Homeopathy (2017) 106, 160–170
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http://dx.doi.org/10.1016/j.homp.2017.06.003, available online at http://www.sciencedirect.com

Structural and thermal analyses of zinc and lactose in homeopathic triturated systems

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Background: **A series of different experimental approaches was applied in *Zincum metallicum (Zinc met.)* samples and lactose controls. Experiments were designed to elucidate the effect of zinc trituration and dynamization on physicochemical properties of homeopathic formulations, using lactose as excipient.**
Methods: ***Zinc met.*** **potencies (*Zinc met* 1–3c) were triturated and dynamized using lactose as excipient, according to Brazilian Homeopathic Pharmacopoeia. Lactose samples (LAC 1–3c) were also prepared following the same protocol and used as controls. The samples were analyzed structurally by Atomic Absorption Spectroscopy (AAS), X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) with Energy Dispersive X-ray Spectroscopy (EDX) and Scanning Electron Microscopy (SEM), and thermodynamically by Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC).**
Results: **AAS analysis detected 97.0 % of zinc in the raw material, 0.75 % (*Zinc met* 1c) and 0.02% (*Zinc met* 2c). XRD analysis showed that inter-atomic crystalline spacing of lactose was not modified by dynamization. Amorphous and crystalline lactose spheres and particles, respectively, were observed by TEM in all samples, with mean size from 200 to 800 nm. EDX obtained with TEM identified zinc presence throughout the amorphous matter but individualized zinc particles were not observed. SEM images obtained from dynamized samples (LAC 1c and *Zinc met* 1c) with electron backscattering could not identify zinc metal grains. The dynamization process induced Derivatives of Thermal Gravimetric (DTg) peak modification, which was previously centered near 158°C to lactose, to a range from 140 to 170°C, suggesting the dynamization process modifies the temperature range of water aggregation. Thermal phenomena were analyzed and**

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Received 7 July 2016; revised 19 June 2017; accepted 21 June 2017

visualized by Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) statistics. Both indicated that fusion enthalpy of dynamized samples (DynLAC 1-3c; DynZn 1-3c) increased 30.68 J/g in comparison to non-dynamized lactose (LAC; $p < 0.05$). ***Conclusions:*** **Our results suggested no structural changes due to the trituration and dynamization process. However, TG and DSC analyses permit the differentiation of dynamized and non-dynamized groups, suggesting the dynamization process induced a significant increase in the degradation heat. These results call for further calorimetric studies with other homeopathic dilutions and other methodologies, to better understand the dynamics of these systems.** *Homeopathy* (2017) **106**, 160–170.

Keywords: Physicochemical; Homeopathy; *Zincum metallicum*; Lactose; Trituration

Abbreviations: XRD, X-ray diffraction; TEM, transmission electron microscopy; EDS, energy dispersive X-ray spectroscopy; SEM, scanning electron microscopy; AAS, atomic absorption spectroscopy; TG, Thermogravimetry; DTg, derivatives of thermal gravimetric ANALYSIS curves; DSC, differential scanning calorimetry; SE detector, Secondary electrons detector; BSE, electron backscattered; PCA, principal component analysis

Introduction

Homeopathic medicines are prepared through processes of dilution and shaking or succussion (called "dynamization" or "potentization" in the homeopathic pharmacopoeia), using exact quantities of active ingredient mixing and inert excipient (lactose).[1] Early in the 19th century, a prior trituration process was credited with the ability to render even insoluble substances in soluble. Initially, plant extracts were not triturated. However, in 1835, Hahnemann concluded that all substances should be hand-triturated, to the 3c and 6× potencies.[2] In 1842, 3c trituration was also adopted as the required starting point for the manufacture of LM potencies.[3]

Since Hahnemann's initial steps in the trituration process, its peculiar features were investigated by a number of experimental approaches, such as spectroscopic measurements and NMR analyses. Other approaches, including quantum physics and nanoscience,[4–6] may increase our understanding of physicochemical properties of solid homeopathic medicines. Botha and Ross reported statistically significant differences in NMR chemical shifts, as well as relative integration values of some signals from triturated and non-triturated homeopathic samples.[4]

Some aspects of homeopathic drugs include speculative hypotheses such as the one proposed by Molski,[5] in which potentization belongs to the class of quasi-quantum phenomena, playing an important role both in biological systems and homeopathy. Another such hypothesis identifies nanoparticles (NPs) at homeopathic solid dilutions, as reported by Chikramane *et al*.[6] These authors demonstrated that the hydroxyl groups in lactose could interact with the metal particles by hydrogen bonds, as shown by specific infrared stretching/vibrational frequencies. Non-covalent interactions with lactose seem to stabilize metal particles, including formation of NPs.[6]

Also, it has been speculated that NPs can occur in homeopathic products as a result of trituration and subsequent shaking in glass containers.[7,8] In fact, presence of nanostructures in homeopathic solutions were detected in highly diluted samples.[7,8]

The physicochemical nature of homeopathic medicines is still far from being clarified, as too are the properties of NPs produced by the dynamization process. Some reports suggest changes in physicochemical properties, with potential biological implications of homeopathic dilutions.[9–15] Further developments in basic research are highly desirable, and one important challenge will be the development of theoretical and experimental methods able to yield consistent and reproducible results.[16]

The need for a better understanding of dynamized systems motivated the *International Research Group on Very Low Dose and High Dilution Effects* (GIRI) to develop a multicentric project using a single metal, zinc, prepared by a unique laboratory (UFRJ, Rio de Janeiro, Brazil), following the same Pharmacopeia (Brazilian Homeopathic Pharmacopea). Research on *Zincum metallicum* can make a valuable contribution, as shown in recent studies performed by GIRI's researchers, with animal,[17–20] wheat seed[19] and physical models[16,21] indicating that this initiative introduces new important knowledge about dynamized systems.

Zinc is an essential microelement required for various cellular functions, including cerebral depression,[22] acting as antioxidant, structural constituent in numerous proteins, and participating in cognitive development, immune response, thymus activity, and others.[23,24]

This paper aims to consider how traditional homeopathic manufacturing process modifies the physicochemical properties of *Zinc met*, comparing findings with dynamized and non-dynamized lactose. Then, different experimental tools were employed in order to characterize and increase the understanding of dynamized solid systems. This research is

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