CHARACTERIZATION OF ARSENIC TRIOXIDE NANOPARTICLES IN ULTRA-DILUTIONS OF ARSENICUM ALBUM AT 12C, 30C AND 200C STRENGTHS USING RAMAN SPECTROSCOPY TECHNIQUE

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ABSTRACT

In recent researches, scientists have evidenced that Arsenic trioxide nanoparticles in prepared magnetic materials exhibited therapeutic effects and also explored the efficacy of Arsenic trioxide nanoparticles for the treatment of various disease conditions. Previous studies also show that the prepared ultra-dilutions of Arsenicum album generate nanoparticles which exhibit antimicrobial properties at nano-level. In Homoeopathic ultra-dilutions, the pharmacological actions can be illustrated by the affinity of generated nanoparticles to the target organs. This was scientifically proven through various methods such as spectroscopic, conductometric, thermoluminescence and model simulations. This study aims at characterizing ultra-dilutions of Arsenicum album in three different strengths, namely, 12C, 30C and 200C, using Raman spectroscopy technique. The prepared samples are characterized using EzRaman-N-Analyzer system. Various scattering profiles obtained by the Raman analysis of ultra-dilutions of Arsenicum Album in three different strengths suggest that the number of molecules acting as Raman scattering points increase with serial dilution. The study conclude that ultra-dilutions of Arsenicum Album possess unique characteristics exhibiting their uniqueness at nanoscale in three different scales in unique fashion which can be ascribed to their unique physical and chemical properties arising due to the process of potentization, evident from the results obtained from Raman spectroscopy.

KEYWORDS: Arsenicum album, Characterization, Homoeopathy, Raman Spectroscopy, Ultra dilutions

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INTRODUCTION:

Arsenic trioxide is very useful in treating malignant and tumorous conditions though it is very toxic with severe side effects when used unjudiciously. The reach of these nanoparticles to the targeted spot in the body can be attributed to their cytotoxic effects on cells of healthy body.\(^{(1)}\) The effects produced by Arsenic trioxide nanoparticles in Arsenicum album in lower molecular doses are totally opposite to that produced by high molecular doses as a result of the potentization process. The results are consistent with the observations obtained from a study conducted.\(^{(2)}\)

The average diameter of Arsenic trioxide nanoparticles along with drug loading, entrapment efficiency and zeta potential have been estimated through many studies and scientists have found that these nanoparticles can be used in biomedicine.\(^{(3)}\)

One such study was conducted in which elemental analysis of Ars alb 30C (a Homoeopathic medicine) was done to determine absolute concentration of Arsenic element and whether any impurity was present in the medicine and proved that the concentration levels of the Arsenic element were well below the limit defined by WHO in drinking water and that it can be used as medicine without side effects.\(^{(4)}\)

Another study was conducted in which Arsenicum album (a homoeopathic medicine) possesses great potential for ameliorating arsenic induced elevated ANA titre and other haematological toxicities by producing relevant changes in haematological parameters.\(^{(5)}\)

Studies have been conducted previously to study the vibrational changes at ultra-molecular level and correlating them with the electrical properties in ultra-dilutions at different strengths using Raman spectroscopy technique\(^{(6)}\). Formation of colloidal nano-bubbles including various gases through Temperature-pressure process in ultra-dilutions were illustrated using this technique. This supports a particular phenomenon which is known as “epitaxy”\(^{(7)}\). Raman spectroscopy is a non-destructive promising technique especially used for analysis in pharmaceutical industries.\(^{(8)}\) It provides specific information regarding the biochemical composition of the sample to be analysed.\(^{(9)}\)

In this study, I would like to prove that ultra-dilutions of Arsenicum album (a homoeopathic medicine) contain nanoparticles of Arsenic trioxide in three different scales of
dilution (12C, 30C, 200C), prepared according to method advised by Dr. Samuel Hahnemann (Founder of Homoeopathic system of medicine), using Raman spectroscopy technique.

MATERIALS AND METHODS

This is an experimental study done to characterize Arsenic trioxide nanoparticles in ultra-dilutions of Arsenicum album in three different strengths (12C, 30C and 200C).

Method of Preparation of Sample for Characterization

In nanotechnology, two strategies are generally employed for fabricating materials at the nanometre scale. They are ‘Top – down’ and ‘Bottom – up’ methods. The Top – down approach produces nanoparticles by deconstructing larger materials with the use of lithographic tools which is employed in this study.  

In Top-down technique, the substances are broken down at nano-level and includes wet-milling and homogenisation under high pressure. This technique provides high fidelity and high controllability to be used as “biomedicines”.

Brief of Procedure

The procedure for preparation of samples starts with 9.9g of sugar of milk taken and divided into three parts in the ratio of 1:3:5. First part of sugar of milk was added with 0.1g of Arsenic trioxide fine powder and mixed well and triturated thoroughly for 6 min. It is then scraped off from mortar and pestle with spatula and stirred well for 4 min. The mixture was then rubbed with pestle for 6 min and again stirred 4 min. Second part of sugar of milk was added and same procedure of rubbing and stirring was followed after which third part of sugar of milk was added. Same procedure of rubbing and stirring was followed and 1st potency (1C) prepared. Same procedure if followed for subsequent potencies upto 3C.

Cork fitted in a phial was removed and 0.065g (1 grain) of 3rd trituration of drug was put into the phial. 50 minims (3.1ml) of purified water was poured and stirred for the substance to dissolve after which 50 minims (3.1ml) of dilute alcohol was added and corked. 2 strokes are given and fourth potency was prepared.1minim (0.062ml) of the fourth potency and 99 minims (6.14ml) of alcohol was added and 2 succussions are given after which 5th potency was prepared.1 minim (0.062ml) of the preceding potency was added to 99 minims (6.14ml) of alcohol to prepare the subsequent potencies. Same procedure followed till the preparation of 12C, 30C and 200 scales of dilution (Fig 1).
Fig 1: Preparation of Ultra-dilutions of Arsenicum album upto 200th potency.

Outcome assessment

Characterization of Arsenicum Album

In the typical Raman analysis experiment the Arsenicum Album samples with varying dilution including 12C,30C and 200C were taken in a pyrex cuvette and subjected to raman scattering using a laser source with photon beam having 785nm wavelength with a 300mW power and 7 cm$^{-1}$ spectral resolution and the scattering output was detected using the photodetector kept at -30°C. The EzRaman-N-Analyzer system was used to perform the experiments.
RESULTS AND DISCUSSION

The results (Fig 2-5) revealed that the various scattering profiles obtained by the raman analysis of ultra-dilutions of Arsenicum Album 12C, 30C and 200C. The obtained results suggest that the number of molecules acting as the raman scattering points increases with serial dilution. The resolved raman spectra shown in Fig (3), (4) and (5) at various wave length clearly indicates that the raman scattering intensity increases with dilution process.

![Fig 2: Raman spectra of various ultra-dilutions of Arsenicum album](image-url)
Fig 3: Raman spectra of various ultra-dilutions of Arsenicum album in the range of wave numbers 850 nm to 950 nm

![Raman spectra of various ultra-dilutions of Arsenicum album in the range of wave numbers 850 nm to 950 nm](image)

Fig 4: Raman spectra of various ultra-dilutions of Arsenicum album at wave numbers 1065 nm and 1107 nm scattering points

![Raman spectra of various ultra-dilutions of Arsenicum album at wave numbers 1065 nm and 1107 nm scattering points](image)

Fig 5: Raman spectra of various ultra-dilutions of Arsenicum album at wave number 1493 nm

![Raman spectra of various ultra-dilutions of Arsenicum album at wave number 1493 nm](image)
Studies have been conducted previously on ultra-dilutions of Arsenicum album which revealed the presence of microparticles along with presence of measurable silicon.\(^{(14)}\) Certain findings from researches prove that the inflammatory action of ultra-dilutions of Arsenicum album can be attributed to its certain independent effects other than that of silica.\(^{(14)}\) Another evidence was that the ultra-dilutions of Arsenicum album beyond Avogadro’s constant produced physiological changes on cells which confirms the theory of hormesis, though there are no particles of the original substance present.\(^{(15)}\) This was supported by the fact that the molecules of active substances act as nucleation centres as they amplify the formation of supramolecular structures and imparting order to the solvent\(^{(16)}\).

Raman spectral analysis was an effective technique especially in comparative studies to monitor the subtle effects produced at the molecular level after inducing nanoparticles.\(^{(17)}\) Each potency leaves its signature on Raman spectra.\(^{(18)}\) Studies have been conducted previously on Arsenic trioxide using Raman spectroscopy technique to study its reaction at nano-level in a 30 molar KOH hydroflux at very high temperature (200 degree celsius) and the research showed that Arsenic trioxide acts as electron donor and that it was being oxidized to Arsenate.\(^{(19)}\)

The present study was intended to characterize nanoparticles in ultra-dilutions of the Arsenicum album at three different scales of dilution (12C, 30C and 200C) prepared by method advised by Dr. Samuel Hahnemann.

The intensity of the Raman scattering was proportional to this polarizability change. Therefore, the resultant Raman spectrum or the scattering intensity was a function of the frequency shifts and depends on the rovibronic states of the molecule. The above figures depict various scattering profiles obtained by the Raman analysis of ultra-dilutions of Arsenicum album 12C, 30C and 200C. The obtained results suggest that the number of molecules acts as the Raman scattering points increases with serial dilution. This may be due to the formation of large number of nano supra molecular scattering clusters in Arsenicum album due to the serial dilution process. For the first time in the world we experimentally validated the presence of Raman active supra molecular clusters in Arsenicum album 12C, 30C and 200C. This confirms that the ultra-dilutions have different physicochemical properties due to its supramolecular chemistry which was evident from the intensity variation in Raman profiles of the samples subjected to analysis at 785nm scattering wave length. The resolved Raman spectra shown in figure 3, 4 and 5 at various wave length clearly indicates that the Raman scattering intensity increases with dilution process. It was
due to the increase in Raman active supramolecules in Arsenicum album during ultra-dilution process. It was proposed that the nanomolecular environment of the constituents present in Arsenicum album was highly influenced by the hydrogen bonding and other rovibronic states of the molecules present in the samples. This was the first report in the world which experimentally validate the unique physicochemical properties of the material constituent present in ultra-dilutions of Arsenicum album using Raman analysis.

CONCLUSION

Even though we recorded the intensity variation of Raman profiles of constituent molecules present in Arsenicum album, we have to do fingerprinting of Raman modes of Arsenic oxide, water, ethanol, hybrid systems and its supra molecular functional derivatives using higher end resolved Raman spectroscopic analysis methods.

In conclusion we report a novel characterization and standardization approach using Raman spectroscopic methods to scientifically prove that the ultra-dilutions of Arsenicum album 12C, 30C and 200C have individual physicochemical characteristics and supramolecular chemistry.

This may be the reason for using these dilutions for different clinical indications based on individual susceptibility and symptomatology in human subjects during therapeutic interventions. It can be ascribed to its unique biochemical or biophysical functionalities which may arise due to its unique supramolecular characteristics evident from the Raman profiles. More extensive studies have to be carried out to explore further aspects and confirmation of molecular entities present in Arsenicum album for reinstating systems of homeopathy as an advanced system of personalized precision medicine.

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CONFLICT OF INTEREST

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There was no conflict of interest.

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