Original Article

An evaluation of Avogadro’s number in the light of HRTEM and EDS studies of high dilutions of *Ferrum metallicum* 6, 30, 200, 1M, 10M and 50Mc

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Abstract
As a therapeutic tool high dilutions (HDs) are always at the center of controversies due to problems to validate them as a function of Avogadro’s number. Nevertheless, homeopathy is practiced around the world as a complementary and alternative medicine. The present study sought to evaluate HDs of homeopathic drug *Ferrum metallicum* (*Ferr*) 6, 30, 200, 1M, 10M and 50Mc, all of which except for 6c surpass Avogadro’s number. Using HRTEM and EDS it was conclusively shown that: 1) all the investigated HDs of *Ferr* contained plenty of nanoparticles (NPs); 2) the size of NPs were within the quantum dots (QD) size range, except for 50Mc, in which larger particles were found (12.61nm); 3) NPs contained iron in various weight percentages; 4) the weight percentage of iron was highest in HDs 10Mc and 50Mc.

Keywords: Homeopathy, High dilutions, *Ferrum metallicum*, Nanoparticles, HRTEM, EDS, Quantum dots, Avogadro’s number

Introduction
Homeopathy was established in the late 18th century by the German physician Samuel Hahnemann. For his experiments, Hahnemann prepared medicines from a wide variety of natural products.1 Homeopathy has been widely used around the globe for more than 200 years with its popularity fluctuating from time to time. While there are misgivings about its scientific validity, all along its history homeopathy showed therapeutic effectiveness in the cure of chronic and acute diseases. Such effectiveness kept it alive as an alternative mode of therapy despite the ill-defined scientific explanations about its plausibility.

The fundamental reason for this situation is the high dilutions (HDs) and ultra-low doses used in homeopathy, whereby the physical existence of one single molecule of the starting material becomes hard to prove.2 The dilution factor in homeopathic HDs exceeds Avogadro’s number (6.023 x 10^{-23}) by several orders of magnitude, and thus one might not expect to find any measurable remnant of the starting material.1 For instance, the dilution factor achieved in *Ferrum metallicum* (Ferr) 6c is 10^{-12}; and 10^{-100000} in HD 50000(50M)c.3 Hahnemann formulated a special method of preparation of homeopathic medicines by first triturating minerals and metals and then diluting and agitating the solution in a fixed and systematic order (potentization). The homeopathic pharmaceutical technique may actually be a crude manual method to
generate ‘top down’ nanoparticles (NPs) of the source material.\(^4\)

Since the nature and therapeutic actions of HDs represent a major challenge to the known physicochemical laws, it is expected that the recent advent of nanoscience and related technology might help solve the mystery of HDs.

The aim of the present study was to investigate the presence of iron NPs in Ferr 6c, 30c, 200c, 1Mc, 10Mc and 50Mc by means of high-resolution transmission electron microscopy (HRTEM)\(^5\) and energy dispersive spectroscopy (EDS).\(^6\)

**Material and methods**

**Samples and preparation:** Ferr is a commonly used homeopathic drug prepared from iron powder.\(^7\) The method of preparation of Ferr in centesimal scale is based on a dilution factor of 1:100 (See also appendix online).\(^8,9\) The samples for the study were procured from Bakson Drugs and Pharmaceuticals Pvt. Ltd, New Delhi, India. The selected HDs were individually sonicated in a sealed bottle for 20 minutes at 50Hz. One micro-drop of the sonicated solution was extracted from the middle of the bottle with a micropipette and placed on the TEM grid and left to dry overnight under infrared light. The grid was placed in the TEM chamber. The particles and agglomerates were identified, focused, TEM images were acquired and the particle size was measured. The elementary composition of the particles was identified and their weight percentage measured by means of EDS.

**Instruments:** In the present study, after a detailed analysis of the drug source material and equipment, we decided to perform TEM and EDS for the analysis of the homeopathic HDs.

Homeopathic drugs prepared from metals and minerals were studied with HRTEM (Jeol TEM 2100 with operating voltage 200kV and 200 mesh carbon coated copper grid) and EDS (Oxford Instruments INCA equipment). HRTEM is able to focus NPs in nanometer range. EDS served to analyze the elementary composition of the identified NPs. Use of this equipment allowed detecting the NPs with the smallest size and to analyze their elementary composition.

**Study setting:** The study was conducted at International and Interuniversity Center for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, India.

**Results**

The elementary composition of NPs in various HDs of Ferr are described in Table 1.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ferr 6c</th>
<th>Ferr 30c</th>
<th>Ferr 200c</th>
<th>Ferr 1Mc</th>
<th>Ferr 10Mc</th>
<th>Ferr 50Mc</th>
</tr>
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<tbody>
<tr>
<td>Fe</td>
<td>0.58</td>
<td>0.52</td>
<td>2.22</td>
<td>0.44</td>
<td>4.57</td>
<td>11.94</td>
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<tr>
<td>C</td>
<td>86.20</td>
<td>21.02</td>
<td>8.51</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>13.22</td>
<td>99.48</td>
<td>76.76</td>
<td>2.16</td>
<td>95.43</td>
<td>88.06</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>Hf</td>
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<td>-</td>
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<td>4.47</td>
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</tr>
<tr>
<td>Mg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Si</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.98</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1:** Elementary composition of particles in various HDs of Ferr.
Sample 1: Ferr 6c
The following images of Ferr 6c were acquired using TEM (Figures 1 and 2):

![Figure 1: Ferr 6c under 50-nm magnification](image1)

![Figure 2: Ferr 6c under 10-nm magnification](image2)

The particle size of Ferr 6 varied from 1.98 to 4.17 nm (minimum-maximum). The particles measured were within the of quantum dot (QD) size range. Many discrete and clear particles were seen in various fields. In some areas the particles were seen together forming islands. The particles were homogenous in nature. Few agglomerates were also seen. Presence of carbon and copper in EDS studies might be considered as contaminants originated in the TEM grid.

Sample 2: Ferr 30c
The following images of Ferr met 30c were acquired using TEM (Figures 3 and 4):

![Figure 3: Ferr 30c under 50-nm magnification](image3)

![Figure 4: Ferr 30c under 20-nm magnification](image4)

The particle size of Ferr 30 varied from 1.99 to 3.66 nm. Many discrete particles of QD size were seen all across the fields. The particles were homogenous. Few agglomerates were also seen.
Sample 3: Ferr 200c
The following images of Ferr 200c were acquired using TEM (Figures 5 and 6):

![Image 1](image1)

**Figure 5: Ferr 200c under 20-nm magnification**

The particle size of Ferr 200c varied from 2.35 to 5.62 nm. The particles size was slightly larger compared to 30c, but within the QD size range. The particles were mostly homogenous and grouped together. Under 2-nm magnification, lattice formations began to appear. Heterogeneous particles and agglomeration of particles were noticed in some fields.

Sample 4: Ferr 1Mc
The following images of Ferr met 1Mc were acquired using TEM (Figures 7 and 8):

![Image 2](image2)

**Figure 7: Ferr 1Mc under 50-nm magnification**

The particle size of Ferr 1Mc varied from 1.15 to 5.38 nm. The particle size remained close to the one of Ferr 200c. Many homogenous particles and few agglomerates were noted.

![Image 3](image3)

**Figure 8: Ferr 1Mc under 5-nm magnification**

EDS evidenced the presence of various elements. As the energy levels of boron and carbon are close, and EDS measurements are based on energy dispersion possibly the...
presence of boron is that of carbon. Silica may appear from glass leaching, while copper and carbon are components of the TEM grid. The presence of magnesium and hafnium requires further analysis and evaluation.

**Sample 5: Ferr 10Mc**
The following images of Ferr 10Mc were acquired using TEM (Figures 9 and 10):

![Figure 9: Ferr 10Mc under 10-nm magnification](image1)

The particle size of Ferr 10Mc varied from 2.74 to 5.91 nm. The particle size is almost similar to the ones in Ferr 1Mc and well within QD size. Many particles were seen. Agglomerates were less frequent compared to the lower HDs. Lattice formation unique to metals was seen under 2-nm magnification. The weight percentage of iron was much higher compared to the lower HDs.

![Figure 10: Ferr 10Mc under 2-nm magnification](image2)

**Sample 6: Ferr 50Mc**
The following images of Ferr 50Mc were acquired using TEM (Figures 11 and 12):

![Figure 11: Ferr 50Mc under 20-nm magnification](image3)

![Figure 12: Ferr 50Mc under 10-nm magnification](image4)
The particle size of *Ferrum metallicum* 50Mc varied from 5.34 to 12.61 nm. The particles size was comparatively larger compared to all the other HDs. Agglomerations were common, matrix formations were seen in some fields. The weight percentage of iron was the highest (11.94%).

**Discussion**

Many hypotheses were put forward to explain the mechanism of action of the high-diluted homeopathic medicines on biological systems considering Avogadro's number as an undeniable scientific fact. Therefore, all the hypotheses assume that it is impossible to find anything of the starting material in HDs. Some among of the most prominent hypotheses are water memory theory\(^\text{12}\), clathrate theory\(^\text{13}\), the silica hypothesis\(^\text{14,15}\), and that homeopathic drugs carry specific signals/information that might act as trigger for turning on or off some relevant genes\(^\text{2}\).

The present study evidenced presence of NPs of iron in all the tested HDs of *Ferrum metallicum*. Previous authors attempted some explanations for the fact that NPs are retained even at dilutions above Avogadro's number. Chikramane et al. used TEM, ICP-AES (inductively coupled plasma–atomic emission spectroscopy) and SAED (selected area electron diffraction) in the analysis of homeopathic drugs of metallic origin up to HD 200c.\(^\text{1}\)

Chikramane et al.\(^\text{1,16}\) suggested that once the bulk concentration is below a threshold of a few nanograms/milliliter (ng/ml) at the end of each dilution step, all of the NPs levitate to the surface and are accommodated as a monolayer at the top. This dominant population at the air-liquid interface is preserved and carried to the subsequent step, thereby giving raise to an asymptotic concentration. Thus, all dilutions are only apparent and not real in the terms of the concentration of the starting material.

In the present study, the iron solution was extracted from the middle of the bottle immediately after sonication, which removed all possible chances of NPs levitate to the surface and form a monolayer. Therefore, it is reasonable to assume that in nanoscale and in the process of preparing homeopathic HDs the laws based on the assumptions of linearity collapse. The chaos created in the bottle during the process of preparing homeopathic HDs seems to generate and sustain the particles infinitely. Therefore, evaluating the presence of NPs in HDs on the basis of Avogadro's number is irrational.

**Conclusion**

Using HRTEM and EDS it was conclusively shown that: 1) all the tested *Ferrum metallicum* HDs contained plenty of nanoparticles (NPs); 2) the size of NPs was well within QD size, except for 50Mc, in which larger particles were found (12.61 nm); 3) NPs contained iron in various weight percentages; 4) the weight percentage of iron was highest in the higher HDs, like 10Mc and 50Mc. Further studies in this area are required.

**Limitations**

HRTEM and FESEM are highly sophisticated methods to identify and locate NPs in homeopathic drug solutions of metallic and mineral origin and plant and organic origin, respectively. EDS is a reliable method for qualitative analysis of the elementary composition of NPs, but it is not a perfect quantitative method for analysis of elementary composition. Even though the quantitative values measured by means of EDS are not perfect, the marginal percentage of error is not a sufficient reason to put the values into question.
Conflict of Interest: None declared.

Acknowledgment: The author expresses its gratitude to International and Inter university Center for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, India and Center for Nanoscience and Engineering, Indian Institute of science, Bangalore, India for extending their support to the present study.

Financial support: The study received no funding from any agency.

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Received: April 18 2015. Accepted: September 1 2015.