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ORIGINAL PAPER

Polycrystalline structures formed in evaporating droplets as a parameter to test the action of *Zincum metallicum* 30c in a wheat seed model

Maria Olga Kokornaczyk[1,*], Stephan Baumgartner[2,3] and Lucietta Betti[1]

[1]*Department of Agricultural Sciences, University of Bologna, Viale Fanin 44, Bologna 40127, Italy*
[2]*Society for Cancer Research, Kirschweg 9, 4144 Arlesheim, Switzerland*
[3]*Institute of Integrative Medicine, University of Witten-Herdecke, Gerhard-Kienle-Weg 4, 58313 Herdecke, Germany*

Background: **Polycrystalline structures formed inside evaporating droplets of different biological fluids have been shown sensitive towards various influences, including ultra high dilutions (UHDs), representing so a new approach potentially useful for basic research in homeopathy. In the present study we tested on a wheat seed model *Zincum metallicum* 30c efficacy versus lactose 30c and water.**
Materials and methods: **Stressed and non-stressed wheat seeds were watered with the three treatments. Seed-leakage droplets were evaporated and the polycrystalline structures formed inside the droplet residues were analyzed for their local connected fractal dimensions (LCFDs) (measure of complexity) using the software *ImageJ*.**
Results: **We have found significant differences in LCFD values of polycrystalline structures obtained from stressed seeds following the treatments ($p < 0.0001$); *Zincum metallicum* 30c lowered the structures' complexity compared to lactose 30c and water. In non-stressed seeds no significant differences were found.**
Conclusions: **The droplet evaporation method (DEM) might represent a potentially useful tool in basic research in homeopathy. Furthermore our results suggest a sensitization of the stressed model towards the treatment action, which is conforming to previous findings.** *Homeopathy* (2016) **105**, 173–179.

Keywords: Ultra high dilutions (UHDs); Droplet evaporation method (DEM); Crystallization; *Zincum metallicum*; Wheat seeds

Introduction

Generally, experimental models applied in basic research regarding the efficacy of ultra high dilutions (UHDs) can be divided into physical and biological models. Physical models mainly concentrate on studies regarding water structure and how this structure gets modified following the subsequent dilution and succussion steps applied during the UHD preparation process.[1–4] Complementary, biological models[5–14] study the influence of UHDs on living organisms, and include *in-vitro* models involving e.g. cell-cultures[6,7] or plants[8–12] and *in-vivo* models involving animals.[13,14] In biological models, as an efficacy measure of the tested UHD different parameters get measured, serving for the comparison of the treated experimental conditions vs. control groups. In most cases the choice of such parameters depends on the experimental model and kind of organism it involves, as also on the tested UHD and its expected action on the organism. For instance, in *in-vitro* studies on basophils treated with UHD of

*Correspondence: Maria Olga Kokornaczyk, Department of Agricultural Sciences, University of Bologna, Viale Fanin 44, Bologna 40127, Italy.
E-mail: maria.kokornaczyk@unibo.it, Stephan.Baumgartner@uni-wh.de, lucietta.betti@unibo.it
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histamine, basophil activation has been found to respond to the UHD's action[7]; whereas in studies on a plant model involving duckweed treated with UHD of a plants growth hormone (gibberellic acid), growth parameters (e.g. the foliar surface) represented the proper parameter.[8]

Recently some studies appeared proposing polycrystalline structures formed during evaporation induced crystallization processes as a new parameter able to assess UHDs efficacy. It has been shown in two different plant models that UHD treatments have modified the polycrystalline structures obtained from evaporating solutions prepared from treated plants vs. controls.[15–17] In particular, in[15,16] wheat seeds, previously stressed with a ponderal concentration of arsenic trioxide (As_2O_3), were watered with arsenic trioxide 45th decimal dilution (As45x) or water as control; subsequently, the wheat seed leakage were analyzed by means of the droplet evaporation method (DEM).[18,19] During evaporation polycrystalline structures are formed, which complexity degree, measured by means of the local connected fractal dimension (LCFD), differed significantly between the As45x treated seeds and controls.

As reported in,[16] the LCFD of the polycrystalline structures showed to be also sensitive towards the number of strokes applied following each dilution step during the As45x preparation: As_2O_3 pretreated seeds watered with As45x treatments prepared with growing stroke numbers created polycrystalline structures characterized by significantly higher LCFD values. Furthermore, positive correlations found between the LCFD values and germination tests indicate that LCFD might be sensitive to the seeds vitality. In[17] instead, four days old cress seedlings watered with *Stannum metallicum* 30c or water as control were analyzed by means of the copper chloride crystallization method.[17,20–22] The experimentation was performed in two independent laboratories. The results showed that 15 second-order texture variables of the resulting crystalline patterns differentiated significantly the two experimental conditions.

In addition to plants, the copper chloride crystallization method may be also applied on blood. There are some older studies indicating that this method may revel differences in blood from a cancer patient with addition of low doses of plant extracts vs. the homeopathically untreated controls.[22] Theoretically, crystallization based methods, being applicable to a very wide range of biological substances and fluids[23–27] might be a useful tool in investigations of all kinds of biological models applied in basic research on homeopathy.

Other studies indicate that polycrystalline structures obtained by means of DEM can be used to study the water's structure[28] and the structure of UHD's,[29] and thus might also serve as an useful parameter for investigations performed on physical models. Since the choice to use polycrystalline structures formed during evaporation-induced crystallization as an outcome parameter does neither depend on the UHD's expected action nor on the specific model in use, polycrystalline structures might constitute a 'first line' parameter able to show if, generally, the UHD treatment has induced some kind of modification into the model.

All studies concerning the evaporation induced crystallization applied to test UHDs[15–17,27] and also studies with the copper chloride crystallization method applied to test food quality[19,20] reported on a strong day-factor influence upon the crystallization results. However, until now no plausible explanation of such a phenomenon is known.

Efficacy tests of *Zincum metallicum* UHDs applied on different models are the topic of many recent studies[30–35] performed within the International Research Group on Very Low Dose and High Dilution Effects (GIRI)[35] indicating on a wide application area of this preparation. Two studies performed on animal models showed that treatments with *Zincum metallicum* UHDs: (i) increased the survival rate and improved the clinical state of mice infected by *Trypanosoma cruzi*[31] and (ii) in a multigeneration study improved reproducibility and behavioral parameters in F1 mice generation in case of *Zincum metallicum* 200c, whereas *Zincum metallicum* 5c reduced the number of births.[32]

In a study performed with a physical model,[34] aiming at nanoparticle tracking during first dilution steps, the authors analyzed *Zincum metallicum* 2c and 3c in respect to lactose 2c and 3c and unsuccussed, undiluted water. The findings suggest that the modification in 100−300 nm particle concentrations caused by the dilution step from 2c to 3c was different in *Zincum metallicum* dilutions in respect to the lactose controls: in *Zincum metallicum* the average count of nanoparticles increased only slightly (from 4.7×10^8 in 2c to 5.1×10^8 in 3c particles/ml), whereas in lactose this difference was much bigger (1.3×10^8 in 2c to 7.5×10^8 in 3c particles/ml). However, a count of 4×10^7 particles/ml was also obtained in laboratory ultrapure water suggesting that careful controls must be applied in research on nanoparticle content of solutions.

In the present study we analyzed wheat seeds treated with *Zincum metallicum* 30c (Zm), lactose 30c (L), and undiluted unsuccussed water (C) by means of DEM. Since in our previous study[15] it was observed that there is a significant increase in the UHD action in the stressed model (compared to the non-stressed model), also in the present experiment we tested the treatments on both stressed (s-seeds) and non-stressed wheat seeds (ns-seeds). Moreover, the here reported experimentation inclined us to state a hypothesis on possible lunar influences on the crystallization process, which might contribute to explain the high significance of the day factor in this and similar studies.

Materials and methods

Wheat seeds

Whole, undamaged wheat seeds (*Triticum aestivum* L. cv. Inallettabile, harvesting year 2010) were used for the experiment. The distinction between s- and ns-seeds was based upon different storing conditions from the harvest until the analysis day (4 years): the ns-seeds were kept in controlled conditions at 5°C, under vacuum, and in the dark, whereas the s-seeds were kept in lab at varying temperature, humidity, and light conditions.