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Original Research Article

Nano Pharmacological Aspect of Homeopathic Drugs - A Comparative Study of Different Scales of Ultra-High Dilutions Based on HRTEM Analysis and NP Characterization of Homeopathic Drug Natrum Muriaticum 6C – CM and LM1 - LM30

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Abstract: The author's earlier publications have shown the presence of nanoparticles (NPs) of the original drug material in all the higher dilutions of homeopathic drugs; viz, *Lycopodium* and *Ferrummetallicum*. The present study is to compare the two different scales of potencies discovered by Samuel Hahnemann; with the help of HRTEM and EDS. It is found that:- i) all the high dilutions (HDs) of *Natrummuriaticum (Nat mur)* contain NPs; ii) the size of NPs is within quantum dots (QD) size range, except for 6C, where larger particles are found (14nm); iii) NPs contain Sodium (Na) and Chlorine (Cl)in various weight percentages; iv) The smallest NPs in higher concentration are present in 50 millesimal (LM) scale; v) The weight percentage of Na and Cl is very high (more than 99%) in all the potencies of LM scale except in LM6.The combined weight percentage of Na and Cl is less than 1% in all centesimal scale of potencies except in CM, where it is 1.09%; vi) The presence of elements other than Na and Cl is many in centesimal scale. The presence of other elements has been negligible in LM scale and vii) The comparative analysis shows that LM scale HDs are rich in the NPs than in centesimal scale HDs.

Keywords: Homeopathy, High dilutions, *Nat mur*, Nanoparticles, HRTEM, EDS, Quantum dots, Avogadro's number, Angstrom, Centesimal scale, 50 millesimal scale (LM scale)

INTRODUCTION

Historically the science of Homeopathy has been subjected to wide criticism on account of the theoretical limitations of 'dilution factor' based on Avogadro's number (6.023 x 1023) [1]. Owing to the ultra-high dilutions of highly potentized homeopathic drugs, their efficacy in treating diseases have been often questioned [2]. Homeopathic potencies beyond 12C [3] cross the limit of Avogadro's number and hence they are considered as placebo solutions without the trace of even a single molecule of the original drug substance in such high dilution [4]. The study by Chikramane *et al* has proved the presence of physical entities in the form of nanoparticles in a few metallic drugs[5] at the level of 6C, 30C and 200C potencies. Recent publications of this author have revealed the presence of nanoparticles in all the centesimal potencies of *Lycopodiumclavatum* i.e. 6C, 30C, 200C, 1M, 10M, 50M and CM, Carbon and Oxygen are the universal elementary constituents of the nanoparticles (NPs) [6]. Further, the presence of NPs in the centesimal scales up to 50M potencies of *Ferrummtallicum* i.e. 6, 30, 200, 1M, 10M and50M has been demonstrated, where the majority of particles have been within quantum dot (QD) size (Nanoparticles of less than 10 nm size) and the weight percentage of iron has been higher in higher dilutions, 10M and 50M [7].

The trituration (pulverising) and succussion procedures in classical homeopathic remedy preparation have been manual methods that generate "top down" nanoparticles of the source material [8]. With the help of High Resolution Transmission Electron Microscopy (HRTEM) [9] and Energy Dispersive spectroscopy (EDS) [10] researcher has demonstrated the presence of nanoparticles in all the potencies of the drug *Natrummuriaticum* [11] (*Nat mur),* in centesimal scale as well as in 50 millesimal scale. The EDS study has helped to analyze the elementary composition of nanoparticles and to demonstrate the presence of Sodium and Chlorine in various proportions in all the potencies. This paper is intended to present the research findings of both centesimal and 50 millesimal scale of potencies and to compare these two scales of ultra-high dilutions. Analysis using TEM and EDS has been helpful to identify the particles and their elementary composition [12]. Therefore, it has been decided to study the sample drugs using TEM and EDS.

MATERIALS AND METHODS

Samples and preparation

Nat mur is a commonly used homeopathic drug prepared from common salt (NaCl). The method of preparation of *Nat mur* in centesimal scale [13] is on a dilution factor of 1:100 and that of 50 millesimal scale is on a dilution factor of 1:50000. The process is called potentisation [14].

The dilution factor obtained in various potencies of *Nat mur* is as follows [15];

Table 1:The dilution factor achieved in various centesimal potencies of Nat mur

Potency	Dilution factor
6C	10^{12}
30C	10^{60}
200C	10^{400}
1M	10^{2000}
10M	10^{20000}
50M	10^{100000}
CM	10^{200000}

Table 2: LM scale in comparison with centesimal scale (approximate values)

Potency	Similar potency in centesimal	Dilution factor
lm1	5C	10^{-10}
lm6	17C	10^{-34}
lm12	31C	10^{-62}
lm18	45C	10^{-90}
lm24	60C	10^{-120}
lm30	73C	10^{-146}

The samples of centesimal scale have been procured from Willmar Schwabe India (P) Ltd, New Delhi, and 50 millesimal scale from Bahola Labs, Pondicherry, India. Both are GMP certified homeopathic pharmaceutical companies. The glass wares used in the preparation of both scale of HDs are pharmaceutical grade soda lime glass. In the preparation of lm scale separate glass vials are used from LM1 to LM 30 with 100 succussions at every step. In the preparation of centesimal scale, independent glass wares are used up to 200C, whereas from 200C to CM same glass ware has been used in the mechanised potentiser giving 10 succussions in every step. These drugs are being stored and sold in the similar soda lime glass ware.

The selected HDs in manufacturer's sealed bottle have been individually sonicated [16] for 20 minutes at 50Hz. One micro-drop of each solution has been taken from the middle of the bottle with the help of a micropipette, and the same placed on the TEM grid and left to dry overnight under infrared light. The grid has been placed in the TEM chamber. The particles and agglomerates have been identified, focused, TEM images taken and the particle size measured. The elementary composition of the particles has been identified and their weight percentage measured by means of EDS.

Instruments

In the present study, it has been decided to use HRTEM and EDS for the analysis of the homeopathic HDs of Nat mur. Jeol TEM 2100 with operating voltage 200kV and 200 mesh carbon coated copper grid has been used. For EDS, Oxford Instruments INCA equipment has been used. Resolution of the HRTEM used has been 0.14nm. EDS (mode spot size varying from 0.5 nm to 25 nm) served to analyze the elementary composition of the identified NPs. Use of this equipment has helped to detect the NPs of 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

A. ***Nat mur* centesimal scale of potencies**

Sample 1 *Nat mur* 6C

HRTEM images of *Nat mur*6C

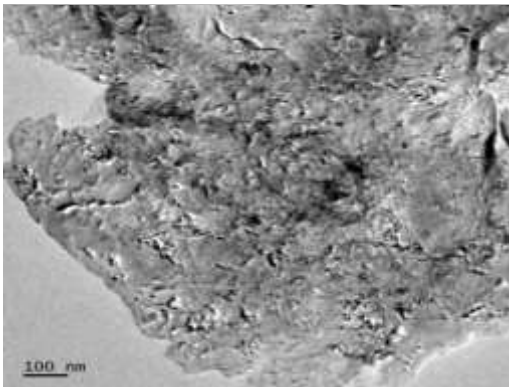

Fig-1: *Nat mur* 6C in 100nmmagnification

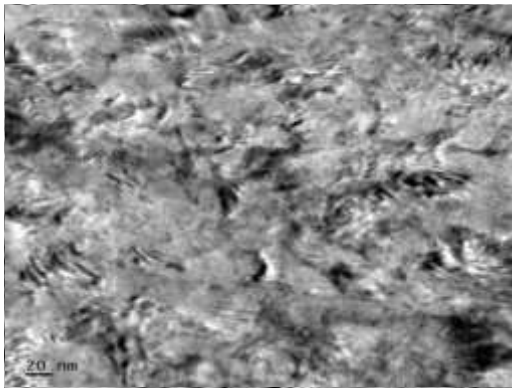

Fig-2: *Nat mur*6C in 20nm magnification

The figures show the nanoparticles of *Nat mur*6C. The figure 1 shows agglomeration of particles. The figure 2 shows that the field is packed with particles. The size of the particles varies from 1nm (minimum) – 14 nm(maximum).

Sample 2 *Nat mur*30C

HRTEM images of *Nat mur*30C

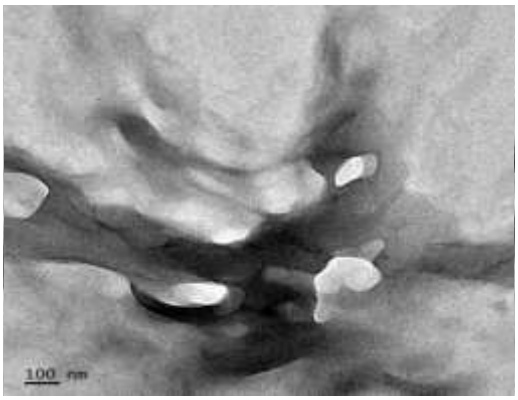

Fig-3: *Nat mur*30C in 100nm magnification

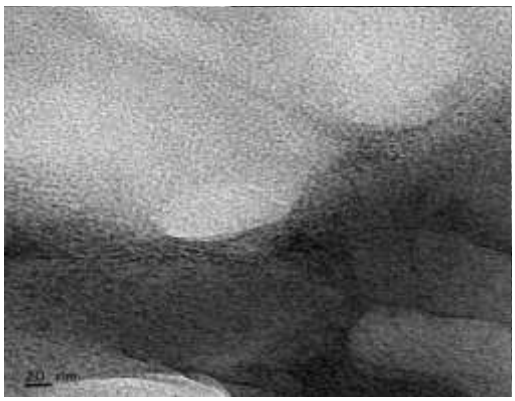

Fig-4: *Nat mur*30C in 20nm magnification

The figures show the nanoparticles present in 30C potency which is entirely different in morphology compared to 6C. The size of the particles varies from 1nm – 3nm.

Sample 3 *Nat mur*200C
HRTEM images of *Nat mur*200C

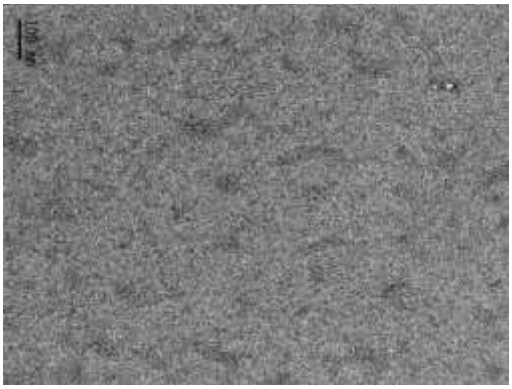

Fig-5: *Nat mur*200C in 100nm magnification

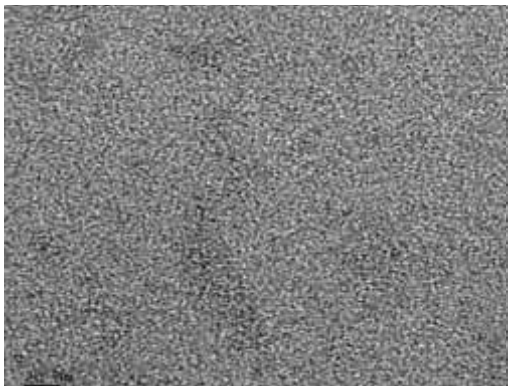

Fig-6: *Nat mur*200C in 10nm magnification

The field is filled with plenty of nanoparticles and clusters. The size of the particles varies from 1nm-3nm.

Sample 4 *Nat mur* 1M
HRTEM images of *Nat mur* 1M

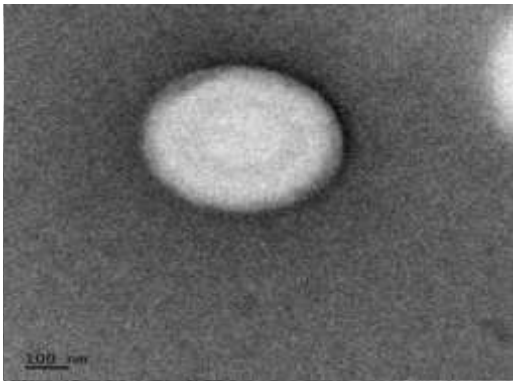

Fig-7: *Nat mur*1M in 100nm magnification

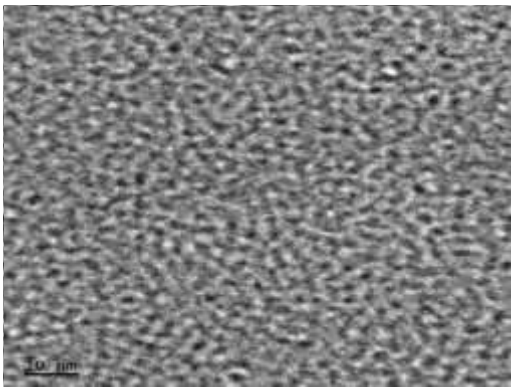

Fig-8: *Nat mur*1M in 10nm magnification

The figures show the presence of nanoparticles in a packed form in abundance. Many clusters are seen. The size of the particles varies from 0.65nm – 1.3nm. Agglomerates are rarely seen.

Sample 5 *Nat mur* 10M

HRTEM images of *Nat mur* 10M

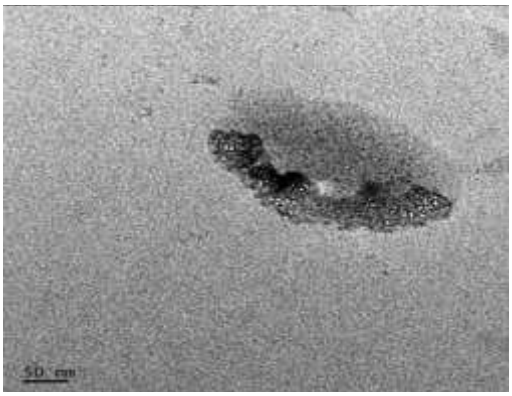

Fig-9: *Nat mur*10M in 50nm magnification

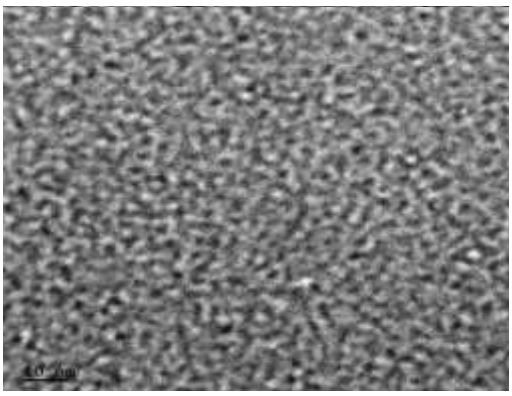

Fig-10: *Nat mur*10M in 10nmmagnification

Numerous closely packed particles and many clusters are seen. Large numbers of particles in various fields are generally well dispersed. The size of the particles varies from 0.65nm to 1.3nm. Few agglomerates were seen.

Sample 6 *Nat mur* 50M
HRTEM images of *Nat mur* 50M

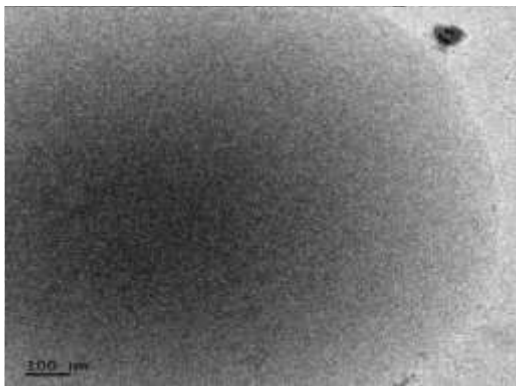

Fig-11: *Nat mur*50M in 100nm magnification

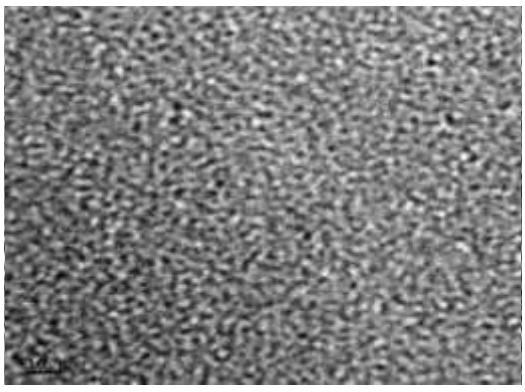

Fig-12: *Nat mur*50M in 10nm magnification

Figures show the presence of many clusters in addition to nanoparticles throughout the field. The 10nm scale magnification show the field full of closely packed particles. The size of the particles varies from 0.9nm – 1.3nm. Field view of 50M was somewhat similar to 10M potency. Agglomerates are seen sparingly.

Sample 7 *Nat mur*CM
HRTEM images of *Nat mur*CM

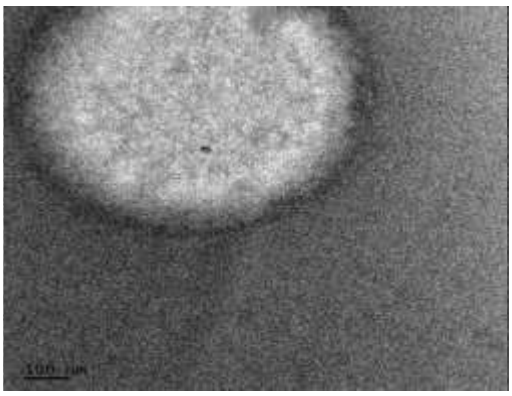

Fig-13: *Nat mur* CM in 100nm magnification

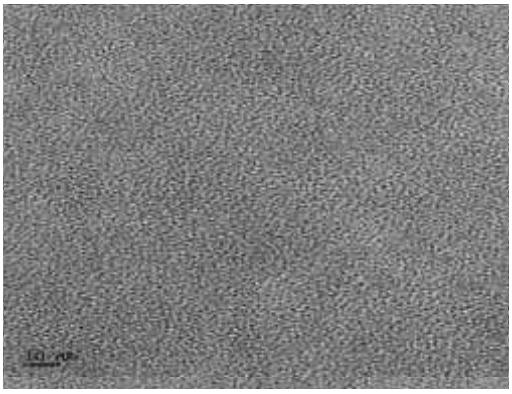

Fig-14: *Nat mur* CM in 10nm magnification

Particles distributed all over the field and many clusters are seen. Plenty of particles are seen all over which are uniform in size and are stable on EDS. No agglomerates are seen in CM potency but clusters were plenty. The size of the particles varies from 0.76nm – 1.15nm. Compared to all other potencies of centesimal scale CM has the smallest particles. In all the potencies of centesimal scale the particles seen stable on EDS.

B. ***Nat mur* 50 millesimal scale of potencies**
Sample 8 *Nat mur*LM1
HRTEM images of *Nat mur*LM1

Fig-15: *Nat mur*LM1 in 100nm magnification

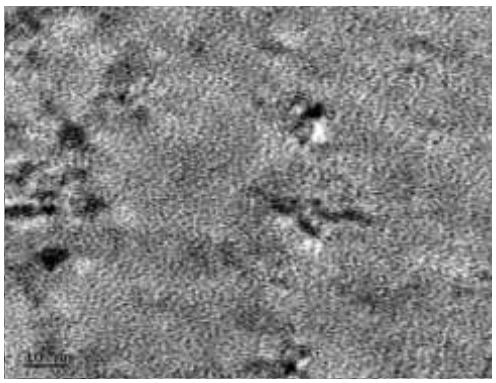

Fig-16: *Nat mur* LM1 in 10nm magnification

Plenty of particles are seen in all the fields. Large numbers of clusters were seen. Particle size varies from 0.69 nm – 6 nm.

Sample 9 *Nat mur LM*6
HRTEM images of *Nat mur*LM6

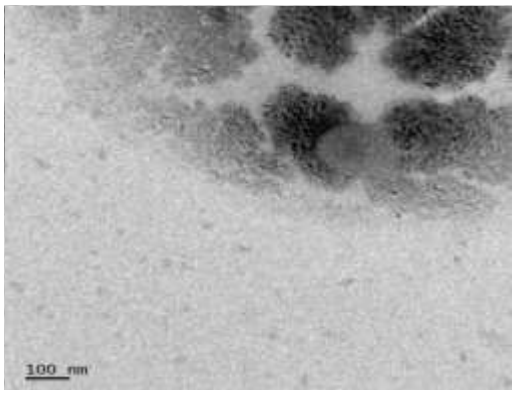

Fig-17: *Nat mur* LM6 in 100nm magnification

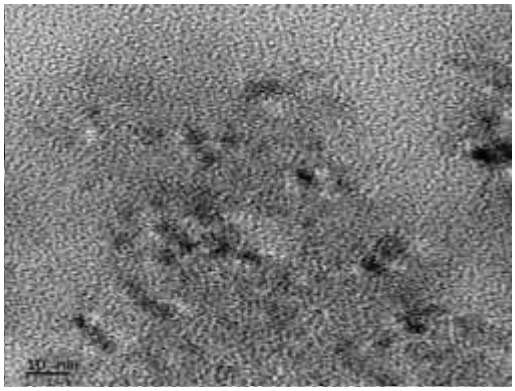

Fig-18: *Nat mur* LM6 in 10nm magnification

Plenty of particles and clusters are seen. Particle size varies from 0.86 nm – 3.3 nm.

Sample 10 *Nat mur*LM12
HRTEM images of *Nat mur*LM12

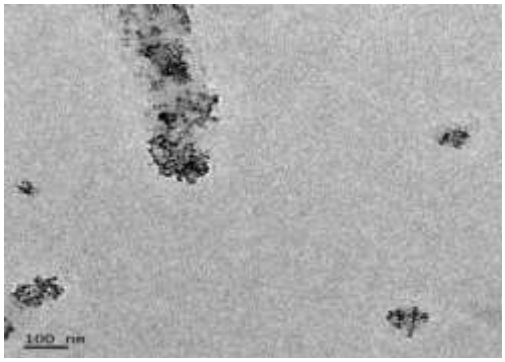

Fig-19: *Nat mur* LM12 in 100nm magnification

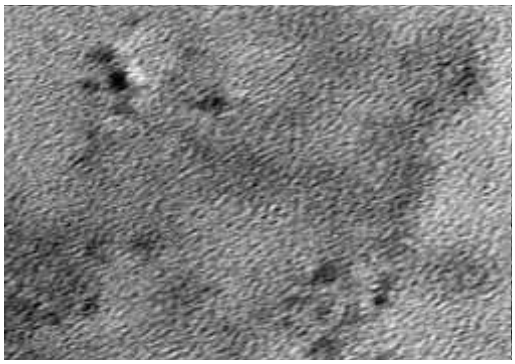

Fig-20: *Nat mur* LM12 in 10nm magnification

Plenty of particles and few clusters are seen. Size of particles 0.75 nm – 6 nm

Sample 11 *Nat mur*LM18
HRTEM images of *Nat mur*LM18

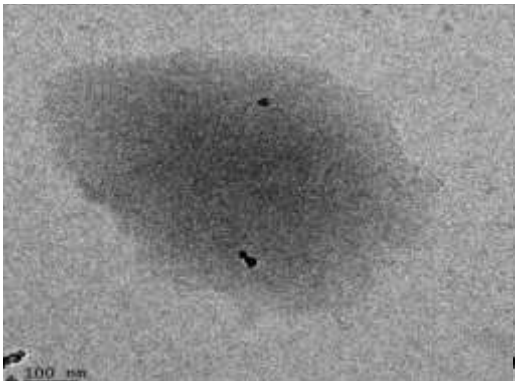

Fig-21: *Nat mur* LM18 in 100nm magnification

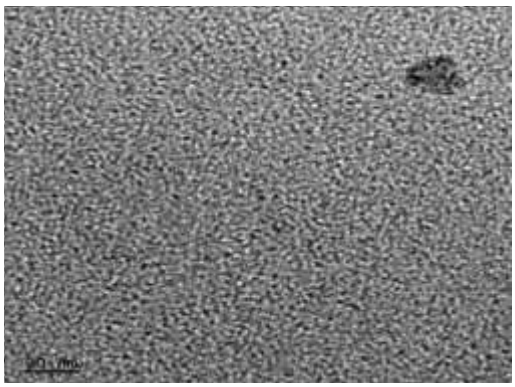

Fig-22: *Nat mur*LM18 in 20nm magnification

Plenty of particles and clusters are seen. Particle size varies from0.66 nm – 3.5 nm.

Sample 12 *Nat mur*LM24
HRTEM images of *Nat mur*LM24

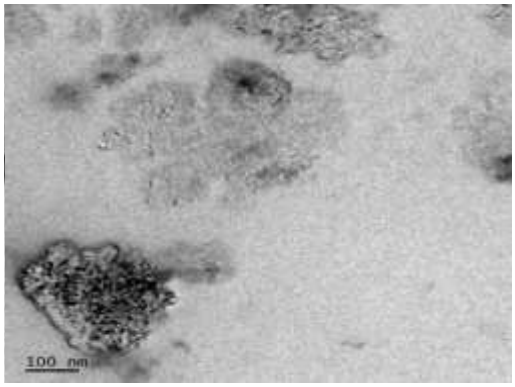

Fig-23: *Nat mur* LM24in 100nm magnification

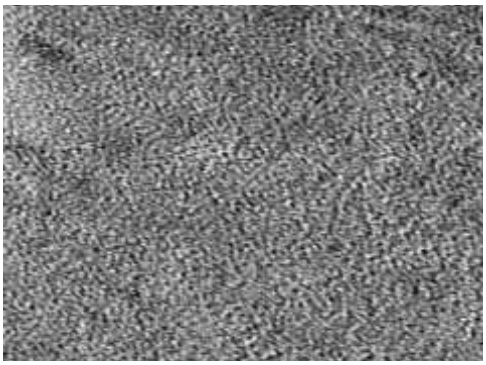

Fig-24: *Nat mur* LM24in 10nm magnification

Plenty of particles are seen. Clusters are more than in earlier potencies. Particle size varies from0.44nm – 1.1 nm.

Sample 13 *Nat mur*LM30
HRTEM images of *Nat mur*LM30

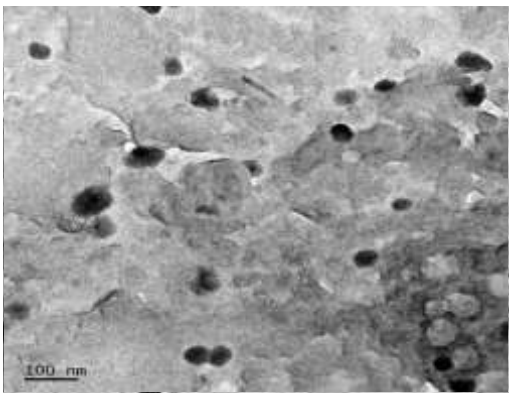

Fig-25: *Nat mur* LM30in 100nm magnification

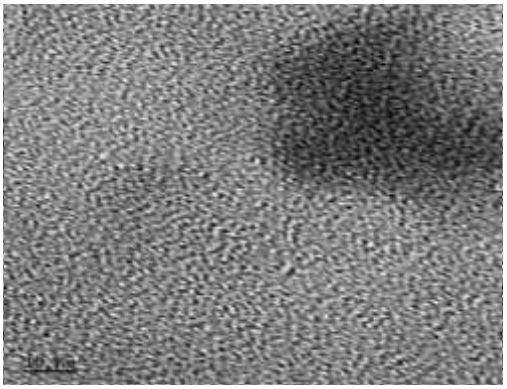

Fig-26: *Nat mur* LM30in 10nm magnification

Clusters are plenty and more than in LM 18 and LM24. Enormous particles are seen. Particle size of *Nat mur*LM 30: 0.23 nm – 1 nm.

In all the LM scale HDs, particles have been stable on exposure to EDS except in one field of LM24, where the particles in a branched structure have started dancing on exposure to energy beams of EDS.

Elementary composition of *Nat mur* 6C –CM

Table 3: Comparative weight percentage of various elements present in Nat mur6C – CM potencies

	Na	Cl	C	Cu	Fe	Cr	Ni	Ca	Sb	I	N	Ti
6C	0.16	0.04	75.18	8.35	10.70	3.25	2.32					
30C	0.33	0.13	71.27	8.74				7.17	10	2.36		
200C	0.31	0.15	73.30	3.47							17.32	5.45
1M	0.02	0.16	93.74	6.08								
10M	0.61	0.07	90.61	8.71								
50M	0.23	0.15	96.79	2.83								
CM	0.60	0.49	92.69	6.22								

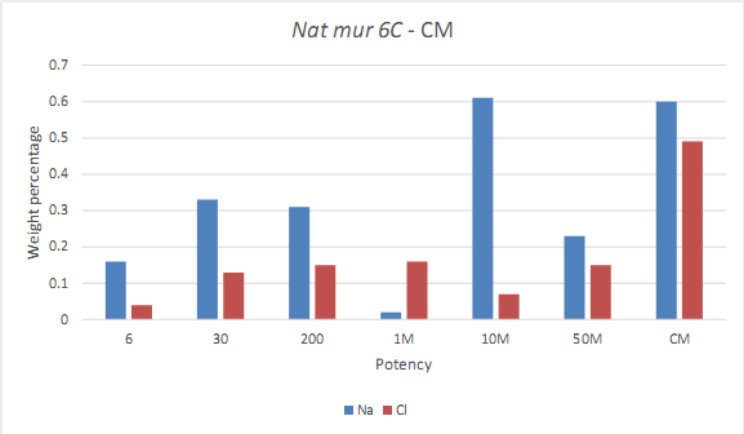

Fig-27: Comparative weight percentage of Sodium and Chlorine present in 6C, 30C, 200C, 1M, 10M, 50M and CM potencies

As evident from the table and bar diagram above the presence of Sodium is the highest in 10M potency i.e.0.61% and next is the CM potency i.e.0.60%. In *Nat mur* 30C Na 0.33% and Cl 0.13%. In 200cH, Na is 0.31% and Cl 0.15%.But in 1M Na 0.02% and Cl 0.16%. Here the presence of the element Cl is more than Na. In 50M Na is 0.23% and Cl 0.15%. However the presence of Cl is the highest in CM potency i.e.0.49%. Na and Cl are present in all the potencies *Nat mur*.

From the present study in HRTEM it is evident that all centesimal potencies of Nat mur contain nanoparticles. Further the study of the particles with EDS reveals the elementary composition of the particles. Four elements commonly observed in all the potencies of centesimal scale are Sodium, Chlorine,

Carbon and Copper. Few other elements seen in 6C, 30C and 200C potencies are Fe, Cr, Ni, Ca, Sb, I, N and Ti. The presence of Na and Cl in all the samples confirms the presence of the elements of Nat mur as nanoparticles in all the potencies of the drug. Since the TEM grid is made up of Carbon and Copper, their presence in the EDS analysis is a possible outcome. The presence of other elements like Fe, Cr and Ni in 6C, Ca, Sb and I in 30C and N and Ti in 200C potencies needs further evaluation.

Elementary composition of *Nat mur*LM 1 – LM 30

Table 4: Comparative weight percentage of various elements present in *Nat mur LM*1– LM30

Potency	Elements				
	Na	Cl	Cu	Si	Pm
LM 1	96	3.95			0.05
LM 6	23.62	27.68		48.70	
LM 12	98.50	1.30	0.20		
LM 18	98.36	1.50	0.14		
LM 24	98.44	1.56			
LM 30	92.60	7.12	0.28		

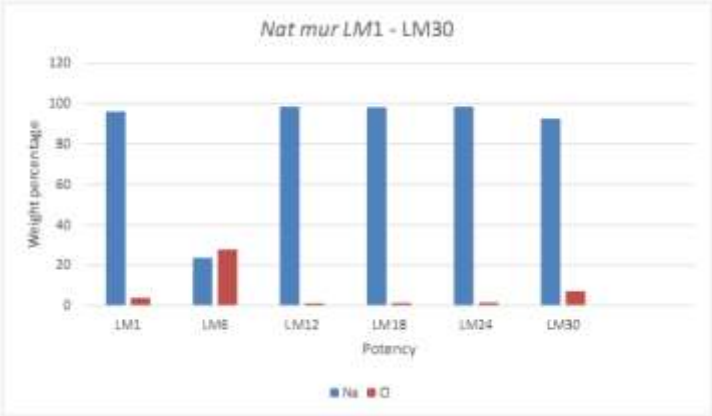

Fig-28. Comparative weight percentage of Sodium and Chlorine present in LM1, LM6, LM12, LM18, LM24 and LM30 potencies

In contrast to the complex elementary composition identified in NPs of centesimal scale, LM scale showed only two elements consistently in all potencies, i.e. sodium and chloride. Other elements seen are Pm, Si and Cu, in which Pm and Cu were insignificant. Si was seen only in lm6. Interestingly sodium and chloride formed nearly 100% of elements in all potencies except in LM6.

These facts open up some interesting observations

i. High weight % of C and Cu shows that TEM grid is poorly covered with NPs in all centesimal scale potencies. Therefore, C and Cu in the TEM grid are well exposed to the electron beams of EDS.
ii. Very high percentage of Na and Cl(more than 99% in all potencies except LM6)show the thick deposition of NPs in the TEM grid in all the potencies of lm scale. Therefore, C and Cu in the grid are not exposed to the electron beams of EDS.
iii. Multitude of elements is seen in all centesimal scale potencies, but presence of elements other than Na and Cl is insignificant in LM scale.

The size of Nanoparticles and the presence of agglomerates/ aggregates/ cluster in various potencies of *Nat mur*6C – CM

Table 5: The size of Nanoparticles and agglomerates/ aggregates/ cluster in various potencies of *Nat mur* 6C– CM

Potency	Particle size	Agglo/ aggre/ cluster
6C	1 – 14 nm	Agglomerates many
30C	1 – 3 nm	Agglomerates few
200C	1 – 3 nm	Agglomerates very few, clusters few
1M	0.65 – 1.3 nm	Aggregates very few, Clusters many
10M	0.65 – 1.3 nm	Agglo very few, aggre very few, clusters more than 1M
50M	0.9 – 1.3 nm	Aggre very few, clusters more than 10M
CM	0.76 – 1.15 nm	Clusters plenty like 50M

The analysis of the nanoparticles of the centesimal scale of HDs shows that the size of particles varies from 1nm to 14nm in 6C potency. In 30C the size of particles has remained 1-3 nm. In 200C particle size has remained 1nm to 3nm which is the same as that of 30C. 1M potency is unique because the size of small particles becomes smaller than 1nm and varies between 0.65nm to 1.3nm. In 10M potency particles are almost equal in size as that of 1M and in 50M potency the size of particles varies from 0.9nm to 1.3nm. Smaller size particles are seen in CM potency with size varying between 0.76nm to 1.15nm. The maximum number of finest particles are seen in CM potency.

Agglomerates are seen in 6C, 30C, 200C and 1M potencies, in a reducing order. Few aggregates are seen in 1M, 10M and 50M potencies. Clusters appearing from 200C have been steadily increasing in all the higher potencies and finally the maximum number of clusters are noticed in CM potency. It is to be noted that 50M and CM potencies are very similar as far as the particle characteristics and clusters are concerned.

The size of Nanoparticles and the presence agglomerates/ aggregates/ cluster in *Nat mur*LM1-LM30

Table 6: The size of Nanoparticles and agglomerates/ aggregates/ cluster in various potencies of *Nat mur*LM1 – LM30

Potency	Particle size	Agglo/ aggre/ cluster
LM 1	0.69nm – 6nm	Few agglomerates, aggregates and clusters
LM 6	0.86 nm – 3.3nm	Few agglomerates, aggregates and clusters
LM 12	0.75 nm – 6 nm	Agglomerates few, clusters few
LM 18	0.66 nm – 3.5 nm	Clusters
LM 24	0.4nm – 1.1 nm	Clusters more than LM18
LM 30	0.23 nm – 1 nm	Clusters plenty, more than LM 24

The analysis of the size of NPs in LM scale shows that smaller NPS in LM1-LM30, and the smaller ones were less than 1nm size and their size is systematically reduced to 0.23nm in LM30. The largest particles are seen in LM1 and LM12 as 6nm. The NPs in the fields are unique with plenty of particles all over. Agglomerates and aggregates are seen in LM1, LM6 and LM12 potencies. Clusters are seen from LM1 onwards and they become more clear and increased in number from LM1 to LM30.

A comparative analysis of the particle size of LM scale and centesimal scale shows that NPs in LM scale are much smaller than the NPs in centesimal scale.

DISCUSSION

From the HRTEM analysis it is evident that all homeopathic potencies in centesimal as well as in 50 millesimal scales of *Nat mur* contain nanoparticles. The study of the samples with EDS reveals clearly the elementary composition of the particles.

According to Chikramane *et al* [17] "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 rise to an asymptotic concentration. Thus, all dilutions are only apparent and not real in the terms of the concentration of the starting material." But in the present study the researcher has taken the sample from the middle of the bottle immediately after sonication, which removed all possible chances of NPs levitating to the surface and forming a monolayer.

It is noted that the size of nanoparticles in the 6 potency of *Nat mur* ranges between 1 to 14nm.In the 30C and in the 200C, they are between 1 to 3nm and that in all other potencies, contain particles smaller than 1.3nm. The nanoparticles smaller than 10nm are denoted as quantum dots [18]. A comparative analysis of 50 millesimal scale of potencies shows that from LM1 – LM30, every NP is within QD size. The largest particle is only 6nm and the smallest is less than 1nm.Among them the larger one is 0.86nm in LM6 and the smaller is 0.23nm in LM30.

The study of Witt *et al* [19] 2006, reported contamination of the initial ABD (Aqua bi destillata) increased and element concentrations changed far more when stored in brown glass bottles than in HDPE (High Density Polyethylene) bottles. Effects were strongest during initial storage time and during the first potentisation step from mother tincture to C1; subsequent potentising steps produced no relevant changes. They concluded that potentising accelerates material exchange between container and solvent, mostly during succussion. Further, they suggested that the contamination in potencies cannot prevent clinical efficacy. On the contrary, it mightbe *necessary* for producing an active potency. Also, they proposed the possibility that the presence of contaminants may be one factor for the generation of physical and biological effects of the potency and the contaminant

concentration may even determine what might be called the 'impact' of a potency's clinical action.

The current study results brings out different outcome in centesimal and 50 millesimal scales regarding the concentration of Na and Cl as well as the level of contaminants. Centesimal HDs up to 200C shows variety of elements in NPs, which may corroborate the findings of Witt et al that the contamination effects are strongest in the initial potentisation steps. After 200C only four elements are detected in the NPs of HDs, in which Na and Cl are the ingredients of *Nat mur* and C and Cu might well be the outcome of the exposure of EDS to the TEM grid, which is made up of copper wires on carbon base. The elementary composition of LM scale from LM1-LM30 shows remarkable presence of Na and Cl. Contaminants seen are only Pm in LM1 and Si in LM6. Other HDs of LM scale are free from contaminants as in 1M and above in centesimal scale.

The proposal of Witt et al that the 'contamination in potencies cannot prevent clinical efficacy' seems plausible, but their suggestion that 'contaminants might be necessary for producing an active potency' and 'contaminant concentration may even determine what might be called the *impact* of a potency's clinical action seems to be far stretched. Absence of contaminants in majority of HDs of *Nat mur* in centesimal and LM scale of potencies is a clear proof against these hypotheses.

Ives *et al* [20] did enzyme assays with acetylcholine esterase to analyse serially succussed and diluted (SSD) solutions prepared in glass and plastic containers. The elemental analysis of SSD water preparations made in glass vials showed that Boron, Silicon and Sodium were present at micromolar concentrations. Kiel *et al* [21] demonstrated the presence of Na as a leaching effect. The consistent presence of Na and Cl in all the HDs of Nat mur and particularly the high concentration in all lm scales and exclusive presence of Na and Cl in all HDs of centesimal scale above 200C (considering C and Cu are part of TEM grid) proves beyond doubt that the presence of Na and Cl is not just a variety of leaching effect but a sure ingredient of Nat mur HDs.

Heribert Mollinger *et al* [22] studied to test whether homeopathic preparations produce symptoms than placebo in healthy volunteers and they concluded that homeopathic remedies produce different symptoms than placebo. Demangeat [23] proposed that the formation of nano bubbles (NBs) during the preparation of homeopathic dilutions under atmospheric pressure cannot be ignored and he postulated that superstructures result from a nucleation process of NBs around the solute, with shells of highly organised water (with ions and silicates if any) which protect the solute against out-diffusion and behave as nucleation centres for further dilution steps. The proposition seems plausible, yet a careful look in to the dilution steps in the preparation of centesimal scale with 1:100 ratio adopted in every step and the 1:50000 in LM scale is an enormous dilution and the large number of NPs and the presence of the starting material in NPs require more sophisticated scientific research and conclusion. Similarly the experimental evidence reported by Elia et al[24] revealed the presence of supramolecular aggregates hundreds of nanometres in size in extremely diluted solutions at ambient pressure and temperature, and in the solid state. They hypothesised that formation of water aggregates occurs in extremely diluted solutions. These observations are of high value and needs to be corroborated with the observation of NPs of the starting material in all potencies of different homeopathic HDs [5,6,7].

The study results of Konovalov *et al* [25] is of great importance. Konovalov and others demonstrated the presence of formation of nanometer - sized molecular assemblies (nanoassociates) in low-concentration aqueous solutions, which were prepared by serial dilution. They proved that, for formation of nanoassociates, a definite structure of dissolved substance is necessary. In blank experiments (without dissolved substance), in which water was diluted with water, formation of nanoassociates did not happened. Initial presence of dissolved substance is necessary for the formation of nanoassociates i.e. no dissolved substance – no effect at all. They also reported that the sizes of nanoassociates in a series of cases reach 400nm. They further proved that along with dissolved substance, external electromagnetic fields are also essential for the formation of nanoassociates, the specific solution preparation procedure is also equally important. Konovalov and associates suggests that the formation and rearrangement of nanoassociates in solutions of different concentration can be considered as a major factor controlling the physicochemical and probably, specific biological properties of diluted aqueous solutions. The demonstration of Nps and their elementary composition in the earlier publications of the author and others [5,6,7] and the same in the current paper proves that Dr. Hahnemann's homeopathy indeed is a type of nanomedicine discovered 200 years prior to the current idea of nanomedicine. *All these new findings demands consistent and serious investigation in to the many unknown facets of homeopathic drugs and their medicinal action.*

The study of Stovbun S V *et al* [26] reported that Phenazepam was shown to possess activity in ultra – low doses only in disperse state, in the form of nanoparticles with a diameter < 100-300 nm, similarly they have shown Panavir possesses pharmacological activity in ultra – low doses and appears as nanoparticles with a diameter of 200 – 300nm, which

have uncompensated negative surface charge and polymer nature. All these studies confirm the possible therapeutic efficacy of nanoparticles, which support the credence of homeopathy it deserves.

Nat mur is a commonly used homeopathic drug in a variety of acute and chronic allergic conditions like eczema, seasonal allergic rhinitis, Asthma, Food allergy and Chronic allergic rhinitis [27]. Common salt (NaCl) in its raw form has never showed curative effect in any of these diseases. But the same produces curative effect with HDs. The homeopathic process of potentisation plays a vital role in curing diseases. The criticism against homeopathy has been based on the Avogadro's limits of dilutions. But it has become more or less irrelevant consequent on the recent discoveries.

It has been found that the structure and functions of an enzyme can be altered by the nanoparticles of drugs. It helps to regulate the protein activity in modulating cellular processes such as signal transduction, DNA replication and metabolism. Protein dysfunction is closely related to human diseases and disorders, and that the ability to regulate enzyme functions and protein-protein interactions provides a promising strategy for effective therapy. The nanoparticles of drugs have some definite advantages over small organic molecules as follows.

1) Nanoparticles have large specific surface areas enabling adequate protein binding and biological interactions.

2) Nanoparticles can enter into the cells easily, in contrast to biological molecules [28].

The above explains well the broader and deeper range of action of *Nat mur*HDs in different diseases. Khuda-Bukhsh*et al* [29] has proposed a hypothesis, based on various evidences; that potentized homeopathic medicines could act regulating gene expression. The possible pathways and sites of action have also been discussed by them. According to this hypothesis, homeopathic remedies carry specific 'signals' that can be identified by specific receptors and can act as a trigger to turn 'on' or 'off' some relevant genes, initiating a cascade of gene actions to alter and correct the gene expressions that went wrong to produce the disorder/diseases. The recent microarray analysis of SantukumarSaha*et al* [30] has confirmed that homeopathic remedies in ultra – high dilutions can trigger altered gene expressions, through epigenetic modifications. Bellavite*et al* [31] in 2012 showed by transcription analysis that HDs of *Gelsemiumsempervirens* could alter the expression profiles of genes in neurocytes. Marotti*et al* [32] in 2014 demonstrated a change in the expression profiles of wheat seedling while treating with ultra-high dilutions of Arsenic trioxide used as a homeopathic remedy against symptoms of arsenic poisoning. In 2014 Bigagli *et al* [33] also demonstrated the effects of homeopathic *Apismellifica* preparations on altered gene expression profiles of human prostatic cells. The ability of *Nat mur* HDs to act curatively in many allergic disorders and autoimmune diseases well supports these observations.

Recently, some nanomaterials, such as fullerene derivatives, gold nanoparticles, rare earth nanoparticles and ferromagnetic nanoparticles, have been found to exhibit strange enzyme – like activity. At first, it seems counterintuitive to imitate natural enzymes with nanomaterials differently. Most of the natural enzymes, which are proteins, have exact amino acid sequence with well-defined tertiary structures. But most of the nanomaterials are not atomically uniform in size and shape. Proteins are soft materials while nanomaterials can be hard with crystalline cores. However, they do share certain similarities, such as overall size, shape and surface charge, enabling nanomaterials to mimic natural enzymes [34].

It is often found that cancer cells have simultaneous resistance to multiple drugs with different chemical structures and action. This phenomenon is commonly referred to as the multiple drug resistance (MDR) of cancer. The development of MDR contributes to significant treatment failure in patients with metastatic cancer. Although it is now increasingly realized that MDR, inherent or acquired, is developed by a variety of mechanisms; MDR has mainly been explained by over expression of ATP-binding cassette (ABC) transporters in resistant cancer cells. Therefore, significant effort in combating MDR has been towards developing drugs that can inhibit these transporters to sensitize resistant cancer cells. Over the past two decades, several generations of transporter inhibitors have been tested in clinical trials. However, these clinical trials have generated disappointing outcome, largely due to the toxicity and low specificity of the inhibitors. Hence, addressing drug resistance still remains a priority. The study reported by Hammond and colleagues provides amble evidence that layer by layer nanoparticle therapeutics can be used to treat drug resistant tumors in a xenograft mouse model. They suggested that nanoparticles hold potential for overcoming multidrug resistance in cancer [35]. In homeopathic clinical practice *Nat mur*HDs shows good palliative effect in leukemias [36].

The exclusive presence of Na as observed in many particles as in LM12, LM18 and LM 24, points to the ionized forms of Na in these potencies. Yun Long Wu, *et al* [37] reported that a change in the cell membrane's local phase is closely related to the nanomaterials' surface charge. Negatively charged nanoparticles are bound to a fluid area on the membrane, induced gelation, whereas positively charged nanoparticles turned gelled areas into a fluid

state for easier penetration. Ionized particles in the dimensions of Quantum Dots in *Nat mur* HDs might be an important reason for its curative action in a wide variety of acute and chronic diseases.

The observation that Quantum Dots (QD) can localize to the cell's nuclear compartment, has led the researchers to investigate their potential genotoxicity. If QDs cause DNA mutation without the cell death, their effect is propagated through future generations of cells and can ultimately lead to disease. This observation of Kim M Tsoi *et al* [38] reveals two possibilities, i.e. QDs can be genotoxic or they can influence the genetic material to correct the errors leading to diseases. The relief/cure in homeopathic drug therapy is most probably due to the ability of NPs and QDs in the homeopathic drug potencies to generate epigenetic modifications and regulating gene expressions.

In fabricating materials at the nanometer scale, nanotechnologists typically employ two general strategies: bottom-up and top-down. The physical top-down approach employs the use of photons (optical lithography), electrons (electron beam lithography) and ions (ion beam lithography) while the chemical top-down strategy relies essentially on chemical reactions that are brought about by chemical etchants (acids and bases) or by application of heat [39]. These methods of preparation of nanoparticles seem to make 'bare' or 'crude' nanoparticles, nanoaggragates and nanocrystals. Some of them may be toxic according to the type of starting (basic) material used. An examination of the manufacturing process of homeopathic HDs reveals a totally different method discovered by Dr.Hahnemann in the beginning of 19^{th} century. In the homeopathic manufacturing process the crude starting materials are mixed and ground (triturated) with sugar of milk (a neutral medium) in an exact proportion for a fixed period initially, and later by the process of succussion. In order to make higher potencies, the previous potency and the dispensing alcohol (another neutral medium) are taken in 1:99 ratio in a bottle and give 10 strong succussions on a hard elastic top. This unique method of preparation of homeopathic drugs eliminates any possibility of toxicity of nanoparticles. The clinical experience of 200 years in homeopathy shows no evidence of nanotoxicity in highly potentized homeopathic drugs.

Investigation of chaos control was largely limited to nonlinear dynamical systems in the classical realm. In the paper 'Harnessing quantum transport by transient chaos' Rui Yang *et al* [40] show that chaos could be used to modulate or harness quantum mechanical systems. They focus on quantum transport through nanostructures, a problem of considerable interest in nanoscience. In homeopathic method of potentization, the process of succcussion of medicinal solutions to make their potencies is a classical method of chaos used to modulate a quantum mechanical system. The presence of nanoparticles of small size and QDs in all the potencies of *Nat mur* is a clear evidence of a quantum mechanical system created by chaos and well preserved within the bottle labelled as homeopathic drug potencies. The curative effect of *Nat mur* HDs in patients clearly suggests that homeopathic preparation of *Nat mur* HDs is a viable way to harness quantum behaviour of nanoparticles.

Massive presence of quantum dots in *Nat mur* potencies shows the ability of the drug to influence the human organism in; physical, biochemical, enzymatic and genetic modulations. *Nat mur* as a commonly used homeopathic drug around the globe by every homeopathic physician is well known for its ability to cure many a disease. Recently Bell IR *et al* [41] demonstrated by using Nanoparticle Tracking Analysis (NTA) the presence of about 2 billion NPs per millilitre in 200C potency of *Argentum metallicum*. Bell IR [42] again demonstrated the presence of $> 4 \times 10^8$ NPs/ml in 6C, 30C and 200C potencies of *Gelsemiumsempervirens*. Exclusive presence of QDs of smallest dimensions in every potency of *Nat mur*centesimal and 50 millesimal scale of potencies is an important finding of this study. The view of the HRTEM grid fields suggests that the number of NPs are much higher in *Nat mur* HDs than the numbers shown by Bell IR in *Argentum metallicum* and *Gelsemium sempervirens* potencies.

CONCLUSIONS

From the study conducted by the investigator relating to the two different scales of HDs of *Nat mur*, it has been observed that 50 millesimal potencies are rich with more finer and subtle nanoparticles. Abundant nanoparticles exist in higher HDs. The following observations are also made in the study

i. LM scale is highly rich with the presence of a number of nanoparticles
ii. NPs in LM scale are much smaller than the NPs in centesimal scale
iii. Appearance of many elements other than Na and Cl in centesimal scale calls for further study.
iv. Na and Cl are the major elements detected in LM scale of potencies

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