

A Nano study: Fineness in action of Homoeopathic medicine- *Natrum muriaticum*

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ABSTRACT- *Natrum muriaticum*, a mineral based remedy which is considered as natural medicine in which the nano-quantities of medicinal substances are used effectively to cure the diseases. The two-century old Homoeopathic system of medicine and the most recent science Nano-technology can be interrelated together as both gain access to the cellular level and their compartments including the nucleus. In this study our aim was to investigate a possible Nano science mechanism of action of homoeopathic medicine. *Natrum muriaticum* is prepared through a characteristic process known as potentization, where serial dilutions are performed with strong strokes at each step of dilution. *Natrum muriaticum* belongs to mineral group of remedies prepared by "common salt" derived from the ocean. *Natrum muriaticum* mother solution and potencies are capable of precipitating silver nanoparticles from silver nitrate at ambient temperature. It is a polychrest remedy found in all tissues of our body. The nanoparticles were characterized by UV spectrophotometer, SEM and by FTIR functional group of medicine. The synthesized silver nanoparticles were generally found to be effective as antimicrobial against some important pathogen like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella sp.* and *Lactobacillus acidophilus* and also as an antioxidative agent with 83.3% of inhibition. The nanoparticles were characterized by UV spectrophotometer where the peak was obtained at the range of 233 nm for mother solution, 240nm for 30c potency, 193nm for 200c, 374nm for 1M and 195.0nm for 10M. Scanning electron microscope (SEM) image of *Natrum muriaticum* mother solution and 30CH showing nanomaterials as cuboidal in shape in 200nm. The work was concluded as *Natrum muriaticum* contains nanoparticles even in higher dilutions beyond Avogadro's number, hence it can be qualified as nanomedicine.

KEY WORDS- NATRUM MURIATICUM, POTENTISATION, SILVER NANOPARTICLE, FOURIER TRANSFORM INFRARED MICROSCOPE (FTIR), UV SPECTROMETER, SCANNING ELECTRON MICROSCOPY(SEM)

INTRODUCTION-

Homoeopathy is the system of medicine which was discovered by Dr. Samuel Hahnemann in 1796 which is based on Natures Law "Similia Similibus Curantur" Let likes be treated by likes". [12] Homoeopathic medicines are prepared through a characteristic process known as potentization.[15] Homoeopathic potentization is a mathematico-mechanical process for the reduction, according to the scale, of crude, inert or poisonous medicinal substances to a state of physical solubility, physiological assimilability and therapeutic activity.[7] The dynamicity of the potencies might act on the vital force of the patient in its curative action. Homeopathically made nanoparticles would initiate adaptive changes in an organism.[4] The recent advent of nanoscience and

related technology with advanced electron microscopy are capable of changing all the past perception about homoeopathy and its drug action. Nanomedicine and nanotechnology accept and realize that particles smaller than the size of 100nm possess properties entirely different from that of particles of larger size. They are capable of showing new chemical and physical properties of bigger dimensions. It is observed that these particles smaller than a critical size have different electronic structure, conductivity, reactivity, melting temperature and mechanical properties.

A nanoparticle is a very small particle made from a specific source material. Because of their smaller sizes many experts limit consideration to nanoparticle in the size range of 1-100nm.[16] Nanoparticle have unique physicochemical and biological property including high catalytic activity and DNA absorption which differ from bulk material dose. Likewise, homoeopathic Nano-particles have abilities to arouse biological signals to penetrate through cellular structures and to stimulate the body's own immune responses.[16]

Natrum muriaticum is a remedy prepared from table salt. It acts on blood and lymphatic system, alimentary tract, genital organs, urinary organs, skin and mind. It is a remedy for certain forms of intermittent fever, anemia, chlorosis, many disturbances of alimentary tract, hypothyroidism, hyperthyroidism, eczema, allergic rhinitis and diabetes. *Natrum muriaticum* has been extensively proved both in lower and higher potencies.[5]

MATERIALS AND METHODS-

PREPARATION OF MOTHER SOLUTION-

As common salt is soluble in small quantities of distilled water, it belongs to class VA, according to the old method of preparation of mother solution. To prepare 100ml of *natrum muriaticum* mother solution, 10gm of pure non iodized sea salt was taken and 90ml of distilled water was added to a clean phial. Only 1/4th of phial was filled. Then the phial was covered with a cork and solution was mixed.

SYNTHESIS OF SILVER NANOPARTICLE PREPARATION:

Homoeopathic mother solution *Natrum muriaticum* was used for the synthesis of silver nanoparticles. 100mg of silver nitrate was added to 20 ml of distilled water. Vigorously stirred with 5ml of mother solution. A change in the color of solution to be observed after 30 minutes for mother solution. Similarly, synthesis of silver nanoparticles was done for different potencies 30CH, 200CH, 1M and 10M of *Natrum muriaticum*.

CHARACTERISATION OF SILVER NANOPARTICLES:

The synthesized particles were characterized using UV spectroscopy, FTIR analysis and Scanning electron microscopy (SEM). The UV –spectroscopy analysis was used to study the absorption peak of synthesized particles. The FTIR analysis was used to study the functional groups present in the synthesized nanoparticles. The SEM technique was employed to visualize the size and shape of silver nanoparticles.

1. UV SPECTROPHOTOMETER:

The reduction of pure Ag⁺ ion was monitored by measuring the UV-VIS spectrum of the reaction medium at 5 hours after diluting a small amount of the sample into distilled water, UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

2. SCANNING ELECTRON MICROSCOPY (SEM):

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. SEM technique was employed to visualize the size and shape of silver nanoparticles. The pattern of dependence of the morphology and size of the Nano sized objects is described.

3. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):

The Fourier transform infrared spectroscopy is used to study the infrared absorption of particles. IR spectra were recorded using KBr pellets on a Perkin – Elmer GX FTIR spectrophotometer.

ANTIOXIDANT ACTION:

Antioxidant property was determined by DPPH assay.

The Antioxidant activity was evaluated by 2, 2- diphenyl-picrylhydrazyl (DPPH) inhibition assay method. A volume of 0.1mM solution of DPPH was used while mother solution and potencies (1.25, 5, 2.5 µl volumes) were used for evaluation of antioxidant activity. Ascorbic acid was taken as a standard control and alcohol as negative control in antioxidant activity protocol. Total phenolic content was measured by Folin-Ciocalteu reagent assay. Total phenolic content of mother solution and potencies were measured in comparison with ascorbic acid.

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

ANTIMICROBIAL ACTION:

Preparation of microbial cultures and isolation of *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus anthracis* and *Staphylococcus aureus* were used from P.G department of Alva's college and microscopic examination was done for the confirmation and were maintained in slants.

ANTIMICROBIAL ASSAY:

Petri dishes were plated with Nutrient Agar media and allowed to solidify for 30 minutes. The organisms were spread on surface of the media using sterile swab stick. Cork borer (7mm) was used to bore wells in media. The silver nanoparticle extract in different potencies (30C, 200C, 1M, 10M, Mother solution) were dispensed into the wells using a micropipette. A positive control of acetone was kept and the extract was allowed to diffuse for 1 hour at room temperature. Then the plates were incubated at 37°C for 24 hrs. Zones of inhibition were measured in centimeters.

RESULT-

Synthesis of nanoparticles from homoeopathic mother solution “*natrum muriaticum*”

The silver Nano particles were synthesized by the reduction of silver ions. This was shown by the change in color of solution from transparent to white opaque solution. The color change in reaction mixture was observed immediately.



Figure 1: color change indicates the formation of nanoparticles

Characterization of synthesized nanoparticles

UV-visible Spectroscopy:

The change in the color was visually observed which indicates the presence of silver nanoparticles. The change in the color is mainly because of surface plasma resonance. The UV-visible spectroscopy is an important technique to study the metal nanoparticles.

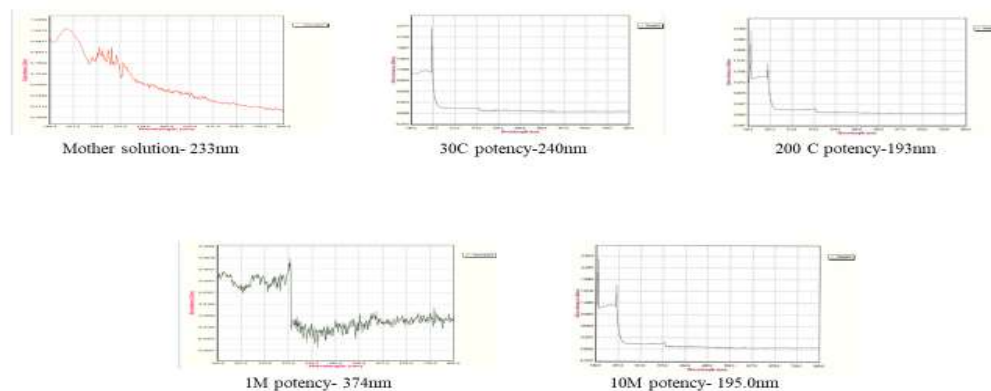


Figure 2 indicates UV spectrophotometer results of the samples

SEM –

The scanning electron microscopy was used to observe the surface morphology Figure 3 and 4 showed particles were cuboidal in shape up to 200nm.

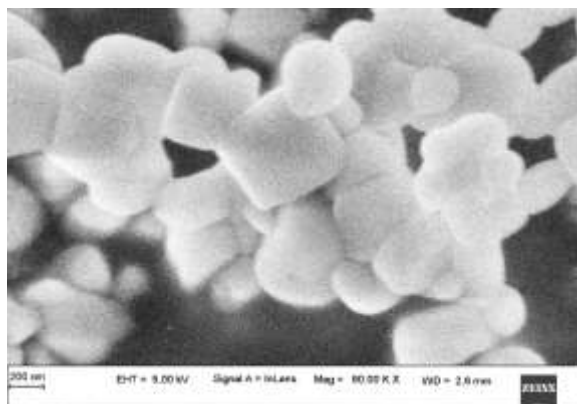


Figure 3: *Natrum muriaticum* mother solution showing nanomaterials

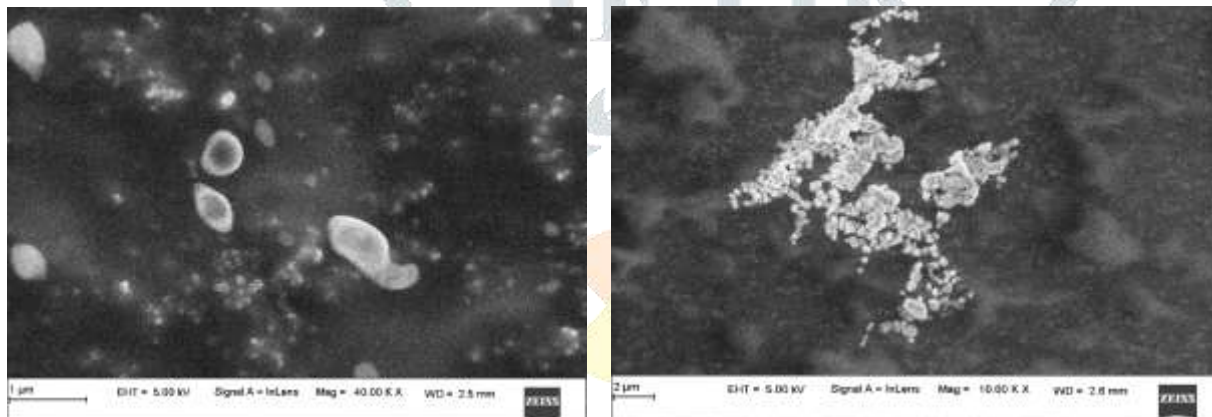


Figure 4: *Natrum muriaticum* 30CH showing nanomaterials

FTIR-

The IR spectrum for sample of SNPs showed bands at 3358.07cm^{-1} , 2922.16cm^{-1} , 2852.72cm^{-1} , 1728.22cm^{-1} , 1359.82cm^{-1} , 1165.00cm^{-1} , 1029.99cm^{-1} , 862.18cm^{-1} , 719.45cm^{-1} , 538.14cm^{-1} for figure 5.

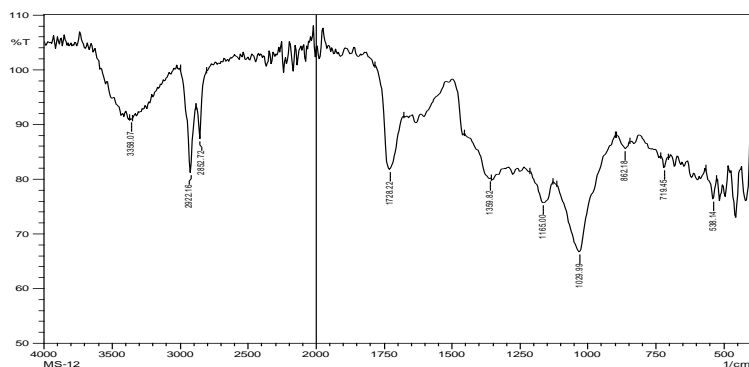


Figure 5: FTIR spectra of powdered SNPs of mother solution

The band at 3358.07cm^{-1} represents N-H O-H bonds. The band at 2922.16cm^{-1} corresponds to C-H bond. The band at 2852.72cm^{-1} showed C-H bond. The band at 1728.22cm^{-1} represents C=O bond. The band at 1359.82cm^{-1} corresponds to C-O-C bond. The band at 1165.00cm^{-1} showed C-O-C bond.

The IR spectrum for sample of SNPs showed bands at 3408.22cm^{-1} , 2922.16cm^{-1} , 2852.72cm^{-1} , 1732.08cm^{-1} , 1359.82cm^{-1} , 1159.22cm^{-1} , 1033.85cm^{-1} , 1033.85cm^{-1} , 719.45cm^{-1} , 459.06cm^{-1} for figure 6.

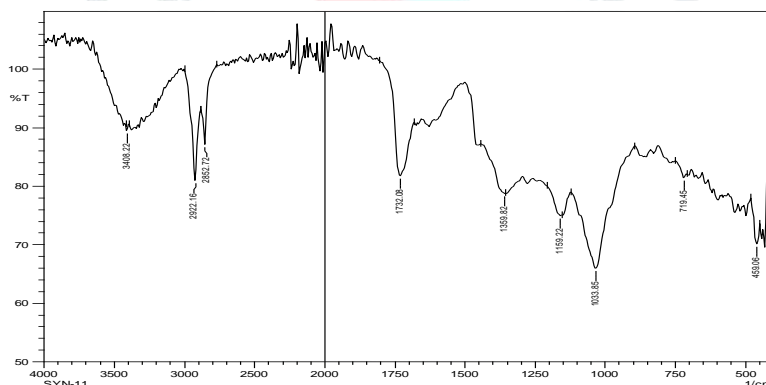


Figure 6: FTIR spectra of powdered SNPs of synthesized mother solution

The band at 3408.22cm^{-1} represents stretch of N-H O-H bond. The band at 2922.16cm^{-1} corresponding to C-H bond. The band at 2852.72cm^{-1} showed 2852.72cm^{-1} C-H bond. The bond at 1732.08cm^{-1} represent C=O bond. The band at 1359.82 represents C-O-C bond.

ANTIOXIDANT ACTIVITY-

Tab 1 represents the antioxidant activity of natrum muriaticum samples. The maximum percentage of inhibition was shown by NM30C(syn), NM200C, NM200C, NM 1M(syn), NM 10M and NM10(syn).

Tab 1

S no.	Samples	% of inhibition
1.	NM MS	83.3% \pm 1 or 2
2.	NM MS (syn)	83.3% \pm 1 or 2
3.	NM 30C	83.3% \pm 1 or 2
4.	NM 30C(syn)	91.6% \pm 1 or 2
5.	NM 200C	91.6% \pm 1 or 2
6.	NM 200(syn)	91.6% \pm 1 or 2
7.	NM 1M	83.3% \pm 1 or 2
8.	NM 1M(syn)	91.6% \pm 1 or 2
9.	NM 10M	91.6% \pm 1 or 2
10.	NM 10M(syn)	91.6% \pm 1 or 2

ANTIMICROBIAL ACTION-

Tab 2a. represents antimicrobial activity of Natrum muriaticum in all potencies (MS,30C,200C,1M and 10M)

Tab 2b. represents antimicrobial activity of synthesized Natrum muriaticum in all potencies (MS,30C,200C,1M and 10M) The zone of inhibition in *Escherichia coli* was observed maximum in NM200C and NM30C(syn). The zone of inhibition in *Klebsiella sp.* was observed maximum in NM 10M(syn). The zone of inhibition in *Lactobacillus acidophilus* was observed maximum in NM30C(syn) and NM1M(syn). The zone of inhibition in *staphylococcus aureus* was observed maximum in NM30C and NM1M(syn).

Tab 2a.: -

S no.	Microorganism	MS	NM 30C	NM 200C	NM 1M	NM 10M
1.	<i>Escherichia coli</i> ATCC 25992	0.5cm	0.5cm	1 cm	0.5cm	0.5cm
2.	<i>Klebsiella sp.</i>	0.5cm	0.5cm	0.5cm	0.4cm	0.5cm
3.	<i>Lactobacillus acidophilus</i> MTCC 10307	0.5cm	0.5cm	0.5cm	0.5cm	0.5cm
4.	<i>Staphylococcus aureus</i> ATCC 29213	0.5cm	1 cm	0.8cm	0.6cm	0.5cm

Tab 2b.: -

S no.	Microorganism	MS (syn)	30 C(syn)	200C(syn)	1M(syn)	10M(syn)
1.	<i>Escherichia coli</i> MTCC 10307	0.6cm	1.3cm	1.2cm	1.2cm	1cm
2.	<i>Klebsiella sp.</i>	0.9cm	0.5cm	0.6cm	0.6cm	1cm
3.	<i>Lactobacillus acidophilus</i> MTCC 10307	1.1cm	1.2cm	1cm	1.2cm	0.6cm
4.	<i>Staphylococcus aureus</i> ATCC 29213	0.5cm	1.2cm	1.2cm	1.4cm	0.8cm

DISCUSSION-

Natrum muriaticum is a commonly used homoeopathic remedy in a variety of acute and chronic allergic conditions like eczema, seasonal allergic rhinitis, asthma, food allergy and chronic allergic rhinitis. Common salt (NaCl) in its raw form has never showed curative effect in any of these diseases. But the same produces curative effect with higher dilutions. The homoeopathic process of potentization plays a vital role.[8] Homoeopathy can be perceived as like nanomedicine as it uses minute sub molecular doses. However, the philosophy, scientific principles and terms of homoeopathic use are clearly delineated as an established, independent medical science and thoroughly documented.[11] Toumey compared homoeopathic medicines Aurimune, argued that nanomedicines differ from homoeopathic medicines. The major difference is the use of a known amount of medicine in case of nanomedicines compared to homoeopathic medicines. But in preparation of homoeopathic medicine measured quantity of medicine and vehicle is taken. It may be argued that what matters here is the 'size' of the possible encrypted information.[2] The color change in the reaction mixture was observed visually. By UV spectrometer peaks we had come to conclusion that reduction has taken place in the solution. The main difficulty in arriving at a rational explanation stems from the fact that homoeopathic medicines are used in extreme dilutions, including dilution factors exceeding Avogadro's number. By this study using Scanning Electron Microscope the size and shape of the particle in nanometer has been obtained from mother solution and by FTIR, the functional groups in Homoeopathic medicine natrum muriaticum are studied. Antimicrobial action was studied on organisms *S. aureus*, *Lactobacillus*, *E. coli* and *klebsiella* and a positive result with transparent circumference around the wells with potencies has been observed, which confirms that bacteria were unable to grow in and around homoeopathic medicines. Many recent studies have proved that nanotechnology has a new therapeutic modality in silver particles for use in medicine.[17] In this investigation the biosynthesized silver particles were shown to have antioxidant property of 83.3%.

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