

Effect of Different Potencies of Nanomedicine Aconitum Napelles on Its Spectral and Antibacterial Properties

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ABSTRACT: The homeopathic drug *Aconitum napelles*, a drug of aconitum species, is used extensively for its anti-inflammatory and vasodilatory action. We report here our study on how the increase in the potency of the drug affects its spectral characteristics, namely UV-Vis spectra, Fourier Transform Infrared Radiation (FTIR) spectra, Raman spectra and Field Emission Scanning Electron Microscopy (FESEM). The absorption peaks of the UV-Vis and FTIR spectra indicate definite signature of decrease in the size of the drug aggregates with increase in potency of the drug. This is confirmed in the FESEM study. Raman spectra indicate that there is no associated change in chemical structure due to potentization.

Using *Staphylococcus aureus*, a gram positive bacteria and *Escherichia coli*, gram negative bacteria, we have also shown here how the change in potency of the drug affects its antibacterial property. A possible explanation of these effects has been put forwarded at the molecular level.

KEYWORDS: *Aconitum napelles*, nano associate, spectral characteristics, high dilution, antibacterial property

I. INTRODUCTION

Truly, homeopathy is the third-most commonly used system of healing in the planet and is being practiced for more than two centuries because of their negligible side effects, low cost, easy availability and easier applicability. These medicines are micro dosed natural substances derived from plants, animals or mineral sources. The basic ingredient when diluted and succussed have specific therapeutic effectiveness. Interestingly, contrary to allopathic medicines, homeopathic medicines are more potent at higher dilution. Their dilution, which is an index of their potency, plays a crucial role in their therapeutic and medicinal applications.

The homeopathic medicine is prepared by an iterative process of centesimal (1: 100) dilution of the mother tincture and shaking (succussion). The process is repeated until the very low molarity repetitive succussed diluted liquid (VLMRSDL) is obtained when the composition of the solution becomes identical to that of the solvent used for its preparation. As potency 1C means dilution 10^2 and Avogadro number is 6.02×10^{23} , the initial solute is virtually no longer present in the medicine beyond potency of 12 C (i.e., dilution by a factor of 10^{24}). But there are ample evidences of people being cured by homeopathy at potency of 200C (i.e. dilution by a factor of 10^{400}). To explain these curious phenomena, several hypotheses have been forwarded, ranging from liquid memory, clathrate formation etc.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

The silica hypothesis suggest the presence of physical entities [1], whereas the liquid memory hypothesis is forwarded by others [2, 3].

At such high dilution, the physical properties also get modified significantly from the original liquid. This is reflected in the measured values of NMR, UV-Vis spectroscopy, dielectric behavior, electrical conductivity and heat of mixing measurements of the resulting VLMRSDL [4,5]. Quantum field theory analysis of experimental data for VLMRSDL especially to explain their UV-Vis, FTIR, NMR studies have also been done. The study indicates that succussion excites rotors and phonons that are responsible for the change [6]. In a different context, T. Maity et al. have shown that a characteristic set of resonance frequencies exists for different dilutions of the homeopathic drug *Cuprum metallicum* [7].

The fact that these medicines are active at such extreme dilution and that one medicine of “zero” concentration is different from another medicine of concentration “zero”, have drawn the skepticism and curiosity of the conventional scientific community, leading to the popular belief that perhaps these medicines have only a placebo effect! To decipher this apparently strange effect, extensive research work is going on and several hypotheses have been forwarded. Elia et al [8] have given experimental evidence of stable water nanostructures at high dilution at standard pressure and temperature. Konovalov et al [9] have argued that formation of nanoassociates, which are nanosized molecular assemblies, are responsible for the observed physicochemical and biological properties at high dilution. Using *Aconitum napelles*, it was reported for the first time in 2010, that at higher potency the size of the drug-aggregates reduces, making it more permeable to the liposomal membrane [10]. By 2011, it was confirmed by several research groups that at extreme dilution homeopathy emerges as nanomedicine [11-14] thus confirming the idea of formation of nanoassociate, as has been proposed by Konvalov et al [9].

Here we report our study on how the change in potency of this drug affects its spectral characteristics, namely UV-Visible spectra, Fourier Transform Infrared Radiation (FTIR) spectra, Raman spectra and Field Emission Scanning Electron Microscopy (FESEM). The absorption peaks of the spectra of the drug at different potencies (6C, 30C and 200C) show definite signature of decrease in the size of drug aggregates with increase in potency of the drug. This is also confirmed in the FESEM study.

Using *Staphylococcus aureus* (*S. aureus*), gram positive bacteria and *Escherichia coli* (*E. coli*), a gram negative bacteria, we have also shown here how the change in potency affects the antibacterial property of this drug. The effect is more significant in case of *E. coli* and hence this study is quite important as though most *E. coli* strains are harmless, but some virulent strains can cause serious food poisoning in their hosts.

Aconitum napelles is a homeopathic drug which is commonly used as anti-inflammatory and vasodilatory agent and has also been studied for its ability to target voltage-gated sodium ion channels [15]. We have reported earlier that when this hydrophobic drug is administered to liposomal membrane, then with increase in dilution of the drug, the rigidity of the membrane increases. From this observation, it was inferred that at higher dilution, which is equivalent to higher potency, the size of the drug aggregates decreases, resulting into higher membrane permeability and thus higher rigidity

II. MATERIALS AND METHODS

Aconitine, a crystalline drug comes from the roots of plants of the genus *Aconitum* [16]. The molecule consists of one benzene ring and other hydrophobic structure, conferring the hydrophobic nature to the drug. It is the principal alkaloid in the *Aconitum napelles* root. The freshly prepared drug *Aconitum napelles* at different potencies had been a gift from Dr. D.S. Bhar of Hahnemann Publishing Company, Kolkata and were used without further purification.

For the antibacterial study, two different bacteria, *S.aureus*, a gram positive bacteria (MTCC Strain No. 96) and *E.coli*, a gram negative bacteria (MTCC Strain No.1652) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, a concern of Government of India.

Peptone, beef extract, yeast extract powder, sodium chloride, agar-agar were obtained from HIMEDIA Laboratories Pvt. Ltd. India and were used without further purification. Milli Q water was used throughout the experiment.

International Journal of Innovative Research in Science, Engineering and Technology

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Raman set up consisted of the spectrometer (Lab RAM HR Jovin Yvon) and a Peltier cold CCD detector. An air cooled argon-ion laser (Ar^+) with wavelength 488 nm was used as excitation light source.

The absorption spectra were taken by a Perkin Elmer UV-Vis spectrophotometer (Lambda-25, Perkin Elmer, USA) at room temperature ($\sim 25^\circ C$).

Fourier Transform Infrared Radiation (FTIR) spectra were taken on 100 FTIR spectrometer (Perkin Elmer), scanning over the range $4500-500\text{ cm}^{-1}$ at a resolution of 4 cm^{-1} with the background scan subtracted.

Field Emission Scanning Electron Microscopy (FESEM) was performed with gold coating the samples for conforming morphology and size by using Inspect F50 SEM, FEI Europe BV.

Antibacterial activity of *Aconitum napellus* was studied using spread plate technique, where *S.aureus* and *E.coli* were treated with the drug at different potencies (6C, 30C, 200C). The strains were grown in bacterial nutrient broth (peptone 0.5%, beef extract 0.1 %, yeast extract 0.2%, NaCl 0.5%) for 24 hours at $37^\circ C$ then subcultured with various drug potencies (6C,30C,200C) for 24 hrs. Bacteria (20 μ l double diluted) were then spread over nutrient agar plate. Nutrient broth with agar 1.8% was used as the solidifying agent. After overnight growth (~ 12 hours), colonies were observed and compared against control plates of both the strains.

IV. RESULT AND DISCUSSION

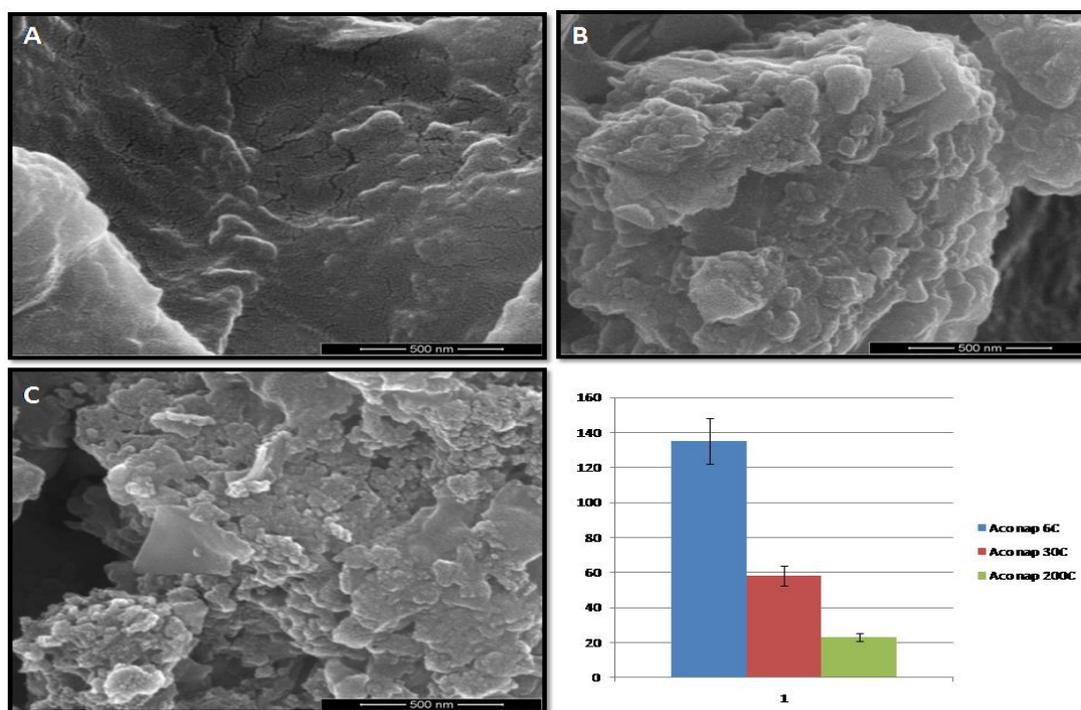


Fig.1: FESEM images of the drug at different dilutions. (A) 6C; (B) 30C; (C) 200C and the bar diagram represents the particle size change with standard deviation.

The FESEM diagram (Fig.1) shows how the poly dispersive drug-aggregates change to mono-dispersive ones with decrease in aggregate size. This happens due to the repeated succession of the drug. The figure shows that at 6C potency of the drug, the average dimension of the drug aggregate lies between 120 nm to 150 nm. The range of dimension of the drug aggregates is within 51 nm to 64 nm for 30C potency, while it reduces to 20 nm to 25 nm for 200C potency of the drug.

International Journal of Innovative Research in Science, Engineering and Technology

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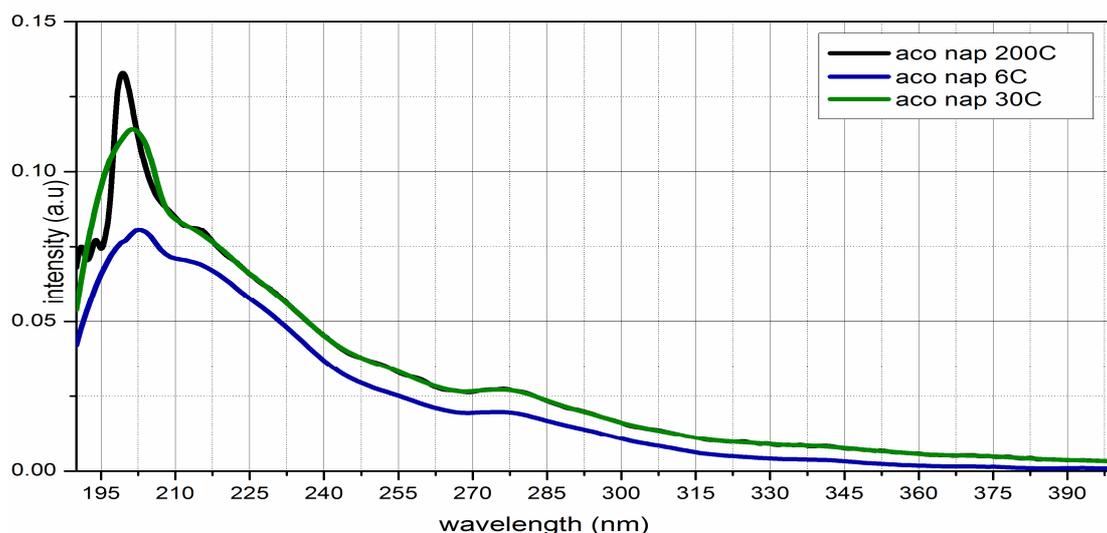


Fig.2: UV-VIS absorption spectra of the drug at different dilutions. (a) 6C; (b) 30C; (c) 200C.

In the UV-Vis absorption spectra (Fig.2), several peaks were obtained within a range of 190- 390 nm. According to Segarra-Marti et al [16], this broad band is typical for structured water. The band appears in other structured waters also, e.g. homeopathic medicine as studied by Elia et al [8].

Two prominent absorption maxima are observed around 200 nm and 277 nm for all potencies. With increase in potency there is an increase in magnitude in the absorption peak, along with a blue shift, while the peak sharpens.

Due to increase in potency the size of the drug aggregate decreases, as has been indicated in their increase in penetration in the liposomal membrane [12]. This inference is also confirmed from the FESEM images (Fig.1). The decrease in size gives rise to an increase in the ratio of surface to bulk atoms and the size distribution changes to mono dispersive ones, thus increasing the absorption of radiation, which in turn increases the height of the absorption peak. With the decrease in size the gap between the associated energy levels increase, leading to the blue shift as observed in the UV-Vis absorption spectra. Using gold nanoparticles He, et al [17] have shown that similar results are obtained where the absorption spectra of the gold nano particles depend upon the dimension of the particles and there are gradual blue shifts with decrease in diameter of the particles. Ghosh et al have shown that in case of the homeopathic medicine *Cuprum metallicum*, the peak height is a function of the potency of the drug and with increase in potency there is an associated blue shift [18]. The drug aggregate is polydisperse in nature for lower dilution and approaches mono dispersive nature as the dilution increases thus enhancing the sharpness of the peaks.

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(An ISO 3297: 2007 Certified Organization)

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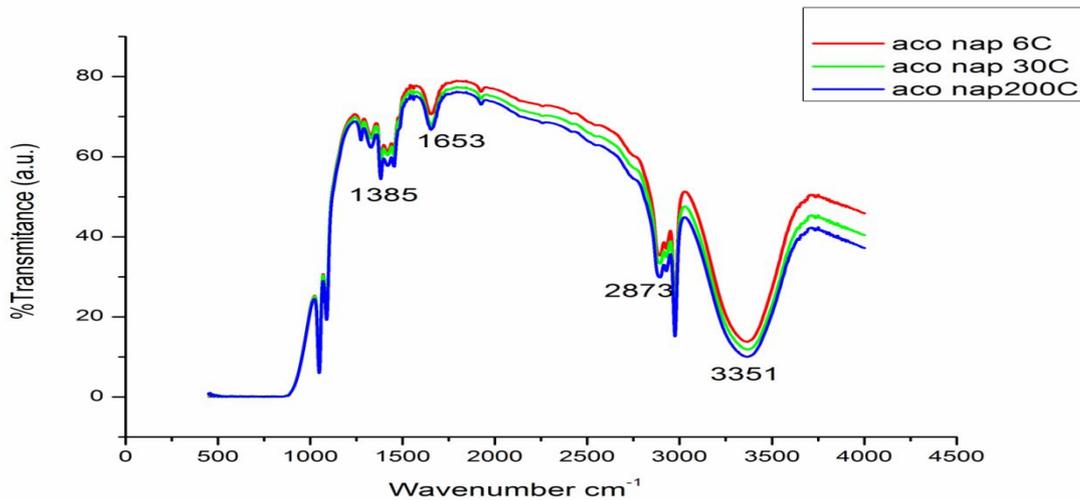


Fig.3: FTIR absorption spectra of the drug at different dilutions of 6C; 30C; 200C

Comparison of FTIR spectra (Fig. 3) between different potencies of the drug reveals that peaks for all the three potencies are obtained at the same position, but the peak intensity and sharpness increase with increase in potency.

The band at 1385 cm^{-1} corresponds to phenol or tertiary alcohol, OH bend, the band at 1653 cm^{-1} corresponds to C=C stretch, the band at 2873 cm^{-1} corresponds to methyl C-H asym/sym stretch and the band at 3351 cm^{-1} corresponds to normal polymeric OH stretch and hydroxyl group, H bonded OH stretch. [19] It appears that due to succession the bond strength of OH bond and H bond change with potency and hence there is a minor blue shift..

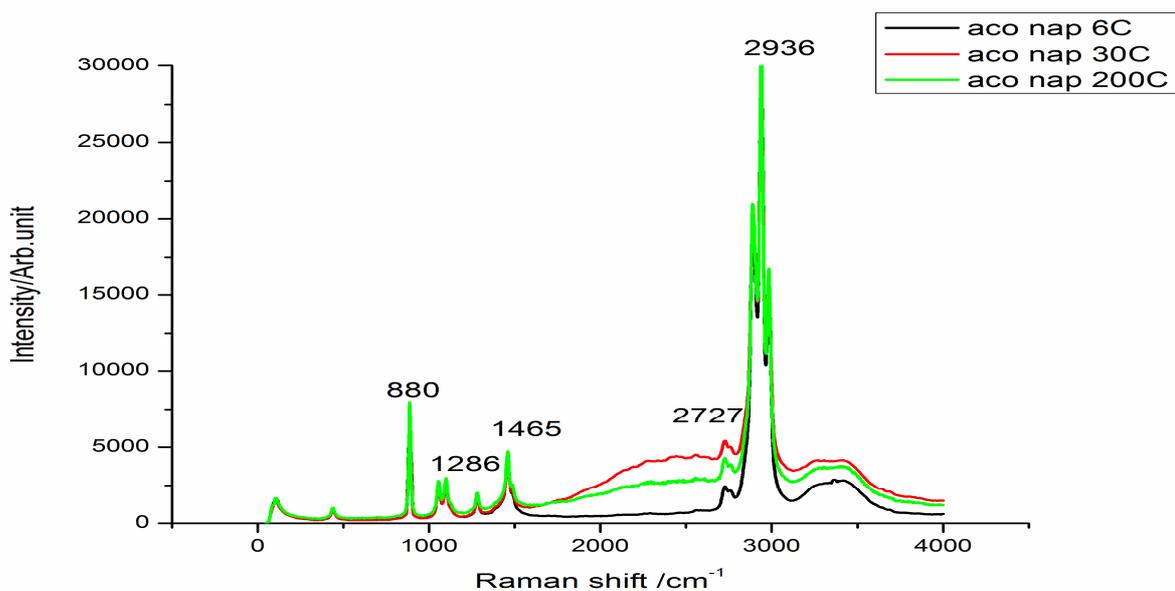


Fig. 4: The Raman spectra at three potencies of the drug 6C; 30C; 200C

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In figure 4, the Raman spectra are almost identical except the region from 2000 to 3700 cm^{-1} where C–H stretching and OH vibrations are present [20, 21]. The result implies that only the C–H and OH bonds are modified as the potency changes. From the Raman spectra, the size effect cannot be explained. However, it reveals that there is no change in the chemical structure of the drug due to potentiation.

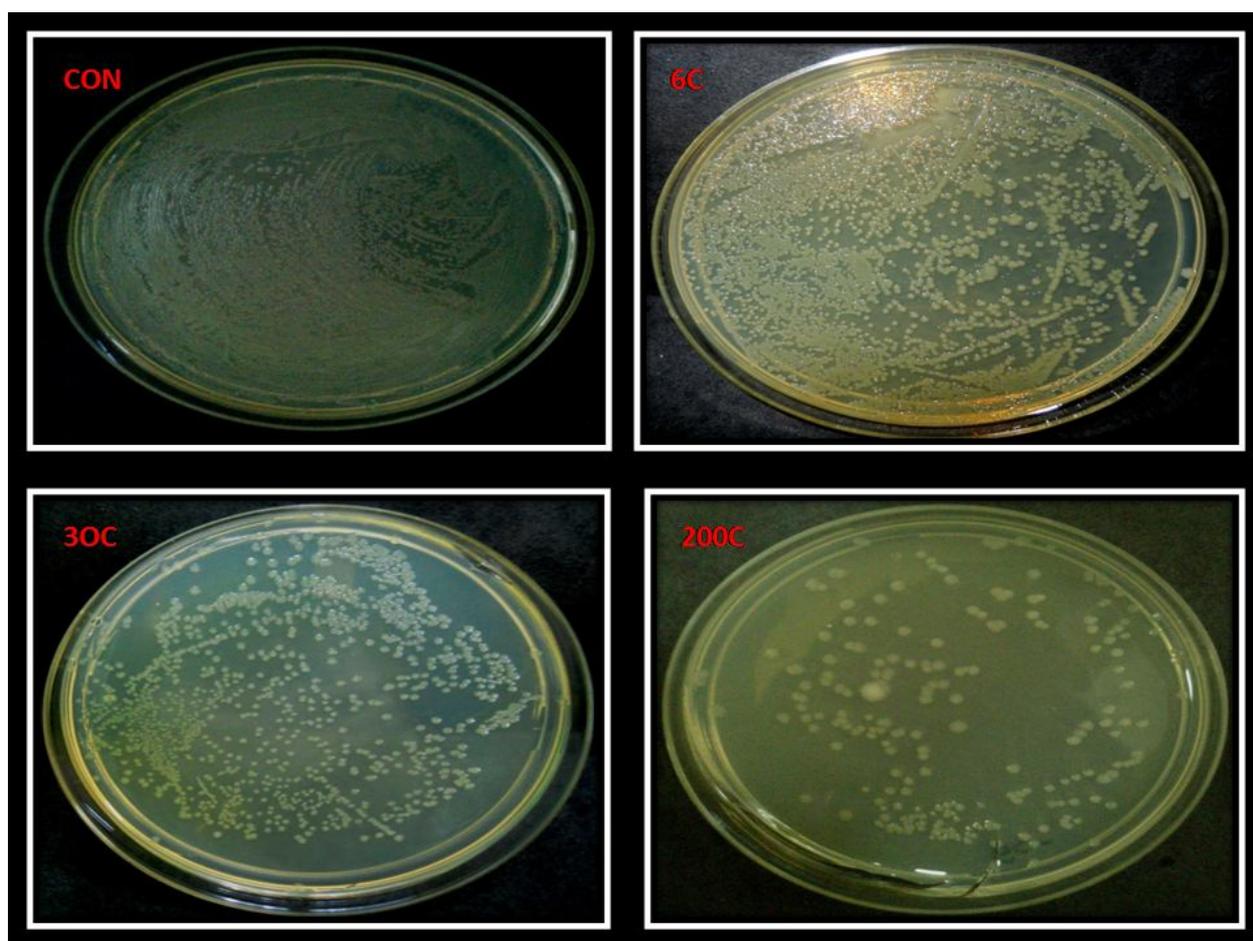


Fig. 5: Antibacterial activity of *Aconitum napellus* on *E.coli* at three different potencies

The drug being hydrophobic in nature has a natural tendency to penetrate in the lipid moiety of the membrane [22] and as the size of the drug-aggregates decrease with increase in potency [10], the effect is expected to be more prominent at higher potencies.

With respect to that, in figure 5 we found images of antibacterial activity of the aconitum napellus on gram negative bacteria *E.coli*. Here, 200C potency of the drug showed maximum inhibition effect on the gram negative bacteria, which also signified that more the potency, more will be the antibacterial effect of aconitum napellus on *E.coli*.

E. coli is a gram negative bacteria and has thin peptidoglycan layer. So secondary metabolites can either penetrate the cell wall and inhibit central dogma pathway or rupture the cell wall and inhibit the growth of the bacteria. The entry of the drug being more at higher potency, we observe higher antibacterial effect in case of *E.coli*.

However, in case of *S. aureus*, a gram positive bacteria they have thick peptidoglycan layer and they can produce potent protein toxin and express cell surface protein that bind and inactivate antibody and other foreign materials. As *Aconitum napellus*, contains many secondary metabolites, namely aconitine, mesaconitine, hyaconitine, jesaconitine

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and some of them are toxic in nature, so perhaps the gram positive bacteria detects the toxic and express cell surface protein that bind with the toxic metabolite and inactivates it. That is why the drug has restricted penetration through the membrane of the gram positive bacteria, causing insignificant effect.

V.CONCLUSION

The study indicates that with increase in potency, there is formation of nano sized molecular assemblies of the drug *Aconitum napelles*, the so called nanocomposites which are responsible for the observed changes in the physicochemical and biological properties at high dilution:

VI.ACKNOWLEDGEMENT

We gratefully acknowledge receiving the freshly prepared drugs at different potencies from Dr. D. S. Bhar. Stimulating discussions with Dr. A. Singh, Dr. T. Yinin, Dr. D. S. Bhar, Dr. A. Kar, Dr. N. Bala and Dr. S. Manna are gratefully acknowledged.

Funding and Support: No grant was available for conducting this work. Equipment facilities were obtained from different departments of the University and neighbouring Institutes.

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