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Conference Presentation

Detection of nanostructures in solutions of *Zincum metallicum* and the vehicle lactose

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Abstract

Background: Nanoparticles (NP), because of their size (< 1μm) and high relative surface area, are highly reactive forms of their source material with biological, chemical, optical, electromagnetic, and thermal properties different from larger bulk forms of the same material. It has been speculated that NPs can occur in homeopathic products as a function of trituration and shaking into glass containers. Moreover, the presence of sugars additives like lactose and of silicates leaked from the glassware are reported to stabilize the nanoparticles.[1] Up to now some authors observed nanoparticles in highly diluted samples[2, 3], but further studies are needed to know the nature of the NPs. Actually, nanostructures in the solutions may derive from the source materials but also from stuff contaminations and also may be constituted by nano-bubbles.[4, 5] Moreover, the mechanism by which the nanostructures can be formed and the effect of serial dilution/succussions of the NPs suspension should be studied.[6] Many tools are available to analyze the nanostructures both in solutions or in dried samples and these give complementary information about the concentration, stability, structure and chemical nature of the NPs. In the present report we describe preliminary observations obtained by the nanoparticle tracking analysis (NTA) in *Zincum metallicum* (*Zinc met.*) solutions at low-grade dilution/dynamization and their lactose controls. This study is part of a formal Brazilian-Italian inter-university collaboration.

Materials and methods: *Zinc met.*/lactose triturated powder (1cH) and the corresponding vehicle lactose was prepared at the Pharmacy Faculty of Rio de Janeiro UFRJ (BR) following the Brazilian Homeopathic pharmacopoeia guidelines. Before the analysis, the *Zinc met.* powder or lactose (1cH) were dissolved in ultrapure water (1% w/v) (Sigma), transferred into clean sterile soda-lime glass tubes and succussed with a DinaA mechanical shaker for 7.5 sec (150 strokes) to obtain the centesimal dilution 2cH. The final assay mixture was

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done by ultrapure sterile water (Sigma) containing 1:10 (v/v) test samples. NPs in the suspensions were analyzed by nanoparticles tracking analysis (NTA) by using the instrument NanoSight LM10 (Malvern). Zeta-potential of *Zinc met*. suspensions and controls was measured by the Zetasizer Nano ZS (Malvern) instrument, by using a combination of electrophoresis and Laser Doppler Velocimetry. Samples from bottom visible sediments were suspended in pure water and analyzed by scanning electron microscope (SEM) under high vacuum associated with an Energy-dispersive X- ray (EDX) probe for microanalysis.

Results: The *Zincum met.* 2cH solution contained small visible metallic debris which were observed after spontaneous sedimentation. Scanning electron microscopy followed by EDS microanalysis showed that those particles (10-500µm) were made by zinc (Figure-I).

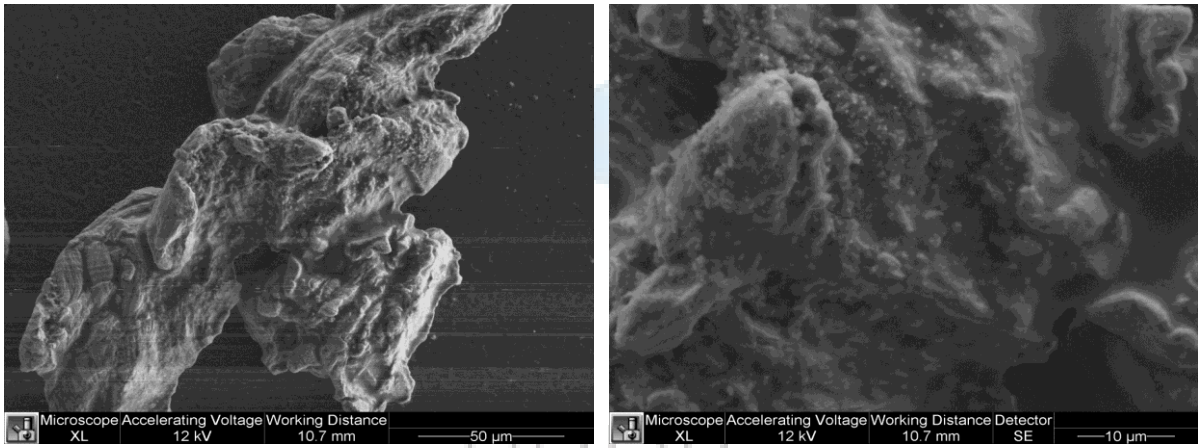

Figure-I: SEM of *Zincum met.* 2cH microscopic particles.

NanoSight analysis of the whole *Zinc met.* 2cH solution showed the presence of particles with nanometric size (100-300nm) (**Figure-II**) at the concentration of $4.7x10^9$ particles/ml. Nanostructures with 100-300 nm size were also detected in the vehicle lactose 2cH, at the concentration of $1.3x\ 10^9$ particles/ml. Poly-dispersion of the NPs is indicated by the presence of multiple peaks in the size/concentration graphs. The highest peaks were detected at 105 nm and 115 nm size in *Zinc met*. 2cH and lactose 2cH, respectively. Ultra-pure water used for NTA analysis contained $3.8\ x10^7$ particles/ml, with the highest peak of 185 nm.

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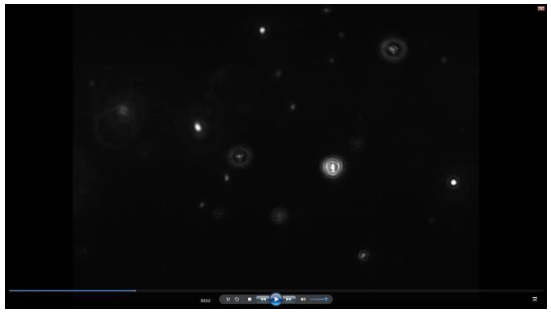
(A)  (B) 

Figure-II: Screen shots from nanoparticle tracking analysis (NTA) videos obtained with Nanosight LM10 of *Zincum met.* 2cH (A) and Lactose 2cH (B) suspensions.

Zeta-potential of *Zinc met.* suspension 2cH and control lactose 2cH, measured with the Zetasizer Nano ZS (Malvern) instrument were -15.5 mV and -36.8 mV, respectively ($p<0.01$).

Discussion: This study was carried out on samples of *Zinc met.* triturated according to the traditional homeopathic pharmacopoeia. The microscopic and microfluidic analysis of the products from the first step of the preparation provides some preliminary insights of the chemical and physical features of a metallic homeopathic drug. Electron microscopy observation showed the presence of small microscopic debris resulting from the trituration procedure, while on EDX microanalysis a substantial amount of nanosized structures, with max peak around 100 nm were also detected. Surprisingly, also in lactose control samples and even in ultrapure water a substantial amount of nanoparticles was detected, although at a lower concentration. These results suggest that nanostructures form easily in solvents (water and lactose) widely used in homeopathic pharmacopoeia. While the role of those nanostructures in the pharmacological activity of diluted drugs remains to be clarified, our findings indicate that careful controls must be applied in such kind of research. *Zinc met*. solution has less negative zeta-potential values than the corresponding vehicle. The magnitude of the zeta-potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. This may suggest a physico-chemical difference in the stationary layer of fluid attached to the dispersed particles. Further studies are ongoing to determine the possible changes of these analytical parameters in solutions made at higher degrees of triturations and dilutions.

Conflict of interest: None declared.

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Keywords: nanostructures, zincum metallicum, lactose, high dilution, trituration, succussion

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