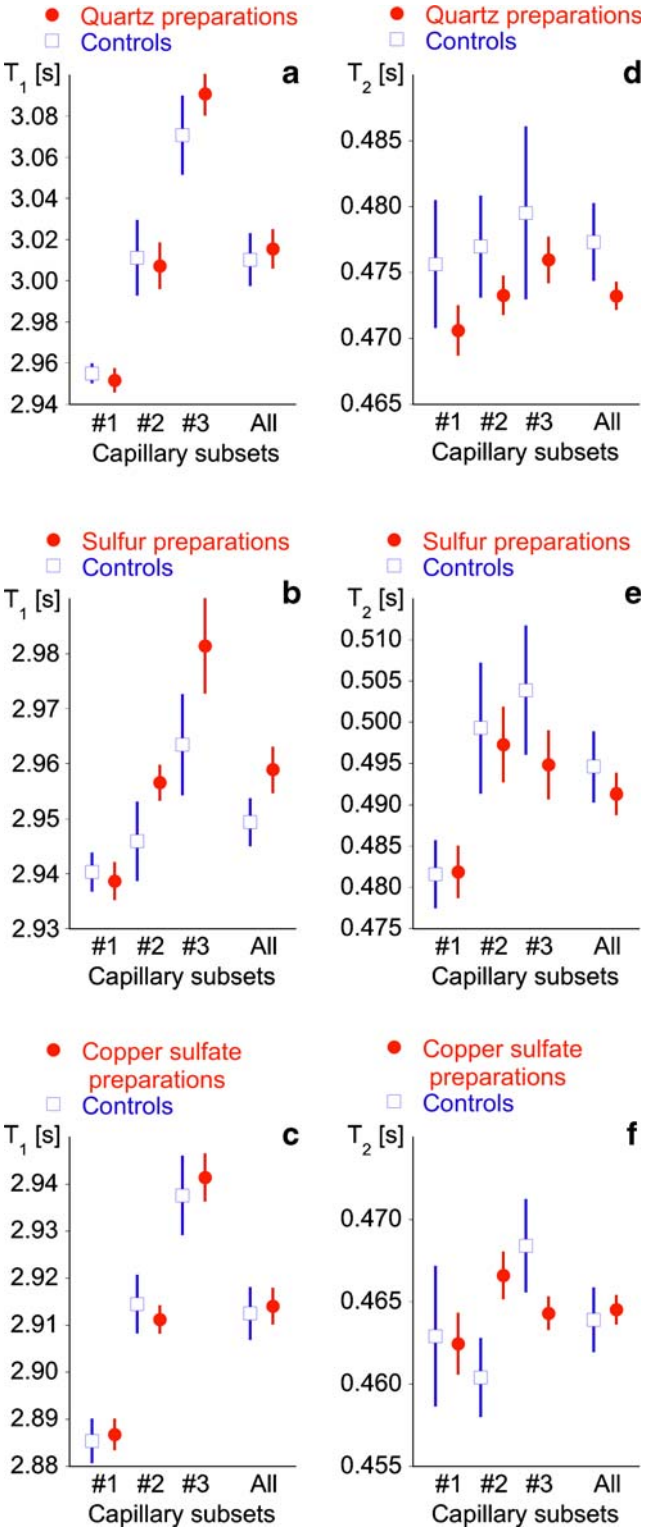

Fig. 2 Mean relaxation times T_1 (seconds; *left*) and T_2 (seconds; *right*) at 600 MHz for homeopathic preparations of quartz (**a**, **d**), sulfur (**b**, **e**), and copper sulfate (**c**, **f**) and corresponding controls (independent samples of analogously agitated potentization medium) as a function of the three capillary subsets and of the entire data set (mean±standard error)

The general increase of T_1 in the course of time is most probably due to degassing of paramagnetic oxygen (O_2) from the sample fluid into the capillary void after sealing. However, there were no statistically significant differences in capillary filling volume between homeopathic preparations and corresponding controls. Thus, randomization was successful, and differences in capillary filling height leading to different O_2 degassing cannot be responsible for the differences in T_1 between homeopathic sulfur preparations and controls.

Disturbing influences of some of the other "unwanted" side effects (nos. 1–8 and 10 of above) cannot be excluded, though we aimed at minimizing all these effects. According to our considerations, artifacts due to factors nos. 1, 2, 3, 7, 8, and 10 (contamination with dust during sample preparation; leaching from the dilution vessel walls; varying ethanol content; changes in pH; traces of the substance diluted; effects of paramagnetic substances other than O_2) are either improbable or negligible, while an influence due to factors no. 1, 4, 5, or 6 (contamination with dust due to the use of capillaries as NMR measurement vessels, which could not be cleaned with reasonable effort; leaching from the capillary vessel walls; microbial contamination; contamination with flame gases) can be neither excluded nor quantitatively estimated. In any case, however, a *systematic* error—i.e., a systematic difference between control samples and homeopathic preparations—seems improbable due to the blinded sample handling and measurement, the randomized dilution and measurement vessel allocation, and the randomized measurement sequence.

Assuming that all systematic errors can be excluded, we are left with a variety of explanations, which might be responsible for the differences between control samples and homeopathic sulfur preparations.

One can raise the hypothesis that homeopathic sulfur preparations influence leaching and/or silica hydrogel formation. One indication toward this direction can be found in the investigation of Demangeat et al. (2004) who measured a 10% excess of Si in homeopathic preparations of SiO_2 compared to potentized dilution medium. The observation that different correlations were in many cases higher (better) for the control samples than for the homeopathic preparations is also compatible with the hypothesis that homeopathic preparations interact with some leaching process.

If one wants to explain the T_1 increase for homeopathic sulfur preparations in the context of dipolar ^1H spin coupling (for a detailed discussion, see ESM S12), we see in principle the following free parameters: (a) the rotational correlation time τ_c, (b) the intramolecular spin–spin distance b, (c) the density of spins N, and (d) the self-diffusion coefficient of water D. In order to obtain an increase in T_1 for homeopathic sulfur preparations, a

Table 3 Results of analysis of variance (ANOVA) with two independent factors: (a) capillary subset (nos. 1, 2, and 3) and (b) preparation (control, homeopathic preparation)

Sample set	Effect	Df effect	Df error	*F*	*p* level
I: Sulfur T_1 (all data)	Capillary subset	2	74	6.4545	0.0026
	Preparation	1	74	4.3899	0.0396
	Interaction	2	74	0.3383	0.7141
I: Sulfur T_1 (without outliers)	Capillary subset	2	74	7.6135	0.0010
	Preparation	1	74	3.9868	0.0495
	Interaction	2	74	0.0992	0.9057

Dependent variable is T_1 from the sulfur data set, obtained from measurements in Zurich at 500 MHz 1 year after sample preparation

decrease in τ_c or N or an increase in b or D is needed (of all parameters, the intramolecular spin–spin distance b has the strongest influence on T_1 because it influences T_1 in the sixth potency). This essentially corresponds to an increase in molecular rotational and/or translational motion or a decrease in density.

All these hypotheses are quite unconventional, but scientific in the sense that they are amenable to empirical testing and thus also to falsification.

Conclusions

There are a number of conclusions that can be drawn from this study. In order to securely exclude unintended artifacts (e.g., influences from the dilution or measurement vessels) and also to achieve a precise characterization of single dilution levels, it will be necessary to replicate the findings in several independent production lines, as done, e.g., by Demangeat et al. (1992) and Demangeat et al. (2004). In such replications, it would be favorable to use larger measurement vessels, which can be easily cleaned, or a larger number of capillaries in order to better randomize any side effects. In this context, a comparison between the use of caps or sealing (both closure methods have advantages and drawbacks) could be performed. In order to facilitate the interpretation and to reduce side effects, it would be better to simplify the system and to use preparations of pure water only (without ethanol). As a consequence, it would be advantageous to work entirely under sterile conditions and easier to use lower field strengths. In addition, the effects of O_2 removal should be evaluated.

It is of ultimate importance to use control samples of analogously shaken potentization medium in order to be able to exclude unspecific effects (i.e., not related to the initial mother tincture) such as potentization vessel wall leaching, plasticizer release from pipette tips, air suspension/dissolution (also leading to pH changes), and radical formation due to cavitation. Analogously, controls in future studies should be comparable regarding lactose content. In addition, blinding and randomization are very important to exclude any other systematic errors.

Complementary investigations necessary or useful for relaxation time data interpretation would involve the measurement of macroscopic physical characteristics such as density and viscosity. The latest and exhaustive review of Becker-Witt et al. (2003) does not list any such investigations. Measurements of the self-diffusion constant D by NMR experiments involving magnetic field gradients

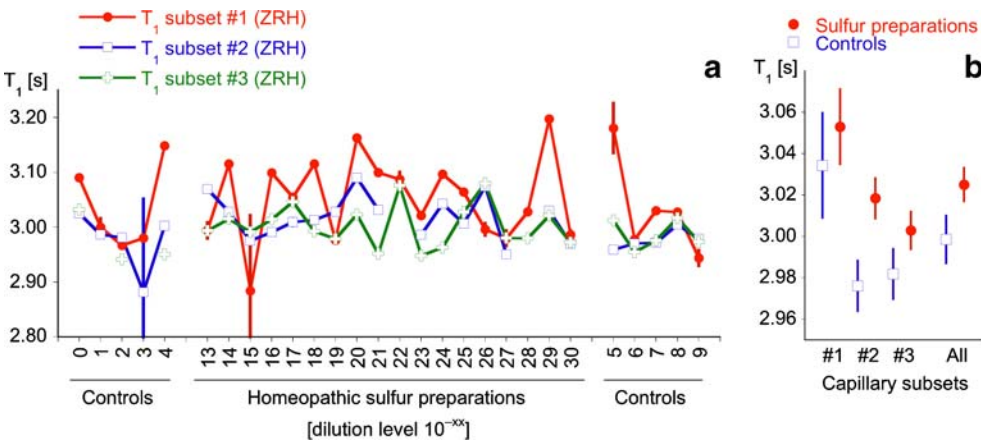

Fig. 3 Relaxation time T_1 (seconds) at 500 MHz (ETH Zurich) for homeopathic preparations of sulfur and corresponding controls: measurement of samples of set II 1 year after preparation (mean±standard error). For each sample, three independent capillaries were measured (*left side*, **a**; mean of three measurements) and the corresponding average calculated (*right side*, **b**; as a function of the three capillary subsets (nos. 1, 2, and 3) and of the entire data set)

could help to separate rotational from translational motion effects. The hypothesis of enhanced leaching through homeopathic potencies could be easily tested in experiments with glass particles at higher temperatures.

Our observation of increased T_1 relaxation times for homeopathic sulfur preparations corresponds approximately to the result of the study of Demangeat et al. (1992), who investigated homeopathic preparations of SiO_2 and analogously potentized dilution medium and observed elevated T_1 relaxation times at 4 MHz for the preparations SiO_2 6*c*, 9*c*, 12*c*, and 15*c* in a similar order of magnitude (1–2%). No difference was seen in T_2 relaxation time. Demangeat et al. (2004) observed similar effects concerning T_1 in a recent study for other frequency ranges (0.02–4 MHz) for SiO_2 15*c* and 21*c*. According to the authors, the elevated T_1 values might suggest decreased correlation times τ_c and correspondingly increased molecular motion of the water molecules in the homeopathic preparations. This interpretation may remind of the original idea of Hahnemann (1921) who suggested that homeopathic procedures lead to a "dynamization" of the remedy.

Recent investigations of Anick (2004) and Aabel et al. (2001) with high-field ^{1}H NMR spectroscopy did not yield any evidence for *stable* water clusters (life span > ms) within liquid homeopathic remedies. These results do not contradict the hypothesis of a decrease in translational or rotational correlation time because the latter characterize water molecule *dynamics*. They are also not in conflict with the leaching hypothesis or presumed density changes.

Given the fact that there are many double-blind clinical trials suggesting specific effects of homeopathic preparations (for references, see "Introduction"), it seems very interesting, important, and challenging to us to further explore possible specific physicochemical characteristics of homeopathic preparations. Can science arrive at firm and trustworthy experimental evidence that homeopathic preparations bear specific properties, i.e., properties relating to the substance diluted even though no more of its molecules can be expected to be present? And if yes, how could such "memory effects" be explained? In all investigations, however, great attention must be given to any possible spurious effects in order not to misinterpret experimental data.

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References

Aabel S, Fossheim S, Rise F (2001) Nuclear magnetic resonance (NMR) studies of homeopathic solutions. Br Homeopath J 90:14–20

Abragam A (1961) Principles of nuclear magnetism. Oxford University Press, Oxford

Anick DJ (2004) High sensitivity 1H-NMR spectroscopy of homeopathic remedies made in water. BMC Complementary and Alternative Medicine 4:15

Anonymous (2004) Homöopathisches Arzneibuch 2004 (HAB). Deutscher Apotheker Verlag, Stuttgart

Atmanspacher H, Römer H, Walach H (2002) Weak quantum theory: complementarity and entanglement in physics and beyond. Found Phys 32:379–406

Barnes J, Resch KL, Ernst E (1997) Homeopathy for postoperative ileus? A meta-analysis. J Clin Gastroenterol 25:628–633

Bastide M, Lagache A (1997) A communication process: a new paradigm applied to high-dilution effects on the living body. Alternative Therapies in Health and Medicine 3:35–39

Baumgartner S, Heusser P, Thurneysen A (1998) Methodological standards and problems in preclinical homoeopathic potency research. Forschende Komplementärmedizin 5:27–32

Becker-Witt C, Weisshuhn TER, Lüdtke R, Willich SN (2003) Quality assessment of physical research in homeopathy. J Altern Complement Med 9:113–132

Bloembergen N, Purcell EM, Pound RV (1948) Relaxation effects in nuclear magnetic resonance absorption. Phys Rev 73:679–712

Cazin JC, Cazin M, Chaoui A, Belon P (1991) Influence of several physical factors on the activity of ultra low doses. In: Doutremepuich C (ed) Ultra low doses. Taylor & Francis, London, pp 69–80

Demangeat JL, Demangeat C, Gries P, Poitevin B, Constantinesco A (1992) Modifications des temps de relaxation RMN à 4 MHz des protons du solvant dans les très hautes dilution salines de silice/lactose. Journal de médecine nucléaire et biophysique 16:135–145

Demangeat JL, Gries P, Poitevin B, Droesbeke JJ, Zahaf T, Maton F, Piérart C, Muller RN (2004) Low-field NMR water proton longitudinal relaxation in ultrahighly diluted aqueous solutions of silica-lactose prepared in glass material for pharmaceutical use. Appl Magn Reson 26:465–481

Elia V, Niccoli M (2000) New physico-chemical properties of water induced by mechanical treatments. A calorimetric study at 25°C. J Therm Anal Calorim 61:527–537

Franks F (ed) (1972) Water—a comprehensive treatise. Plenum, New York

Funk W, Dammann V, Vonderheid C, Oehlmann G (1985) Statistische Methoden in der Wasseranalytik. Weinheim, VCH Verlagsgesellschaft

Funk W, Dammann V, Donnevert G (1992) Qualitätssicherung in der Analytischen Chemie. Weinheim, VCH Verlagsgesellschaft

Hahnemann S (1921) Organon der Heilkunst (6. Auflage). Heidelberg

Jacobs J, Jonas WB, Jimenez-Perez M, Crothers D (2003) Homeopathy for childhood diarrhea: combined results and metaanalysis from three randomized, controlled clinical trials. Pediatr Infect Dis J 22:229–234

Jonas WB, Linde K, Ramirez G (2000) Homeopathy and rheumatic disease. Rheum Dis Clin North Am 26:117–123

Kratky KW (2004) Homöopathie und Wasserstruktur: Ein physikalisches Modell. Forsch Komplentarmed Klass Naturheilkd 11:24–32

Lasne Y (1986) Propriétés des Solutions "Homéopathiques"—Mesure da la Relaxation Magnétique T2. Université Claude Bernard, Lyon

Lüdtke R, Wiesenauer M (1997) Eine Metaanalyse der homöopathischen Behandlung der Pollinosis mit Galphimia glauca. Wien Med Wochenschr 147:323–327

Milgrom LR (2002) Patient-practitioner-remedy (PPR) entanglement. Part 1: a qualitative, non-local metaphor for homeopathy based on quantum theory. Br Homeopath J 91:239–248

Rey L (2003) Thermoluminescence of ultra-high dilutions of lithium chloride and sodium chloride. Physica A 323:67–74

Taylor MA, Reilly D, Llewellyn-Jones RH, McSharry C, Aitchison TC (2000) Randomised controlled trial of homoeopathy versus placebo in perennial allergic rhinitis with overview of four trial series. Br Med J 321:471–476

Vickers AJ, Smith C (2006) Homoeopathic Oscillococcinum for preventing and treating influenza and influenza-like syndromes. Cochrane Database Syst Rev 3: CD001957

Weingärtner O (1990) NMR-Features That Relate To Homoeopathic Sulphur-Potencies. The Berlin Journal on Research in Homoeoepathy 1:61–68

Weingärtner O (2003) What is the therapeutically active ingredient of homeopathic potencies? Homeopathy 92:145–151