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NANOCHARACTERISATION OF HOMOEOPATHIC ULTRA-DILUTIONS OF PLUMBUM METALLICUM

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ABSTRACT

Homoeopathic system of medicine uses drugs prepared by the process of potentisation, by which crude medicinal substance become immeasurably small and penetratingly efficacious and remedial. Dr.CFS Hahnemann (1745 – 1842) could not demonstrate these particle and this bring forth severe criticism from and outside the homoeopaths. This stood as an obstacle to acceptance of Homoeopathy by scientific world and public at large. The situation was changed on demonstration of nanoparticles in some of the potencies of some homoeopathic drugs by using one or two nano-technological procedure. Hence, it was attempted to undertake the material characterisation of different potencies (6C, 12C, 30C, 200C 1M, 10M, 50M, and CM) of Homoeopathic drug – Plumbum metallicum, with different nano-characterisation procedures - Field emission scanning electron microscopy (FESEM) with energy dispersive spectroscopy (EDS), atomic force microscopy (AFM), x-ray photoelectron spectroscopy (XPS), high resolution transmission electron microscopy (HRTEM) with selected area electron diffraction (SAED) and dynamic light scattering (DLS). The samples were prepared without any pre-concentration and pre -treatment of drugs. The investigation has demonstrated the presence of nanoparticles of plumbum met along with other elements in all dilutions. This opens an opportunity for further studies in the standardisation of homoeopathic drugs as well as understanding its mode of action.

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INTRODUCTION

Dr C.F.S Hahnemann established Homoeopathic system of medicine in 1796. This system of medicine is based on the basic principles of *similia similibus curanteur*, *single remedy* and *minimum dose*. He explained the state of health, disease and cure on the background of *vitalistic philosophy*. Further he introduced a new method of preperation of drugs called - "potentisation or dynamization". Usually decemal (1:10 dilution), centesimal (1:100 dilution) and 50 millicemal (1:50,000 dilution) potencies are used in practice. They are marked as "d", "CH" and "0/1" respectively. 30 CH, 200CH, 1M, 10M, 50M, and CM are the commonly used potencies in daily clinical practice.

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REVIEW OF LITERATURE: Homoeopathic medicines are prepared by the peculiar process of serial dilution and succussion and is termed *potentisation or dynamisation* by which the medicinal powers of crude substances are activated. The products are termed as potencies or dilutions in different degrees. Medicinal powers of even inert substances are activated by this process[1] Sugar of milk, Alcohol or water is usually used as the media for dilution. The concept of dynamization was hinted by Dr C.F.S Hahnemann in 1829 and was fully introduced in 1833 [2]. Dr Hahnemann might have foreseen the presence of *smallest particles and changes produced by the mechanical action by rubbing and shaking.* But he could not demonstrate the nature of these smallest particles due to unavailability of suitable instruments. This provoked opposition from inside and outside of Homoeopathy and stood as an obstacle to scientific approval of Homoeopathy as a system of medicine. In the light of development in science this matter is less relevant now and

scientific investigation have demonstrated that all matter is made of energy or power[3]. On the background of theory of structure formation and structure conservation in water, formation of sustained static and dynamic structures in aqueous solutions is considered to be probable method of storage information in associated liquids. In academic research circles, most criticism of the validity of Homoeopathy focused on the issue of ultra-high dilutions where concentrations below the Avogadro limit are applied. This criticism placed promoters of homoeopathy in a very difficult situation due to lack of well accepted fundamental scientific grounds by the peer-reviewed academic community. The Geometrical models or dynamic models forwarded were not accepted as it was not proved by scientific parameters[4]. Reproduction of the results of Nuclear Magnetic Resonance (NMR) study by Conte *et al* on potentised and unpotentised *Nitric acid* dilutions were not possible. It was reported that difference between T2 values for successed and unsuccessed *nit-ac* solutions was very little[5]. Prashanth Satish Chikrmane et al [6] succeeded in demonstrating Nano- particles of the starting materials and their aggregates in 6C, 30C and 200C potencies of Zincum met, Cuprum met, Stannum met and Aurum met by TEM analysis. They estimated concentration of the starting material by ICP-AES. Medicines from different bottles were concentrated and pre-treated for the study. It was suggested that the comminution process involved in the trituration of raw materials with lactose and successions applied to liquid media might have resulted in the production of particles in different sizes and shapes. Further studies conducted under the supervision of third author has demonstrated presence of nanoparticles in 30C, 200C, 1M, 10M, 50M and CM potencies of *Aurum metallicum, Lycopodium clavatum* [7]*, Natrum mur and Psorinum*. Identification of size, size distribution, zeta potential, stability[8] and aggregation[9] of Nano particles in solutions can be assessed by Dynamic light scattering (DLS). Estimation of size in relation to intensity, volume and number, z-average etc are possible by DLS. Zeta potential helps to estimate the electrostatic affinity between nanoparticles and thereby to predict their stability [10]. With its surface charge, nanoparticles attracts a thin layer of oppositely charged ions to its surface. This double layer of ions carries the nanoparticle while it diffuses in the solution. The Zeta potential of the particles ranges from +100 mV to -100 mV. Nano particles with zeta potential values more than +25 mv and lower than -25 mv indicates higher degree of stability. Assessment of Zeta potential is useful for understanding the state of the nanoparticle surface and for predicting the long-term stability of the nanoparticle. Study of surface nature of Nano particles and long-term stability prediction can be done with assessment of zeta potential[11].

It was suggested that difference in larger mean size of nanoparticles present in verum Gelsemium 30C and most stable zeta potential of 200C potency may be due to verum botanical nature and contaminants from natural cork stopper from *Quercus suber*. It was also suggested that the manufacturing materials and methods in the preparation of Homoeopathic medicines generates nanoparticles.[12] The main pathway for entry of nanoparticles into the cell is receptor-mediated endocytosis. Optimal size of the nanoparticle for endocytosis is 25-50nm. It was suggested that a threshold size for single nanoparticle exists below which they could not be encapsulated. The nanoparticle size, ligand density and surface tension of membrane determines the dynamics of the receptor mediated endocytosis of single nanoparticles. Different pathways are observed in endocytosis of multiple nanoparticles. Small particle aggregates into cluster, intermediate sized aggregates into pearl – chain like arrangement and larger ones are independently internalized. The size of the NPs, distance between the particles and dose of particles determines the internalization pathways and accordingly four different pathways - synchronous internalization, asynchronous internalization, pinocytosis-like internalization, and independent internalization- are observed.[13]. Accurate quantification of NP internalization is necessary to study the physical and chemical properties on uptake into and clearance from the target cells.[14] Identifying the characteristics - size, shape, character, surface features, surface charge - of the nanoparticles present in homoeopathic drugs may help in understanding the cellular transport and mode of action apart from the standardisation of the drugs. Individual studies for detection of nanoparticles using some potencies of the Homoeopathic medicines with one or two procedures have given promising result. Hence it was proposed to conduct the material characterisation of different potencies of one medicine – Plumbum metallicum – with different nano characterisation procedures that can be helpful for further studies.

METHODOLOGY

Present study was conducted using field emission scanning electron microscope (FESEM) with EDS (energy dispersive spectroscopy), atomic force microscopy (AFM), x-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS) and high resolution transmission electron microscope (HRTEM). The drug Plumbum metallicum manufactured by the Dr.Willmar Schwabe India Pvt Ltd (WSI) according to the Homoeopathic Pharmacopeia of India was procured from market. This study was conducted without any pre – concentration and pre – treatment of the drug and no natural cork is used. Dry film on mica sheet or silicon wafer or respective grids were used for AFM, HRTEM, FESEM and XPS after sonication. Alcoholic solutions of different potencies are used for analysis of DLS studies and for the estimation of zeta potential. Hence, all the analysis was conducted in its natural state. FESEM analysis were carried out using Carl Zeiss Ultra 55 FESEM equipped with Oxford, EDS analyser. Approximately 5-7 ml of drug solution is taken in a vial, sonicated for 180 seconds. This drug solution is taken into micropipette and one drop is casted on aluminium foil and dried. Plumbum met 6C, 12C, 30C, 200C, 1M, 10M, 50M and CM were analysed using FESEM with EDS.AFM analyses of plumbum met 6C, 12C, 200C, 1M, 50M and CM potencies were carried out on Multimode SPM (Veeco Nanoscope V) (The probe used for imaging was antimony doped silicon cantilever with a resonant frequency of 300 kHz and spring constant of 40 Nm^{-1}) Samples were prepared by drop casting 2 µL solution of the sample on a freshly cleaved mica surface and dried under air. Imaging was done under ambient conditions in tapping mode. XPS analysis were carried out by AXIS ULTRA analyser. The drug solution is sonicated for 180 seconds and one drop (~0.25ml) casted on silicon wafer cleaned by isopropyl alcohol. X-Ray source used was monochromatic Aluminium (Al). DLS analysis for Z-average and Zeta potential determination were carried out by electro- chemical Trace analysis system- Malvern, after sonication for 15 minutes. HRTEM analyses were conducted with 30C, 200C, and 10M potencies using JEOL – JEM 2100 analyser. Drug solution is sonicated for 10 minutes and 2 drop casted on copper grid and dried. Then analysed under operating voltage of 200 kV, camera length 499.6 mm and resolution 0.25nm. Analysis of these potencies were conducted to estimate the size of the nanoparticles and to confirm their crystal structure using SAED pattern determination.

RESULTS AND DISCUSSION

FESEM images with EDS analysis has shown presence of different elements like oxygen, aluminum, sodium, silica, Sulphur, chlorine, potassium, calcium, zinc, magnesium, iron, and manganese along with Plumbum in different proportions in different potencies as shown in Fig-1.

The distribution of different elements in different potencies are demonstrated in Fig .2. The weight% of Plumbum met in different potencies are given in Fig.3. There is variable distribution of Plumbum met (weight%) in different potencies from 0.09% to 37%. FESEM study with EDS analysis of 6C, 12C, 30C, 200C 1M, 10M, 50M, and CM potencies have demonstrated the presence of different elements including Plumbum met in different concentrations. Apart from the presence of starting substance Plumbum met FESEM spectrum analysis have shown presence of oxygen, aluminum, silicon, chloride, potassium, calcium, zinc, sodium, iron, magnesium, sulphur, carbon, and manganese in different potencies. They might have aroused from the sugar of milk used as a base for the preparation of medicine or from the instruments used for the preparation of potencies during succession and the resultant shearing force or from the grids used for the analysis. Weight % of the original drug substance, Plumbum met particles, in these potencies are variable (Fig-3). Contrary to the common belief of reduction in quantity of the original substance as potentization advances, highest presence (weight %) is seen in 10M potency and 200C potency and lowest in 50m and CM potencies. FESEM results confirm the formation of agglomeration of nanoparticles. AFM analysis of 6C, 12C, 200C, 1M, 50M and CM potencies has also demonstrated particles with variable distribution of height in different potencies. The details of 2D (particle height profile in inset) and the 3-D images are given in Fig -4. The height of the particles varies from 4 - 50nm. AFM analysis have shown particles with varying height (Fig-4). This may be due to presence of agglomeration as observed in FESEM analysis. Apart from this, AFM analysis demonstrates more number of particles, in 200C in confirmation with the finding of highest presence of atomic and weight percentage in FESEM analysis and the height of the particle are reduced and they are closely dispersed. XPS analysis of Plumbum met with varying potencies viz. 6C, 12C, 30C, 200C, 1M, 10M, 50M and CM are given in Fig–5(a). Analysis of XPS spectrum shows variation in mass concentration percentage ranging from 1.32 to 5.39 (Fig- 5 b) and is maximum in 200C. XPS analysis of 6C, 12C, 30C, 200C, 1M, 10M, 50M and CM potencies has further confirmed the presence of original medicinal substance Plumbum met in all potencies as given in Fig-5a. XPS spectrum analysis has demonstrated the presence of other elements like carbon, oxygen along with Plumbum met. The percentage of mass concentration obtained for different potencies are shown in Fig- 5b. Table 1 shows the overall XPS data of the analysis with atomic weight percentage.

Table 1. XPS analysis of Plumbum met at different potencies showing the FWHM values and atomic concentration percentage

Potency	Position BE(eV)	FWHM (eV)	Atomic Conc.(%)
6C	133.6	5.151	0.15
12C	134.6	7.120	0.24
30C	134.9	6.394	0.20
200C	133.5	6.180	0.39
1M	134.0	6.020	0.30
10M	134.6	3.680	0.09
50M	134.6	6.446	0.17
CM	133.6	5.451	0.16

Table 2. Z-average and zeta potential of Plumbum met in different potencies

	Z-average (nm)	Zeta potential (mV)
6C	854	-30.3
12C	1326	-58.6
30C	704.9	-7.43
200C	1300	-15.1
1M	787.7	-21.7
10M	697.3	-20.5
50M	631.2	-8.73
CM	1161	-8.99

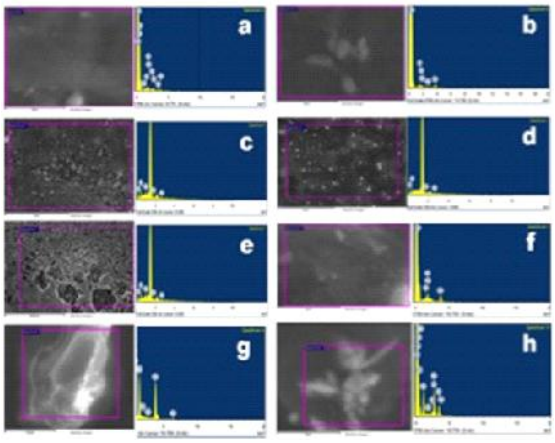

Fig. 1. FESEM images and EDS analysis of (a) Plumbum met 6C (b) Plumbum met 12C (c) Plumbum met 30C (d)

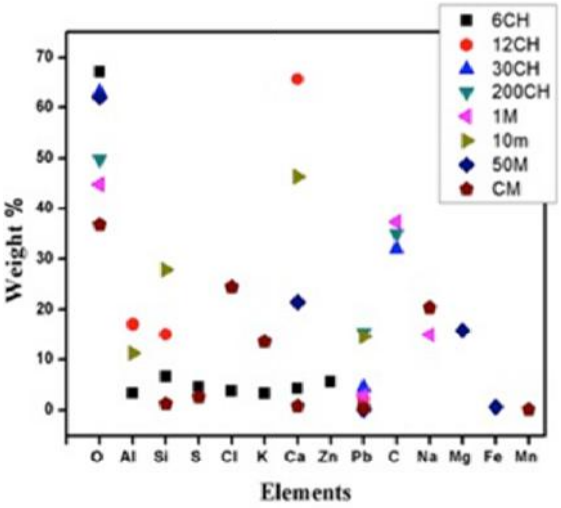

Fig. 2. Distribution of different elements from FESEM and EDS analysis

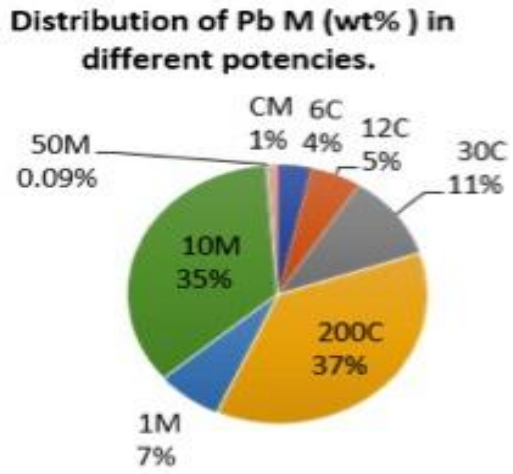

Fig. 3. Elemental distribution of Pb M (weight %) in different potencies by EDS analysis

The Z-average of different potencies of Plumbum met varies differently. Z- Average varies from 631.2 nm to 1300 nm. The increased size of these particles can be attributed to the agglomeration due to the surface charge. The Zeta potential also shows a similar nature with variation from -7.43mv to -58.6mv and is given in Table–2. These demonstrate particles varying size and stability. Among the different potencies analysed, 6C and 12 c have shown a stable zeta potential value (Table – 2).

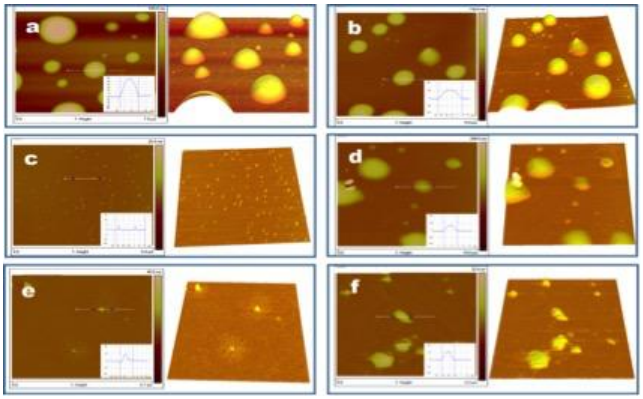

Fig. 4. AFM 2D(particle height profile in inset) and 3D images of (a) Plumbum met 6C, (b) Plumbum met 12C, (c) Plumbum met 200C, (d) Plumbum met 1M, (e) Plumbum met 50M, (f) Plumbum met CM

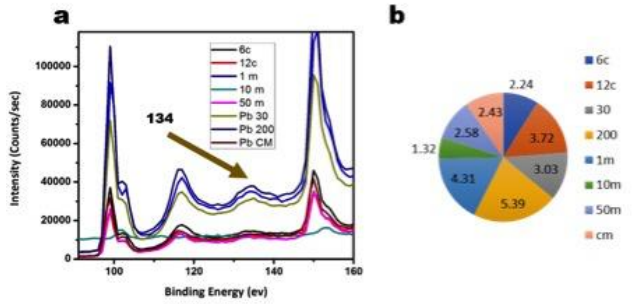

Fig. 5. (a) XPS spectrum of different potencies of Plumbum met (b) Distribution of mass concentration (%) in different potencies

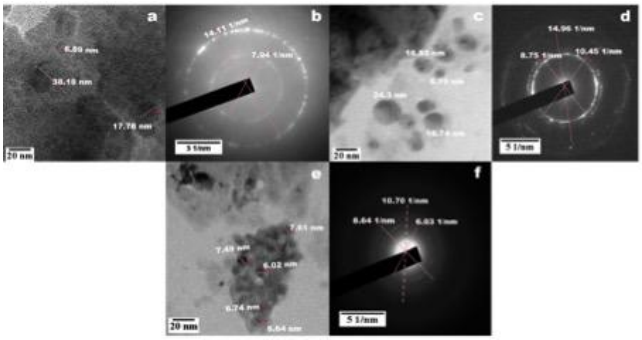

Fig. 6. HRTEM and SAED pattern of (a and b) Plumbum met 30C, (c and d) Plumbum met 200C, (e and f) Plumbum met 10M

HRTEM images and SAED pattern of Plumbum met 30C, 200C, and 10M potencies are given in Fig-6 (a to f). The size of the particles varies from 6.89 nm to 38.18nm in 30 C potency, from 8.19nm to 24.3nm in 200C potency, and between 6.02nm to 7.61nm in 10M potency. HRTEM study in 30, 200, and 10m potencies have shown the presence of particles in varying sizes. Clusters and agglomeration of these particles are also seen. The size of the particles varied from 5.65nm to 42.93 nm diameter. The selected area diffraction patterns from TEM analyses were investigated. Fig 6(b) shows the SAED pattern of Plumbum met with 30 CH. The d-spacing from SAED pattern at 0.142 nm corresponds to (112) plane, 0.249 nm corresponds to (101) of lead (Pb) as per the ICSD Ref code 00-023-034. In sample 200 C given in Fig 6(d), 0.149 nm corresponds to (103) and 0.125 nm to (202) of Pb (Ref code 00-023-0345), spacing 0.195 nm corresponds to (102) and 0.251 nm to (101) of Pb (ICSD Ref code 00-023-0345)). In sample 10 M (f) 0.251 nm corresponds to (101), and 0.195 nm corresponds to (102) of Pb. According to the mode of preparation of homoeopathic drugs the mass concentration of the original material have to be reduced as potentisation increases. But, contrary to this, all investigations in this study shows that there is no inverse relation with the mass concentration and degree of potentisation.

The mass concentration, weight%, Z- average and zeta potential are differently present without any correlation to the degree of potentisation.

CONCLUSION

Present study establishes the presence of Nano particles of original starting material - Plumbum metallicum - in all potencies, opening a new avenue for the characterisation. Particles of starting material along with other elements are present in varying sizes, height, concentrations, stability, and surface charge. All potencies have shown varying degree negative charges. Contrary to common belief, the mass concentration, size and surface charges are not correlated directly to the degree of potentisation (serial dilution and succession). These observations have to be confirmed by further repeated experiments using same medicines, other drugs and drugs prepared by different firms.

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Glossary of abbreviations:

AFM - Atomic force microscopy.
C- Centesimal scale of preperation of Homoeopathic medicine. (Potencies of 1000C and above are usually labelled with Roman numeral M and with the centesimal 'C' indicator implied (since all such high potencies are centesimal dilutions)1M = 1000C; 10M = 10,000C;
50M = 50,000C;CM = 100,000C).
CH- Centesimal scale of homoeopathic medicine prpared according to Hahnemannian Method.
DLS-Dynamic Light Scattering.
d- decemal scale of prperation of Homoeopathic medicine.
EDS- Energy Dispersive Spectroscopy.
FESEM- Field Emission Scanning Electron Microscopy.
FWHM –Full Width at Half Maximum
HERTEM - High Resolution Transmission Electron Microscopy.
ICP-AES – Inductively Coupled Plasma – Atomic Emission Spectroscopy.
ICSD- Inorganic Crystal Structure Database.
NMR- Nuclear Magnetic Resonance.
NP- Nano particle.
Pb, Pb M – Plubum Metallicum.
SAED - Selected Area Electron Diffraction.
SPM- Scanning Probe Microscope.
STIC- Sophiscated Test and Instrumentation centre.
TEM- Transmission Electron Microscopy.
WSI- Dr.Willmar Schwabe India Pvt Ltd.
XPS- X-ray Photoelectron Spectroscopy.

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