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Comparison of homeopathic globules prepared from high and ultra-high dilutions of various starting materials by ultraviolet light spectroscopy

Sabine D. Klein *, Ursula Wolf

Institute of Complementary Medicine, University of Bern, 3012 Bern, Switzerland

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ABSTRACT

Objective: Homeopathic globules are commonly used in clinical practice, while research focuses on liquid potencies. Sequential dilution and succussion in their production process has been proposed to change the physico-chemical properties of the solvent(s). It has been reported that aqueous potencies of various starting materials showed significant differences in ultraviolet light transmission compared to controls and between different dilution levels. The aim of the present study was to repeat and expand these experiments to homeopathic globules.

Methods: Globules were specially produced for this study by Spagyros AG (Gümligen, Switzerland) from 6 starting materials (*Aconitum napellus*, *Atropa belladonna*, phosphorus, sulfur, *Apis mellifica*, quartz) and for 6 dilution levels (6x, 12x, 30c, 200c, 200CF (centesimal discontinuous fluxion), 10,000CF). Native globules and globules impregnated with solvents were used as controls. Globules were dissolved in ultrapure water, and absorbance in the ultraviolet range was measured. The average absorbance from 200 to 340 nm was calculated and corrected for differences between measurement days and instrumental drift.

Results: Statistically significant differences were found for *A. napellus*, sulfur, and *A. mellifica* when normalized average absorbance of the various dilution levels from the same starting material (including control and solvent control globules) was compared. Additionally, absorbance within dilution levels was compared among the various starting materials. Statistically significant differences were found among 30c, 200c and 200CF dilutions.

Conclusion: This study has expanded previous findings from aqueous potencies to globules and may indicate that characteristics of aqueous high dilutions may be preserved and detectable in dissolved globules.

1. Introduction

Highly diluted remedies are applied in homeopathy and anthroposophically extended medicine. Several modes of action and models have been discussed on how the production of these aqueous solutions by sequential dilution and succussion, also termed potentization, might feature their structural properties.[1] It has been proposed that a network of hydrogen bonds develops around non-polar solutes that remains with successive dilution, even when the molecules of the starting material have disappeared,[2,3] thus giving the water a more organized state.[4] Additionally, nanobubbles have been discussed to contribute to these supramolecular structures,[5] which are assumed to disappear upon heating.[4]

Differences in the structure of the water in these homeopathic preparations may be reflected in small but measurable changes in physico-chemical properties. Accordingly, methods such as ultraviolet (UV) spectroscopy,[2,6–12] nuclear magnetic resonance spectroscopy,[4,13–17] calorimetry[18] or thermoluminescence[19,20] have been employed to investigate possible differences between homeopathic preparations and respective controls.

It has been found that dilution and succussion can lead to the introduction of contaminants, e.g., trace elements such as Si, Li, Na, Mg.[9,21] This can be avoided when dilutions are prepared very carefully (e.g., by washing all flasks and pipettes with the same solution used to prepare the dilutions) or under clean room conditions.[16,22]

* Corresponding author at: Institute of Complementary Medicine, University of Bern, Fabrikstrasse 8, 3012 Bern, Switzerland. Fax: +41 31 6324262.
E-mail addresses: sabine.klein@ikom.unibe.ch, kontakt@sabineklein.ch (S.D. Klein).

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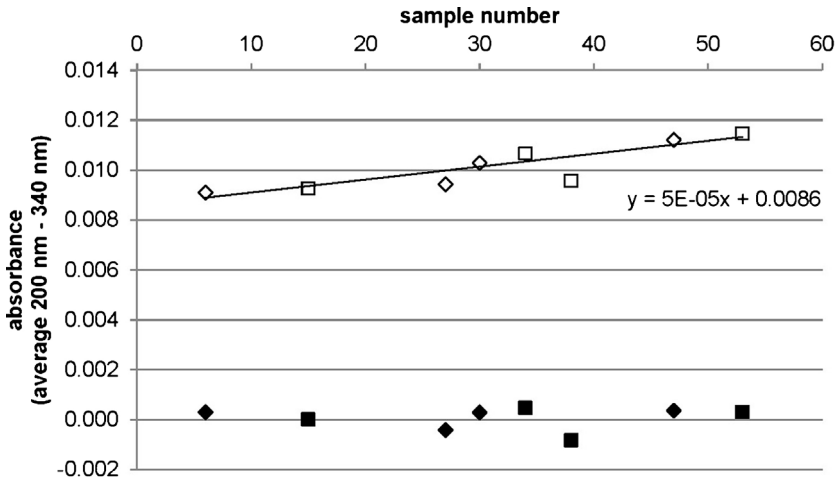

Fig. 1. Obtaining normalized absorbance by correcting for instrumental drift.
A typical example from one measurement day is shown. Diamonds represent native globules, and squares represent solvent control globules. Open symbols show the average absorbance from 200 nm to 340 nm. Samples were measured in a randomized order, and plotting absorbance versus sample number revealed an instrumental drift over time. The intercept of the linear regression (measure for the differences between measurement days) and the slope (measure for the instrumental drift) were used to correct the values, yielding the normalized absorbance (filled symbols). The same equation was then used to normalize the absorbance of the various dilution levels measured on the same day (not shown). A normalized absorbance can thus have positive or negative values.

This is crucial for experiments testing for differences between high dilutions and controls. Our group reported that high and ultra-high dilutions of various starting materials showed significant differences in UV light transmission to controls and between different dilution levels, all the more so when these dilutions were produced under controlled conditions.[10–12]

In clinical practice, globules of potentized starting materials are commonly applied besides aqueous dilutions. In a preliminary study, we found differences in UV absorbance between verum and placebo globules of *Aconitum napellus* 30c or calcium carbonate/quercus e cortice 6x dissolved in water.[23] These globules had been produced for clinical trials and not for laboratory experiments. It was unclear whether the differences in UV absorbance originated from specific characteristics of the starting materials, from differences in the production of verum and placebo globules, and/or other unknown interference factors.

Therefore, the aim of the present study was to repeat and expand our previous experiments with globules produced under controlled conditions accurate for comparison of UV absorbance, i.e., sucrose globules and ethanol from the same batch were used to minimize the introduction of possible artifacts.

2. Methods

2.1. Globules

The globules were specially produced for this study by Spagyros AG (Gümligen, Switzerland) and differed only in the starting materials of the potentized dilutions. Sucrose globules and ethanol from the same batch were used to minimize the introduction of possible artifacts. The following starting materials were investigated: *A. napellus*, *Atropa belladonna*, phosphorus, sulfur, *Apis mellifica*, and quartz (2 plants, 2 non-metal elements, 1 animal, and 1 mineral).

Dilution was performed in a 43% ethanol/57% water mixture until the last step, for which 73% ethanol/27% water was used. Manual succussion was performed vertically with 30 strokes in each step.

200CF and 10,000CF dilutions were produced by discontinuous fluxion in a machine with water as dilution medium. In this single glass technique, the potentisation vessel is alternately filled with the dilution medium and emptied again in each step, while a defined amount of liquid remains in the vessel. The operating mode of the machine has been described.[24] The final three steps were manually diluted in 30% ethanol/70% water, 43% ethanol/57% water and 73% ethanol/27% water and succussed with 30 strokes.

Two kinds of controls were generated in the experiments: native globules and globules impregnated with a succussed 73% ethanol/27% water mixture (solvent control globules).

The globules were produced in July 2012 and the measurements were carried out from September 2012 to March 2013. Vials were coded to display the starting material but the dilution level and the controls were blinded. Coding was unblinded only after completion of all measurements and preliminary description of the data by box plots. The vials were stored in aluminum boxes, each box containing the same dilution level of the 6 starting materials.

2.2. UV absorbance measurements

Globules were gently dissolved in ultrapure water (arium® pro VF, Sartorius Stedim AG, Goettingen, Germany) at 10 mg/ml in Fiolax® test tubes. Samples were prepared in quadruplicates, 19–22 h prior to the measurements to allow complete dissolution, wrapped individually in aluminum foil and stored in the dark at room temperature. Absorbance of the samples in the UV range (from 190 to 340 nm) was measured in a randomized order with a Shimadzu UV-1800 double beam spectrophotometer (Reinach, Switzerland) equipped with an auto sampler CETAC ASX-260 (Omaha, USA; as described previously in Ref.[10]). This wavelength range has previously proven to be better suited to detect differences than visible light or near infrared light.[25] The spectrophotometer had been switched on 2 h prior to the measurements for sufficient warming up. Globules of each starting material and corresponding controls were freshly dissolved and measured on 5 independent days.

2.3. Data analysis

Due to the high absorbance of sucrose below 200 nm, the main component of the globules used, only absorbance values at 200 nm and higher were included in the analysis and one value for each nm was recorded. For each sample the mean of the absorbance values from 200 nm to 340 nm was calculated. Fig. 1 shows how